Recycling of Domestic Wastewater Treated by Vertical-Flow Wetlands for Irrigating Crops

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ABBREVIATION

Al	Aluminium
ANOVA	Analysis of Variance
APHA	American Public Health Association
As	Arsenic
B	Boron
Be	Beryllium
BOD_5	Biochemical Oxygen Demand after five days
Ca	Calcium
Cd	Cadmium
CFU	Colony Forming Unit
Cl	Chlorine
Co	Cobalt
COD	Chemical Oxygen Demand
COSHH	Control Substances Hazardous to Health
Cr	Chromium
Cu	Copper
CWs	Constructed Wetlands
DW	Deionised water
Ec	Electrical conductivity
EC	European Communities
E-Coli	*Escherichia Coli*
ET	Evapotranspiration
FAO	Food and Agricultural Organisation
FC	Faecal Coliform
Fe	Iron
FWSFCWs	Free Water Surface Flow Constructed Wetlands
H^+	Hydrogen ion
HCO_3	Bicarbonate
HF	Horizontal flow
HFCWs	Horizontal flow Constructed Wetlands
HNO_3	Nitric acid
HRT	Hydraulic retention time
IBM SPSS	International Business Machine Statistical Package for Social Sciences
ICP	Inductively Coupled Plasma
IPCC	International Panel on Climate Change
IWA	International Water Association
K	Potassium
Li	Lithium
Mb	Molybdenum
Mg	Magnesium
MHPRC	Ministry of Health of the People's Republic of China
Mn	Manganese
N	Nitrogen
n.a	Not applicable
n.d	Not detected
Na	Sodium

NaOCl	Sodium hypochlorite
NF	Nano Filtration
NH_4-N	Ammonia-Nitrogen
Ni	Nickle
nm	Not measured
NO_3-N	Nitrate-Nitrogen
NTU	Nephelometric Turbidity Unit
µs/cm	Microsiemens per centimetre
mg/l	Milligrams per Litre
P	Phosphorus
PAHs	Polycyclic Aromatic Hydrocarbons
Pb	Lead
pH	Power of Hydrogen
PO_4-P	Otho-phosphate-phosphorus
PSDS	Product Safety Data Sheet
RO	Reverse Osmosis
RZM	Root Zone Method
SAR	Sodium Adsorption Ratio
Se	Selenium
SF	Surface Flow
SFCWs	Surface Flow Constructed Wetlands
SLR	Surface Loading Rate
SO_4	Sulphate
SS	Suspended Solids
SSF	Subsurface Flow
SSFCWs	Subsurface Flow Constructed Wetlands
TC	Total Coliform
TDS	Total dissolved Solids
TN	Total Nitrogen
TP	Total Phosphorus
TPH	Total Petroleum Hydrocarbons
TSS	Total suspended solids
TW	Tap water
TW+F	Tap water spiked with fertiliser
TW+WW	Tap water spiked with tap water
UK	United Kingdom
UNESCO	United Nations Educational, Scientific and Cultural Organization
UOW	University of Wisconsin
USA	United States of America
USEPA	United States Environmental protection Agency
VF	Vertical Flow
VFCWs	Vertical Flow Constructed Wetlands
WB	World Bank
WW	Wastewater
Zn	Zinc

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Publications in Terms of Google Scholar Citations (14 January 2017; http://scholar.google.co.uk). Total citations: 35. H-Index: 4. I10-Index: 1

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EXECUTIVE SUMMARY

Due to water scarcity in many semi-arid countries, there is considerable interest in recycling various nutrient-rich wastewater streams, such as treated urban wastewater, for irrigation in the agricultural sector. The aim is therefore to assess if domestic wastewater treated by different sustainable wetland systems (some contaminated by diesel spills) can be successfully recycled to irrigate commercially grown crops such as Sweet Pepper (California Wonder; cultivar of *Capsicum annuum* Linnaeus Grossum Group) and Chilli (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') grown either in compost or sand within a laboratory environment. The objectives were to assess the suitability of the irrigation water for long-term growth when using recycled wastewater, the impact of different treated wastewaters as a function of the wetland type, the impact of treated wastewater volume for irrigation, the suitability of different growth media for vegetable growth irrigated with treated wastewater, the effect of a diesel oil spill on the suitability of the recycled wastewater for vegetable irrigation, the economic return of various experimental systems in terms of marketable yields, the impact of differently treated wastewater on soil and fruit mineral and microbial contamination as a function of the wetland type as well as its operation and management, and the possibility of regenerating *Capsicum annuum* using the mother plant's seed and irrigation with recycled wastewater treated by constructed wetlands to obtain a new cultivar adapted to urban wastewater. Vertical-flow constructed wetlands treated the domestic wastewater well, meeting the irrigation water quality standards for most water quality parameters with exception of phosphorus, ammonia-nitrogen, potassium and total coliforms, which showed high values significantly ($p < 0.05$) exceeding the thresholds set for irrigation purposes.

The growth of both Sweet Pepper and Chilli fed with different treated and untreated wastewater types was assessed. A few plants suffered from either a shortage and/or excess of some nutrients and trace minerals. The overall growth development of Sweet Peppers was poor due to the high concentrations of nutrients and trace minerals. However, a high Sweet Peppers yield in terms of economic return (marketable yield expressed in monetary value) was linked to raw wastewater and an organic growth medium, while the plants grown in organic medium and irrigated with outflow from wetlands of large aggregate size, high contact and resting times, diesel-spill contamination and low inflow loading rate produced the best fruits in terms of their dimensions and fresh weights, indicating the role of diesel in reducing too-high nitrogen concentrations. In contrast, Chillies did reasonably well but the growth of foliage was excessive and the harvest was delayed. High Chilli yields in terms of economic return were associated with tap water and an organic growth medium, and a wetland with a small aggregate size and short contact time and long resting time with a low inflow loading rate, while the best fruit quality in terms of length, width and weight was observed for plants grown in organic media and irrigated with outflow water from wetlands containing small aggregates with long contact and resting times and fed with a high inflow loading rate (undiluted wastewater), releasing more nutrients into their effluent resulting in a greater marketable profit. Low fruit numbers correlated well with inorganic growth media. Filters contaminated with hydrocarbon were usually associated with a substantially lower Chilli marketable yield than those filters lacking hydrocarbon pollution. Chilli generations were grown successfully when using wastewater treated by constructed wetlands and organic soil. High Chilli generation yields in terms of economic return were associated with wetlands containing small aggregates with long contact and resting times and fed with a high inflow loading rate (undiluted wastewater), releasing more nutrients into their effluent producing the best fruit quality in terms of length, width and weight resulting in a greater marketable profit. Chilli generation plants were grown with considerably shorter heights and produced abundant fruit numbers which were harvested earlier than their mothers due to the reduction of irrigation water volume applied on them compared to their mothers. However, excessive nutrients applied on mother plants via irrigation water resulted in better fruit quality in terms of dimensions and weights compared with their generations, leading to a greater marketable profit. Findings indicate that nutrient concentrations supplied to the crops by a combination of compost and treated wastewater are usually too high to produce a good harvest. However, as the compost was depleted of nutrients after about ten months, the harvest increased for pots that received pre-treated wastewater. The productivity of crops in terms of harvest was independent of the wastewater consumption volume, but may have depended on the water quality. A high yield was related to the most suitable provision of nutrients and trace elements. The mineral content of the organic soil was significantly higher than that for the inorganic soil, before and after irrigation with treated wastewater. No substantial mineral contamination was observed in the soils due to irrigation with treated wastewater. Slight to moderate zinc contamination was detected in harvested fruits based on common standards for vegetables. No bacterial contamination was detected for fruits harvested from plants irrigated with wetland outflow water. In contrast, fruits harvested from those plants irrigated with preliminary treated wastewater showed high contamination by total coliforms, *Streptococcus* spp. and *Salmonella* spp., especially for fruits which were located close to the contaminated soil surface. However, findings indicate that vegetables receiving wastewater treated with wetlands can be considered as safe compared to those receiving only preliminarily treated wastewater. The project contributes to ecological sanitation understanding by closing the loop in the food and water chain. Findings will lead to a better understanding of the effects of different wetland treatment processes on the recycling potential of their outflow waters.

CHAPTER 1

INTRODUCTION

1.1 Overview

This chapter gives an introduction and overview of the water scarcity problem around the world and discusses the factors contributing to this problem. The introduction also provides arguments for using alternative water resources to alleviate this problem. Furthermore, the rationale, aim and objectives of the study, as well as the outline of the research, are presented.

1.2 Background and motivation

Globally, fresh water scarcity is a developing problem and natural water resources are becoming inadequate to fulfil demand. This problem is present all over the world e.g. southern Europe, the Middle East, Australia, the southern states of the USA and North Africa.

According to Kivaisi (2001), rainfall is the main water source around the world which produces around 40,000 to 45,000 km^3 every year supporting the rapidly increasing population, which is expected to increase by 85 million yearly as reported by Stikker (1998), leading to decline in water supply and subsequently to water conflicts. According to Alcamo, Döll, Kaspar, and Siebert (1997) and Alcamo, Henrichs, and Rösch (2000), 1.8 billion people will experience absolute water scarcity, and two thirds of the world will be living under water-stressed conditions by 2025, while almost half the world will live under conditions of high water stress by 2030 (Scheierling et al., 2011).

Moreover, about 80 countries around the world are expected to be suffering from serious shortage in water supply every year (Gleick, 1993). According to Stikker (1998), the number of countries facing water scarcity during the last four decades, most of which are developing countries, is expected to increase to 34 by the year 2025 (Table 1.1).

Table 1.1: Countries experiencing water scarcity in 1955, 1990 and 2025 (projected), based on availability of less than 1000 m^3 of renewable water per person per year (adapted from Stikker (1998))

Countries in water scarcity category			
In 1955	In 1990	By 2025 under all UN population growth projections	By 2025 only if they follow UN medium or high projections
Malta	Qatar	Libya	Cyprus
Djibouti	Saudi Arabia	Oman	Zimbabwe
Barbados	United Arab Emirates	Morocco	Tanzania
Singapore	Israel	Egypt	Peru
Bahrain	Tunisia	Comoros	
Kuwait	Cape Verde	South Africa	
Jordan	Kenya	Syria	
	Burundi	Iran	
	Algeria	Ethiopia	
	Rwanda	Haiti	
	Malawi		
	Somalia		

In addition to human population growth, industrial and agricultural activities expansion, global warming and climate changes are other reasons contributing to the water scarcity problems in many regions worldwide. However, the present situation of water scarcity in the world is mainly due to the forces of increasing population and economic development (Huang & Xia, 2001).

This is especially evident for the world's fastest growing cities which typically are located in low-income developing countries and characterised by poor water infrastructure and unsatisfactory wastewater treatment (Varis & Somlyódy, 1997).

As the population increases, the need for food and water will continually grow.
As a result, the actual consumption of water will quickly approach the limits of the resources available and, subsequently, agricultural land will become rare (FAO, 2003). This will be the main factor limiting development and consequently will be a major economic, social, and political challenge in such regions.

Furthermore, climate change has the potential to impose additional water resources pressures in some regions. The rise in temperature associated with climate change leads to a general reduction in the proportion of precipitation falling as snow, and a consequent reduction in many areas in the duration of snow cover.

This has implications for the timing of streamflow in such regions, with a shift from spring snow melt to winter runoff (Arnell, 1999). As a result, significant reductions in precipitation, or major alterations in the timing of wet and dry seasons may occur in some regions of the world. The second assessment report of the Intergovernmental Panel on Climate Change (IPCC) cautioned that global warming would lead to increases in both floods and droughts (Houghton, 1996).

However, many environment, economy and society aspects are dependent upon water resources and changes in the hydrological resource which may severely affect environmental quality, economic development and social well-being (Alcamo et al., 1997). Climate change, however, is just one of the pressures facing water resources and their management over the next few years and decades (Stewart, 2012).

Generally, there are both supply-side and demand-side pressures. The supply-side pressures include climate change (reducing or increasing the amount of water available), and also include environmental degradation, for example the accumulation of organic and inorganic pollutants resulting from different sources, such as domestic, agricultural and industrial, in the surface water, ground waters and plants, leading to degradation in water quality which negatively impacts the receiving ecosystem (Ijeoma &Achi, 2011).

On the other hand, the demand-side pressures include population growth, leading to increased demands for domestic, industrial and agricultural (particularly irrigation) water resulting in sharply increased in wastewater characteristics such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), turbidity, and increase in discharge of various types of pollutant such as: nitrogen compounds (i.e. ammonia nitrogen and nitrates), petroleum hydrocarbons, heavy metals like cadmium, chromium, nickel, lead, copper and zinc, and microbes (faecal coliform, *E-Coli* and salmonella).

These pollutants will cause deterioration in water quality in the receiving water course making these sources are unsuitable for drinking, irrigation and aquatic life. However, climate change may affect the demand side of the balance as well as the supply side (Arnell, 1999).

Due to this water scarcity problem around the world, it is essential to think about non-conventional water resources for satisfying the increased rates of demand for fresh water. Some countries around the world have made significant steps toward desalination of seawater for meeting the urban demands for their people.

However, desalination methods require large amounts of energy, which is costly both in environmental pollution and in money terms, making this technology limited for domestic purposes (Karagiannis & Soldatos, 2008). Use of different natural water resources, like river, rain, and drainage water or drainage water blended with fresh water, are other alternative options for irrigation purposes in many countries (Pedrero, Kalavrouziotis, Alarcón, Koukoulakis, & Asano, 2010).

Moreover, wastewater is concluded as an available alternative option to overcome the shortage in water supply resulting from previous discussed reasons, particularly population growth (Bichai, Polo-Lopez, & Ibanez, 2012; Noori, Mehdi, & Norozi, 2013, 2014; Almuktar & Scholz, 2015).

However, due to the varying nature of wastewater (in terms of mineral load, organic and biological constituents) the reuse of such water should be monitored regularly to assess potential risks which may affect the whole environment (FAO, 2003).

Inadequate provision of sanitation and wastewater disposal facilities leads to environmental and public health problems, with around 1.8 million people dying every year from several related diseases (Nellemann, Baker, Bos, Osborn, & Savelli, 2010).

Adequate reuse of wastewater is a necessity to protect public health, the environment and water resources. Direct disposal of untreated wastewater to land and water bodies has a negative impact on human health (Khurana & Pritpal, 2012).

Because of this, wastewater treatment and recycling methods will be vital to provide sufficient fresh water in the coming decades, since our water resources are limited (FAO, 2003) Wastewater reclamation, recycling, and reuse has evolved due to the increasing of pressure on water resources.

The feasibility of producing the specific quality of the reclaimed water to fulfil multiple water use objectives is now of real importance (Asano & Levine, 1996). Understanding the principles of urban wastewater reuse as an alternative and reliable source of water supply and analysis of the cost of wastewater reclamation are essential (Asano, 1994; Mujeriego & Asano, 1999).

1.3 Purposes of treated wastewater reuse

The treated wastewater produced as effluent from sewage systems of urban communities represents another non-conventional renewable water source, which could be an attractive and cheap option to be used for several purposes including agricultural land irrigation, aquaculture, landscape irrigation, urban and industrial uses, recreational and ecosystem uses, and artificial recharging of ground water (Asano, Burton, Leverenz, Tsuchihashi, & Tchobanoglous, 2007).

More than 70% of water over all the world is consumed for irrigation purposes (UNESCO, 2003). Therefore, the application of treated wastewater for agricultural irrigation has much potential (Meda & Cornel, 2010), especially when incorporating the reuse of nutrients like nitrogen and phosphorous in particular, which are important for plant production (Norton-Brandao et al., 2013).

Furthermore, the use of wastewater for irrigation purposes is another non-conventional water resource option which is widely implemented in lower income countries and in arid and semi-arid high income countries due to the high stress on water resources (Smit, Nasr, & Ratta, 1996; WB, 2000; FAO, 2003).

Estimation studies of using wastewater for agricultural use around the world indicate that around 20 million hectares of agricultural land is irrigated by both treated and untreated wastewater (Jiménez & Asano, 2008). The use of wastewater for agricultural purposes is by far the most established application, and the one with the longest tradition.

Since approximately, 70% of world water use, including all the water diverted from rivers and pumped from underground, is used for agricultural irrigation (Pedrero et al., 2010) then the reuse of treated wastewater for purposes such as agricultural and landscape irrigation will reduce the amount of water that needs to be extracted from natural water sources as well as reducing discharge of wastewater to the environment.

Furthermore, the proper management of reusing wastewater in agriculture could reduce the overall toxicity to both soil and crops as well improve the water resources shortages

(FAO, 2003). Treated wastewater reuse in agriculture is a common practice in the Mediterranean countries and other arid and semi-arid regions which are confronting increasing water shortages, supporting renewable agriculture and food systems. Also there is considerable interest in the long-term effects of treated wastewater on crops planned for human consumption (FAO, 2003; Pedrero et al., 2010).

1.4 Technologies applied in wastewater treatment and reuse for irrigation

Conventional wastewater treatment systems are energy intensive and include mechanical treatment components which require heavy investment and incur high operational costs.

Studies have shown that existing wastewater treatment systems in most of the developing countries failed to treat wastewater adequately because of high maintenance costs, lack of local expertise and poor governance (Mustafa, 2013). Moreover, the current water sources are contaminated because of the discharging of untreated sewage and industrial wastewater into surface waters resulting in water quality deterioration and contamination of drinking water sources which adversely impacts irrigation, fish production and recreation (Kivaisi, 2001).

Based on that, water pollution is one of the main threats to public health especially in developing countries. Therefore, it is important to protect the existing water sources by treating wastewater discharges from human activities and to reuse wastewater to combat water scarcity.

Developing a mix of strategies that increase supply, manage demand, and reduce long-term pressures on water is more urgent than ever before, as population pressures are continuing to increase. However, strategies to deal with water shortages depend on local conditions, including topography, the extent of water scarcity, available financial resources, and technical and institutional capacity (Cosgrove & Rijsberman, 2000). It is essential to adopt wastewater treatment technologies which can sufficiently treat wastewater in the long run (such as sustainable technologies).

Combination of high-technology wastewater treatment systems seems inappropriate since it is techno-economically infeasible, as discussed above. Hence, there is a great need to develop suitable, inexpensive and rapid wastewater treatment and reuse techniques in the present century instead of traditional and costly treatment systems (Kumar, Imran, Tawfik, Arunima, & Shilpi, 2012).

Table 1.2 shows the current technologies applied in urban wastewater reuse for irrigation with their treatment abilities in terms of salinity, pathogens, nutrients and heavy metals, since they are considered as the main groups of pollutants of concern in treated effluent (Norton-Brandão, Scherrenberg, & van Lier, 2013). Moreover, the advantages and disadvantages of these technologies are also listed below.

Table 1.2: Wastewater treatment technologies for irrigation purposes (abilities, advantages and disadvantages).

Technology	Abilities			Advantages	Disadvantages
	Salinity	Pathogens	Nutrients and heavy metals		
Oxidants					
Sodium hypochlorite (NaOCl)	No electrical conductivity removal (Norton-Brandão et al., 2013)	High bactericidal action (Bixio & Wintgens, 2006)	Nitrate removal (NO_3) of 10% and ortho-phosphate- (PO_4) removal of 18% (Üstün, Solmaz, Çiner, & Başkaya, 2011)	Low operating costs (Bixio & Wintgens, 2006)	High operability, high formation of by products, moderate investment costs (Bixio & Wintgens, 2006)
Ozone	-	High bactericidal action (Bixio & Wintgens, 2006)	-	Low formation of by-products (Bixio & Wintgens, 2006)	High operability, moderate operating costs, High investment costs (Bixio & Wintgens, 2006)
Ultraviolet treatment	-	High bactericidal action (Bixio & Wintgens, 2006)	-	Low formation of by-products, low operating costs (Bixio & Wintgens, 2006)	High operability, moderate investment costs (Bixio & Wintgens, 2006)
Photo catalysis with TiO_2	-	High inactivation of coliforms (Rojas-Higuera et al., 2010)	-	Likely use of renewable energy in the case of solar photo catalysis, no formation of by-products, use of inexpensive catalysts and facilities (Lydakis-Simantiris, Riga, Katsivela, Mantzavinos, & Xekoukoulotakis, 2010)	Lack of residual bactericidal action and slow kinetic behaviour (Lydakis-Simantiris et al., 2010)

Table 1.2 (cont.)

Technology	Abilities			Advantages	Disadvantages
	Salinity	Pathogens	Nutrients and heavy metals		
Constructed wetlands and ponds	No removal of electrical conductivity (Pedrero, Albuquerque, Amado, Marecos do Monte, & Alarcón, 2011)	Bacterial removal between 1 and 6 log units (Feigin, Ravina, & Shalhevet, 2012)	Ammonia (NH_4) removal > 70%, total phosphorous (TP) removals > 65% (Zhai, Xiao, Kujawa-Roeleveld, He, & Kerstens, 2011); removal in the range of 55% for chromium (Cr) (Arroyo, Ansola, & de Luis, 2010), between 25% and 35% for nickel (Ni), between 25% and 87% for zinc (Zn) and 9% for copper (Cu) (Galletti, Verlicchi, & Ranieri, 2010), 33% for cadmium (Cd) and 75% for cobalt (Co) (Pedrero et al., 2011)	Low maintenance costs and energy usage, no formation of by-products (Brissaud, 2007; Ghermandi, Bixio, Traverso, Cersosimo, & Thoeye, 2007)	Large footprint, efficiency depending on meteorological conditions (Brissaud, 2007; Ghermandi et al., 2007)
Medium filtration	-	Faecal coliform removal between 0.6 and 1.5 log units (Li, Yu, Liu, & Ma, 2012)	Achievement of final concentrations of 5 mg/L of total nitrogen (TN) and 4-10 mg/L of ortho-phosphate-phosphorous (PO_4-P) (Metcalf, 2003)	Low investment costs, low operating costs (Y. Li et al., 2012)	Low removal of faecal coliform (Y. Li et al., 2012)

Table 1.2 (cont.)

Technology	Abilities			Advantages	Disadvantages
	Salinity	Pathogens	Nutrients and heavy metals		
Membranes filtration	Removal or preservation of nutrients according to the pore size; -Reverse osmosis (RO) allows removals of 90% of Electrical conductivity, (Jacob et al., 2010), -Nano filtration (NF) rejects only divalent cations allowing most monovalent ions, which include nutrients, to pass and hardly alters the salinity (Chang, Lee, Oh, & Kim, 2005)	Bacterial removals higher than 5 log units (Lazarova, Savoye, Janex, Blatchley Iii, & Pommepuy, 1999)	Removal of 83% of sodium (Na) and 80% of chlorine (Cl) (Oron et al., 2008) as well as nutrients; removes sodium ions and divalent cations simultaneously (Chang et al., 2005); allows removals in the range of 75% for chromium (Cr) and > 80% for arsenic (As) (Fatone, Bolzonella, Battistoni, & Cecchi, 2005)	Simultaneous disinfection and removal of electrical conductivity (Norton-Brandão et al., 2013)	High investment costs, high operating costs (Lazarova et al., 1999)
Electrolysis	-	Effective disinfection with low current charges (Rodrigo, Cañizares, Buitrón, & Sáez, 2010)	-	Effective in killing a wide spectrum of microorganisms (Drogui, Elmaleh, Rumeau, Bernard, & Rambaud, 2001)	Formation of significant amounts of perchlorates (Bergmann, Rollin, & Iourtchouk, 2009)

Table 1.2 shows that compared to conventional treatment systems, constructed wetlands (CWs) seem to be the technology of the highest ability in terms of pollutants removal and have advantages in terms of low maintenance cost and required energy. Furthermore, constructed wetlands have a strong prospective for application in developing countries (Kivaisi, 2001).

Constructed treatment wetlands are engineered wastewater purification systems that encompass biological, chemical and physical processes, which are all similar to processes occurring in natural treatment wetlands.

They are implemented for environmental pollution control to treat a variety of wastewaters including industrial effluents, urban and agricultural runoff, animal wastewaters, sludge and mine drainage (Sani, Scholz, & Bouillon, 2013; Scholz, 2010; Vymazal, 2011), and petroleum wastewaters (Scholz, 2008; Tang et al., 2010; Wallace et al., 2011; Al-Baldawi et al., 2014; Vymazal, 2014) and have recently been applied successfully to treat domestic wastewater (Scholz, 2010; Dong et al., 2011; Sani et al., 2013; Paing et al., 2015).

Furthermore, constructed wetlands have a higher rate of biological activity compared with conventional wastewater treatment systems which allows conversion of many of the pollutants in the wastewater into non-toxic by-products or essential nutrients that can be reused for additional biological activity.

Constructed wetlands have been used for secondary and also in some cases for tertiary levels of treatment and reuse. For example, they have been successfully used to treat wastewater to meet standards developed by regulatory bodies (Kadlec & Wallace, 2008). In developed countries there is a motivation to control micro-pollutants in wastewater while developing countries are still struggling to control macro-pollutants (organic material, nutrients and pathogens). However, constructed wetlands have been shown to successfully control organic material, nutrients and pathogens (Mustafa, 2013).

Constructed wetland technology is a viable option that not only reduces nutrients but also has a role in disinfection, rendering the treated wastewater to be used as a resource to irrigate crops, playing arenas, gardens or golf courses. Constructed wetlands are accomplishing distinction as an active and low cost alternative for treatment of wastewater in both the developed and developing world (Greenway, 2005). Recently, some large-scale wetland systems have also been successfully applied to treat domestic wastewater (Dong, Wiliński, Dzakpasu, & Scholz, 2011).

According to Belmont et al. (2004) and Wang et al. (2005), treatment of urban wastewater using wetlands technology has been reported to be suitable for irrigation of plants due to meeting the specification of national guidelines. Moreover, constructed wetland systems showed high efficiency in removing most contaminants in domestic wastewater including chemicals (organic materials, heavy metals and trace elements, etc.) and microorganisms (bacteria, viruses, parasites, etc.) as reported by Kivaisi (2001) and Gross et al. (2007).

1.5 Selection of plants irrigated with treated wastewater

Many vegetables have the potential to grow well on recycled wastewater. However, there is the potential for some vegetables, such as lettuce and cabbage, to become contaminated by microbes, because their edible leaves are too close to the ground receiving the treated wastewater. Moreover, accumulation of heavy metals and trace elements in the soil irrigated with wastewater will present a high risk of mineral contamination of grown vegetables, such as carrots and potatoes.

Therefore, it makes sense to select vegetables where the edible fruit is located far away from the ground. This may include peppers, tomatoes, maize, eggplants, beans, lentils and peas. Moreover, the amount of required irrigation water should be considered when choosing the plants to be irrigated with wastewater. Therefore, choosing plants which can be grown in low water consumption is highly recommended.

The next step in selecting suitable vegetables is to decide on easy-to-grow and relatively cost-effective plants with high nutritional value. Finally, the environmental conditions for growing the selected crops should be considered to achieve the best results in terms of growth and production (FAO, 2003). Many vegetables may fit these conditions in particular geographical settings, such as Chillies and Sweet Peppers.

1.6 Justification, aim and objectives

There are many studies involving irrigation of plants with different treated and untreated wastewater (Yang H X, 2002; Cheng X J, 2003; Chen Y et al., 2004; Domłnguez-Mariana E et al, 2004; Huang Y Y et al., 2005; Jun-Feng et al., 2007; Jiménez & Asano, 2008).

However, most research on irrigation with treated wastewater deals with traditional treatment processes which are known to be highly cost effective (Pollice et al, 2004; Oron et al., 2008; Rebhun & Jayakody, 2008; Botti et al., 2009; Hyun & Lee, 2009; Media & Cornel, 2010; Nikaido et al., 2010; Ayni et al, 2011; Batarseh et al., 2011; Cano et al., 2011; Martinez et al., 2011; Mrayed et al., 2011). According to the literature, due to the high efficiency of wetlands in treating wastewater, there is an interest in recycling the effluent for different purposes particularly in the agricultural field (Kivaisi, 2001; Lopez et al., 2006; Masi & Martinuzzi, 2007; Allio et al., 2008; Morari & Giardini, 2009; Cirelli et al, 2012).

Moreover, traces of hydrocarbons from diesel spills associated with urban runoff or industrial effluent are a more recent challenge (Blanchard et al., 2001; Blanchard et al., 2004; Tao et al., 2004; Charalabaki et al., 2005; Busetti et al., 2006; Sanchez et al., 2007; Chung et al., 2008; Xiao et al., 2008; Manoli & Samara, 2009; IARC, 2010; Scholz, 2010; Garcia-Delgado et al., 2012).

Despite the numerous studies on recycling of urban wastewater treated with different technologies for irrigation purposes, there are few long-term and controlled studies involving domestic wastewater due to health and safety concerns. Moreover, there are few studies in the literature on recycling of domestic wastewater treated by wetlands in general and vertical flow ones in particular.

According to Morari and Giardini (2009), there are few studies if any giving attention to long-term evaluation of wetlands effluent suitability, mainly in vertical flow (VF) ones, for irrigation purposes. Moreover, no studies have been undertaken to monitor the impact of different wetland system designs on treated domestic wastewater (contaminated and uncontaminated with diesel) and the subsequent effect on plant growth, productivity and safety in terms of human consumption.

In this study, effluent from different types of wetland systems treating domestic wastewater was selected to irrigate vegetables grown in the laboratory (controlled environmental conditions). Some of the wetlands received standard wastewater while the others received wastewater that was subject to a one-off diesel fuel spill.

The treated wastewater from all wetland types was recycled for the irrigation of Bell Peppers and Chillies, which are commonly seen as two popular, relatively expensive and easy-to-grow vegetables with high nutritional value; also they can be grown in greenhouses in the UK (Nickels, 2012).

Furthermore, some plants were irrigated with other water types for comparison such as deionised water (DW) to check the sufficiency of nutrients in the media for growing the plants, tap water (TW) and tap water spiked with fertiliser (TW+F) as traditional irrigation water sources, tap water spiked with wastewater (TW+WW) considering the wastewater as an organic fertiliser, and raw wastewater (WW) to study the effect of nutrient and element concentrations on plant growth before and after treatment.

This study will provide the scientific justification for integrating treatment wetlands into agricultural food production. Moreover, it will fill gaps in knowledge and understanding by assessing the impact of different wetland (some contaminated with diesel) system designs in terms of their suitability in providing irrigation water for example crops, which should be safe for human consumption, lead to a good economic return and whose corresponding water management should not result in soil contamination.

Therefore, the overall aim of this study is to assess if vegetables can be grown successfully on recycled domestic wastewater treated by constructed wetlands. The corresponding key objectives related to the growing of Sweet Pepper and Chilli is to assess:

- the suitability of the irrigation water for long-term growth when using recycled wastewater,
- the impact of different treated wastewaters as a function of the wetland type,
- the impact of treated wastewater volume for irrigation,
- the suitability of different growth media for vegetable growth irrigated with treated wastewater,

- the effect of a diesel oil spill on the suitability of the recycled wastewater for vegetable irrigation,
- the economic return of various experimental systems in terms of marketable yields,
- the impact of differently treated wastewater on soil and fruits mineral and microbial contamination as a function of the wetland type as well as its operation and management, and

- the possibility of regenerating *Capsicum annuum* using the mother plant's seed and irrigation with recycled wastewater treated by constructed wetlands to obtain a new cultivar adapted to urban wastewater.

1.7 Thesis outline

This thesis report starts by reviewing the problem of water scarcity worldwide and discusses the potential reasons contributing to it. An overview of alternative water resources, such as wastewater, and the possibility of treatment by a wetlands system is reported. In this study, the assessment of treated wastewater for irrigation purposes has been investigated compared to the thresholds.

The impact of treated wastewater using wetlands on growth and productivity of example crops, and the risk of harvest contamination by heavy metals, trace elements and microbes has been studied as well. Moreover, the impact of environmental conditions on productivity and harvest of plants has been investigated. Finally, the potential use of treated wastewater for producing a new cultivar of the examined crops and their safety for human consumption has been studied. This report is divided into the following sections:

- Chapter one overviews background and motivation for the study, purposes of wastewater reuse, technologies for treated wastewater for irrigation use, plants types to be irrigated with treated wastewater, justification, aim and objectives and thesis outline.

- Chapter two contains a comprehensive literature review which includes the latest advances in wastewater recycling for crop irrigation. Constructed wetland components and different classes with their impact on quality of treated wastewater are presented. Moreover, the treated wastewater quality for irrigation in terms of numerous contaminants (sediments, heavy metals and trace elements, microbes, organic materials and salinity) is discussed according to the standards.

- The main portion of this chapter is devoted to the published studies on treating domestic wastewater using wetland systems and the potential reuse for irrigation of different plants. Possibility of contamination different crops irrigated with treated wastewater is also discussed based on previous studies.

- Chapter three gives a description of the materials, tools and experimental set-ups undertaken in this study. This chapter explains the operation methods of the wetland systems in the greenhouse in terms of filter designs and physical arrangement. Moreover, growing vegetables in the laboratory under controlled conditions at different stages is explained. Water, soil and vegetables sampling and quality analysis, calibration of equipments used in the laboratory and statistical methods used in data analysis are presented in this chapter as well.

- Chapter four presents the results of treated water quality analysis using different designs of wetland systems in terms of aggregate size, contact and resting times and inflow loading rate. Moreover, the hydrocarbon contamination in the outflow water of some filters and its impact on the growth of Chilli and Sweet pepper under laboratory conditions is investigated in terms of vegetative growth, fruit production and marketable yield assessment.

- Chapter five discusses the potential contamination of soil and fruits irrigated with recycled domestic wastewater treated by vertical flow constructed wetlands. This study investigates contamination by trace elements, heavy metals and various types of bacteria, transferring via application of treated wastewater, and the potential risk of human consumption and to public health.

- Chapter six presents an experimental work on growing a new generation of crops using seeds produced from original plants irrigated with recycled domestic wastewater treated by vertical flow constructed wetlands.

In this chapter the new generation growth and productivity compared with the mother plants are presented. Moreover, investigation of the possibility of mineral and microbial contamination of the new generation harvest is discussed.

- Chapter seven contains the conclusion and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

Almuktar, S. A. A. A. N, **Abed, S.N., & Scholz, M.** Wastewater treatment and recycling for mitigation of a global water crisis - A critical review. *Water research*

Almuktar, S. A. A. A. N, **Abed, S.N., & Scholz, M.** Wastewater management for irrigation purposes- A critical review. *Agricultural Water Management*

2.1 Overview

This chapter aims at giving a review of the relevant literature that concerns the topic of this study. The main focus of this chapter is on constructed wetlands technology to treat wastewater for subsequent reuse. The standard requirements of treated wastewater for irrigation reuse are discussed in detail. However, this chapter is divided into the following sections: 2.1 introduces the chapter, 2.2 describes constructed wetlands development history, 2.3 talks about constructed wetlands classification, while 2.4 shows the constructed wetlands design and operation. The impact of environmental factors on constructed wetlands behaviour, wetland crucial value, and selection of vertical flow wetlands for treating wastewater are presented in sections 2.5, 2.6, and 2.7, respectively. Moreover, recycling of treated wastewater for irrigation reuse, and standards for irrigation water quality are discussed in sections 2.8 and 2.9. Methods used for irrigation by wastewater with proper selection are presented in sections 2.10 and 2.11, while scheduling of irrigation water amount and selection of crops for irrigation with wastewater are detailed in sections 2.12 and 2.13. Lastly, the potential impacts of wastewater reuse for irrigation are discussed in section 2.14 and the chapter summary is presented in section 2.15.

2.2 Constructed wetlands historical development

Constructed treatment wetlands are engineered wastewater purification systems that encompass biological, chemical and physical processes, which are all similar to processes occurring in natural treatment wetlands. They are implemented for environmental pollution control to treat a variety of wastewaters including industrial effluents, urban and agricultural runoff, animal wastewaters, sludge and mine drainage (Scholz, 2010; Vymazal, 2011; Sani et al., 2013), petroleum wastewaters (Scholz, 2008; Tang et al., 2010; Wallace et al., 2011; Al-Baldawi et al., 2014; Vymazal, 2014;) and domestic wastewater (Scholz, 2010; Dong et al., 2011; Sani et al., 2013; Paing et al., 2015).

According to Kadlec and Knight (1996), the use of natural wetlands as a suitable means for management of wastewater and sewage has been undertaken since 1912. This will subsequently lead to deterioration of such wetlands, due to the accumulation of nutrients, resulting in serious pollution. However, the first study on treatment of wastewater by wetlands planted with macrophytes was undertaken by a German scientist called Kathe Seidel in 1952 who carried out her research in the Max Planck Institute, Germany (Seidel, 1965).

According to Vymazal (2005), during the period from 1952 to 1956, Seidel performed a number of experiments in utilising macrophytes to treat different types of wastewater such as phenol wastewater (Seidel, 1955, 1965, 1966), dairy wastewater (Seidel, 1976) or livestock wastewater (Seidel, 1961). Moreover, in the early 1960s, this German scientist carried out more experiments involving growing wetland plants in wastewater of different originality, attempting to improve the behaviour of different wastewater treatment plants of low efficiency such as septic tanks and pond systems (Vymazal, 2005).

For example, in order to improve the anaerobic system of a septic tank, Seidel combined two methods for sewage to flow in, vertically via an infiltration bed and horizontal by an elimination bed (Seidel, 1965) producing a new system of wetlands known as “hybrid” which were revived at the end of 20^{th} century as reported by Vymazal (2005, 2011a, 2014). However, the vertical flow wetlands begun by Seidel in Germany are considered as the original types according to Cooper et al. (1996) and Vymazal (2005, 2009, 2011a, 2014).

However, following the original design of vertical flow wetland systems, interest in them began to reduce, later recovering after observing their high efficiency in nitrification compared to that of horizontal wetland systems, leading to designers being discouraged from using the latter one. Moreover, in Europe, the horizontal subsurface flow constructed wetlands were reported to be noticeably used more than the vertical ones (Vymazal, 2005, 2014).

Furthermore, a new design of horizontal wetlands system was developed by Seidel and Kickuth in the 1960s using Root Zone Methods (RZM). However, this new design was different from the previous one created by Seidel as it contained a humid substrate mainly of mud. The latter design was firstly used for treating urban sewage in Germany (Kickuth, 1978, 1981; Brix, 1987; Vymazal, 2009).

Investigations have been carried out into the use of constructed wetland systems for treated wastewater in Europe and the United States of America since the 1950s and 1960s, respectively. In the USA, the studies were expanded during the periods of 1970 to 1990 (USEPA, 2000), while in the United Kingdom, the popularity and acceptance of constructed wetlands was recorded in the mid-1980s when water engineers became aware of the root zone method (Cooper et al., 1996).

Moreover, due to the ability of constructed wetland systems in treating wastewater discharged from small communities, water authorities accepted this system in small villages of populations ranging from 50 to 1000 capita. However, this system suffered from numerous problems which needed to be sorted out leading the researchers to pursue the option of subsurface vertical flow systems instead (Cooper et al., 1996).

Nowadays, using wetland systems to treat different types of wastewater is globally widespread as reported by Hoffman et al. (2011); Abou-Elela et al. (2013); Vymazal (2014) and Wu et al. (2014). However, in developing countries, especially those of tropical and subtropical climate conditions like Nigeria and Tanzania, this technology is still unpopular (Neue et al., 1997). This is because of lack of knowledge about the substantial role of wetlands technology in controlling environmental pollution (Kimani, Mwangi, & Gichuki, 2012; Abou-Elela et al., 2013) and the absence of practical knowledge to advance such research technology on the basis of geography (Kivaisi, 2001).

As a result, full understanding of the role of wetlands in controlling environmental pollution and enhancing ecology is essential (Mohamed, 2004; Kamau, 2009; Al-Baldawi et al., 2014, 2015). However, in some developing countries, such as China (Xinshan, Qin, & Denghua, 2010; Meng et al., 2014; Song et al., 2015) and India (Sheoran & Sheoran, 2006; Sharma et al., 2013), the application of wetlands technology has been practised since the 1990s, with increasing advanced research and investigation studies in this technology.

2.3 Constructed wetlands classification

Generally, classification of constructed wetlands is dependent on three main factors: water level in the system, which accordingly categorises the constructed wetlands as free water surface flow (FWSF CWs) or subsurface flow (SSF CWs); macrophytes; and the movement direction of the water in the system (Kadlec & Knight, 1996; Langergraber et al., 2009; Hoffman et al, 2011; Vymazal, 2014). According to the water movement direction in the system, the constructed wetlands may be classified into vertical and horizontal types (Figure 2.1) which could be combined in one single system (hybrid) in order to get high pollutants removal efficiency (Vymazal, 2014). Moreover, constructed wetlands may also be classified according to their objectives into: habitat creation, flood controlling or wastewater purification, as reported in some recent studies (Vymazal 2013a; Vymazal, 2014; Stefanakis et al., 2014).

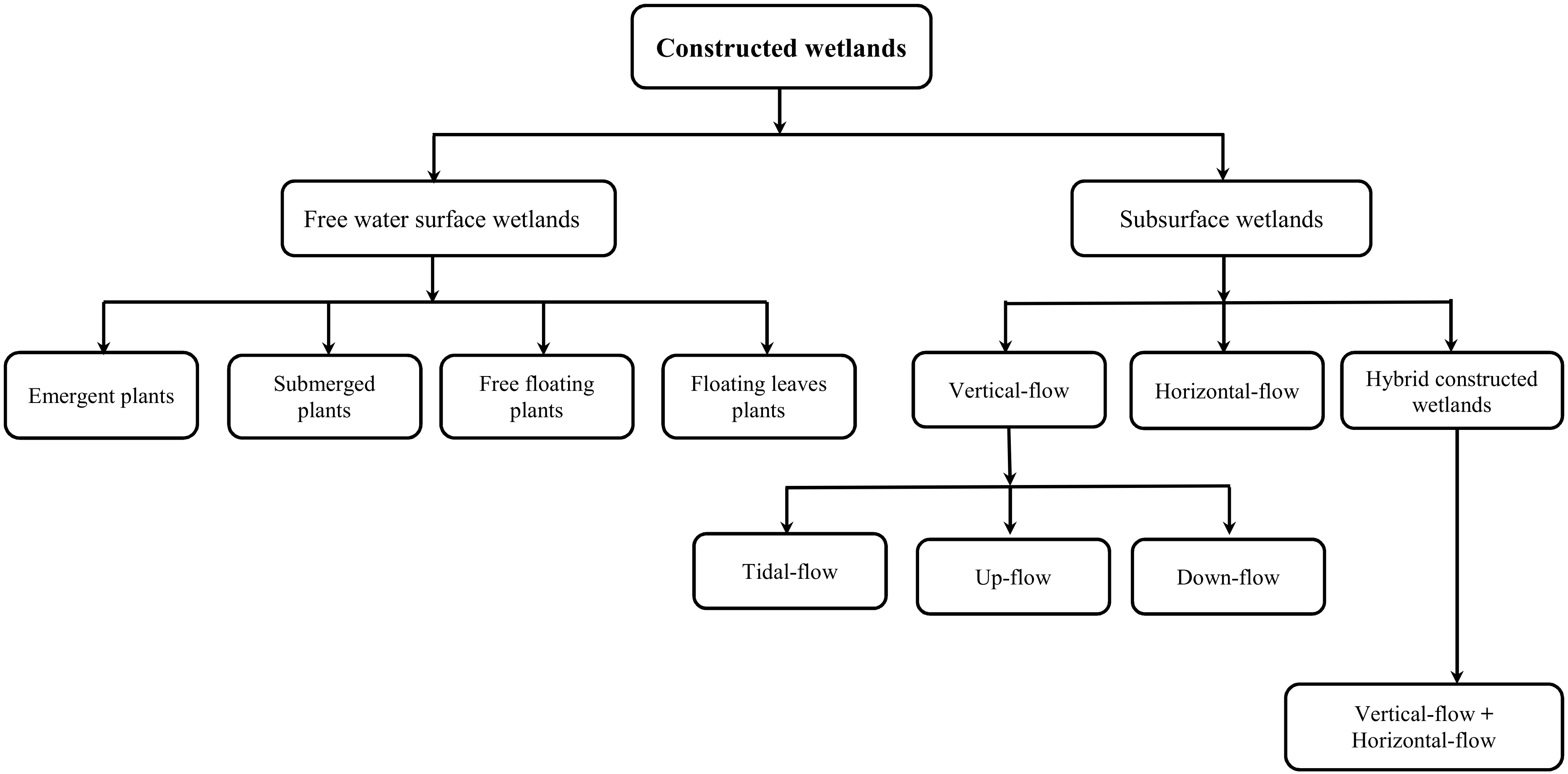

Figure 2.1: Constructed wetlands classification.

FWSF CWs are comprised of an exposed aquatic area covered with different types of plants such as emergent, free floating, floating leaved, bottom rooted or submersed macrophytes (Figure 2.2).

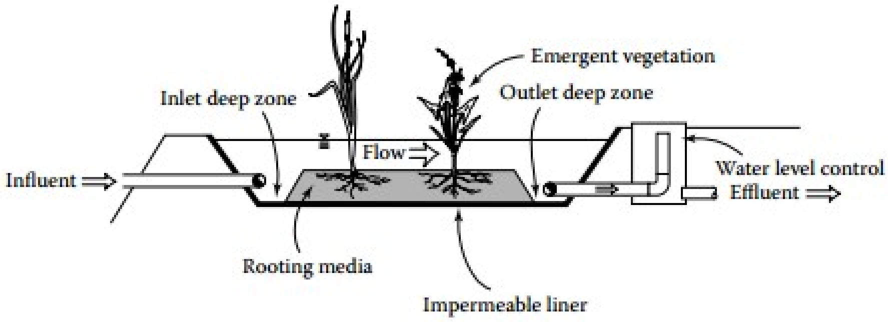

Figure 2.2: Free water surface-flow constructed wetlands configuration (adapted from Kadlec and Wallace (2009)).

According to Vymazal et al. (1998, 2006) and Wu et al. (2014), the operation of free water surface constructed wetlands is similar to that of natural ones. This system consists of a sealed shallow pool in order to prevent wastewater leakage to the underground aquifer with a substrate of 40 cm thick soil for establishing the macrophytes, as discussed by Stefanakis et al. (2014). In FWSF CWs, the wastewater is flooded from the top then flows horizontally on the system media producing water depth ranging from 20 to 40 cm and up to 80 cm as reported by Vymazal et al. (2006) and Akratos et al. (2006). Moreover, treatment processes such as sedimentation, filtration, oxidation, adsorption and precipitation will occur as the wastewater passes through this wetlands system (Kadlec & Wallace, 2009). Since FWSF CWs closely simulate natural wetlands (Kadlec & knight, 1996), a high wildlife variety is expected (insects, molluscs, birds, mammals, etc.). Moreover, these types of wetlands require a large land area with high potential for exposure to humans (International Water Association [IWA] Specialist Group, 2000).

Because of this, FWSF CWs are infrequently used for secondary treatment as there is a high potential for human exposure to pathogens (USEPA, 2000). As a result, this type of wetlands is usually used in advanced treatment for the effluent from tertiary treatment process such as lagoons, trickling filters, activated sludge systems, etc. (Figure 2.3).

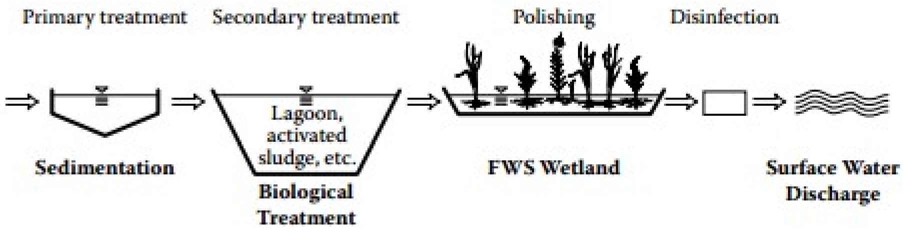

Figure 2.3: Typical application of a FWSF wetland for municipal wastewater treatment (adapted from Wallace and Knight (2006)).

However, in terms of treatment efficiency, FWSF CWs are reported as good for suspended solids, biochemical oxygen demand, nitrogen, heavy metals and pathogens (Vymazal, 2007; Kadlec & Wallace, 2009; Tsihrintzis & Gikas, 2010).

On the other hand, the subsurface flow constructed wetlands (SSF CWs) system consists of macrophytes planted on substrates of sand or gravel, allowing flooding of the system with wastewater which will pass through by gravity, improving treatment processes (Knowles et al., 2011). The substrate arrangement in this system will provide an effective path that enhances the role of microorganisms in the system to treat various types of pollutants and allowing processes such as filtration and adsorption to occur (Hoffman et al., 2011).

Although a sand substrate for SSF CWs was used initially in Europe and more recently practised all over the world, gravel substrates for this system are used in numerous countries, namely North and South Africa, New Zealand, Asia and Australia. However, Fan et al. (2012, 2013) and Nivala et al. (2013) reported that SSF CWs treatment systems show high efficiency in terms of nitrogen and carbon compounds removal due to high oxygen availability in their substrate. Moreover, this type of wetlands shows good efficiency in small areas compared to that occupied by surface flow constructed wetlands (SF CWs) as reported by Hoffman et al. (2011) and Stefanakis et al. (2014).

The vertical flow constructed wetlands (VF CWs) system was initially established and utilised by the German scientist, Seidel, in the early 1960s as reported by Vymazal and Kröpfelová (2011). This type of wetland became popular for use after understanding the drawbacks of the horizontal type in terms of nitrification incapability of the wastewater due to limitation of oxygen availability in this system bed (Cooper, 1999; Stefanakis et al., 2014).

In the VF CWs the wastewater is applied intermittently (Figure 2.4) in cycling of filling and draining the substrate media leading to a high rate of oxygen transfer in the system (Vymazal & Kröpfelová, 2008; Wallace, 2013; Li et al., 2015). The applied wastewater floods the system and is then allowed to drain by gravity (Zhao et al., 2004). As a result, air will enter the system pores and improve the aeration and biological treatment processing (Vymazal et al., 2006; Fan et al., 2012; Song et al., 2015).

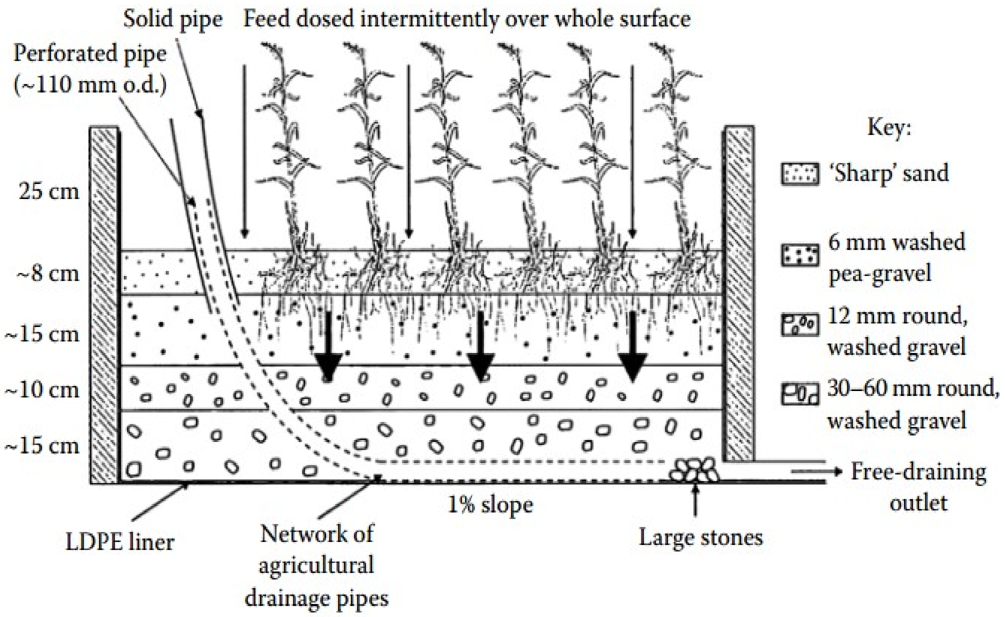

Figure 2.4: Typical arrangement of a VF constructed wetland (Adapted from Cooper et al. (1999)). LDPE, Low Density Polyethylene.

However, vertical flow constructed wetlands are reported to have high efficiency in terms of treating different types of pollutant in the wastewater. For example, Brix and Arias (2005), Prochaska et al. (2007) and Paing et al. (2015), indicated that VF CWs can remove chemical oxygen demand, biochemical oxygen demand and suspended solids well from the wastewater. However, VF CWs are reported to be poor in terms of phosphorus removal due to insufficient interaction between wastewater and system media. Moreover, many studies have shown that VF CWs perform well in terms of nitrification (Langergraber et al., 2007; Zhi et al., 2015), while others indicated their insufficiency in denitrification (Scholz, 2010; Vymazal & Kröpfelová, 2011). However, the denitrification in this system could be improved by a discontinuous loading regimes amendment as discussed by Weedon (2003); Arias, Brix, and Marti (2005) and Weedon (2010).

In this wetland type, the substrate media contain sand or gravel of a size which increases with depth (Vymazal et al., 2006). This substrate is prepared with a depth of 45 to 120 cm from top to bottom and slope ranging from 1 to 2% in order to enable treated wastewater to be drained and collected easily from the system outlet. Moreover, the discontinuous application of wastewater in vertical flow constructed wetlands will provide the system with more oxygen due to air being sucked in while draining the treated wastewater out of the system by gravity (Stefanakis et al., 2014). Moreover, this operation will be enhanced when aeration pipes are inserted in the system leading to improvement in the nitrification processes and organic matter removal when compared with the horizontal flow constructed wetlands system (Vymazal, 2007; Kadlec & Wallace, 2009; Stefanakis et al., 2014). The application of VF CWs is beginning to be developed in many areas of the world, such as Asia and Africa (Kivaisi, 2001; Abou-Elela et al., 2013; Wu et al., 2014), while it was originally developed in Europe, mainly in Germany, Austria, the UK and the USA (Kadlec & Wallace, 2009).

Another type of subsurface flow wetlands is the horizontal flow one (HSSF CWs) in which the wastewater moves horizontally through the system substrate, plants roots and rhizomes toward the system outlet (Vymazal, 2009, 2014). In this system, the treatment of the wastewater which is flooded enduringly to the system, occurs due to the interconnection of biological, chemical and physical processes as wastewaters pass through the aerobic, anaerobic and anoxic zones of the system (Kadlec & Knight, 1996; Vymazal, 2014). According to Brix (1987), the oxygen available in the system substrate is provided by roots and rhizomes in the aerobic zone. HSSF CWs are planted with reeds which are established in the system substrate (Figure 2.5) containing gravel or sand, or both, underneath which the applied wastewater passes from the system inlet toward the outlet (Vymazal et al., 2006).

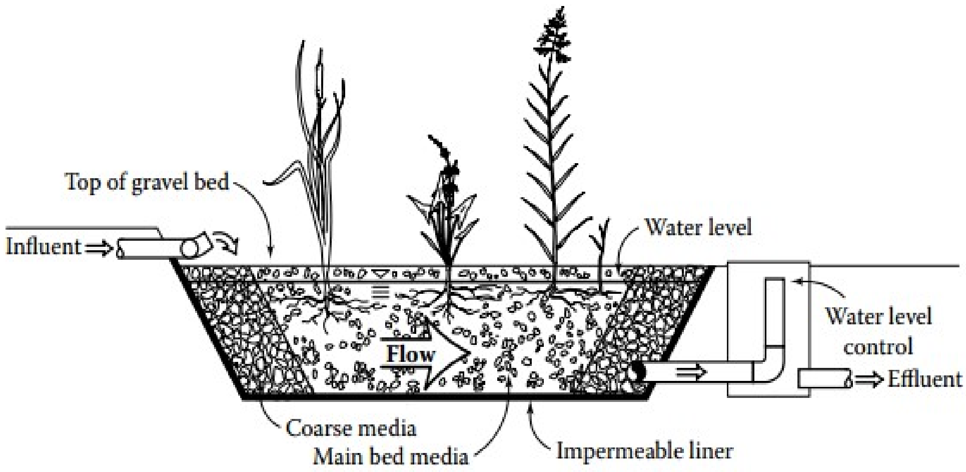

Figure 2.5: Schematic of horizontal subsurface flow constructed wetlands (adapted from Wallace and Knight (2006)).

In this system, the substrate depth ranges from 30 to 80 cm (Akratos & Tsihrintzis, 2007) depending on the macrophytes types and their root depths with a slope of 1 to 3% supporting the gravitational flow of the applied wastewater. Moreover, the bottom of the system is sealed with an impermeable membrane avoiding leakage of the wastewater to the aquifer (Kadlec & Wallace, 2009). Moreover, proper design of HSSF CWs will allow the wastewater to be invisible at the surface of the system media and will enable it to remain about 5 to 15 cm under the surface (Vymazal et al., 2006). This will reduce the possibility of human exposure and limit the wild life habitats and mosquito breeding (Kadlec & Wallace, 2009). However, macrophytes roots and porous media in this system are responsible for biomass development and subsequently enhancing the organic matter and suspended solids removal from the contaminated water (Akratos & Tsihrintzis, 2007; Gikas et al., 2010; Vymazal, 2014). Compared with SFCWs systems, HF constructed wetlands require a smaller land area but incur high investment costs as reported by Tsihrintzis et al. (2007). Moreover, HSSF CWs systems have been applied in Europe and the USA (Vymazal, 2014). Although HSSF CWs are reported to be poor in terms of ammonia nitrogen removal, they can treat nitrate nitrogen well due to the anoxic and anaerobic conditions available in HSSF CWs which limit the nitrification of NH_4-N, but favour NO_3-N denitrification (Tuncsiper, 2009; Zhange et al., 2014). In contrast, due to the availability of aerobic conditions in the VSSF CWs system, the NH_4-N is removed well through nitrification processes, while NO_3-N is not, as the denitrification is absent in this system (Zhange et al., 2014). In other words, HSSF CWs are known to be good in denitrification but poor in nitrification, while VSSF CWs show contrary performance (Vymazal & Kröpfelová, 2011; Vymazal, 2014). This has led researchers to develop a combined SSF CWs system consisting of both HSSF CWs together with VSSF CWs (Figure 2.6) aiming to obtain higher nitrogen removal (Vymazal, 2005; Ayaz et al., 2012; Vymazal, 2014).

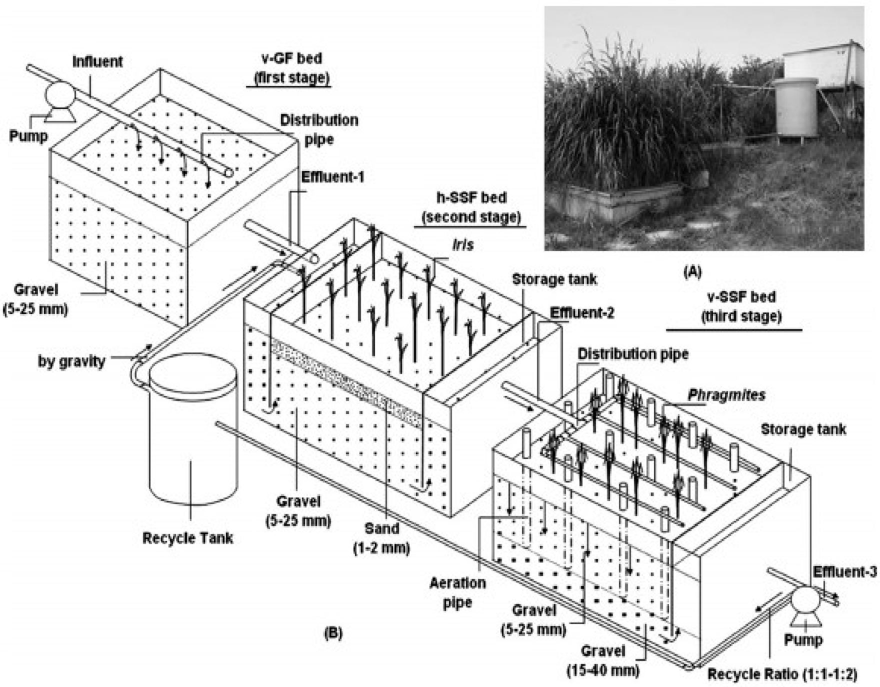

Figure 2.6: Hybrid constructed wetlands arrangement (adapted from Tuncsiper (2009)).

The first hybrid constructed wetland system was developed by the German scientist, Seidel, between 1960 and 1969 and after that a few similar systems were developed in France between 1980 and 1989 and then in the UK between 1990 and 1999 (Vymazal, 2005). Currently, the use of this combined wetlands system is widespread around the world due to its efficiency in removing ammonia, nitrate and total nitrogen from many types of wastewaters (Vymazal, 2005, Ye & Li, 2009; Vymazal & Kröpfelová, 2011; Ayaz et al., 2012). Moreover, many studies have indicated that a hybrid wetlands system could be used to treat different types of wastewater such as winery wastewaters (Serrano et al., 2011), pharmaceuticals (Reyes-Contreras et al., 2011), oil field produced water (Alley et al., 2013), grey water (Commino, Riggio, & Rosso, 2013) and industrial effluents (Vymazal, 2014).

2.4 Constructed wetlands sustainable design and operation

2.4.1 Constructed wetlands vegetation

Wetland plants are known as macrophytes which are commonly used plant species in treatment wetlands (Vymazal, 2002; Stefanakis et al., 2014). Macrophytes are considered as a significant part of the wetland (natural and constructed) system (Scholz, 2006, 2007, 2010; Villa et al., 2014), as the presence or absence of those plants will characterise the definition of the wetlands (Saeed & Sun, 2012) as green technology (Stefanakis et al., 2014). Macrophytes can absorb pollutants from the wastewater and accumulate them in their tissue in addition to providing the microorganisms in the system with a complimentary growing environment as discussed by Vymazal (2002). Moreover, wetland macrophytes are responsible for transferring the oxygen from their roots to the rhizosphere zone around them providing an aerobic condition to enhance the contaminants degradation in the system (Moshiri, 1993).

For example, in an intermittent loading system for vertical flow constructed wetlands, the macrophytes roots dissolve the organic matter in the wastewater and subsequently prevent the substrate clogging by producing holes for the water to pass through. Furthermore, growth of macrophytes in a wetlands substrate will stabilise the media leading to improvement in the hydraulic conductivity of the system, reduce clogging probability while providing suitable conditions for microbe growth, nutrient observing and release more oxygen to the water as reported by Li et al. (2008) and Stefanakis et al. (2014). The role of macrophytes and the impact of various species of wetland plants on the treatment efficiency are still disputed (Scholz, 2006). However, some earlier studies stated the substantial impact of macrophytes on wetland treatment systems in terms of contaminant removal. For example, Akratos and Tsihrintzis (2007) studied the reduction percentage in chemical and biochemical oxygen demand in planted wetland and control systems. Their results showed that the reduction percentage in the planted wetlands of 89% was greater than that of the controlled system which showed a reduction percentage of 85%. Total suspended solids and biochemical oxygen demand reduction percentages were observed to be higher in the planted filter (90% and 75%, respectively) of the subsurface flow system compared to those in the controlled system which showed reduction percentages of 46% and 63%, in that order (Karathanasis, Potter, & Coyne, 2003). In Greece, a study was carried out to determine the reduction percentage of polycyclic aromatic hydrocarbons (PAHs) from urban wastewater using constructed wetlands and a gravel filter (Fountoulakis et al., 2009). The results showed that the planted filter led to a reduction percentage of 79% which was higher than that for the gravel filter of 73.3%. Another study showed that the removal efficiency of pharmaceuticals in a planted wetland system was higher than that in an unplanted one (Verlicchi & Zambello, 2014).

On the other hand, there are some studies which have indicated that there is no substantial impact of wetlands macrophytes in terms of pollutant removal in both planted and unplanted systems. For example, some researchers found that there was no difference in biochemical oxygen demand removal efficiency by constructed wetland systems during different times of plant growth (Scholz & Xu, 2002; Scholz, 2006), while other researchers found that there was no substantial difference in removal efficiencies in systems planted with different plant types like reeds, duckweed and algae (Balizon et al., 2002).

According to Kadlec and Knight (1996), a number of points should be considered when choosing wetland plants. For example, the chosen macrophytes should be waterlog, anoxic and hyper-eutrophic condition tolerable, local species and widely available in the country. In addition, perennial plants which live for more than two years or grow for two seasons are preferable for constructed wetlands sustainability. Similarly, Wu et al. (2015) recommended that plants selected to be used in wetlands should be tolerant to hyper-eutrophic and waterlogged-anoxic conditions with capability for absorption of wastewater pollutants in addition to climate change adaptation.

2.4.1.1 Macrophytes used in constructed wetlands

Wetland plants can be categorised under four main classes namely, emergent plants, floating leaves macrophytes, submerged plants and freely floating macrophytes as detailed below:

1. Emergent macrophytes are known to be stabilised in the substrate and are usually observed above the water surface. Moreover, these plant types are grown in a water depth of 50 cm or more above the soil (Saeed & Sun, 2012; Vymazal, 2011). Macrophytes such as: *Acorus calamus, Carex rostrate, Phragmites australis (*common reeds*), Scirpus lacusris and Typha latifolia* are examples of these plant types (Saeed & Sun, 2012) as well as *Iris spp.*

(Iridaceae), Juncus spp. (Juncaceae) and *Eleocharis spp. (Spikerush)* as reported by Wu et al. (2015).

2. Floating leaves plants are fixed in the saturated substrate with a water depth ranging from 0.5 to 3.0 metres and have leaves over the water surface such as *Nymphaea odorata*, *Nuphar lutea, Nymphoides peltata, Trapa bispinosa and Marsilea quadrifolia* plants (Saeed & Sun, 2012; Wu et al., 2015).
3. Submerged macrophytes require aerated water for good growth. Moreover, the plants tissues responsible for photosynthesis processes are covered with water. However, these types of plant are mainly used to polish secondary treatment plants as stated by Saeed and Sun (2012). *Myriophyllum spicatum, Ceratophyllum demersum, Rhodophyceae, Hydrilla verticillata, Vallisneria natans,* and *Potamogeton crispus* are examples of these plants (Wu et al., 2015).
4. Freely floating plants float on the water surface and have the ability to remove nitrogen and phosphorous from the wastewater through denitrification processes and subsequently combine them in their biomass. Moreover, these plants can remove suspended solids from wastewater as reported by Moshiri (1993). *Lemna minor, Spirodela polyrhiza, Eichhornia crassipes, Salvinia natans and Hydrocharis dubia* are examples of these types of macrophytes, as indicated by Wu et al. (2015).

However, there have been many studies undertaken to find the most popular plants used in wetlands worldwide. For instance, a survey on common emergent macrophytes used in free water surface flow constructed (FWSF CWs) was undertaken by Vymazal (2013b). His results showed that *Phragmites australis* is the most popular plant in Europe and Asia, while *Typha latifolia* was recorded as the most used species in North America. In Africa, *Cyperus papyrus* is commonly used while, *Phragmites australis* and *Typha domingensis* and *Scirpus validus* are the most popular plants in Central/South Americas and Oceania, respectively.

Regarding the plant types used in subsurface flow constructed wetlands (SSF CWs), a review study undertaken by Vymazal (2011) showed that *Phragmites australis* is the most commonly used species globally that is mainly used in Europe, Canada, Australia, Asia and Africa.

Furthermore, *Typha* species such as *latifolia, domingensis, orientalis* and *glauca* are classified as the second most commonly used plants in SSF CWs found in North America, Australia, Africa and East Asia. In addition, *lacustris, validus, californicus* and *acutus* which are classified as *Scirpus* species are commonly used in North America, Australia and New Zealand (Vymazal, 2011). However, *Phragmites australis (Cav.) Trin. ex Steud* (common reeds) (Figure 2.7) are reported as the most commonly used species of wetland plants (IWA Specialist Group, 2000; Scholz, 2006; Vymazal, 2014).

Figure 2.7: The *Phragmites australis* (common reeds).

2.4.1.2 Macrophytes tolerance to wastewater

Plant tolerance is another crucial factor which should be considered when choosing the specific plant types for constructed wetlands as some plants may suffer due to the treating of various types of pollutants present in the wastewater resulting in limitation in both plant survival and treatment efficiency. This mainly occurs when applying a high load of wastewater or treating wastewater that contains abundant toxic contaminants (Surrency, 1993). Moreover, environmental stresses like eutrophication can damage wetland plants by inhibiting their growth or even causing their disappearance, with a direct effect on wetland treatment performance. According to Xu et al. (2010) excessive ammonia in wastewater, for example, can lead to physiological damage of plants and subsequent limitation of nutrient up-take by macrophytes.

However, visual symptoms linked to ammonia abundancy can be observed as leaves chlorosis, growth destruction, and root sinking as well as depression in plant yield (Xu et al., 2010). Based on this, several studies have been undertaken to evaluate the tolerance of wetland plants to different levels of contaminants available in wastewaters. For example, *T. latifolia* was reported to be stressed at ammonia concentration ranging between 160 and 170 mg/l (Surrency, 1993), while *Scirpus acutus* was noted as the only species among five types that was negatively affected by ammonia concentrations ranging between 20.5 and 82.4 mg/l under a field study experiment undertaken by Hill et al. (1997).

Moreover, the impact of increased concentrations of ammonia reaching 400 mg/l on three types of wetland plants was studied by Li et al. (2011). Their results showed that the three types were significantly different in their ammonia tolerance and *Zornia latifolia* was recorded to have the highest tolerance of the three. The physiological response of *P. australis* to different chemical oxygen demand (COD) concentrations was investigated by Xu et al. (2010). Their results showed that COD concentrations of more than 200 mg/l can affect the plant metabolism processes, while concentrations of more than 400 mg/l can result in obvious *P. australis* physiological changes. Also, *Arundo donax* and *Sarcocornia fruticose* were reported to be very effective in removing high salinity, as well as organic matter, nitrogen and phosphorus, from wastewater (Calheiros et al., 2012), while *Typha angustata* was observed to remain alive under high chromium levels of 30 mg/l for a duration of 20 days, showing an outstanding accumulation ability (Chen et al., 2014). Moreover, *P. australis* was noted to tolerate and remove the antibiotics available in wastewaters up to concentrations ranging between 0 and 1000 mg/l (Liu et al., 2013).

These studies are essential not only in understanding the tolerance of different types of wetlands but also to provide good information about selection of the most tolerant species for treating wastewater using construction wetlands.

2.4.1.3 Macrophytes pollutants removal capacity

Plants have an important role in wetland systems which can directly affect the wastewater quality by improving various removal processes and straight consuming of nitrogen, phosphorous and other elements (Ong et al., 2010; Liu et al., 2011; Ko et al., 2011).

Moreover, wetland plants can accumulate toxic material in their tissue such as heavy metals and antibiotics (Liu et al., 2013). Several studies have been undertaken to investigate wetland plants up-taking capacity. For example, Wu et al. (2013) performed a study of four emergent plants uptake capacity in a wetland system treating contaminated river water. The authors' results reported a nitrogen and phosphorous net uptake capacity of 6.50 to 26.57 g N/m^2 and 0.27 to 1.48 g P/m^2, respectively. However, the plants uptake capacity may differ depending on many factors such as types of wastewater, loading rate, hydraulic retention time, weather conditions and system arrangements as stated by Saeed and Sun (2012).

Furthermore, Greenway and Woolley (2001) stated that wetland plants can remove a high percentage of nitrogen and phosphorous ranging from 15 to 80% and 24 to 80% of N and P, respectively, while Wu et al. (2013) found these percentages could be lower and ranged between 14.29–51.89% and 10.76–34.17% for total nitrogen and total phosphorous removal, respectively. With respect to heavy metal removal, Ha et al. (2011) studied the accumulation capacity of indium, lead, copper, cadmium and zinc in *Eleocharis acicularis* plants.

Their results reported that these types of plants had an outstanding ability to accumulate metals available in the wastewater. However, Yadav et al. (2012) concluded that bioaccumulation of heavy metals is different based on plant species but also on the specific part of the plant, as the metals can be removed by the underground biomass more effectively than the over ground one.

2.4.2 Constructed wetland substrates

The media used in constructed wetlands is named substrates or aggregates. Wetlands media could be sand, gravel, rocks or organic material such as soil and compost which provide the primary support for the wetlands plants and microorganism growth, enhancing biodegradation of wastewater pollutants, in addition to its impact on system hydrology mechanisms (Tietz et al., 2007; Meng et al., 2014). Moreover, substrates can remove contaminants from the wastewater by ion exchange / non-specific adsorption, specific adsorption / precipitation and complexation (Dordio & Carvalho, 2013; Ge et al., 2015). However, the chemical composition of wetland media can affect the system efficiency. For example, soil of low nutrient content will lead the plants in the system to uptake the nutrients from the applied wastewater directly. Also, the gravel substrate in the system should be washed from time to time to enhance the filtration rate and reduce the clogging of system media. Furthermore, using a gravel substrate with a reed system will improve the nitrification process rates, while the use of soil media with a reed system in the wetlands will increase the denitrification rate as discussed by Markantonatos et al. (1996). Moreover, substrate size has an important role in the system mechanism as it may affect the surface area for growing the biofilm in addition to the system pores blockage probability.

For instance, Meng et al (2014) reported that an excessively large aggregate size will reduce the surface area for microorganisms to grow, while Brix and Arias (2005) indicated that the small-sized-grain media will support the growth of biofilm by increasing the available surface area with a high possibility of clogging the system pores. Furthermore, Hoffman et al. (2011) and Meng et al. (2014) concluded that the hydraulic loading rate in wetland systems particularly subsurface flow types can be affected directly by wetland aggregate porosity, as the clogging of wetlands media is a common problem in such systems affecting the system performance, especially when using unsuitable media pores for the applied organic load.

However, the media which is used in the wetland is dependent on the purpose for which the wetlands are designed. This media can be varied from fine grain to field stones. Using coarse grained media in the wetland systems will increase the hydraulic conductivity and reduce the probability of system clogging while the fine media will remove the suspended solids and turbidity well with a high potential for clogging to occur in the system (Sundaravadivel & Vigneswaran, 2009).

Under saturation conditions, the pores in the wetland substrate will be filling with water instead of air. In this case the dissolved oxygen available in the water will be consumed by microbes. Since this oxygen will be more than that restored during the circulation phase, the media will become anoxic. Moreover, the substrate will be anaerobic under inundation conditions (Scholz, 2006, 2010; Stefanakis et al., 2014). Using a sand and gravel mixture in the wetland substrate is recommended as this can improve the system behaviour in terms of hydraulic conductivity and contaminants removal (Stottmeister et al., 2003).

Moreover, using fine grained instead of large-grained media in a wetland system is preferable as it will provide better conditions for microorganism growth and subsequently improve pollutant biodegradation (Dordio & Carvalho, 2013) but at the same time the fine aggregate will lead to clogging the media (Brix & Arias, 2005; Song et al., 2015). Langergraber et al. (2003) reported that using compound layers of gravels arranged by size increment from the top is suggested, however, their results showed that the clogging in such a system is highly likely.

Other studies showed that using an anti-sized reed bed system instead of the traditional mono-sized is very effective in terms of pollutant removal from heavily contaminated wastewater (Sun, Zhao, & Allen, 2007), while Song et al. (2015) indicated that the using of large-size packing media will result in high removal of chemical oxygen demand, ammonia and nitrogen while reducing the chance of system clogging as evaluated in their vertical flow wetland systems. Moreover, several studies have been undertaken to assess the impact of different substrate media used to improve contaminant adsorption capacity.

For example, Menge et al. (2014) confirmed the results obtained from previous studies (Saeed & Sun, 2011; Tee et al., 2012; Saeed & Sun, 2013) which assessed using different media substrates, such as organic mulch and rice husk, on system efficiency. The results showed that these substrates enhanced the nitrogen removal due to organic carbon content. However, these results contradicted others regarding the use of expensive media to improve the wetland system performance. For instance, using granular activated carbon did not increase the adsorption capacity of constructed wetland media as shown by Scholz and Xu (2002).

Moreover, using zeolite and bauxite substrates did not show a substantial enhancement in wetland system efficiency as reported by Stefanakis and Tsihrintzis (2012). Table 2.1 displays the most common substrates used in constructed wetland systems.

Table 2.1: Common substrate types used in constructed wetland systems with source.

Substrate type		
Natural material	**Industrial by products**	**Artificial products**
Sand (Saeed & Sun, 2013)	Slag (Cui et al., 2010)	Activated carbon (Ren et al., 2007)
Gravel (Calheiros et al., 2008)	Fly ash (Xu et al., 2006)	Light weight aggregates (Saeed & Sun, 2012)
Clay (Calheiros et al., 2008)	Coal cinder (Ren et al., 2007)	Compost (Saeed & Sun, 2012)
Calcite (Ann et al., 1999)	Alum sludge (Babatunde et al., 2010)	Calcium silicate hydrate (Li et al., 2011)
Marble (Arias et al., 2001)	Hollow brick crumbs (Ren et al., 2007)	Ceramsite (Li et al., 2011)
Vermiculite (Arias et al., 2001)	Moleanos limestone (Mateus et al., 2012)	
Bentonite (Xu et al., 2006)	Wollastonite tailings (Hill et al., 1997)	
Dolomite (Ann et al., 1999)	Oil palm shell (Chong et al., 2013)	
Limestone (Tao & Wang, 2009)		
Shell (Seo et al., 2005)		
Shale (Saeed & Sun, 2012)		
Peat (Saeed & Sun, 2012)		
Wollastonite (Brooks et al., 2000)		
Maerl (Saeed & Sun, 2012)		
Zeolite (Bruch et al., 2011)		

2.4.3 Constructed wetland microorganisms

The constructed wetland is a formidable system supporting the growth of microbial communities which play an important role in removing various types of wastewater pollutants during the biological processes, in addition to the physical (sedimentation, filtration) and chemical (oxidation, reduction, precipitation, volatilisation) processes as well as macrophytes uptake undertaken in the constructed wetland system (Scholz, 2006, 2010).

According to Kadlec and Night (1996), Paredes et al. (2007), Kadlec and Wallace (2009) and Shao et al., 2013, bacteria, fungi, algae and protozoa can be considered as the main groups of microorganisms available in the aerobic and anaerobic zones of the wetland system. The important role of microorganisms in the constructed wetlands system is due to their microscopic size allowing contact and feeding of the pollutants via their enzymes (Francis, 1996; Truu, Juhanson, & Truu, 2009).

However, in the wetland system, the interaction of biological, chemical and physical processes results in the treat of organic pollutants and transformation of nitrogen, phosphorous and heavy metals (Scholz, 2006, 2010). For example, organic matter in the wetland system is removed by aerobic and anaerobic degradation processes, while nitrogen can be removed via microbial metabolism such as ammonification, nitrification, denitrification and anammox processes (Meng et al., 2014).

Moreover, the biodegradation of organic matter is mostly linked to autotrophic bacteria which produce organic particles from inorganic carbon, like carbon dioxide, and heterotrophic bacteria that obtain their growth requirement from organic compounds, as well as protozoa and fungi (Kadlec & Wallace, 2009). According to Ainesworth et al. (1973), all fungi obtain their nutrition and energy requirements for growth from organic matter (heterotrophic).

However, most fungi utilise saprophytic nutrition which mainly depends on dead organic matter degradation. Fungi are plentiful in wetland systems and play a significant role in water quality treatment. Moreover, fungi are environmentally important in wetlands because they arbitrate a substantial proportion of carbon and nutrients recycling in wetland and aquatic ecosystems.

Furthermore, Fungi can symbiotically live with algae and higher plants, increasing their ability for nutrients sorption from air, water, and soil. However, presence of fungi in wetland systems containing toxic metals and chemicals will limit the production of algae and higher plants as the recycling of nutrients in such systems will be condensed. In the wetland ecosystem, fungi grow naturally on dead plant litter layers (Kadlec & Wallace, 2009).

However, the interaction between microorganisms, substrate and plants of a wetland system can directly affect the pollutant removal ability of the constructed wetland system (Scholz, 2010). For example, the plants in the wetland system consist of two parts, above and underground biomass, providing a large surface area for the microbial growth necessary for microbial processes in the wetland system (Brix, 1997). Moreover, as wetland macrophytes grow and die the falling of leaves and stems produces multilayers of litter (organic debris). This will create a porous substrate layer providing a substantial area for microbial attachment which will directly affect the function of water quality improvement (Brix, 1997). Moreover, the wetland plants will transfer the oxygen through their hollow tissue and release it from the roots to the rhizosphere zone supporting the aerobic degradation of organic matter and nitrification process as discussed by Brix (1997).

However, microorganisms in wetland systems can be highly active if suitable conditions and adequate nutrients are available for growth and survival, otherwise they will become dominant as reported by Truu et al. (2009). According to Meng et al. (2014), the chemical biodegradation undertaken in the wetland system by microorganisms consists of a complex series including biochemical processes which differ according to the microbe groups.

The role of wetlands is significantly affected by the microorganisms and their metabolism since these microbes which naturally live in water, media, or roots of wetland plants, can consume organic matter and nutrients and subsequently reduce, break down or completely remove various pollutants from the wastewater (Wetzel, 1993; Faulwetter et al., 2009; Truu et al., 2009).

Microorganism groups in constructed wetland systems can be divided into internal and external microbes which are characterised according to their activities (Truu et al., 2009). For example, the internal group responsible for metabolic activity flourish and live in wetland systems contributing to pollutants treatment, while pathogens in inflow wastewater, which are considered as external microbes, have no important impact in the wetland ecosystem as they do not survive since the wetland system is antagonistic to external microbes (Vymazal, 2005).

2.4.4 Constructed wetlands hydrology and surface loading rate

The continuous or discontinuous inundation of the wetlands system substrate which is linked to anaerobic conditions and provides a place where biogeochemical operations occur is named hydrology (Scholz, 2010). In wetland systems, hydro period and depth of flooding are the main two parameters of wetlands hydrology (Gosselink & Turner, 1978) which can directly affect nutrients, oxygen amounts, and pH as well as the wetland stability as discussed by Scholz and Lee (2005) and Scholz (2006; 2010).

The time when the wetland media is water logged is defined as the hydro period which can be affected by many factors such as topography, geology, groundwater, subsurface soil features, and climate conditions. Moreover, hydraulic retention time (HRT), can be defined as the average time for which water remains in the wetlands. HRT is a very crucial factor in wetland design and performance evaluation (Ghosh & Gopal, 2010), mainly in solids settling, macrophytes uptake and biochemical processes (Stefanakis et al., 2014). Several studies have been undertaken to monitor the impact of HRT on treatment efficiency of wetland system. For example, Calherios et al. (2009) studied the relation between HRT and chemical oxygen demand removal efficiency. The authors' results showed that with decreasing HRT, the effluent chemical oxygen demand concentrations will increase. These results were confirmed by Trang et al. (2010) who observed the reduction in organic matter and nitrogen removal efficiency with the reduction of HRT in their system due to less contact time of contaminants in the wetland. This drop in removal efficiency was observed in biochemical oxygen demand and total suspended solids as well under short HRT conditions (Weerakoon et al., 2013).

The impact of wetland design and operation variables on the treatment efficiency of domestic wastewater was assessed by Dong et al. (2011). The authors' reported that their wetland system showed high performance in removing contaminants. Their system achieved 98, 94, 92, 90, 96, 97 and 96% removal efficiency for variables of biochemical oxygen demand (BOD), suspended solids (SS), chemical oxygen demand (COD), nitrate-nitrogen (NO_3-N), total nitrogen (TN), ammonia nitrogen (NH_4-N) and ortho-phosphate-phosphorus (PO_4-P), respectively. However, Dong et al. (2011) concluded that these results were achieved because of the high hydraulic retention time of about 92 days.

However, HRT is the only operational factor which can be controlled in the wetland systems. For instance, critical biochemical oxygen demand removal efficiency can be obtained at HRT of below 1 day, while this efficiency will be enhanced at HRT of about 7 days as reported by Reed and Brown (1995). Based on this, HRT is an important factor that affects the efficiency of the wetland system treatment which is normally decided by designers (Weerakoon et al., 2013). Despite the advantage of improving the treatment efficiency when increasing the HRT, this can be considered as a main disadvantage in large wetland areas particularly when the land availability is restricted (Deblina & Brij, 2010).

In wetlands, the surface loading (SLR) rate is mainly dependent on influent concentration and flow. However, SLR is difficult to control as the influent compositions vary significantly. Increasing of influent flow will lead to an increase in SLR and a decreasing in HRT (Scholz, 2010). However, the treatment efficiency of wetlands is a function of both hydraulic loading rate and HRT as reported by Rousseau et al. (2008) and Abou-Elela et al. (2013). For example, in the case of high hydraulic loading rate and low HRT, the pollutants in the wastewater will pass quickly through the wetland substrate without adequate contact time for biodegradation processes resulting in low treatment performance.

Other researchers have stated that ammonia nitrogen can be removed well under long HRT regardless of the maturity of wetland plants, while the chemical oxygen demand variable has been observed to be unstable through experiments involving wetlands with mature macrophytes (Stefanakis & Tsihrintzis, 2012; Zhi et al., 2015). However, long resting time can also enhance the nitrification and biodegradation processes by supporting the system with an artificial aeration time.

Moreover, the organic matter contents in vertical flow constructed wetlands (VF CWs) influents along with the applied contact time in the system can directly affect the biodegradation processes (Stefanakis et al.,2014). Accordingly, biodegradable organic matter will be oxidised rapidly due to availability of oxygen in the system bed, while the intractable matter will be incompletely degraded due to inadequate hydraulic contact time.

Furthermore, Tietz et al. (2007); and Stefanakis and Tsihrintzis (2012) indicated that the organic matter breakdown mainly occurs in the top layers of a wetland system, predominantly in the 10–20 cm upper layers due to high availability of oxygen and microbe density in these layers. Flooding depth in a natural wetland varies between +2 m and –1 m (mean value of +1 m) based on the ground surface (Scholz, 2010).

The impact of water depth on treatment efficiency has been investigated by several authors. For example, Aguirre et al. (2005) studied the impact of flooding depth on organic matter removal efficiency by using two subsurface horizontal flow constructed wetlands of different water depths (0.27 and 0.5 m). Their results showed that the shallow system gave better performance than the deep one mainly in terms of biochemical oxygen demand which showed removal of 72 to 85% in shallow wetland, and 51 to 57% in the deep one, suggesting that metabolism pathways may differ with varying water depth.

The same observation was reported regarding removal of pathogens in horizontal subsurface flow constructed wetlands which showed better removal of total coliform and *E.coli* in shallow systems (Morato et al., 2014). Contrary to this, greater water depth is suggested to increase the contact time resulting in improving the treatment efficiency (Kadlec & Wallace, 2008). However, the actual water depth in the wetland system is mainly dependent on the maximum depth of plant roots, which in turn is dependent on the selected wetland system plant types. As a result, the selected plant types will determine the substrate depth in the wetland bed, which should not be very deep otherwise the plant roots will not reach the system bottom leading to anaerobic conditions in this zone which is devoid of roots (Scholz, 2010). Furthermore, the water depth in the wetland is directly linked to the availability of oxygen in the system as the upper layers will be aerated by atmospheric diffusion while inside the system, diffused oxygen from the plant roots will achieve the aeration. This means that the bottom layers of the system which are not reached by roots will lack oxygen resulting in anoxic or anaerobic conditions in these zones. Table 2.2 summarises some design and operation recommendations for treating wastewater using constructed wetlands, while Table 2.3 provides an overview of constructed wetlands design and operational parameters in developing countries.

Table 2.2: Design and operation recommendations for treating wastewater using constructed wetlands (adapted from Wu et al. (2015)).

	Design criteria	
Parameter	FWSF CWs	SSF CWs
Bed size (m^2)	Larger if available	<2500
Length to width ratio	3:1–5:1	<3:1
Water depth (m)	0.3–0.5	0.4–1.6
Hydraulic slope (%)	<0.5	0.5–1
Hydraulic loading rate (m/day)	<0.1	<0.5
Hydraulic retention time (day)	5–30	2–5
Media	Natural media and industrial by-product preferred, porosity 0.3–0.5, particle size <20 mm (50–200 mm for the inflow and outflow)	
Vegetation	Native species preferred, plant density 80% coverage	

Note: FWSF CWs, free water surface flow constructed wetlands; SSF CWs, subsurface flow constructed wetlands.

Table 2.3: Overview of constructed wetland design and operational parameters in developing countries.

Location	Wastewater type	Wetland design and operation				References
		Plant species	Dimension (L x W x D) (m x m x m)	HLR (m^3/day)	HRT (day)	
Free water surface flow constructed wetlands						
Peradeniya, Sri Lanka	Municipal WW	*Scirpusgrossus* *Typhaangustifolia*	25.0 x 1.0 x 0.6	13	18h	Jinadasa et al. (2006)
Nyanza, Kenya	Sugar factory WW	*Cyperuspapyrus* *Echinochloa pyramidalis*	3.0 x 20.0 x 0.4	75 mm/d	-	Bojcevska and Tonderski (2007)
Taihu, China	Lake water	*Typha angustifolia*	20.0 x 1.5 x 0.8	0.64 m/d	-	Li et al. (2008)
Putrajayacity, Malaysia	Storm water	*Phragmiteskarka* *Lepironiaarticulata*	1.5 x 0.7 x 0.8	0.17–0.63	-	Sim et al. (2008)
Shanghai, China	River water	*Phragmites australis*	800 m^2 x 0.75 m	1800	10	X.Li et al. (2009); M.Li et al. (2009
EI, Salvador	Municipal WW	*Typha angustifolia*	48.9 x15.0 x 0.6	151.4	9.8	Katsenovich et al. (2009)
Liaohe, China	Oil-produced WW	*Phragmites australis*	75.0 x 7.5x 0.25	18.75, 37.5	15; 7.5	Ji et al. (2007)
Petchaburi, Thailand	Municipal WW/	*Typha angustifolia*	4.0 x 1.0 x 1.5	6-150 mm/d	2; 5	Klomjek and Nitisoravut (2005)
Subsurface horizontal flow constructed wetlands						
Egypt	Greywater	*Phragmites australis*	1.1 x 1.0 x 0.4	-	5	Abdel-Shafy et al. (2009)
	Blackwater	*Phragmites australis*	1.1 x 1.0 x 0.4	-	10	
Juja, Nairobicity, Kenya	Municipal WW	*Cyperus papyrus*	7.5 x 3.0 x 0.6	-	-	Mburu et al. (2013)
	Municipal WW	*Cyperus papyrus*	7.5 x 3.0 x 0.6	-	-	
Dares Salaam, Tanzania	Municipal sludge	*Typha latifolia*	4.2 x 1.4 x 0.6	0.683	2.5	Kaseva (2004)
	Municipal sludge	*Phragmites mauritianus*	4.2 x 1.4 x 0.6	0.683	2.5	
Dongying, Shangong, China	Municipal WW	-	35.2ha x 0.5	50,000	1.8	Wang et al. (2006)
	Industrial WW	-	35.2ha x 0.5	50,000	1.8	
Mother Dairy Pilot Plant, India	Municipal sludge	*Phragmites australis*	69 x 46 x 0.3	43.05 l/ m. d	5.15	Ahmed et al. (2008)

Table 2.3 (cont.)

Location	Wastewater type	Wetland design and operation				
		Plant species	Dimension (L x W x D) (m x m x m)	HLR (m^3/day)	HRT (day)	References
Shatian, Shenzhen, China	Municipal WW	*Cannaindica*	80 x 30 x 1.5	-	11.5	Shi et al. (2004)
	Municipal WW	*Thaliade albata*	58 x 20x 1.6	-	8	
Dhaka, Bangladesh	Tannery WW	*Phragmites australis*	1.3 x 1.0 x 0.8	6 cm/d	4.8	Saeed et al. (2012)
	Tannery WW	*Phragmites australis*	1.3 x 1.0 x 0.8	6 cm/d	12.5	
Taihu, Zhejing, China	Lake water	*Typha angustifolia*	20.0 x 1.5 x 1.0	0.64 m/d	-	Li et al. (2008)
Peradeniya, Sri Lanka	Municipal WW	*Scirpus grossus*	1 x 25 x 0.6	-	18	Tanaka et al. (2013)
	Municipal WW	*Hydrilla verticillata*	1 x 25 x 0.6	-	18	
Futian, Shenzhen, China	Municipal WW	*Kandelia candel*	2 x1 x 0.75	-	1; 2; 3	Yang et al. (2008)
	Municipal WW	*Aegiceras corniculatum*	2 x1 x 0.75	-	1; 2; 3	
Wuhan, China	Municipal WW	-	3.0 x 0.7x 1.0	130 l/d	-	Zhang et al. (2010)
EI, Salvador	Municipal WW	*Phragmites australis*	18.3 x 7.3 x 0.6	151.4	-	Katsenovich et al. (2009)
Can Tho University, Vietnam	Municipal WW	*Phragmites vallatoria*	12 x 1.6 x 1.1	31 mm/d	-	Trang et al. (2010)
				62 mm/d	-	
				104 mm/d	-	
				146 mm/d	-	
Subsurface vertical flow constructed wetlands						
Beijing, China	Municipal WW	*Salix babylonica*	1.5 x 0.8 x 1.0	0.12 m/d	-	Wu et al. (2011)
Jinhe River, Tianjin, China	River water	*Typha latifolia*	0.196 m^2 x 1.3 m	0.8 m/d	-	Tang et al. (2009)
Shanghai, China	Municipal WW	-	-	0.76 m^3/m^2.d: 0.04 m^3/m^2.d	-	Wang et al. (2006b)
Kampala, Uganda	Municipal WW	*Cyperus papyrus*	0.58 m^2 x 0.82 m	0.064	5	Kyambadde et al. (2004)
Wuxi, China	Livestock WW	*Phragmites communis*	2.0 x 2.0 x 1.0	0.4	-	He et al. (2006)
	Livestock WW	*Phragmites typhia*	2.0 x 2.0 x 1.0	0.4	-	
Guangzhou, China	Municipal WW	*Cyperus alternifoliu*	5.0 x 3.0 x 1.8	0.45m^3/m^2.d	18	Chan et al. (2008)
Chiang Mai, Thailand	UASB effluent	*Scirpusgrossus Linn*	2.0 x 2.0 x 1.4	3; 6; 12 cm/d	-	Kantawanichkul et al. (2003)
Wuhan, China	Municipal WW	*Typha orientalis*	1.0 x 1.0 x 1.0	250 mm/d	1.2	Chang et al. (2012)
	Municipal WW	*Canna indica*	1.0 x 1.0 x 1.0	250 mm/d	1.2	

Table 2.3 (cont.)

Location	Wastewater type	Wetland design and operation				
		Plant species	Dimension (L x W x D) (m x m x m)	HLR (m^3/day)	HRT (day)	References
		Sub- surface hybrid constructed wetlands				
Yongding River, China	Lake water	-	7.3 hm^2	0.58 m^3/m^2.d	34.26 h	Liu et al. (2007)
Texcoco, Mexico	Municipal WW	*Phragmites communis*	8.8 x 1.8 x 0.6	2.88	2.3	Belmont et al. (2004)
Nepal	Municipal WW	*Phragmites karka*	8.0 x 9.5 x 0.5	0.13 m d	-	Singh et al. (2009)
	Municipal WW	*Canna latifolia*	10.0 x 7.5 x 0.6	0.13 m d	-	
Turkey	Municipal WW	*Iris australis*	1.5 x 3.5 x 0.4	60 l/ m^2 d	-	Tunçsiper (2009)
	Municipal WW	*Phragmites australis*	1.5 x 3.5 x 0.32	60 l/ m^2 d	-	
Ningbo, China	Municipal WW	*Taxodium ascendens*	8 x 6 x 1	16 cm/d	5.4	Ye and Li (2009)
	Municipal WW	*Zizania aquatica*	7 x 5 x 3	32 cm/d	2.7	
Bogotá Savannah, Columbia	Municipal WW	-	4354 m^2 x 0.6 m	40 cm/d	0.6	Arias and Brown (2009)
	Municipal WW	-	17,416 m^2 x 0.5 m	10 cm/d	4.5	
Jakarta, Indonesia	Laboratory WW	*Typha sp.*	3.0 m^2 x 0.4 m	250 l/d	1	Meutia (2001)
	Laboratory WW	*Lemna sp.*	3.0 m^2 x 0.4 m	250 l/d	1	
Koh Phi, Thailand	Municipal WW	*Canna, Heliconia*	2300 m^2 x 0.7 m	400	-	Brix et al. (2011)
	Municipal WW	*Papyrus*	750 m^2 x 0.6 m	400	-	

Note: UASB: Up-flow anaerobic sludge blanket (UASB).

2.4.5 Constructed wetlands influent feeding mode

Influent feeding mode is reported as another crucial design factor that can affect the performance of a wetland system (Zhang et al., 2012). Wetlands can be fed in different ways such as continuous, batch or intermittent modes. These modes may affect the oxidation / reduction conditions as well as the oxygen to be transferred and diffused in the system resulting in treatment efficiency modification. Accordingly, several studies have been performed to investigate the impact of feeding mode on wetland system treatment efficiency.

Wu et al. (2015) stated that the batch feeding mode generally showed the best performance compared to the continuous one as the former can provide more oxygen in the treatment system. These results were confirmed by Zhang et al. (2012) who performed a study to compare the removal efficiency in tropical subsurface flow constructed wetlands operated using batch and continuous modes. Their results showed that ammonia was removed with an efficiency of 95.2% in the batch mode operated system which was significantly higher than that obtained from the continuous mode of 80.4% removal efficiency. Moreover, feeding the system intermittently can improve the removal of nitrogen and organic matter as reported by Saeed and Sun (2012).

It was indicated that the intermittent feeding system showed noticeable improvement in ammonium removal efficiency compared to that of the continuous one when a comparison study between two feeding systems was performed on subsurface flow constructed wetlands (Caselles-Osorio & García, 2007). On the other hand, the continuous feeding mode enhances the removal of sulphate compared to the intermittent ones as reported by Wu et al. (2015).

The impact of intermittent feeding mode and different duration of dry time on the treatment efficiency of vertical flow constructed wetlands was investigated by Jia et al.

(2010). The authors' results stated that compared to the continuous feeding system, the intermittent one showed lower chemical oxygen demand (COD) and total phosphorous (TP) removal efficiency with high ammonium reduction ($\geq$ 90%) due to the high oxygen available in the system during the intermittent feeding operation. This agrees with the results obtained from Jia et al. (2010) who studied the influence of continuous and intermittent feeding operation on nitrogen removal of free water surface flow and subsurface flow constructed wetlands. Authors' results showed that in subsurface flow constructed wetlands, the intermittent feeding operation significantly improved ammonium removal while no significant impact was observed in the free water surface constructed wetland system.

2.5 Impact of environmental factors on constructed wetlands behaviour

2.5.1 Wastewater pH

Wastewater pH is an important factor that may affect the performance of wetlands mainly in terms of nitrogen and organic matter removal. For example, substantial alkalinity consumption during the nitrification process leads to a significant drop in pH values of the system, subsequently affecting denitrification rates as discussed by Kadlec and Knight (1996). However, the optimum pH value for the denitrification process can range between 6.0 and 8.0, while the highest rate occurs at a pH value of 7.0 to 7.5 as reported by Saeed and Sun (2012). Moreover, Vymazal (2007) noted that the slower rate of denitrification process can occur at a pH value of 5.0, while insignificant denitrification rate can be observed at pH values below 4.

Wastewater pH value is also important for organic matter, mainly for anaerobic degradation processes (Saeed & Sun, 2012). This is because of the high sensitivity of the bacteria responsible for the formation of methane gas in the system to pH values;

the bacteria can only survive in pH values between 6.5 and 7.5. As a result, the anaerobic degradation process will not complete if the pH value is not in this range leading to volatile fatty acid accumulation in the system and a subsequent drop in the pH value killing all methanogens available in the wetland system as reported by Copper et al. (1996) and Vymazal (1999).

2.5.2 Temperature

Several studies have been undertaken to monitor the impact of weather temperature on wetland treatment processes (Zhang et al., 2014). For example Lim et al. (2001) and Trang et al. (2010) studied the wetland behaviour in tropical conditions and they found that there is a significant impact of higher operation temperature on improving the treatment process in less time, mainly associated with the rate of organic matter degradation, nitrification and denitrification processes. According to Demin and Dudeney (2003) and Katayon et al. (2008), a high rate of nitrification process can be achieved at a temperature range between 16.5 and 20 °C, while very slow rates occur at temperatures of 5 to 6 °C and above 40 °C as reported by Hammer and Knight (1994), Werker et al. (2002) and Xie et al. (2003). However, the ammonification process will occur optimally at a temperature range of 40 to 60 °C (Vymazal, 2007). Moreover, Tuncsiper (2009) reported that ammonia nitrogen and nitrate nitrogen removal efficiencies were 7% and 9%, respectively greater in summer than the winter in a constructed wetland system.

This because of the direct link between microbe activity and temperature in the wetlands and the subsequent impact on pollutants removal efficiency, which will generally decline at low temperature due to the reduction in microbial activities (Zhang et al., 2014). In shanghai, another study was undertaken to investigate the impact of seasonal temperature on the performance of constructed wetlands (Song et al., 2009). The authors' results showed that the treatment efficiency clearly depended on the temperature. For example, they found the removal efficiency of chemical oxygen demand was higher in summer and spring (66.3 and 65.4%, respectively) than in winter and autumn (59.4 and 61.1%, respectively). Also, they found the removal efficiency of ammonia nitrogen and total phosphorous was higher in summer (54.4 and 35.0%, respectively) than in winter (32.4 and 28.9%, respectively). On the other hand, Li et al. (2008) did not find significant differences in chemical oxygen demand removal efficiency in different seasons, while a noticeable difference in removal of nutrients was recorded in summer compared to winter. However, the negative impact of low temperature on nitrogen and organic matter removal in constructed wetlands was confirmed by Ruan et al. (2006), Akratos and Tsihrintzis (2008), Zhang et al. (2011) and Zhao et al (2011).

From the literature, it seems the wetland treatment efficiency in tropical regions is higher than that in temperate regions due to differences in the climate temperature promoting better plant growth leading to higher up-taking by macrophytes (Kivasi, 2001; Diemont, 2006; Katsenovich et al., 2009; Bodin, 2013). Moreover, high temperature will increase the microbial activity and subsequently increase the microbiological removal mechanisms. For example, the removal efficiency of organic matter will increase in high temperature as the rate of aerobic and anaerobic degradation will be increased as well.

On the other hand, high temperature will increase the ammonification rate and plant litter break-down releasing ammonia nitrogen and phosphorous from the tropical wetland sediments. As a result, the concentrations of these nutrients in the effluent will be higher than in the influent meaning negative removal efficiency in these wetlands.

2.5.3 Availability of oxygen

In subsurface flow constructed wetlands, the availability of oxygen is an important environmental factor which has a direct impact on treatment performance of the system as it controls the nitrification and aerobic degradation of organic matter (Saeed & Sun, 2012). However, in horizontal subsurface flow constructed wetlands which have a saturated substrate of constant water logging, there is insufficient oxygen availability leading to inhibition of nitrification processes (Cerezo et al., 2001; Ramirez et al., 2005), while in vertical flow constructed wetlands, the intermittent feeding mode of wastewater and unsaturated substrate will enhance the air diffusion and subsequently increase the availability of oxygen in the system as discussed by Sun et al. (1998) and Noorvee et al. (2007) and this will result in promoting the nitrification and aerobic degradation of organic matter (Saeed & Sun, 2012).

However, the denitrification and anaerobic degradation of organic matter is promoted in horizontal flow constructed wetlands, despite the lack of oxygen availability (Rousseau et al., 2004), indicating the effectivity of these systems in nitrate nitrogen and organic matter treatment (Saeed & Sun, 2012). On the other hand, the rate of oxygen transfer in vertical flow constructed wetlands was quantified to be 28.0 g $O_2/m^2.d$ (Cooper, 2005) but can be increased by forced aeration leading to improve the nitrification processes as reported by Saeed and Sun (2012).

Moreover, Ong et al. (2010) studied the impact of available oxygen on wetland treatment efficiency by comparing the results obtained from two vertical flow constructed wetlands, one aerated by forced aeration and other non-aerated. The results showed that the aerated system had higher nitrogen and chemical oxygen demand removals (90 and 94%, respectively) compared to those from the non-aerated system

(59 and 90%, respectively) indicating a significant impact of forced aeration on nitrogen removal efficiency but not on organic matter.

These results were confirmed by Stefanakis and Tsihrintzis (2012) who observed high removal efficiency of organic matter and nitrogen in their wetland system due to improving of system bed aeration. Enhancing aeration of the wetland substrate contributes strongly in removal of petroleum hydrocarbons in wastewaters, with efficiency of 100% as reported by Wallace et al. (2011). Regarding the vertical flow constructed wetlands, as the wastewaters are applied intermittently then drained vertically from the system by gravity, this will provide the wetland media with a high amount of oxygen supporting the aerobic biodegradation processes of the organic matter (Vymazal, 2007; Stefanakis & Tsihrintzis, 2012; Fan et al., 2013 and Zhi et al., 2015).

2.6 Wetlands crucial value

Natural wetlands, which the constructed wetlands are simulated from, have numerous important values mainly for people who have lived close to them throughout human history, during which the wetlands can be considered as a vital economic resource. Despite that, people have begun to realise the impotency of wetlands for their society during the last 50 years (Stefanakis et al., 2014; Vymazal, 2014). Wetlands can support various animal and plant life that can supply many ecosystem services for people's growth and development, such as food, water, flood control, and fuel wood, while supplying environmental biodiversity that can improve water quality, in addition to the social services of aesthetic and recreation improvement which wetlands can supply (Scholz, 2006, 2010).

Furthermore, wetlands can provide other values, such as reduction of global warming by absorbing carbon dioxide, in addition to providing indirect food chain support by producing different animals which can be consumed by humans (Stefanakis et al., 2014), as well as controlling erosion and pollutant degradation (Minga et al., 2007). However, because of the vital role of wetlands in controlling pollution, some scientists have named them "Earth's kidneys" as they catch and retain most of the contaminants passed through them before they can be received by a surface water body (Kadlec & Knight, 1996; Cui et al., 2012). Moreover, wetlands are named "biological supermarkets" by some scientists because of their highly natural production as indicated by Barbier, Acreman, and Knowler (1997), Mitsch and Gosselink (2000) and Chen and Lu (2003).

However, wetland values have been classified by different scientists. For example, wetland value classification of Vymazal et al. (1998) and Cui et al. (2012) was based on the following:

- Hydrological values: flood and erosion control and recharging of ground water;
- Climatic values: global warming and carbon fixation;
- Biodiversity and wildlife improvement;
- Research values;
- Aesthetic, recreational and reclamation values;
- Producing energy; and
- Aquaculture system improvement

Moreover, De Groot et al. (2006) and Cui et al, (2012) identified the value of wetland as enhancement of aquifer ground water, flood management, settling of different materials, reducing CO2, heat storing and releasing, reducing of solar radiation and food chain support. Other researchers classified the wetland values as ecological, economic and sociocultural whereby the combination of all of them will give the overall wetlands value (Stefanakis et al. 2014). According to Schuyt and Brander (2004), ecological, sociocultural and economic aspects were reported to be the overall wetlands value, including environmental biodiversity, water provision, water quality improvement, irrigation, flood reduction, wood production and supply of hydroelectric power, in addition to recreation, carbon dioxide balance, weather improvement, education and scientific values.

Regarding the economic viewpoint, several studies have been undertaken to estimate the monetary value of wetlands. For example, Costanza et al. (1997) predicted the monetary value of wetlands around the world and found this value can reach up to US$ 14.9 trillion, while Schuyt and Brander (2004) estimated the annual monetary value of worldwide wetlands to be US$ 70 billion.

Considering all wetland values mentioned above, especially the aptitude of controlling flood events and capability of wastewater purification, wetlands have become significantly recognised, as reported by Stefanakis et al. (2014) and Vymazal (2014). Nowadays, wetlands are known to remove various types of contaminants, such as organic and inorganic material as well as metals and trace elements from wastewater, based on natural physical, chemical and biological processes, and this has motivated researchers to produce constructed wetlands for assessing the impact of different technologies on wetlands performance. The purpose of constructed wetlands is to increase the various processes which occur in the system under controlled environmental conditions for human benefit, such as flood inhibition and water quality enhancement. Assessment of constructed wetlands compared to natural ones has been undertaken by many researchers. For instance, the assessment of subsurface flow wetlands for wildlife habitation was undertaken by Knight et al. (2001). In their assessment, they found that their systems can provide flora and fauna with habitation and diversity as well as recreation provision (water storage and bird watching) and enhancement of the environment aesthetically.

Regarding the ecological value of both types of wetlands, Campbell, Cole, and Brooks (2002) reported that artificial and natural wetlands have the same value. This contradicted with Ghermandi et al. (2010) who indicated that constructed wetlands showed more value than natural ones during their studies comparing 186 different natural and constructed wetlands.

Because of wetlands values in treating wastewater, several studies have been undertaken to assess the recycling of wetland effluent for different purposes, mainly agricultural reuse. For example, Cui, Luo, Zhu, and Liu (2003) carried out an experimental work on vertical flow constructed wetlands treating septic tank effluent in Guangzhou (China) which achieved removal rates for chemical oxygen demand, five-day biochemical oxygen demand, suspended solids, total nitrogen and total phosphorus of 60, 80, 74, 49 and 79%, respectively. After that the treated effluent was used for hydroponic cultivation of water spinach and romaine lettuce. The removal efficiencies of the whole system for chemical oxygen demand, five-day biochemical oxygen demand, suspended solids, total nitrogen and total phosphorus were 71, 98, 97, 86, and 87%, respectively. It was found that using treated effluent for hydroponic cultivation of vegetables could reduce the nitrate content in vegetables. The removal rates for total bacteria and coliforms by using a vertical flow bed system with cinder substrate were between 80 and 90%, and between 85 and 96%, respectively. Lopez et al. (2006) assessed constructed wetlands treating municipal effluents to be reused in agriculture. The authors' results recorded average removal efficiencies for suspended solids, five-day biochemical oxygen demand, chemical oxygen demand, total nitrogen and total phosphorus of 85, 65, 75, 42 and 32%, respectively. Morari and Giardini (2009) evaluated the treatment efficiency of pilot-scale vertical flow constructed wetlands on municipal wastewaters and their suitability for irrigation reuse in Italy. Their results showed that only water quality parameters with high removal efficiencies fulfilled the Italian guidelines for irrigation reuse, whereas parameters with lower efficiencies such as suspended solids and total phosphorus limited the potential water reuse. Furthermore, Cirelli et al. (2012) presented the results of a reuse scenario where tertiary-treated municipal wastewater using a constructed wetland was supplied for irrigation of vegetables in Eastern Sicily, Italy. They found elevated levels of *Escherichia coli* in the irrigation water, which were frequently above the Italian limits of 50 colony forming units (CFU)/100 ml for secondary urban effluents.

2.7 Selection of vertical flow constructed wetlands for domestic wastewater treatment

Due to their ability in different wastewater purification, vertical and horizontal subsurface flow wetlands investigation is spreading worldwide (Abou-Elela et al., 2013; Kantawanichkul & Wannasri, 2013; Paing et al., 2015; Wu et al., 2014). This is because they are cheaply operated with simple operation and maintenance in addition to their environment aesthetic improvement (Scholz, 2006, 2010). Although both vertical and horizontal wetlands can treat wastewater well, some studies have reported better performance of vertical flow wetlands in terms of some water quality parameters than horizontal ones. For instance, researchers showed that the draining of wastewater from vertical flow constructed wetland media enhances the biochemical oxygen demand (BOD) and ammonia nitrogen reduction, supplying outstanding conditions for nitrification processes (Cooper, 1996, Vymazal et al., 2006; Gikas & Tsihrintzis, 2012; Fan et al., 2013; Li et al., 2015) in comparison with those for horizontal flow constructed wetlands of continuously saturated and anaerobic media. Moreover, vertical flow wetlands were reported to perform satisfactorily in terms of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) and particles removal (Brix & Arias, 2005; Prochaska et al., 2007).

Although vertical flow constructed wetlands (VFCWs) are known to be poor in terms of denitrification (Vymazal, 2005), some other studies have stated that the modification in vertical flow constructed wetlands to a discontinuous loading rate system will improve their denitrification processes. Some researchers have stated that total nitrogen (TN), and chemical and biochemical oxygen demands (COD and BOD) were removed from wastewater treated by their vertical flow wetlands at a rate of 69 and 90%, respectively (Arias et al., 2005; Gross et al., 2007). Moreover, suspended solids (SS), biochemical oxygen demand (BOD) and ammonia nitrogen (NH_4-N) were removed at an efficiency of 90% from municipal pre-settled wastewater treated by vertical flow constructed wetlands operated for 10 years at a specific loading rate in the UK (Weedon, 2003, 2010). Stefanakis et al. (2014) appealed that the wetland system was improved when using sand as a main filter substrate with discontinuous application of wastewater considering the intermission as an aeration time for the system, representing the ability of wetlands to treat the wastewater with high standards.

In China, Shen et al. (2015) assessed a wetlands system in which starch blends were used as a source of solid carbon to improve nitrate removal and their results showed that high denitrification rate was achieved with nitrate removal rate of 98%. In addition, 95% removal efficiency of organic matter and ammonia-nitrogen (NH_4-N) was achieved in a wetland system of no modification, as reported by Li et al. (2015). However, vertical flow constructed wetlands (VFCWs) can be considered as the technological art used to control water pollution. Moreover, the small area required for establishing vertical flow constructed wetlands compared to that required for horizontal ones has rapidly increased interest in the former one around the world, as indicated by Abou-Elela et al. (2013), Stefanakis et al. (2014) and Paing et al. (2015). For example, construction of vertical flow wetlands requires 1–2 square metres per capita, while horizontal ones need 5–10 square metres per capita. These unit areas for vertical flow constructed wetlands have been used in many countries including the UK (Cooper & Green, 1998; Cooper, 2005; Weedon, 2010), while in the Czech Republic and Greece they used 1–1.5 square metres per person (m^2/pe) as reported by Vymazal and Kröpfelová (2011) and Stefanakis and Tsihrintzis (2012). Area requirements of 1.6 m^2/pe and 1.5 m^2/pe were used in Germany and the Canary Islands as stated by Olsson (2011) and Vera, Martel, and Márquez (2013). Moreover, in treatment, various types of contaminants including organic, inorganic, suspended solids and others were found to be removed better in vertical flow constructed wetlands than in horizontal ones, as reported by several studies. For instance, in Turkey, Yalcuk and Ugurlu (2009) studied the removal efficiency of vertical and horizontal flow constructed wetlands treated landfill leachate and they stated that the heavy metals such as chromium, copper, zinc, lead and nickel were removed well in VFCWs compared to the HFCWs. Moreover, assessment of the performance of vertical and horizontal flow constructed wetlands treating polluted river water in Vietnam was undertaken by Konnerup et al. (2011). The authors stated that vertical flow constructed wetlands treated the contaminated river water with high efficiency and low environmental harmful effect compared to the horizontal systems. In addition, vertical flow constructed wetlands were reported to remove nitrogen better than horizontal ones during an operation time of 4 years in Boku university (Canga et al., 2011). However, several studies have been undertaken recently in urban wastewater treatment, which suggest the use of vertical flow constructed wetlands rather than horizontal ones to improve water quality. Pandey et al. (2013), for example, studied the performance of two wetland systems treating urban wastewater in Nepal for an operation period of 7 months and their results showed that VFCWs had treatment efficiency higher than horizontal ones. Similarly, vertical flow constructed wetlands were reported to be more effective in treating municipal wastewater than horizontal systems during the long-term study of 3 years undertaken by Abou-Elela et al. (2013).

The probability of clogging (a phenomenon in which media pores are blocked leading to a reduction in the hydraulic conductivity and permeability of the system which directly impacts the whole system behaviour) was assessed in both vertical and horizontal constructed wetland systems. For instance, Knowles et al. (2011) reported that horizontal flow constructed wetlands are more likely to have clogging issues than vertical ones after comparison studies were undertaken between the two systems. These issues mainly depend on the applied hydraulic and solids loads used in both systems, therefore, these loads should be considered carefully when designing the wetland systems. A high hydraulic loading rate will make the HFCWs more likely to clog than the VFCWs, making the latter one the most preferable system. Furthermore, the lack of oxygen in HFCWs (Vymazal, 2014) makes them more prone to clogging in comparison to VFCWs which are known to be rich in oxygen (Hua et al., 2014; Song et al., 2015), making the latter system the most preferable choice.

2.8 Recycling of treated wastewater for irrigation purposes

Water supply and water quality degradation are global concerns. With increasing water demand, the unexpected impacts of extreme events, and climate change, these concerns will be intensively increased. For this reason, marginal water quality will become a highly important component of agricultural water supplies, mainly in water-scarce countries (Qadir et al., 2007). Since about 70% of water around the world is reserved for irrigation purposes (UNESCO, 2003), enabling the application of treated wastewater for agricultural use is of high potential (Meda & Cornel, 2010) even if this amount of treated wastewater can only cover a portion of irrigation requirements, as reported by Norton-Brandao et al. (2013). The scientific basis for the acceptance of wastewater reclamation, recycling and reuse in agriculture has evolved from developments in water and wastewater engineering science coupled with an increasing pressure on water resources management. However, the evaluation of the effects of treated wastewater reuse on crops intended for human consumption is of particular interest (Aiello, Cirelli, & Consoli, 2007; Asano & Levine, 1996; Cirelli et al., 2012).

2.9 Standards for irrigation water quality

The quality of the wastewater mainly depends on the quality of the water source, the nature of the wastes added during use, and the degree of wastewater treatment which has been applied. The water characteristics of importance in agricultural and landscape irrigation are particular chemical elements and compounds that affect plant growth or soil permeability (Pedrero et al., 2010). Wastewater should be sampled and analysed for those constituents before being used for irrigation to define the suitability of the water for agricultural and landscape irrigation (Pettygrove & Asano, 1984).

The potential health risks and environmental impacts resulting from wastewater use for irrigation have been well documented (Angelakis, Bontoux, & Lazarova, 2002). Health and environmental aspects are particularly sensitive issues and important fundamentals, since wastewater effluent must not be used and/or be accepted to replace conventional or possibly other non-conventional water sources for irrigation, unless it is sufficiently treated and safely applied (Gerba & Rose, 2003; Salgot, Vergés, & Angelakis, 2003). The main goals of water reuse in agriculture are to provide an adequate supply of high quality water for agronomists and to warrant food safety (Dobrowolski, O'Neill, Duriancik, & Throwe, 2008). Table 2.4 provides an overview of wastewater parameters and constituents with their potential impact on agricultural use, while Table 2.5 displays various guidelines on quality of irrigation water. In the following subsections, more details regarding these problems are provided.

Table 2.4: Wastewater pollutants, contaminants and their possible impact on agricultural use (adapted and updated from Asano et al. (1985) and FAO (2003)).

Pollutant/ Constituent	Parameter	Impacts
Plant food nutrients	Nitrogen (N), phosphorous (P), potassium (K), etc.	- Excess N: potential to cause nitrogen injury, excessive vegetative growth, delayed growing season and maturity, and potential to cause economic loss to farmer - Excessive amounts of N and P can cause excessive growth of undesirable aquatic species (eutrophication). - Nitrogen leaching causes ground water pollution with adverse health and environmental impacts
Suspended solids	Volatile compounds, settleable, suspended, and colloidal impurities	- Development of sludge deposits causing anaerobic conditions - Plugging of irrigation equipment and systems such as sprinklers
Pathogens	Viruses, bacteria, helminth eggs, faecal coliforms etc.	- Can cause communicable diseases
Biodegradable organics	Biochemical oxygen demand (BOD) and chemical oxygen demand (COD)	- Depletion of dissolved oxygen in surface water - development of septic conditions - unsuitable habitat and environment - Can inhibit pond-breeding amphibians - Fish mortality - humus build-up
Stable organics	Phenols, pesticides, chlorinated hydrocarbons	- Persist in the environment for long periods - Toxic to environment - may make wastewater unsuitable for irrigation
Dissolved inorganic substances	Total dissolved solids (TDS), electrical conductivity (EC), sodium (Na), calcium (Ca), magnesium (Mg), chlorine (Cl), and boron (B)	- Cause salinity and associated adverse impacts - Phytotoxicity - Affect permeability and soil structure
Heavy metals	Cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), arsenic (As), mercury (Hg), etc.	- Bio accumulate in aquatic organisms (fish and planktons) - Accumulate in irrigated soils and the environment - Toxic to plants and animals - Systemic uptake by plants - Subsequent ingestion by humans or animals - Possible health impacts - May make wastewater unsuitable for irrigation
Hydrogen ion concentrations	pH	- Especially of concern in industrial wastewater - Possible adverse impact on plant growth due to acidity or alkalinity - impact sometimes beneficial on soil flora and fauna
Residual chlorine	Both free and combined chlorine	- Leaf-tip burn and damage some sensitive crops, when exceeds 5 mg/l free chlorine - Groundwater, surface water contamination (carcinogenic effects from organochlorides formed when chlorine combines with residual organic compounds)

Table 2.5: Irrigation water quality guidelines (adapted and updated from Norton-Brandao et al. (2013)).

Guideline	Unit	**Westcot and Ayers (1985)**	**WHO (1989)**	**US EPA (2004)**	**ANZECC (2000)**	**SRD (2007)**	**Italian Decree (2003)**	**FAO (1994, 2003) and Pescod (1992)**
Irrigation parameter/type of guideline		Water quality for irrigation	Wastewater quality for agriculture	Reclaimed water quality for irrigation	Water quality for irrigation	Water quality for agriculture	Treated wastewater quality for reuse	Reclaimed water quality for irrigation
Salinity								
Electrical conductivity (EC)	dS/m	0.7–3[a]	-	-	<0.65; 0.65–1.3; 2.9–5.2[i]	3	-	0.7–3; 3[a]
Sodium adsorption ratio (SAR)	-	-	-	-	5-10	6	10	0–15
Sodium (Na)	me/l	-	-	-	-	-	-	0–40
Magnesium (Mg)	me/l	-	-	-	-	-	-	0–5
Calcium (Ca)	me/l	-	-	-	-	-	-	0–20
Carbonate (CO_3)	me/l	-	-	-	-	-	-	0–0.1
Bicarbonate (HCO_3)	me/l	-	-	-	-	-	-	0–10
Chloride (Cl)	me/l	-	-	-	-	-	-	0–30
Sulphate (SO_4)	me/l	-	-	-	-	-	-	0–20
Total dissolved solids (TDS)	mg/l	450–2000[a]	-	500–2000	-	-	-	450–2000[a]
Suspended solids (SS)	mg/l	-	-	-	-	20	10	-
pH	-	6.5–8	-	6	-	-	6–9.5	6.5–8.4
Pathogenicity								
Intestinal nematodes	eggs/l	-	<1[c]	-	-	-	-	-
	eggs/10 l	-	-	-	-	1[l]	-	-
E. coli	CFU/100 ml	-	-	-	-	100	100	-
Faecal coliforms (FC)	CFU/100 ml	-	<1000[c]	-	-	-	-	-
Thermotolerant coliforms	CFU/100 ml	-	-	-	<10; <1000[j]	-	-	--
Total coliforms (TC)	CFU/100 ml	-	-	0–1000[d, e]	-	-	-	-

Table 2.5 (cont.)

Nutrients								
Nitrate-nitrogen (NO_3-N)	mg/l	-	-	-	-	5.5	-	5–30[a]
Ammonia-nitrogen (NH_4-N)	mg/l	-	-	-	-	-	-	0–5
Total Nitrogen (TN)	mg/l	-	-	10[d, f]	5; 25 to 125[k]	10	15	-
Phosphorus (PO_4-P)	mg/l	-	-	5[d, g]	0.05; 0.8 to 12[k]	-	2	0–2
Potassium (K)	mg/l	-	-	-	-	-	-	0–2
Heavy metals and trace elements								
Aluminium (Al)	mg/l	-	-	5; 20[h]	5; 20[h]	-	1	5
Arsenic (As)	mg/l	-	-	0.1;2[h]	0.1;2[h]	0.1	0.02	0.1
Beryllium (Be)	mg/l	-	-	0.1; 0.5[h]	0.1; 0.5[h]	0.1	0.1	0.1
Cadmium (Cd)	mg/l	-	-	0.01; 0.05[h]	0.01; 0.05[h]	0.01	0.005	0.01
Cobalt (Co)	mg/l	-	-	0.05;5[h]	0.05; 0.1[h]	0.05	0.05	0.05
Chromium (Cr)	mg/l	-	-	0.1; 1[h]	0.1; 1[h]	0.1	0.005	0.1
Copper (Cu)	mg/l	-	-	0.2; 5[h]	0.2; 5[h]	0.2	1	0.2
Iron (Fe)	mg/l	-	-	5; 20[h]	0.2; 10[h]	-	2	5
Lithium (Li)	mg/l	-	-	2.5; 2.5[h]	2.5; 2.5[h]	-	-	2.5
Manganese (Mn)	mg/l	-	-	0.2; 10[h]	0.2; 10[h]	0.2	0.2	0.2
Molybdenum (Mb)	mg/l	-	-	0.01; 0.05[h]	-	0.01	-	0.01
Nickel (Ni)	mg/l	-	-	0.2; 2[h]	0.2; 2[h]	0.2	0.2	0.2
Lead (Pb)	mg/l	-	-	5; 10[h]	2; 5[h]	-	0.1	5
Selenium (Se)	mg/l	-	-	0.02; 0.02[h]	0.02; 0.05[h]	0.02	0.01	0.02
Vanadium (V)	mg/l	-	-	0.1; 1[h]	0.1; 0.5[h]	0.1	0.1	0.1
Zinc (Zn)	mg/l	-	-	2; 10[h]	2; 5[h]	-	0.5	2
Boron (B)	mg/l	-	-	-	-	-	-	0.7–3[a]; 0–2

Note: [a] For a slight to moderate degree of restriction on use; [b] For surface and sprinkler irrigation respectively; [c] Irrigation of crops likely to be eaten uncooked, cereal crops, industrial crops; [d] Food crops; [e] Value depends on the state of the USA, treatment degree of the water and type of crop (raw, edible); [f] Parameter only set for the state of New Jersey; [g] Parameter only set for the state of Michigan; [h] Long term and short term irrigation; [i] Sensitive, moderately sensitive and tolerant crops, respectively; [j] Raw human food crops with and without direct contact with irrigation water, respectively; [k] Maximum concentration (mg/l) which can be tolerated for 20 and 100 years, respectively; and [l] Crop irrigation using a system whereby reclaimed water comes into direct contact with edible parts of crops to be eaten raw.

2.9.1 Sediments

Suspended solids (SS) often include volatile and fixed solids which are considered as one of the most common contaminants found in wastewater. Suspended solids are those particles of size larger than 2 microns found in the water. Other particles smaller than 2 microns are considered as dissolved solids. Most of the suspended solids constituents are inorganic material. Moreover, bacteria and algae could also contribute to the total solids concentration.

Turbidity measurements are often used as an indicator of water quality based on clarity and expected total suspended solids in water (FAO, 2003). Water turbidity is dependent on the amount of light dispersed by particles in the water column. With increasing particles in the water the light spreading will increase as well. This explains how turbidity and suspended solids are related to each other (Hannouche et al., 2011). However, turbidity cannot be used for direct measurement of suspended solids in the water. Since turbidity is used to measure the water clarity, it is usually measured as an indicator of how suspended solid concentrations change in the water without providing an exact measurement of solids.

These solids can cause development of sludge deposits and anaerobic conditions especially when untreated wastewater is discharged to the aquatic environment or agricultural area (FAO, 2003). Moreover, excessive amounts of suspended solids will plug the irrigation system especially when using drip and sprinkler systems (Table 2.4). However, clogging frequencies were observed to be greater in drip irrigation systems than in sprinklers systems, as reported by (Capra & Scicolone, 2007).

Kadlec and Knight (1996), Green et al. (1997), Garcia et al. (2010) and Hua et al. (2013) reported that most of these solids can be removed well using constructed wetlands (CWs) technology through sedimentation, settling, adsorption and biological degradation processes performed in CWs systems. Moreover, in surface flow constructed wetlands, solids removal will occur through flocculation, sedimentation and filtration processes undertaken in the system as reported by Kadlec and Wallace (2009). In addition, interaction and adhering of suspended solids with other constituents available in a wetlands system, such as heavy metals and nutrients, pathogens and organic matter, will improve their removal from wastewater (Sundaravadivel & Vigneswaran, 2001).

In subsurface vertical flow constructed wetlands, the removal of solids will depend on characteristics of the substrate, hydraulic load and microorganisms available in the system (Manios, Stentiford, & Millner, 2003). However, vertical flow constructed wetlands have been reported to be very efficient in solids removal due to the surface area provided by the system media leading the particles to settle gravitationally and adhere with substrate and plants surfaces (Song et al., 2015)

2.9.2 Nutrients and minerals

The nutrients in treated wastewater are considered as a valuable fertiliser provided to the crop or landscape production. However, the availability of these nutrients in abundance may exceed the needs of plants causing problems of excessive growth in vegetative parts rather than the flowers and seeds, delayed or uneven maturity, or reduced quality as well as pollution of underground water (Westcot & Ayers, 1985). The nutrients in reclaimed wastewater can support crop growth, but periodic monitoring is required to avoid imbalanced nutrient supply (FAO, 2003). However, more details about these elements are shown in the sections below:

2.9.2.1 Nutrients

Jone (2013) discussed the positive and negative impacts of key nutrients and minerals on plants. The major minerals impacting on the growth of plants are nitrogen (predominantly ammonium), phosphorus, potassium, calcium, magnesium and sulphur. Nitrogen, phosphorous and potassium are essential nutrients for plant growth and their presence will improve the value of the water for irrigation purposes (FAO, 2003). However, when discharged to the aquatic environment, nitrogen and phosphorous will lead to the growth of undesirable aquatic life and if discharged with excessive amounts on land, nitrogen can cause the pollution of ground water as discussed by FAO (2003) and Asano et al. (1985).

Nitrogen concentrations in urban wastewater range between 20 and 100 mg/l based on local people's water use (Pescod, 1992). However, nitrogen can be available in wastewater either organically or inorganically. Amino acids, urea and uric acids can be considered as the main sources for organic nitrogen (Kadlec & Knight, 1996), while inorganic nitrogen can be available as ammonium, nitrite and nitrate (Saeed & sun, 2012). In treated wastewater, the amount of those nitrogen forms is dependent on treatment processes. For instance, nitrogen forms and concentrations can be controlled by proper selection of hydraulic loading rate and periods for flooding and drying in the treatment system (Asano, 1994; Asano & Levine, 1996). Hence, if ammonium is the most common nitrogen form in the wastewater, then short flooding duration with regular drying time such as 2 flooding days and 5 drying days will result in high nitrification rate for the ammonium in the system because of the availability of aeration conditions. On the other hand, long flooding and drying times (e.g. 1 month flooding and 1 month for drying) will lead to ammonium development and absence of nitrification due to the anaerobic conditions (Asano, 1994).

However, if these flooding and drying times are applied intermediately (e.g. 1 to 2 weeks flooding against 1 to 2 weeks drying times) then there will be a chance for aerobic and anaerobic conditions simulated nitrification and denitrification processes in which undertaken by anaerobic bacteria resulting in the conversion of nitrate to nitrogen gas and oxides that return to the atmosphere (Asano, 1994; Asano & Levine, 1996).

In constructed wetlands systems, nitrogen is largely removed by nitrification (oxidation of ammonia to nitrate) and denitrification (reduction of nitrates to nitrogen gas) processes. These two processes can occur simultaneously only in media with aerobic and anaerobic conditions (Cooper et al., 1996). This means insufficient nitrogen removal in wetlands systems if the available oxygen for aerobic biodegradation is inadequate (Scholz, 2010; Fan et al., 2013; Vymazal, 2014). Choudhary et al. (2011) reported that processes of nitrogen eradication in constructed wetlands are nitrification, ammonia volatilisation, fixation, nitrate ammonification, ammonification, denitrification, organic nitrogen interment, anammox (anaerobic ammonium oxidation), plant and microbial uptake and ammonia adsorption. However, among all of these processes, nitrification and denitrification are considered as the main mechanisms for nitrogen removal in constructed wetland systems, while other processes are secondary as they are limited by the substrate adsorption capacity (Song et al., 2010). Moreover, volatilisation, adsorption and plant uptake only contribute in a very small part to nitrogen removal, for example Tuncsiper (2009) and Kantawanichkul et al. (2003) reported that only 4 to 7% of total nitrogen can be up-taken by plants in the wetland systems. This contradicts results observed by Shamir et al. (2001); Healy and Cawley, 2002; Kantawanichkul et al. (2009) and Bialowiec et al. (2011) who quantified a wide range of 0.5 to 40% of total nitrogen could be removed by plants. However, nitrogen levels can be increased in the effluent when plant detritus is decayed (USEPA, 2000).

Generally, the rate of nitrogen removal is mainly dependent on the wetlands type (Vymazal, 2007). For example, single stage wetlands showed low efficiency in total nitrogen elimination compared to combined ones, such as hybrid systems which can completely remove total nitrogen as reported by Vymazal and Kröpfelová (2011) and Ayaz et al. (2012). Moreover, vertical flow constructed wetlands were reported to be more effective in nitrogen removal than horizontal ones due to the good aeration conditions available in the former one resulting from draining the system regularly, unlike the latter one which is known for its lack of oxygen (Neralla et al., 2000; Vymazal, 2007). Because of this, several researchers suggested an intermittent loading mode, promising long distance of flow providing essential organic matter for denitrification process, aiming to remove nitrogen effectively (Luederitz et al. 2001; Vymazal, 2005; Ayaz et al., 2012; Mietto et al., 2015).

Environmental conditions such as temperature are reported to affect nitrogen removal considerably in wetland systems as in higher weather temperature the microbe activity will increase leading to increased nitrification processes and a subsequent high rate of nitrogen removal (Kadlec, 1999; Kuschk et al., 2003; Gikas & Tsihrintzis, 2010, 2012). In addition to temperature, nitrogen removal in wetland systems may be affected by the annual cycle of other parameters such as precipitation, humidity, vegetation growth (Kadlec, 1999), pH, alkalinity, inorganic carbon source, microbial population, ammonia nitrogen concentration and dissolved oxygen (Vymazal, 2007). Contradicting this, Harbel, Perfler, and Mayer (1995) and Reed, Middlebrooks, and Crites (1995) stated that there is no clear relationship between temperature variation and nutrient removal.

Phosphorus in wastewater can be available in concentrations ranging between 5 and 50 mg/l based on local people's water use (Pescod, 1992). In constructed wetlands, phosphorous is transformed to organic and inorganic phosphate biologically. In alkaline soil (pH > 7), phosphate will precipitate with calcium forming calcium phosphate, while in acidic soil (pH < 7), phosphate will react with iron and aluminium oxides in the soil resulting in insoluble compounds. Initially, phosphate is immobilised by soil adsorption then gradually reverts to insoluble forms that allow more absorption of mobile phosphate. However, in media of clean sands with neutral pH, phosphate will be relatively mobile (Asano, 1994; Asano & Levine, 1996). The bioavailability of ortho-phosphate-phosphorus makes it is rapidly and easily consumed by macrophytes and algae (Vymazal, 2007; Choudhary et al., 2011).

According to Vymazal (2007), phosphorus can be removed in wetland systems by several processes such as leaching, mineralisation, fragmentation, desorption, adsorption, and plant and microbial uptake as well as retention of sediment. However, Gikas and Tsihrintzis (2012) stated that consumption of phosphorus by microbes and adsorption via media pores are the most predominant processes to eliminate phosphorus in wetland systems.

Mechanisms by which phosphorous is removed from wastewater differ based on wetland types. For instance, in subsurface vertical flow wetlands, system media can adsorb phosphorous and the substrate type will impact the absorption capacity, while adsorption in free water surface wetlands will be by emergent plants (Vymazal et al., 1998).

Generally, subsurface flow constructed wetlands were reported to be ineffective in terms of phosphorous removal (Vyzamal, 2007). This is because phosphorous removal in these systems mainly occurs by adsorption and/or precipitation in the system matrix

(Kadlec & Knight, 1996) as well as by plant and biomass up-taking (Mander et al., 2003). This is confirmed by Paing et al (2015) who reported that the main mechanism in phosphorous removal is adsorption by the system matrix when they reviewed 169 full-scale subsurface vertical flow constructed wetlands in France.

In subsurface flow constructed wetlands, although plants can uptake phosphorous from the system, they can also release it to the effluent when plant detritus starts to decay (USEPA, 2000), explaining the reason why in some systems phosphorous concentrations in the outflow were greater than the inflow. According to Vohla et al. (2011), removal of phosphorous in subsurface flow constructed wetlands is difficult to sustain due to dependency of the system life on the used filter media (Gruneberge & Kern, 2001). However, Paing et al. (2015) reported a decreasing tendency in phosphorous removal with system age as they observed 47%, 30% and 9% phosphorous removal during the first year, between the second and sixth years and between the sixth and twelfth years, respectively. Moreover, filter media should contain calcium, iron or aluminium to achieve effective phosphorous elimination from the wastewater by precipitation and sorption processes (Arias et al., 2001; Vohla et al., 2007).

Regarding the type of wetlands substrate, use of a gravel bed is not suitable in subsurface flow constructed wetlands (mainly horizontal ones) if phosphorous removal is the main target. This is reported by Kurkusuz et al. (2005) who performed a study to compare two subsurface flow constructed wetlands in terms of phosphorous removal by applying the same hydraulic and organic loads using blast furnace slag and gravel based substrates. They found that blast furnace slag removed phosphorous significantly better than the gravel media showing removal efficiencies of 47% and 4%, respectively. Furthermore, alum sludge was observed to eliminate phosphorous in subsurface flow constructed wetlands effectively showing a monthly removal efficiency ranging between 75% and 94% (Zhao et al., 2011).

Regarding the guidelines of proper wastewater quality for agriculture, FAO (1994) classified the suitability of treated wastewater for recycling in terms of nutrients. For instance, acceptable ranges for ammonia-nitrogen, ortho-phosphate-phosphorous and potassium were between 0 and 5, between 0 and 2, and between 0 and 2 mg/l, respectively (Table 2.5). Furthermore, Pescod (1992) stated that there is no restriction for irrigation water reuse if nitrate-nitrogen values are < 5.0 mg/l. Slight to moderate constraints exist for the range between 5 and 30 mg/l. Severe recycling restrictions are usually imposed for values of more than 30 mg/l.

2.9.2.2 Heavy metals and trace elements

The micro-nutrients that are beneficial for plants in small amounts are (in no particular order) copper, manganese, molybdenum, iron, zinc and iodine. Copper, manganese, molybdenum, iron, zinc and aluminium (in no particular order) are often described as heavy metals (Jone, 2013). In heavily industrial countries, these heavy metals may be considered as a significant problem when recycling treated wastewater. Cadmium (Cd), copper (Cu), molybdenum (Mo), nickel (Ni) and zinc (Zn) are some of those heavy metals which may be available in the wastewater. They pose a serious risk to human health and animals and even the irrigated crops, as they can accumulate in crop tissue and subsequently affect humans or animals feeding on them (FAO, 2003; Bakhshayesh, Delkash, & Scholz, 2014). As a result, in some developed countries, maximum heavy metal loads allowed to be applied to land are listed, as shown in Table 2.6.

Moreover, Pescod (1992) and FAO (2003) recommend limits for trace minerals in reclaimed water to be used for irrigation. Long-term (for water used continuously on all soils) and short-term limits (for water used for a period of up to 20 years on fine-textured neutral alkaline soils) are shown in Table 2.5.

Table 2.6: Maximum allowed metals yearly applied loading for agricultural land (adapted from FAO (2003)).

Country	Maximum applied load (kg/ha)						
	Cd	Cu	Cr	Pb	Hg	Ni	Zn
France	5.4	210	360	210	2.7	60	750
Germany	8.4	210	210	210	5.7	60	750
Netherlands	2.0	120	100	100	2.0	2	400
UK	5.0	280	1000	1000	2.0	70	560

According to FAO (2003), in Near East countries heavy metal and trace elements in wastewater are not considered as a serious problem. This is because of the low concentrations of heavy metals in urban wastewater due to the low levels of industrial activities in addition to the mostly alkaline soil (pH > 7) with high calcium carbonate ($CaCo_3$) in such regions. This alkaline soil will inhibit the activity of heavy metals and reduce their mobility and availability for crops. In such a case, concentration and loading of heavy metals are allowed to be higher than those listed in Tables 2.5 and 2.6, respectively. Therefore, application of wastewater containing heavy metals on calcareous soil is not considered while in the case of acid soils (soil with a pH of less than 7), heavy metals could be a problem to sensitive plants and hence specific management actions should be undertaken (Westcot & Ayers, 1985; FAO, 1994, 2003) such as:

- Apply liming on acid soil to increase the alkalinity and subsequently reduce heavy metals solubility and availability for crops;

- Avoid application of acid fertiliser;
- Select crops with high tolerance to heavy metals;
- Select crops with no bio-magnification properties.

As heavy metals are toxic for plants in high inflow water concentrations, their presence in wastewater may limit the suitability of the wastewater for irrigation (FAO, 1994, 2003). However, hydrogen ion activity (pH) of irrigation water affects metal solubility and its availability for plants. According to Pescod (1992) and FAO (2003), the normal range of pH for irrigation water is between 6.5 and 8.5.

In constructed wetlands, these heavy metals and trace elements can be removed by various mechanisms. For example, Denga, Yea, and Wonga (2004); Galletti, Verlicchi, and Ranieri (2010) and Guittonny-Philippe et al. (2014) reported that these elements can be removed via different physical, chemical, and biological processes performed in the wetland systems. This may include settling, sedimentation sorption, adsorption, complexation, cation and anion exchange, oxidation and reduction, chemical precipitation and co-precipitation as insoluble salts, photo-degradation, phyto-accumulation, biodegradation, microbial activity, and plant uptake. In vertical flow wetlands, these elements are most likely to accumulate in the litter layer at the top of the system, while in horizontal flow wetlands, heavy metals and trace elements tend to accumulate near the system inlet regardless of elimination pathways (Cheng et al., 2002). However, most of those elements available in the wastewater are removed in wetlands through the interaction with system media after being treated by wetland plants which is considered as a polishing system, as stated by Matagi, Swai, and Muganbe (1998) and Guittonny-Philippe et al. (2014). Moreover, in wetland systems, the heavy metals can be removed effectively by settling and sedimentation processes after a series of dynamic transformations performed in the system (Prestes et al., 2006;

Terzakis et al., 2008; Matagi et al., 1998). However, the sedimentation of those heavy metals will occur after agglomeration to bigger particles that can be trapped by wetland sediment as reported by Walker and Hurl (2002). According to Scholz (2006, 2010), wetland macrophytes can also be considered to trap the metal solids available in the wastewater while it passes through the surface of the system plants. Moreover, the accumulation of heavy metals in wetland biomass can be considered as a predominant way to eliminate those metals in wetland systems as reported by Madera-Parra et al. (2015) who agreed with the observation reported by Guittonny-Philippe et al. (2014) showing that the heavy metals in the wetland system can be removed by accumulation in the system sediment as well as in different parts of macrophytes tissue such as roots, stems, leaves and shoots. Furthermore, sorption process in wetland systems which include adsorption, absorption and precipitation reactions can be considered as the main chemical methods for heavy metal removal (Marchand et al., 2010).

Plant uptake is another mechanism for heavy metal removal in wetland systems (Scholz & Hedmark, 2010; Grisey et al., 2012; Guittonny-Philippe et al., 2014) as those elements can be eliminated via roots and rhizosphere immobilisation processes (Ye et al., 2001; Sultana et al., 2014) resulting in absorption of several elements such as lead, zinc, copper and cadmium as indicated by Denga et al. (2004) and their translocation to the underground plant parts as reported by Baldantoni, Lagrone, and Alfani (2009) and Zhang et al. (2010).

However, wetland macrophytes can uptake heavy metals with differing capacities depending of several factors such as plants species, heavy metal levels, sediment chemistry and pH, in addition to the temperature and organic matter content as reported by Sheoran (2004); Sheoran and Sheoran (2006); Liu et al. (2007) and Marchand et al. (2010). Furthermore, several studies have been performed to investigate the role of wetland plants in heavy metal removal. For example, 85% of metal removals were observed to be through wetland media, while the contribution of macrophytes was reported to be less than 3% during a study undertaken by Allende et al. (2014). Moreover, they explained that the co-precipitation of arsenic with iron undertaken in the system media was due to adjustment of pH with the assistance of cation exchange capacity. On the other hand, Liu et al. (2007) concluded in their research that the highest removal of heavy metal in wetlands was associated with the macrophytes through phyto-extraction processes in addition to the metal precipitation provided by the system as reported by Mays and Edwards (2001).

Furthermore, Mungure et al. (1997) stated that the sedimentation in their lab-scale wetland system was the main sink for removal of several metals in addition to the role of plants, showing the accumulation of those metals will be the highest in macrophyte roots compared to that in stems and leaves, indicating the immobilisation of metal by plants roots is the main mechanism for plant metals uptake. Similarly, Yeh et al. (2009) stated that in their wetland system, the plant roots accumulated heavy metals of about 2 to 3 times greater than those in stems and leaves. Furthermore, their system recorded removal efficiency of iron, chromium, nickel and zinc of 83, 82, 69 and 55%, respectively, and they noted that most of the metals were reserved by settling in the system bottom then slightly removed via emergent macrophytes. Moreover, in constructed wetland systems, Khan et al. (2009) concluded that lead removal efficiency via plant uptake will be different with plant species. However, they also observed that system plant roots remove lead more than the aerial parts.

In addition, Khan et al. (2009) reported that metals such as lead and cadmium were mainly retained in sediments at the system bottom indicating that sedimentation is the most common method for heavy metal removal in wetland systems rather than plant uptake. Finally, they stated that constructed wetlands are very effective in heavy metal removal from industrial wastewater and the removal rates showed by the system were Cd > Cr > Fe > Pb > Cu > Ni, confirming that the key removal mechanism was via sedimentation and removal was marginal via plant uptake.

In wetland systems, removal of pollutants could be performed via the interaction between plants and microorganisms as well as through biological processes, as plant root systems can provide good conditions for various types of microbes to survive. Also, these microorganisms will be enclosed within the roots surface and will be transferred through the wetland substrate with the growth of plant roots (Trapp & Karlson, 2001). Moreover, Mastretta et al. (2009) and Sultana et al. (2014) stated that fixation of nitrogen, prevention of pathogen attack and detoxification of contaminants are metabolic functions which can be provided by complementary bacteria for their host. In wetland systems, microbes can also produce siderophores which can interact with various metals with the possibility to either reduce their toxicity or raise their labile properties before uptake by macrophytes as indicated by Sultana et al. (2014).

2.9.2.3 Toxic ions

FAO (2003) stated that the most toxic ions in treated wastewater are sodium, chloride and boron. Toxicity due to a specific ion happens when that ion is taken up by the plant and accumulates in amounts that result in damage or reduced yield. The source of boron is usually household detergents or discharges from industrial plants.

Chloride and sodium also increase during domestic usage, especially where water softeners are used. For sensitive crops, toxicity is difficult to correct without changing the crop or the irrigation water supply. The problem is usually highlighted by severe (hot) climatic conditions (Westcot & Ayers, 1985).

Although there are several guidelines on treated wastewater quality for irrigation, the Food and Agriculture Organisation (FAO) and Westcot and Ayers references are the ones most commonly mentioned in the literature. For example, FAO (1994) recommended the sodium concentrations in irrigation water should not exceed 40 milliequivalent per litre, which will not affect plant growth (Table 2.5).

In contrast, Westcot and Ayers (1985) classified the degree of restriction of sodium concentration in irrigation water when using surface irrigation, in terms of sodium adsorption ratio (SAR), to be less than 3 mg/l for non-restricted use, between 3 mg/l and 9 mg/l for moderate use and severe restriction use if the sodium value exceeds 9 mg/l in the irrigation water. Moreover, FAO (2003) reported that boron exceeding 0.5 mg/l is toxic to sensitive plants.

Pescod (1992) classified boron concentrations in irrigation water according to the degree of restriction on its use: there are no limitations for values of less than 0.7 mg/l, slight to moderate controls for values between 0.7 mg/l and 3.0 mg/l, and severe restrictions for measurements of more than 3.0 mg/l. Table 2.7 lists some plant tolerances to boron concentrations in irrigation water, adapted from Westcot and Ayers (1985).

Table 2.7: Relative tolerance of plants to boron concentrations in irrigation water (adapted and updated from Westcot and Ayers (1985)).

Sensitive (1 mg/l)	Semi-tolerant (2 mg/l)	Tolerant (3 mg/l)
Citrus	Bean	Carrot
Avocado	Bell pepper	Lettuce
Apricot	Tomatto	Cabbage
Peach	Corn	Onion
Cherry	Olivers	Suger beet
Grapes	Radish	Date palm
Apple	Pumpkin	Asparagus
Pear	Wheat	Turnip
Plum	Potato	
Straberries	Sunflower	

Another potential problem with wastewater recycling is the excessive chlorine residue in treated effluent. Excessive chlorine in reused water could affect plant foliage especially when using a sprinkler irrigation system. A residual chlorine concentration value of 1 mg/l will not affect plant foliage while a value of more than 5 mg/l will cause severe damage to plants (FAO, 1994, 2003).

However, most chlorine in reclaimed wastewater is in a combined form that will not cause crop damage. Moreover, ground water contamination is expected due to the toxic effects of chlorinated organics when irrigating with reused wastewater (Pescod, 1992). There are many visual symptoms that could be appeared on plants growing in either deficiency or surplus conditions of nutrients and minerals. These symptoms could be used as a guide to predict the problems which plants may be suffering from. Table 2.8 summarises some of those visual symptoms and their possible causes according to the references. These symptoms could appear either on young or mature/old parts of plant based on the mobility and immobility of the elements. Table 2.9 provides the concentration levels of some trace elements that may cause plant phytotoxicity.

Table 2.8 a: Visual symptoms of plants associated with elements deficiency.

Visual symptoms	**Possible elements deficiency**
Stunted growth	Nitrogen and/or phosphorous (Chemicals, 2014; Kennelly, O'Mara, Rivard, Miller, & Smith., 2012; McCauly, Jones, & Jacobsen, 2011; Silva et al., 2000; Wong, 2005), and/or potassium (McCauly et al., 2011; Silva et al., 2000), and/or calcium (Kennelly et al., 2012; McCauly et al., 2011; Wong, 2005), and/or molybdenum (Chemicals, 2014; Silva et al., 2000), and/or boron (McCauly et al., 2011; Silva et al., 2000), and/or iron and/or zinc (McCauly et al., 2011), and/or copper (Hosier & Bradley, 1999; McCauly et al., 2011; Silva et al., 2000), and/or calcium (Kennelly et al., 2012; McCauly et al., 2011; Wong, 2005), and/or molybdenum (Chemicals, 2014; Silva et al., 2000), and/or boron (McCauly et al., 2011; Silva et al., 2000), and/or iron and/or zinc (McCauly et al., 2011), and/or copper (Hosier & Bradley, 1999; McCauly et al., 2011; Silva et al., 2000).
Few flowers with poor and deformed fruits	Nitrogen and/or phosphorus and/or potassium (Chemicals, 2014; Silva et al., 2000).
Chlorosis	Yellowing or whitening of the green plant tissue, because of a decreased amount of chlorophyll due to deficiency in nitrogen (Hosier & Bradley, 1999; Kennelly et al., 2012; McCauly et al., 2011; Silva et al., 2000), and/or potassium (Chemicals, 2014; McCauly et al., 2011; Silva et al., 2000; Wong, 2005), and/or magnesium (Kennelly et al., 2012; Silva et al., 2000), and/or sulphur (Silva et al., 2000; Wong, 2005), and/or calcium (Kennelly et al., 2012; Wong, 2005), and/or boron (McCauly et al., 2011), and/or iron (Kennelly et al., 2012; McCauly et al., 2011; Silva et al., 2000; Wong, 2005) and/or zinc (McCauly et al., 2011), and/or copper and/or manganese (McCauly et al., 2011; Silva et al., 2000; Wong, 2005).
Burning of leaf margins with midrib remaining green	Potassium (McCauly et al., 2011).
Interveinal chlorosis	Magnesium (Chemicals, 2014; Kennelly et al., 2012; McCauly et al., 2011; Wong, 2005), and/or potassium (Hosier & Bradley, 1999; Kennelly et al., 2012), and/or iron (Hosier & Bradley, 1999; McCauly et al., 2011; Wong, 2005), and/or zinc and/or manganese (Hosier & Bradley, 1999; McCauly et al., 2011; Wong, 2005), and/or copper (Wong, 2005).
Yellowish or reddish-purple and midrib remaining green	Magnesium (Kennelly et al., 2012; McCauly et al., 2011; Silva et al., 2000).
Purplish red colouring	Phosphorus (Hosier & Bradley, 1999; McCauly et al., 2011; Wong, 2005).
Curly small leaves	Phosphorus (McCauly et al., 2011).

Table 2.8 a (cont.)

Visual symptoms	Possible elements deficiency
Necrosis	Death of plant tissue due to deficiency in nitrogen and/or potassium (McCauly et al., 2011; Wong, 2005), and/or magnesium (Kennelly et al., 2012), and/or calcium (Kennelly et al., 2012), and/or boron and/or iron (McCauly et al., 2011), and/or manganese and/or copper (Silva et al., 2000; Wong, 2005) .
Leaf tips brown and necrotic	Phosphorus (McCauly et al., 2011), and/or potassium (Kennelly et al., 2012).
Dark green leaves and stem	Phosphorus (McCauly et al., 2011; Wong, 2005).
Light green to yellowish young leaves	Sulphur (Hosier & Bradley, 1999; McCauly et al., 2011), and/or molybdenum (McCauly et al., 2011; Silva et al., 2000).
Spindly small plant with thin stem	Sulphur (McCauly et al., 2011).
Pale green of entire plant	Sulphur (McCauly et al., 2011)
Fruit blossom end rot	Calcium (Chemicals, 2014; Hosier & Bradley, 1999; Kennelly et al., 2012; Silva et al., 2000).
Premature falling of buds and blossoms	Calcium (Silva et al., 2000).
Distorted young leaves with dark green colour	Calcium (McCauly et al., 2011).
Dry or brittle leaf	Calcium (McCauly et al., 2011).
Weak stem	Calcium (McCauly et al., 2011; Silva et al., 2000).
Death of terminal buds	Boron (Chemicals, 2014; Hosier & Bradley, 1999; McCauly et al., 2011).
Brittle and distorted leaves	Boron (Chemicals, 2014; McCauly et al., 2011).
Brown discoloration	Copper (McCauly et al., 2011).
Thick and curled leaf tips	Boron (McCauly et al., 2011; Silva et al., 2000).
Misshapen flowers and buds	Boron (McCauly et al., 2011).
Poor flowering and seeds	Zinc (McCauly et al., 2011).

Table 2.8 b: Visual symptoms of plants associated with elements surplus.

Visual symptoms	**Possible elements surplus**
Dark green and abundant foliage	Nitrogen (Chemicals, 2014; McCauly et al., 2011; Wong, 2005), and/or zinc (McCauly et al., 2011), and/or Iron (Foy, Chaney, & White, 1978).
Stunting and reducing in branches	Nitrogen (Wong, 2005), and/or molybdenum (McCauly et al., 2011), and/or copper (Chemicals, 2014; McCauly et al., 2011) and/or manganese (Silva et al., 2000) and/or Iron (Foy et al., 1978).
Tall plants with weak stem	Nitrogen (McCauly et al., 2011).
Leaf margins rolling downward	Nitrogen (McCauly et al., 2011).
Lesions on stem	Nitrogen (McCauly et al., 2011).
Yellow-brown leaf discoloration	Molybdenum (McCauly et al., 2011).
Golden yellowish leaves	Molybdenum (McCauly et al., 2011).
Interveinal yellowing	Zinc (McCauly et al., 2011).
Leaves chlorosis and abscission	Manganese (Chemicals, 2014).
Low growth rate	Manganese and/or copper (Chemicals, 2014).
Yellowing and necrosis of leaf tip or margins toward midrib	Boron (Chemicals, 2014; McCauly et al., 2011; Silva et al., 2000).
Chlorosis in plants	Zinc and/or copper (Chemicals, 2014; McCauly et al., 2011), and/or boron and/or manganese (McCauly et al., 2011).
Reddish area close to margins	Boron (Silva et al., 2000).
Necrotic lesions on leaves	Manganese (McCauly et al., 2011; Silva et al., 2000).

Table 2.9: Trace element thresholds for crop production (adapted and updated from FAO (1994, 2003)).

Element	RMC	Remarks
Aluminium (Al)	5.0	In acid soil (pH < 5.5) can cause non-productivity, while in more alkaline soil (pH > 7.0) will rapid the ion and eradicate any toxicity.
Arsenic (As)	0.10	Plant toxicity varies widely, ranges from 12 mg/l to 0.05 mg/l for Sudan grass and rice, respectively.
Beryllium (Be)	0.10	Plant toxicity varies widely, ranges from 5 mg/l to 0.5 mg/l for Kale and bush beans, respectively.
Cadmium (Cd)	0.01	At concentration of 0.1 mg/l in the nutrient solutions, will be toxic to beans, beets and turnip. Conventional concentrations are recommended as cadmium can accumulate in plants to levels that may harm humans.
Cobalt (Co)	0.05	At concentration of 0.1 mg/l in nutrient solution may be toxic to tomato plants and could be inactive at neutral to alkaline solution.
Chromium (Cr)	0.10	Knowledge on plants toxicity is not clear yet, so conservative levels are recommended. Not recognised yet as a necessary element for plant growth.
Copper (Cu)	0.20	At concentrations ranging from 0.1 to 1.0 mg/l in nutrient solution, can be toxic to several plants.
Fluoride (F)	1.0	Normally inactive in neutral and alkaline soils.
Iron (Fe)	5.0	In aerated soil, will not be toxic to plants, but can cause soil acidification and loss of availability of necessary phosphorous and molybdenum. Iron can cause unsightly deposits on plants, equipment and buildings when using overhead sprinkling.
Lithium (Li)	2.5	Tolerable by most crops at concentrations up to 5 mg/l. Lithium is mobile in the soil, toxic to citrus at low limits of less than 0.075 mg/l. It can act similarly to boron.
Manganese (Mn)	0.20	In acid soil, magnesium is usually toxic to several crops.
Molybdenum (Mo)	0.01	Will not cause plant toxicity when available in water and soil with normal concentrations, but can be toxic to livestock when growing forage in soil of high molybdenum concentrations.
Nickel (Ni)	0.20	At concentration of 0.5 mg/l, nickel will be toxic to several plants. Toxicity will be reduced at alkaline or neutral pH.
Lead (Pb)	5.0	At quite high concentration, will constrain growth of plant cells.
Selenium (Se)	0.02	Can cause plant toxicity at concentration of 0.025 mg/l, and livestock toxicity when forage is grown in soil of high selenium availability.
Titanium (Ti)	-	Plants can exclude it effectively. Specific plant tolerance is unknown.
Vanadium (V)	0.10	At relatively low concentrations, vanadium can cause toxicity to most plants.
Zinc (Zn)	2.0	Can be toxic to most plants at varying range of concentrations, toxicity can be reduced at pH of more than 6.0 and in soils of fine texture or organic content.

Note: RMC recommended maximum concentrations (mg/l).

2.9.3 Microbiological content

In addition to the sediments, nutrients and minerals, pollutants that wastewater often contains are pathogens, which can harm human health and the environment (Qadir et al., 2007). Biological agents available in wastewater such as bacteria, protozoa and pathogenic viruses can reach humans either through the mouth by eating contaminated vegetables or via the skin by contact with those pathogens such as *schistosornes* and hookworms. In countries, such as those in the Near East Region, where nematode infections and diarrhoeal diseases are widespread, these agents are considered a serious problem. However, there are many factors which affect the transmission of diseases associated with those pathogens such as:

- Pathogens survival time in water, soil, crops and fish;
- Availability of intermittent host for infection;
- Wastewater application frequency;
- Types of crops to be irrigated with wastewater;
- The way in which the human host is exposed to the contaminated water, soil, crops and fish.

FAO (2003) have listed the survival time for most pathogens in water, soil and crops in different environments within the temperature range of 20 to 30 °C, as shown in Table 2.10.

Table 2.10: Excreted pathogen survival time at temperature of 20–30 °C.

Pathogen type	Survival time (day)			
	In night soil, faeces and sludge	In sewage and fresh water	In the soil	On crops
Viruses				
Enteroviruses	<100 (<20)	<120 (<50)	<100 (<20)	<60 (<15)
Bacteria				
Faecal Coliforms	<90 (<50)	<60 (<30)	<70 (<20)	<30 (<15)
Salmonella spp.	<60 (<30)	<60 (<30)	<70 (<20)	<30 (<15)
Shigella spp.	<30 (<10)	<30 (<10)	-	<10 (<5)
Vibrio Cholerae	<30 (<5)	<30 (<10)	<20 (<10)	<5 (<2)
Protozoa	<30 (<15)	<30 (<15)	<20 (<10)	<10 (<2)
Entamoeba histolytica cysts	<30 (<15)	<30 (<15)	<20 (<10)	<10 (<2)
Helminths				
Ascaris lumbricoides eggs	Many moths	Many moths	Many moths	Many moths

Note: Usual survival time is shown in brackets.

In 1971, the World Health Organization first examined the health concerns of wastewater use in agriculture (WHO, 1973). In 1989 the World Health Organization released the first microbial guidelines (Table 2.5) for irrigation water which confirmed that the total coliforms should not exceed the value of 1000 colony forming units (CFU) per 100 ml, and less than 1 intestinal nematode egg per litre of water sample used for irrigation of crops that are likely to be eaten uncooked based on the results of epidemiological studies of irrigation by wastewater (FAO, 2003; WHO, 1989). According to EPA (1992), the typical bacteria survival time in soil, fresh water and crops is less than 70, 60 and 30 days, respectively. Based on that, wastewater should be treated before use or discharge to the environment (Ongley, 1996).

Constructed wetland systems have been reported to remove various types of pathogens effectively (Scholz, 2006, 2010). Arias et al. (2003), Hansen et al. (2004) and Molleda et al. (2008) demonstrated that in subsurface flow constructed wetlands, pathogens can be removed through different mechanisms, such as antibiotics excretion (Garcia et al., 2013). However, this mechanism cannot be evidenced as reported by Stottmeister et al., (2003).

Moreover, in constructed wetlands, pathogens can be removed directly or indirectly via different processes such as filtration, sedimentation, adsorption, and predation by protozoa and bacteriophages (Kadlec & Wallace, 2008). Investigation of the role of sedimentation on pathogen removal in wetland systems was performed by Karim et al. (2004).

The authors' results showed that statistically, there are no significant differences in faecal coliform and coliphage numbers in effluent water compared to those in the sediment, indicating that macrophyte roots of wetland systems play an important role in pathogen removal. These results agreed with those obtained by Garcia et al., (2013) who reported that *E-Coli* were removed well by wetland plants.

Regarding the impact of wetland types, several studies have been undertaken to investigate the best performance of subsurface vertical or horizontal flow constructed wetlands on pathogen removal efficiency (Garcia et al., 2013). For example, Hansen et al., (2004), Vacca et al., (2005) and Fountoulakis et al., (2009) reported that both systems showed the same total coliform and faecal coliform removal rates of 2 log units.

However, vertical flow constructed wetlands were reported to be more efficient in pathogen removal due to the high aerobic conditions available in such systems (Kadlec & Wallace, 2008). Moreover, the hybrid wetland system showed higher pathogen removal rate of 4 log units, as reported by Bederski et al., (2004) and Masi et al., (2004). In hybrid wetland systems, a lower number of Helminth eggs were observed in the outflow compared to the inflow, as reported by Khatiwada and Polprasert (1999), Karpiscak et al., (2000), Arias et al., (2003) and Molleda et al., (2008) with up to 100% removal efficiency achievable, as indicated by Molleda et al. (2008).

Although subsurface flow constructed wetlands were proved to remove pathogens from wastewater efficiently, there is no information about whether either the single-stage or combined (hybrid) system can treat the wastewater sufficiently to meet required standards (Garcia et al., 2013). Neerunjun (2014) investigated the treatment of his household grey water using a horizontal flow constructed wetland system for a studying duration of 13 weeks in Mauritius. The author's results showed that the effluent of his system did not meet the local standards for irrigation purposes in terms of pathogen requirements. Furthermore, Winward et al. (2008) surveyed the performance of constructed wetlands in grey water pathogen removal. The authors concluded that chemical disinfection is essential for application of grey water reuse after wetland treatment.

2.9.4 Organic pollutants

Organic compounds, which have long bonding structures, usually consist of carbon. The performance of the organic compounds is dependent on their molecular structure, size and shape and the presence of functional groups that are important causes of toxicity. There are many different types of organic pollutants, such as hydrocarbons, polychlorinated biphenyls and pesticides. However, hydrocarbons are the most recent organic pollutants which present particular challenges in recycling of wastewater for irrigation, especially in oil-rich countries.

Murakami, Nakajima, and Furumai (2005) indicated that asphalt/pavement road wear was the major hydrocarbon source of road dust in urban areas. Moreover, Pengchai, Nakajima, and Furumai (2005) concluded that tyre wear and diesel vehicle exhaust and drains were the major sources of hydrocarbons in road dust in heavy traffic areas.

Petroleum hydrocarbons may contaminate irrigation water sources through run-off from impermeable surfaces during water collecting (Moilleron, Gonzalez, Chebbo, & Thévenot, 2002). Hydrocarbons consist of numerous carcinogenic compounds that have been increasing in recent decades in many urban ponds, mainly in areas experiencing rapid urban development (Van Metre, Mahler, & Furlong, 2000). Grease and oil that pollutes a water source will also contain metal contaminates. Hydrocarbon pollution is visually evident at low concentrations and can adversely affect fish and aquatic plants in irrigation water (FAO, 2003). Chinese standards for irrigation water quality (SEPA, 2005) highlight that the total hydrocarbons in irrigation water should not exceed a maximum value of 1.0 mg/l.

Oxygen is a natural component in all water bodies, and it is necessary for all aquatic plant and animal life. The microorganisms which play an important role in cleaning polluted streams need the oxygen to break down complex organic pollutants into simple and harmless chemicals. With increasing pollution levels of water streams, the dissolved oxygen demand will increase due to the competition between microorganisms and other aquatic life. This will lead to adverse results like fish mortality and septic conditions in those aquatic areas (Magaud, Migeon, Morfin, Garric, & Vindimian, 1997). However, biochemical oxygen demand (BOD) and/or the chemical oxygen demand (COD) could be measured as an indirect indicator of the amount of organic material in the water (FAO, 1994, 2003).

Constructed wetland systems have been reported to remove organic matter effectively (Scholz, 2010; Gikas & Tsihrintzis, 2012; Abou-Elela et al., 2013). In these systems the removal of organic matter is predominantly performed through several processes such as aerobic and anaerobic, filtration, sedimentation, volatilisation, adsorption, and microbial metabolism as reported by Karathanasis et al. (2003), Song et al. (2006) and

Stefanakis et al. (2014). In subsurface flow constructed wetlands, both aerobic and anaerobic degradation processes occur simultaneously. During the aerobic processes, a source for oxygen will be the atmospheric diffusion in addition to the oxygen transferred by plants to the rhizosphere inside the system substrate (Cooper et al., 1996). In comparison, the anaerobic processes are performed in the anaerobic zone of the wetlands which are empty of plant roots and located below the maximum plant root depth which is about 0.3 m for Cattails and 0.6 m for common reeds as reported by Saeed and Sun (2012). These types of wetland plants are known for their root system density, providing a huge surface area for growing aerobic microorganisms (Sim, 2003) in addition to the microbes growing around substrate surfaces and forming a microbial film. As a result, when the wastewater passes through the system, it will be in contact with all of these microbes which will convert the organic matter in the water to carbon dioxide and other stable compounds for their survival (Meng et al., 2014).

Furthermore, Meng et al. (2014) stated that the biodegradability of organic matters is the main factor that affects the rate of organics degradation in subsurface flow constructed wetlands. This biodegradability is indicated by the ratio of BOD/COD in the wastewater, reported to range typically between 0.3 and 0.8 for untreated wastewater, with a ratio ≥ 0.5 indicating easy degradation of organic matter and a ratio < 0.3 indicating difficulty in oxidation of organic matter by microorganisms (Meng et al., 2014).

Organic matter removal in constructed wetlands can be assessed via the variation of chemical and biochemical oxygen demand values in the system. Coarse organic matter can be eliminated by entanglement and sedimentation gravitationally in the pores of the wetland substrate as reported by EPA (1993), while soluble organic particles can be removed via the development of microbes in the system media and/or by adhering on wetland plant roots as indicated by Song et al. (2006).

However, dissipation, accretion and cycling of organic matter are the main factors which affect the constructed wetlands function. Accumulation of organic matter in the wetland system will provide the microorganisms with a sustainable carbon and nutrients source. Nevertheless, accumulated organic matter can result in clogging of system media and prevention of water penetration through the substrate resulting in reduction of the hydraulic retention time of wastewater in the system which subsequently affects the treatment efficiency, as reported by Nguyen (2000). This is confirmed by the results observed by Tanner and Sukias (1995), Winter and Goetz (2003), Zhao et al., (2009), Hua et al. (2014) and Song et al. (2015) who noted a linear relationship between system clogging and applied loading rate of chemical oxygen demand and total suspended solids, as an increase in the applied organic load will increase the total suspended solids leading to severe system clogging.

Biochemical processes are an essential mechanism for organic matter degradation in wetlands, and enhancing water quality by gasification or mineralisation, as well as for the formation of organic substances through production of new biomass. For example, these organic matters were reported to contain 45 to 50% carbon which can be used as an energy source for the survival of several microorganisms in wetlands systems (DeBusk, 1999). This carbon will be converted to carbon dioxide in the root zone by system plants providing more oxygen for treatment processes. In addition, organic matter in the wetland system can be removed via other processes such as absorption and/or adsorption which can be performed at a ratio that depends on numerous factors such as, macrophytes, litter, surface media and organic matter properties (EPA, 2000).

Bacteria and fungi also play a vital role in removal of organic matter in wetlands during gasification and mineralisation processes (Choudhary et al., 2011).

Furthermore, volatilisation processes are another mechanism for organic matter elimination in wetland systems. During these processes, contaminates will directly escape from the wastewater to the atmosphere. However, volatilisation may not occur directly in some wetlands, but rather occurs through a pathway of biological processes. For example, wetland macrophytes will absorb the contaminants via their system roots then release them to the atmosphere by a process named phyto-volatilisation (Hong et al., 2001; Ma & Burken, 2003).

In surface flow constructed wetlands, the volatilisation rate of contaminants is expected to be higher than that in subsurface flow systems due to the direct contact of wastewater with the atmosphere in the former system compared to the latter (Kadlec & Wallace, 2009) which may result in air pollution and spreading of contaminants in the environment as indicated by McCutcheon and Rock (2001). However, subsurface flow constructed wetlands, especially vertical ones, were reported to remove organic matter and other pollutants effectively under aerobic and/or anaerobic decomposition processes as well as via assimilation of system microbes and plant uptake (Leonard, Key, & Srikanthan, 2003; Mander et al., 2003; Sun, Zhao, & Allen, 2005; Lee and Scholz, 2006).

2.9.5 Salinity

Irrigation water quality in terms of salinity is assessed by determining the amount and types of salts available in the water (Westcot & Ayers, 1985). Crops are significantly different in their tolerance of salinity conditions (FAO, 2003). Increasing the salinity of treated wastewater will reduce the possibility of irrigation reuse due to the damaging impacts on soil and crops (Maas & Grattan, 1999).

However, there are several approaches suggested by FAO (2003) to overcome the salinity problem when irrigating with treated wastewater:

- Selection of crops tolerant to the wastewater salinity and still commercially viable. Generally, most crops can be successfully grown under salinity of less than 3 dS/m with good management of wastewater. However, with increasing salinity, selection of suitable crops will be difficult and the choice for fodder crops will be highly restricted. Table (2.11) lists the tolerance of some crops based on specific ranges of salinity.
- Selection of crops of high absorbency of salts without toxicity impact such as salt harvesting crops. Sudax, Bermuda grass, sorghum and barely are examples of salt harvested crops.
- Selection of uniformly applied irrigation system with high efficiency and the ability for frequent irrigation. Moreover, using a modern irrigation system with suitable management can result in better crop yield.
- Irrigation scheduling is an essential factor to control salinity as it can be directly affected by the amount and frequency of irrigation water application. For example, using micro-irrigation systems requires high frequency of water application and in this case the salinity of irrigated soil should be conserved at minimum levels.
- Leaching is another possible approach to control salinity but will not be appropriate in the case of a shallow water table, insufficient drainage and water shortage. When irrigating soil for a long time with wastewater, then the total applied salt (salt in) should be equal to that up-taken by plants and taken through leaching (salt out). This approach is very important to select suitable crops and better management of wastewater for irrigation reuse. Moreover, using of salt harvest crops will obtain good results and the cultivation of such crops periodically is highly recommended.

- Soil conditioners such as polymers can be used under certain conditions and for a specific duration. However, these conditions are not recommended for open field crops due to their short half-life and high cost.
- Drainage facilities should be available to avoid waterlogging and salinisation in arid and semi-arid areas. However, the combination of drainage and sufficient scheduled irrigation will allow salts in plant root zones to be accessed via leaching processes.

Furthermore, Westcot & Ayers (1985) reported that the only practical solution to manage the salinity problem is to ensure there is a downward flux for water and salt through the root zone. This will provide good drainage to allow the driving of water and salt under the root zone. Without adequate drainage, irrigation with treated wastewater will not be possible for long-term conditions. However, when drainage water salinity exceeds the thresholds for crops, the blending of treated wastewater with fresh water either before or during irrigation will be a possible solution to reduce the salinity level in irrigation water and extend the volume of water available for irrigation (Rhoades, 1999; Oster & Grattan, 2002). A salinity problem in irrigation water is usually indicated by measuring the electrical conductivity (Ec). However, FAO (2003) recommended that irrigation water salinity should not exceed 3000 µS/cm for vegetable production (Table 2.5).

Regarding wetlands efficiency in terms of salinity removal, Lymbery et al. (2006), performed a study on pilot-scale subsurface flow wetlands incorporating *Juncus kraussii* constructed to investigate the system efficiency in terms of salt and nutrients removal at several concentrations from inland saline aquaculture effluent of Western Australia for a duration of 38 days. The authors' results showed that 44 to 53% of the total sodium chloride (NaCl) was removed by the system indicating the capability of wetlands to remove the salinity from ground water discharged by aquaculture processes. However, the authors reported these results during their short-term experiment and they suggested that for long-term operation, the accumulation of salts in the soil and plants will affect the system efficiency in terms of salt removal. Because of this, using salt-tolerant plants (halophytes) in wetland systems is a suitable alternative for treating water of high salinity as they can remove up to 35,000 mg/l of effluent salinity (Brown et al., 1999).

Table 2.11: Some cultivated crop salinity tolerances (adapted and updated from FAO (2003)).

Irrigation water salinity (dS/m or mg/l)					
< 2 or < 1280	**2–3 or 1280–1920**	**3–4 or 1920–2560**	**4–5 or2560–3200**	**5–7 or 3200–4480**	**> 7 or > 4480**
Citrus	Fig	Sorghum	Soybean	Safflower	Cotton
Apple	Oliver**	Groundnut	Date palm***	Wheat	Barely
Peach	Broccoli	Rice	Harding grass	Sugar beet	Wheat grass
Grapes	Tomato	Beets	Trefoil	Rye grass	
Strawberry	Cucumber	Tall fescue	Artichokes	Barley grass	
Potato	Cantaloupe			Bermuda grass	
Pepper	Watermelon			Sudax	
Carrot	Spinach				
Onion	Vetch				
Beans	Sudan grass				
Corn	Alfalfa				

Note: 1 dS/m=640 mg/l; ** Much higher Ec levels were reported (up to 6 dS/m) for olive in Tunisia; and *** Similar higher Ec levels were reported for date palm trees in Algeria (up to 7–8 dS/m).

2.10 Wastewater irrigation methods

Crop yields and productivity are affected directly by water irrigation which is considered as a vital factor which may impact on agricultural economy in both arid and semi-arid countries. However, there are several irrigation methods available for application of wastewaters as detailed below according to Pescod (1992) and FAO (1994, 2003).

2.10.1 Surface irrigation methods

This is the traditional method of irrigation by wastewater that may include flood irrigation by basin or border which will wet most of the land surface or irrigation by hose basin in which water is supplied by hose. Furthermore, surface methods may include furrow irrigation in which only part of the land will be wetted. Surface methods are the most popular method of irrigation in more than 95% of the irrigated area worldwide. This is because of their low cost and simplicity in understanding and application. Moreover, in developing countries, these irrigation methods are appropriate mainly if the agricultural productivity is not limited by water.

2.10.2 Pressurised irrigation methods

This may include sprinklers, subsurface, drip and bubbler irrigation systems. Sprinkler systems, in which the soil and crops are wetted similarly to when it rains, could be either high capacity, ordinary mini sprinklers or sprayers. Subsurface irrigation systems are not yet applied in irrigation with wastewater.

However, this system will be useful when using wastewater of poor quality and high human risk as the system can provide better health protection particularly when used in combination with trickle irrigation. Moreover, a localised irrigation system with application of consistent flow is named a bubbler system and is known to be better than mini sprinklers and drip system as the latter two may cause clogging in irrigation systems when used for a long time, as reported by Capra and Scicolone (2007)

Furthermore, irrigation can be performed by point or localised systems such as drippers which are characterised as follows:

- Highly efficient system mainly used in areas with water scarcity problems;
- Suitable to manage issues such as irrigation water salinity and alkaline soil;
- Can be considered as the system with the most potential for use of wastewater, especially when purification processes are undertaken to avoid possible clogging problems;
- Can provide minimum contact between wastewater and both farmers and irrigated crops; and
- Will not cause pollution in the atmosphere or area close to the irrigated field.

2.11 Selection of wastewater irrigation methods

There are several factors affecting selection of the irrigation system type in order for it to be used properly. For example, wastewater quality, tradition, crops, skills, and worker ability to manage different types of irrigation methods, as well as the probability of health risk to workers, the public and the environment (FAO, 2003).

Clogging is one of the serious problems which may affect the efficiency of an irrigation system. This problem may happen in all of the system types listed above with the exception of surface irrigation. For example, growth of slimes and bacteria in either the sprinkler head, supply line or emitter orifice in addition to the accumulation of salts and suspended solids will cause a serious clogging issue. However, the drip system is the one which is most likely to experience clogging issues especially when high suspended solids are present, despite its high suitability for use when irrigating with wastewater as it provides good protection of human health and against contamination of plants (Pescod 1992; FAO, 1994, 2003). Table 2.12 shows the evaluation of different irrigation methods based on the most popular issues, while Table 2.13 overviews some factors that will affect the selection of the irrigation method with required wastewater measures.

Table 2.12: Assessment of the suitability of different irrigation methods to use with wastewater.

Parameter	Surface methods		Pressurised methods	
	Furrow	**Border**	**Sprinkler**	**Drip**
Leaves wetting, damage and poor yield	Crops planted on ridge and foliage will not be damaged	Yield will not be seriously affected except some bottom leaves	Substantial yield loss due to severe foliar damage	No leaves injury with this irrigation method
Salt accumulation	Crops will be affected by salts accumulated in ridge	Unlikely to accumulate the salts in root zone as they move vertically		Salt dowel can be created between point of drips as the salts move radially along water movement direction
Maintain soil water potential	Plants will be under stress between irrigations		Not possible during growing season	Possible during growing season and reducing the impact of salinity
Possibility to handle waste water without substantial loss in yield	Fair to medium and satisfactory yield is possible with good management and drainage		Fair to poor with low yield due to plant suffering from foliar damage	Good to excellent with very little decrease in yield

Table 2.13: Factors affecting the selection of irrigation method with wastewater required measures.

Irrigation method	Factors affect the selection	Wastewater special measures
Border (flooding)	Low cost, exact levelling not essential	Full protection for workers, handlers and consumers
Furrow	Low cost, exact levelling may be required	Protection for workers and possibly for handlers and consumers
Sprinkler	Medium efficiency, levelling is not required	Some crops should not be used, mainly tree fruits, distance of minimum 50 to 100 m from roads and houses, odour inconvenience especially when anaerobic wastes are used.
Subsurface (localised)	High cost with high efficiency and greater yield	Filtration is required to avoid clogging of emitters.

2.12 Schedule and amount of irrigation water

In plant root systems, the water is extracted from the soil for growth. However, most of this extracted water escapes to the atmosphere as vapour via the leaves and stem of plants. These processes mainly occur during the day time and are called “transpiration”. Moreover, the same mechanisms occur with the water in the soil surface which is named “evaporation”. As a result, crop water needs include both processes of transpiration and evaporation, known together as “evapotranspiration”. This amount of water may be exceeded for the leaching fraction requirement (FAO, 2003). Plant water requirements can be expressed as mm/day, mm/month or mm/season. Table 2.14 lists some crop water requirements according to FAO (1992). However, with different locations and various environmental conditions, it is very difficult to fix the amount of irrigation water required by plants. This will lead to either surplus or deficiency in wastewater nutrients applied to the same plants, grown in the same soil type but in different places. Moreover, wastewater of a particular quality could be safe in one place but have an adverse impact in another place. For this reason, it is difficult to adjust the fertilisation with wastewater in comparison with the fresh water case.

Table 2.14: Water requirements for some crops (adapted from FAO (1992)).

Crop	Required water (mm/growing period)
Alfalfa	800–1600
Banana	1200–2200
Bean	300–500
Cabbage	380–500
Citrus	900–1200
Cotton	700–1300
Groundnut	500–800
Maize	500–800
Potato	500–700
Rice	350–700
Sunflower	800–1200
Sorghum	450–650
Wheat	450–650

2.13 Selection of crops to be irrigated with wastewater

Many vegetables have the potential to grow well on recycled wastewater. However, there are many points which should be considered during selection of crop types for irrigation with wastewater (Pescod 1992; FAO, 1994, 2003) such as:

- Human health protection in terms of pathogens and heavy metal contamination;
- Water consumption particularly in the case of limited irrigation water (Table 2.14);
- Relatively cost-effective crops;
- High nutritional value crops;
- Easy to grow crops (especially in the case of research purposes);
- Environmental conditions necessary for growing the crops; and
- Required levels of certain nutrients and trace elements for growing the crops.

Regarding pathogen contamination risk, WHO (1989) reported that if the treated wastewater met the guideline for unrestricted reuse (i.e. < 1000 faecal coliforms per 100 ml and < 1 nematode egg per litre), then this water can be used for irrigation of all crops without extra health protection measures. However, if the wastewater for irrigation does not meet WHO guidelines, there are still some possibilities to use it for irrigation of some plants without introducing any risk for consumers. Based on this, crops were categorised into three main groups according to Shuval et al. (1986):

- **Category A** (protection is required for workers only) such as:

- Crops not consumed by humans (e.g. cotton, sisal...);
- Crops processed either by heating or drying (e.g. grains, oilseeds, sugar beet…) or canning before being consumed by humans;
- Sun dried fodder crops which are harvested before animal consumption;
- Landscape and fenced areas without public access.

- **Category B** (extra measures are required) such as:

- Pasturelands and fodder crops;
- Human consumption crops which are not in direct contact with the wastewater, should not be picked off the ground and not irrigated with spraying system (e.g. vineyards and tree crops);
- Human consumption crops which are eaten after cooking (e.g. beetroot, potato, eggplants...);
- Human consumption crops which are eaten after peeling (e.g. water melons, nuts, citrus, bananas….);
- Any crops which are not irrigated with a sprinkler system.

- **Category C** (wastewater should be treated to meet WHO unrestricted guidelines):

- All crops that are eaten uncooked and with direct contact with the wastewater (e.g. lettuce, carrot, fruits irrigated by spraying);
- Landscape areas of public access (e.g. golf course, lawn and parks).

However, there is a potential for some vegetables such as lettuce and cabbage to become contaminated by microbes, because their edible leaves are too close to the ground receiving the treated wastewater. Furthermore, for vegetables where the edible part is grown under ground level, such as carrot, turnip, potato, root beet... etc., there will be a high potential for contamination with heavy metals applied by wastewater as they will accumulate in the soil where those crops grow (FAO, 1972). Therefore, it makes sense to select vegetables where the edible fruit is located far away from the ground to avoid both microbial and mineral contamination. This may include peppers, tomatoes, maize, eggplants, beans, lentils and peas.

In addition, crop cost value is another factor that affects the choice of vegetables to be irrigated with wastewater. Table 2.15 lists the most essential cultivated crops produced around the world, based on statistics derived from FAO (1994). However, from year to year these values may vary significantly due to fluctuation in prices and weather in addition to other factors which may affect the production. Moreover, nutrient values of selected crops should be considered as well as the duration of crop maturity during which plants will be irrigated with the wastewater. Tables 2.16 and 2.17 show the nutrient values and time for growing some crops, respectively.

Table 2.15: Agricultural product values produced by different countries (adapted from FAO (1994)).

Crop	Value in thousand dollar (2012)	Top producing country and value (2011)
Rice	$186,667,648	$49.6 billion (Mainland China)
Wheat	$84,281,536	$13.7 billion (Mainland China)
Soybeans	$65,903,601	$21.8 billion (United States)
Tomatoes	$58,223,483	$17.9 billion (Mainland China)
Sugar cane	$56,903,836	$23.9 billion (Brazil)
Maize (corn)	$55,478,433	$26.4 billion (United States)
Potatoes	$49,681,577	$12.6 billion (Mainland China)
Grapes	$39,494,901	$5.2 billion (Mainland China)
Apples	$31,706,244	$15.2 billion (Mainland China)
Bananas	$29,721,954	$8.4 billion (India)
Mangos,	$23,338,979	$9.1 billion (India)
Onions, dry	$18,121,063	$5.2 billion (Mainland China)
Beans, dry and green	$17,490,000	$6.2 billion (Mainland China)
Olives	$16,450,780	$6.3 billion (Spain)
Chillies and peppers, green and dry	$13,320,000	$7.5 billion (Mainland China)
Oranges	$12,356,000	$4.1 billion (Mainland China)
Cucumbers	$11,580,000	$9.1 billion (Mainland China)
Lettuce,	$10,840,000	$6.3 billion (Mainland China)
Sugar beets	$9,790,000	$1.6 billion (France)
Watermelons	$9,770,000	$7.4 billion (Mainland China)
Carrots, turnips	$7,010, 000	$3.9 billion (Mainland China)

Table 2.16: Nutrient values of some crops (retrieved from USDA (2013)).

Nutritional value per 100 g	Rice	Tomato	Potato	Onion	Beans	Chilli	Pepper	Cucumber	Lettuce	Carrot	Alfalfa
Energy (kJ)	1.527	74	321	166	334	166	84	65	55	173	96
Carbohydrates (g)	80	3.9	17.47	9.34	10.5	8.8	4.64	3.63	2.23	9.6	2.1
Sugars	0.12	2.6	15.44	4.24	-	5.3	2.4	1.67	0.94	4.7	-
Dietary fibres	1.3	1.2	2.2	1.7	-	1.5	1.8	0.5	1.1	2.8	1.9
Fat (g)	0.66	0.2	0.1	0.1	0.5	0.4	0.17	0.11	0.22	0.24	0.7
Protein (g)	7.13	0.9	2	1.1	9.6	1.9	0.86	0.65	1.35	0.93	4.0
Vitamins (mg)											
Thiamine (B_1)	0.0701	0.037	0.08	0.046	-	-	0.057	0.027	0.057	0.066	0.076
Riboflavin (B_2)	0.0149	-	0.03	0.027	-	-	0.028	0.033	0.062	0.058	0.126
Niacin (B_3)	1.62	0.594	1.05	0.116	-	-	0.48	0.098	-	-.983	0.481
Pantothenic acid (B_5)	1.014	-	0.296	0.123	-	-	0.099	0.259	0.15	0.273	0.563
Vitamin B_6	0.164	0.08	0.295	0.12	-	0.51	0.224	0.04	0.082	0.138	0.034
Folate (B_9)	-	-	0.016	0.019	-	-	0.01	0.007	0.073	0.019	0.036
Vitamin C	-	14	19.7	7.4	-	144	80.4	2.8	3.7	5.9	8.2
Vitamin E	-	0.54	0.01	-	-	-	0.37	-	0.18	0.66	-
Vitamin K	-	0.0079	0.0019	-	-	-	0.0074	0.0164	0.102	0.013	0.031
Minerals (mg)											
Calcium	28	-	12	23	-	-	10	16	35	33	32
Iron	0.80	-	0.78	0.21	-		0.34	0.28	1.24	0.3	0.96
Magnesium	25	11	23	10	-	23	10	13	13	12	27
Manganese	1.088	0.114	0.153	0.129	-	-	0.122	0.079	0.179	0.143	0.188
Phosphorus	115	24	57	29	-	-	20	24	33	35	70
Potassium	115	237	421	146	-	322	175	147	283	320	79
Sodium	-	-	6	-	-	-	3	2	5	69	6.0
Zinc	1.09	-	0.29	0.17	-	-	0.13	0.2	0.2	0.24	0.92
Other constituents (g)											
Water	11.61	94.5	75	88.1		88	-	95.23	95.63	88	-
Fluoride	-	-	-	0.0011		-	0.002	0.0013	-	0.0032	-
Lycopene	-	2.573	-	-	-	-	-	-	-	-	-

Table 2.17: Period for different growth stages of some crops and growth requirement (obtained from B&Q plc supplier).

Crop	Growth stages: Sowing	Growth stages: Planting	Growth stages: Harvesting	Growth stages: Total (months)	Growth requirements	Notes
Chilli	March to April	May to July	August to October	8	➢ Warm and humid climate, ➢ Can grow in all soil types	➢ Annual plants ➢ Can be used fresh for cooking or dry ➢ Easy to grow
Pepper	March to April	May to July	August to October	8	➢ Warm soil ➢ Sensitive to an abundance of moisture and ➢ Sensitive to extreme temperatures ➢ Can grow in all soil types	➢ Annual plants ➢ Can be eaten raw or used for cooking ➢ Easy to grow
Tomato	February to April	May to June	July to September	8	➢ Widely grown in greenhouses ➢ Can grow in all soil types	➢ Perennial in its native habitat, and grown as an annual in temperate climates ➢ Can be used fresh for cooking, ➢ Easy to grow
Onion	January to March	April to July	August to October	10	➢ Can grow in all soil types	➢ Annual plants
Cucumber	April to May	June to July	August to October	7	➢ Can be grown in greenhouses or outdoors ➢ Can grow in all soil types	➢ Annual plants
Lettuce	March to July		August to October	8	➢ Hot temperature ➢ Can grow in all soil types	➢ Annual plants ➢ Long owing/harvesting season

Table 2.17 (cont.)

Carrot	March to July	August to December	10	➢ Grow best in full sun but tolerate some shade ➢ Optimum temperature is 16 to 21 °C ➢ Can grow in all soil types	➢ Annual plants
Turnip	April to August	September to December	9	➢ Can grow in all soil types	➢ Annual plants
Alfalfa	Several months	As soon as the buds start to appear	-	➢ Can grow under any conditions ➢ Prefers alkaline and free-draining soils ➢ Drought resistant	➢ Perennial plants ➢ High nitrogen fixation rate in the soil
Clover	Several months	As soon as the buds start to appear	-	➢ Can grow at most times of the year ➢ Adapted to frost and drought	➢ Short lived perennial ➢ High nitrogen fixation rate in the soil

Despite the fact that most crops can grow in all soil types (Table 2.17), the media in which plants grow in has a significant effect on quality, growth and yield of vegetables (Olle, Ngouajio, & Siomos, 2012). For example, Del Amor (2007) studied the effect of three different cultivation methods (organic, integrated and conventional farming) on the growth of sweet pepper under greenhouse conditions. The author's results showed that the fresh weight of plants and total leaves in the organic treatment were significantly reduced compared to the conventional one, explaining the impact of plant nitrate concentrations in the organic cultivation which is directly correlated with growth rate of plants.

However, Del Amor (2007) stated that organic and conventional farming did not show any significant differences in terms of marketable yield, while the integrated farming showed the highest yield in the extra and first class fruit categories. Moreover, fruit firmness and pericarp thickness showed higher values with the organic method with no significant differences compared to the conventional method. The Del Amor (2007) study summarised that adding a proper dosage of organic fertilisation, taking into consideration the capacity of the plants and maintaining yield under nutrient depletion at later stages of development, is highly recommended.

This agreed with the results obtained by Gungor and Yildirim (2013) who studied the effect of different growing media on quality, growth and yield of pepper (*Capsicum annuum* L.) under greenhouse conditions. Peat alone, and a mixture of peat, perlite and sand (volume ratio of 1 to 1 to 1) growing media were used in this study. The results showed that peat growing media could be successfully used to obtain better quality and yield. However, using a mixture media significantly increased length, diameter and weight of fruit compared to peat. On the other hand, the fruit number per plant and yield were higher for peat grown plants than for those grown in the mixture.

Moreover, peat moss and coco-peat alone or mixed with sand led to a better harvest than other media (Rahimi, Aboutalebi, & Zakerin, 2013).

In parallel to the effect of growth media, Table 2.17 shows that environmental conditions in which the plants were grown had a significant influence on the crop yields and quality. For example, light has an important effect on optimum plant growth. Growing plants in insufficient light will increase the plant height, number of nodes and leaf size with inhibition of lateral shoots developing on the main plant stem. Subsequently, fruit set, number of fruits per plant, fruit location on plants, fruit development and yield will be highly affected (Rylski & Spigelman, 1986). Low light intensity may lead to flower inhibition or cause flower abscission (Wein & Zhang, 1991).

Temperature is another factor which could affect the growth of plants. For example, plant height and chlorophyll content could decrease as daily temperature decreases. Moreover, growing plants in low temperature conditions will cause a reduction in plant stem and leaves dry weight and an increase in the content of all minerals and nutrients such as nitrogen, phosphorous, potassium, calcium, magnesium, manganese and copper in different plant parts (Inthichack, Nishimura, & Fukumoto, 2014).

In addition to light and temperature, the air humidity has a significant effect on plant growth. For example, the plant transpiration rate will decrease when air humidity increases. Moreover, the macro-nutrient concentrations in plant leaves will decrease with increasing air humidity, especially for potassium and calcium. Furthermore, macro-nutrient concentrations in growing media will decrease when plants are grown under high air humidity conditions, especially for nitrogen and potassium (Gisleröd, Selmer-Olsen, & Mortensen, 1987). However, humidity values below 50% could have a negative impact on fruit development (Bakker, 1989).

Furthermore, consideration of the required levels of certain nutrients and trace elements for growing the selected crops is quite important, as unfavourable concentrations will be a challenge to the growth of plants fed by recycled pre-treated wastewater. For example, Asfaw, Sime, and Itanna (2012) studied the capability of different vegetable crops, such as onion, carrot, beet root, Swiss chard, tomato and cabbage, to grow under different concentrations of pollutants in wastewater used for irrigation purposes. Findings showed considerable tolerance in growth of vegetable seeds. Untreated wastewater enhanced the germination of some vegetable seeds at relatively low concentrations, whereas higher effluent concentrations were linked to inhibitory effects. Moreover, Boyden and Rababah, (1996) assessed the recycling of nutrients from settled primary domestic wastewater (not disinfected) to produce value-added crops including capsicum and tomato. The crops grown in these systems considerably removed nitrogen and phosphorous from settled primary sewage, and appeared healthy compared to the control using commercial nutrients. Furthermore, Bar-Tal, Aloni, Karni, Oserovitz, et al. (2001) and Bar-Tal, Aloni, Karni, and Rosenberg (2001) studied the effects of the solution nitrogen concentration and the ratio of nitrate nitrogen to ammonia nitrogen ($N-NO_3$: $N-NH_4$) on fruit yield, quality and the incidence of blossom-end rot of bell pepper plants grown in greenhouse conditions. Their results showed that the yield of high quality was increased with the increasing of $N-NO_3$ to $N-NH_4$ ratio and decreased dramatically as the NH_4 concentration in the solution increased to be more than 2 $mmol \cdot L^{-1}$. Moreover, the high NH_4 concentration is the main reason for suppression of calcium concentration in the leaves and fruits and subsequently increased the possibility of blossom-end rot incidence. Production of flat fruits could also be increased with the increasing of ammonia concentration in the solution.

Cheng, Shearin, Peet, and Willits (2004) assessed an integrated system that recycles waste organics and treats wastewater from a swine farm to grow vegetables. An anaerobic digester with ambient temperature was used to treat the swine wastewater and to produce biogas. A trickling nitrification bio-filter was developed to convert ammonium in the effluent into nitrate. The nitrified anaerobic effluent was used as both fertiliser and irrigation water. Moreover, FAO (2003) stated the nutrient requirements for pepper, required for proper canopy formation: nitrogen (90 kg/ha), phosphorus (6 kg/ha), potassium (90 kg/ha), phosphorus pentoxide (14 kg/ha) and potassium oxide (108 kg/ha). The corresponding values for good fruit production are as follows: nitrogen (2.0 kg/ton), phosphorus (0.26 kg/ton), potassium (1.83 kg/ton), phosphorus pentoxide (0.6 kg/ton) and potassium oxide (2.2 kg/ton). Furthermore, Chemicals (2014) stated the required rates of macro-and secondary plant nutrient uptake by pepper plants in greenhouses: nitrogen (390–920 kg/ha), phosphorus pentoxide (200–330 kg/ha), potassium oxide (640–1530 kg/ha), calcium oxide (100–210 kg/ha), magnesium oxide (60–150 kg/ha) and sulphur (40–50 kg/ha).

In addition, Ciju (2013a) reported the following nutrition values for 100 g of fresh and raw green Bell Peppers: phosphorus (20 mg), potassium (175 mg), calcium (10 mg), magnesium (10 mg), iron (0.34 mg) and zinc (0.13 mg). In comparison, Ciju (2013b) reported the following nutrition values for 100 g of sun-dried Chillies: phosphorus (159 mg), potassium (1870 mg), calcium (45 mg), magnesium (88 mg), iron (6.04 mg) and zinc (1.02 mg). Further beneficial elements may include silicon. Other elements such as aluminium should be present in low quantities within the irrigation water.

2.14 Potential impact of wastewater irrigation reuse

There are several advantages associated with wastewater recycling for irrigation including the supply of nutrients and trace minerals to plants, potentially leading to higher yields and a decrease in the demand for inorganic fertilisers (Bichai, Polo-López, & Fernández Ibañez, 2012; Val-Moraes, Marcondes, Carareto Alves, & Lemos, 2011). Furthermore, irrigation by recycled wastewater can increase the productivity of farming by between 100 and 400%, allowing some crops to be grown in regions with unfavourable conditions. However, nutrients found in the wastewater used for irrigation should be checked to take account of the specific fertiliser requirements of crops, ensuring high marketable yields (FAO, 2010).

However, irrigation with wastewater is associated with numerous disadvantages. In this section, the possible impacts of irrigation with wastewater will be reviewed according to the literature including impacts on public health, crops, soil and ground water resources, property values, and ecological and social impacts as explained below:

2.14.1 Impacts on public health

Pathogenic microorganisms and heavy metals are among the main issues affecting human health when irrigating with wastewater. For example, bacteria, viruses and human parasites such as helminth eggs and protozoa are of particular interest as they are difficult to remove from the wastewater and have a substantial impact on human health. These pathogens are responsible for many infectious diseases in both developing and developed countries (WHO, 1989).

Moreover, the risk of pathogenic infection varies with age group. For instance, high instances of infection by hookworm and Ascariasis infections have been reported among children (Cifuentes et al., 2000; Feenstra et al., 2000; Habbari et al., 2000). The pathogenic guidelines for wastewater irrigation are listed in Table 2.5. Furthermore, heavy metals in wastewater can be considered as another issue that may affect human health mainly when consuming crops of high metals accumulation. Based on this, FAO/WHO (2001) recommended the following thresholds for metals in vegetables: cadmium (0.1 mg/kg), cobalt (50.0 mg/kg), chromium (2.3 mg/kg), copper (73.3 mg/kg), iron (425.0 mg/kg), manganese (500.0 mg/kg), nickel (66.9 mg/kg), lead (0.3 mg/kg) and zinc (100.0 mg/kg). In contrast, Chary, Kamala, and Raj (2008) recommended a limit for copper in vegetables of 20 mg/kg, while for lead and zinc the corresponding values were 1 and 50 mg/kg, respectively. Furthermore, The Ministry of Heath of the People's Republic of China (MHPRC, 2005) stated the following maximum levels of contaminants in food: arsenic (0.05 mg/kg), chromium (0.5 mg/kg), cadmium (0.05 mg/kg) and lead (0.1 mg/kg), while the EC (2001a) has set maximum levels for certain contaminants in food: copper (20 mg/kg), lead (0.3 mg/kg), zinc (50 mg/kg) and cadmium (0.05 mg/kg) as shown in Table 2.18. Moreover, Table 2.19 overviews some studies on public health impact of irrigation with wastewater.

Table 2.18: Recommended levels of metals content in vegetables.

Metals (mg/kg)	**Guidelines**			
	FAO/WHO (2001)	Chary et al. (2008)	MHPRC (2005)	EC (2001a)
Arsenic	-	-	0.05	-
Cadmium	0.1	-	0.1[a], 0.2[b], 0.05[c]	0.1[a], 0.2[b], 0.05[c]
Cobalt	50	-	-	-
Chromium	2.3	-	0.5	-
Copper	73.3	20	-	20
Iron	425	-	-	-
Manganese	500	-	-	-
Nickel	66.9	-	-	-
Lead	0.3	1	0.3[a,b], 0.1[c]	0.3
Zinc	100	50		50

[a, b] are the recommended levels in root and leafy vegetables, respectively; [c] for other vegetables.

Table 2.19: Studies on public health impact of irrigation with wastewater.

Year and author (s)	Objectives	Methodology	Findings	Suggestions
Shuval et al. (1986)	Full epidemiological studies on wastewater reuse for irrigation	Modelling of health risk assessment	• Helminths, bacteria and viruses are the most risky pathogens in that order • Predominant standards for wastewater irrigation were very restrictive	• Proposing un restrictive standards for irrigation with wastewater • WHO health guidelines basis
Brosnan &O'Shea (1996)	Impact of accumulative reduction of untreated wastewater discharges on coliform concentrations in lower Hudson Raritan Estuary	Water sampling and analysis for monitoring total coliform and faecal coliform concentrations	• Deterioration in coliform concentrations as the result of decline in wastewater discharges • Infrastructure provision and wastewater distribution system improvements and maintenance, • Abatement of illegal connections, wet weather overflows, and reduced discharge	• Improving water quality • Recreational resource value enhancement • Saving cost on bathing advisories
Olivieri et al. (1996)	Potential health risk assessment related to potable use of advanced treated wastewater	Chemical risk assessment and organisms indicator of raw water supply vs reclaimed water, epidemiological data baseline on reproductive health and neural tube flaw	• Hyacinths & advance treatment were used to generate reclaimed water for portable uses • Reclaimed water for potable use was less than available water supply	• Using Hyacinths system as alternative treatment • Public attitude to reusing potable water • Financial possibility in San Diego

Table 2.19 (cont.)

Year and author (s)	Objectives	Methodology	Findings	Suggestions
Downs et al. (1999)	Monitoring risk in Mexico from exposure to surface and ground water contaminated with untreated wastewater used for irrigation	Pathogens detection risk assessment	• Surface and ground water contaminated with elevated total coliforms levels • Gastrointestinal disease risk due to contamination of water resource with faecal coliform • Infants and children at risk • High possibility of diarrhoea and skin pollution by nitrate • Both inside and outside irrigated area are at risk	• Priority to pathogens risk interference • Assessment of nitrate skin pollution and undertake possible treatment
Cifuentes et al. (2000)	Risk assessment of giardia infections in Mexico agricultural population	Exposure to raw wastewater vs rain-fed of households in agricultural villages	• High risk infection of children • High correlation between infection risk and contaminated drinking water with inappropriate disposal of faeces • No risk recorded for exposure to raw wastewater and agricultural activities	• Provision of primary health care and wastewater treatment units • Issues of human capital formation
Habbari et al. (2000)	Infection transmission of geohelminthic among children in primary school due to irrigation with raw wastewater in Morocco	Occurrence rate of childhood disease in communities irrigated with untreated wastewater vs other with no irrigation with wastewater with the impact of demographic and defensive activities	• Ascariasis occurrence was 5 times higher in wastewater regions • High infection rates were recorded for contact with wastewater and wastewater irrigated lands • No variation in Trichuris infection rate • High risk of geohelminthic infection due to use of raw wastewater in Ben-Mallal	• Sufficient wastewater treatment for irrigation • Water provision and programme of sanitation • Control of wastewater exposure • Public health and educational programmes

2.14.2 Impacts on crops

Generally, treated and untreated wastewaters are widely used for irrigation purposes due to the availability of nutrients and essential elements necessary for plant growth (FAO, 2003). Using wastewater in irrigation will lead to increase the potential yield of most crops reducing the required amount of chemical fertiliser and saving the farmers net cost. However, applying nutrients and elements via irrigation with wastewater to plants which exceeds their requirements, mainly total nitrogen, may lead to excessive vegetative growth and delay in ripening and maturity of the yields, or in some other extreme conditions may lead to the loss of the yield.

Several studies have been undertaken by agronomists trying to quantify the impact of irrigation with wastewater on parameters linked to yield and quality. Agronomists concluded that using treated wastewater in irrigation of plants will increase their potential yield and quality more than what would be otherwise possible. An overview of some of these studies is shown in Table 2.20. In spite of several issues associated with the use of wastewater in irrigation, it still very attractive for use by farmers as it will save fertiliser costs even if in some cases it will not improve the amount and quality of the yield. Excessive nitrogen, phosphorous and potassium available in the wastewater which may exceed the needs of plants will impact negatively on the yield. For example, urea effluent from treatment plants will be a rich liquid fertiliser but if it is highly concentrated then it will impact adversely on corn and rice yields as reported by Singh and Mishra (1987).

Moreover, chemical pollutants available in the wastewater, mainly industrial wastewater, should be taken into consideration when irrigating plants as they will accumulate in plant tissue and then enter the food chain by human consumption. For example, Kalavrouziotis, Robolas, Koukoulakis, and Papadopoulos (2008) conducted an experiment in a greenhouse, to study the effect of treated municipal wastewater (TMWW), compared to ordinary irrigation water, on the macro- and micro-elements and heavy metal content of Brassica oleracea var. Italica (broccoli), and B. oleracea var. Gemmifera (Brussels sprouts) plants, as well as on the physical and chemical properties of the clay loam soil, and its inorganic composition, to examine the possibility of TMWW reuse for irrigation of the above vegetables. The results showed that applied TMWW increased significantly, in comparison to control, the content of some macro- and micro-elements in the soil. Furthermore, the levels of the heavy metals in the edible plant parts were very high causing a high health risk factor, and therefore the TMWW studied, cannot be used for the irrigation of these vegetables.

Moreover, the highly frequent application of wastewater will increase the salinity affecting crops with salt sensitivity. This was confirmed by Zavadil (2009) who studied the effect of irrigation with municipal wastewater on vegetables and crops like lettuce, radish, carrot and potato. Primary treated wastewater and secondary treated wastewater were used in this experiment work, while irrigation with local well water or public water supply was used as a control treatment. The results showed that statistically the primary treated wastewater compared to the secondary treated one, significantly increased the yield of all vegetables and crops. However, irrigation with secondary treated wastewater caused an increase in sodium content in radishes and carrots, while irrigation with the primary treated wastewater led to an increase in the sodium content in the edible parts of all vegetables. Moreover, the results showed that irrigation with this water caused a high bacterial contamination in all vegetables.

Also, the high microbiologically contaminated wastewater causes a reduction in the overall crop yield and quality with high potential for contamination by pathogens and intestinal helminths. However, high yields can be achieved by using pre-treated wastewater for irrigation of various crops under controlled environmental conditions (Zavadil, 2009). Identification of the agricultural, industrial and human sources of microbial contamination from pre- to post-harvest operations of Cantaloupes (*Cucumis melo var. cantalupensis* (Naudin)) grown at ten different farms in southern Texas was undertaken by Materon, Martinez-Garcia, and McDonald (2007). The results indicated that irrigation water contained a wide range of microorganisms that could cause human illnesses and were able to survive on the rind of Cantaloupes before, during and after harvesting.

Moreover, traces of hydrocarbons from diesel spills associated with urban run-off or industrial effluent are a more recent challenge (Scholz, 2010; García-Delgado, Eymar, Contreras, & Segura, 2012) which will affect the irrigated soil and crops. For instance, García-Delgado et al. (2012) undertook a greenhouse study in Spain to assess the effect of treated urban wastewater contaminated with hydrocarbons on soil and pepper quality. They concluded that the wastewater application saved fertiliser (37% nitrogen, 66% phosphorus and 12% potassium) and that the total poly-aromatic hydrocarbons and heavy metals (cadmium, lead and arsenic) within the pepper fruits were low. The highest concentration (lower than the proposed threshold concentration for carcinogenicity) was recorded for phenathrene.

These observations contradicted with those obtained by Khan, Aijun, Zhang, Hu, & Zhu (2008) who carried out a greenhouse experiment of lettuce (*Lactuca satuva* L.) pot planting to assess the concentrations of polycyclic aromatic hydrocarbons and heavy metals (HMs) accumulated in vegetables grown in wastewater-contaminated soils. The results showed that the plant shoots were highly contaminated with polycyclic aromatic hydrocarbons and heavy metals (cadmium, chromium, nick, and lead) which exceeded the guidance limits set by the State Environmental Protection Administration (SEPA), China and the World Health Organization (WHO) indicating the potential health risks associated with cultivation and consumption of leafy vegetables on wastewater-contaminated soils. Moreover, irrigation with wastewater contaminated with hydrocarbons will result in increased populations of microbial communities as reported by Benedek et al. (2013) who studied the impact of long-term total petroleum hydrocarbons (TPH), volatile petroleum hydrocarbons, total alkyl benzenes and polycyclic aromatic hydrocarbons on the structure of bacterial communities of four different contaminated soil samples. They concluded that a very high amount of TPH positively affected the diversity of hydrocarbon-degrading bacteria.

According to the literature, the impact of irrigation with treated wastewater will mainly depend on the degree of treatment and the nature of the crops. Economically, irrigation of crops with good wastewater management and practices will achieve several benefits such as increasing the yields, providing extra irrigation water and saving the cost of chemical fertiliser (FAO, 1994, 2003).

Moreover, many studies have been carried out in California to improve a consistent system for wastewater treatment for producing irrigation water which guarantees production of agricultural crops in association with protection of public health (SDLAC, 1977; Sheikh, Cort, Kirkpatrick, Jaques, & Asano, 1990). A key result of these studies showed that reclaimed wastewater could be successfully used for irrigation of crops, even those which may be consumed uncooked, without opposing environment or health requirements (Sheikh et al., 1990; York, Holden, Sheikh, & Parsons, 2008).

Table 2.20: Overview of wastewater irrigation impact on crops.

Year and author (s)	Objectives	Methodology	Findings	Conclusions
Day et al., (1975)	Impact of irrigation with treated domestic wastewater on growth and yield parameters of wheat	• Well water mixed with normal dosage of NPK vs • well water mixed with simulated NPK dose vs • treated wastewater with no fertiliser	Irrigation with wastewater resulted in: • High wheat yield • High wheat protein content • No change in wheat feed quality in terms of fibre content	Treated wastewater is a high potential source for irrigation that saves fertiliser cost with high yield production
Mortvedt and Giordano, (1975)	Impact of contamination with zinc and chromium tannery wastewater on maize crops	Application of soil highly contaminated with zinc and chromium from domestic wastewater	• High forage production • Zinc available to maize • Low zinc levels with no change of chromium levels in the maize tops • Crops uptake chromium with no impact on their growth	Possibility of irrigation with tannery wastewater under good management
Sidle et al., (1976)	Accumulation of heavy metals in reed grass and maize over time	Long-time of 11 years irrigation with wastewater	• High chromium and zinc levels in reed grass compared to maize • High metals accumulation in irrigated soil • Levels of heavy metals in plants did not pose risk in food chain • Heavy metals were removed via plant uptake	High level of metals in grass may affect sheep feed and animal programme as well as loading and removal of metals modelling to assess the life of land disposal system

Table 2.20 (cont.)

Day and Tuker (1977)	Impact of irrigation with treated domestic wastewater on growth and yield parameters of sorghum	• Well water mixed with normal dosage of NPK vs • well water mixed with simulated NPK dose vs • treated wastewater with no fertiliser	Irrigation with wastewater resulted in: • More forage production with high maturity period and low density of crops • Higher sorghum yields • No differences in protein content with less amino acid • Increasing in sorghum yields compared to the control	Treated wastewater is a high potential source for irrigation that saves fertiliser cost with high yield production
Bole and Bell (1978)	Optimise the utilisation of domestic wastewater irrigation system used for forage production	• Treatment of domestic wastewater using lagoon • Growth and nutrient consumption • Efficiency of alfalfa, reed grass, brome grass, wild rye, and wheat grass	• High alfalfa production compared to other grass specious • Doubling nitrogen production of alfalfa • Excess nitrogen uptake to be more than that supplied for all except wheat grass • Wastewater supplying phosphorous exceeding plant uptake • Alfalfa is the most suitable crop as it has its own nitrogen supplying system (nodules) • Reed grass can be used for optimal wastewater disposal as it can remove most nutrients and survive flooding	• Wastewater provided forage with sufficient phosphorous but not nitrogen • System of forage such as alfalfa and reed grass can be considered for optimisation of wastewater utilisation and disposal

Table 2.20 (cont.)

Marten et al., (1980)	Impact of domestic wastewater on feed quality and yield of maize vs reed grass	Feed quality, dry weight, digestible dry matter of maize vs reed grass were the experiment parameters using two levels of treatment for wastewater for irrigation	• Reed grass less digestible than maize • Maize produced dry and digestible dry matter more than reed grass • Higher protein content in reed grass with low digestible dry matter	• Perennial grass showed better efficiency of wastewater nitrogen removal compared to the maize • High renovation efficiency of wastewater effluent can be achieved with good management of reed grass and maize system
Ajmal and Khan (1985)	Textile factory effluent impact on chemistry of soil and growth of two vegetables: kidney beans and lady's fingers	• Textile effluent diluted to 25, 50, 75 and 100 v/v • Usual irrigation water for control • Impact on kidney beans and lady's fingers	• High BOD, COD, Cl, SO_4, K, Ca, Mg with high alkalinity • Dilution wastewater result to increase elements levels in soil of top more than in subsoil • Na levels in plants increased • Dilution of wastewater to 75 and 100 % inhabit plant growth, while dilution of 50 % enhance growing of plants	• Dilution of textile factory effluent can be used for irrigation without affecting soil properties • Textile effluent is valuable source for irrigation due to nutrient richness • Design of industrial policy is required

Table 2.20 (cont.)

Ali (1987)	Risk assessment of irrigation with domestic water on food crops such as onion alfalfa and summer squash	• Application of sprinklers with secondary treated wastewater mixed with chlorinated wastewater • Treatments with and without fertiliser • Vegetable contamination with faecal coliform counting	• After 24 hr irrigation with sprinklers, no faecal coliforms detected on summer squash • After 15 day irrigation of onion, no faecal coliforms were detected • Irrigation with secondary treated wastewater and chlorination can be used with vegetables which are cooked before eating • Vegetables processed before eating can be irrigated with low level of wastewater treatment	• Guidelines for reuse of wastewater for irrigation in Saudi Arabia
Singh and Mishra (1987)	Impact of irrigation with urea plant outflow on soil properties, rice and corn growth, dry matter and pigment content	Untreated effluent was diluted v/v to 2.5, 5, and 50% vs a control of tap water	• The effluent is of high alkaline • Soil properties are affected negatively when irrigated with effluent of > 10% concentration • High protein content in rice and corn irrigated with effluent concentration of 2.5 and 5% (nitrogen absorption and utilisation) • Effluent of > 10% affected negatively on seeds germination, dry matter and pigment content for both rice and corn	• Urea plant effluent can be used as a source of liquid fertiliser • Diluted urea effluent can be used for irrigation of crops • Pollution and eutrophication control is required

Table 2.20 (cont.)

Misra and Behera (1991)	Impact of irrigation with paper industry effluent on rice growth, carbohydrates and protein content	• Untreated effluent of various dilution vs distilled water • Impact of effluent concentration and exposure time on rice seedlings	• Increasing of effluent concentration and exposure time adversely affect rice growth, carbohydrates and protein content • Protein content is highly affected by effluent concentration and can be considered as bio-indicator for phytotoxicity by the effluent • Paper industrial outflow is not suitable for irrigation	• Regulation for paper industry pollution is required • Phytotoxicity and pollution risk should be evaluated • Eutrophication and pollution control is required
Aziz et al., (1995)	Impact of irrigation with crude oil refinery wastewater on growth and yield of four wheat varieties	• Treated effluent vs a control of ground water • Same dose of fertiliser is used • Assessing growth parameters of shoot length, leaf, fresh and dry weight, • Assessing plant yield parameters of grain yield, protein, and carbohydrate contents	• Treated effluent followed the standards so it is suitable for irrigation • Irrigation with treated effluent did not affect soil properties • Irrigation with treated outflow results in increasing of plant growth, yield, protein and carbohydrate content • Crops showed better performance due to additional nutrients available in the treated wastewater • Response of plants to treated effluent was different from one cultivar to another	• Treated effluent did not affect soil properties and can be used for crop irrigation • Evaluation of long-term impact is required • Policies for industrial pollution and food security are required

Table 2.20 (cont.)

Aziz et al., (1996)	Impact of long-term irrigation with petrochemical refinery wastewater on • Grain and soil heavy metal accumulation • Yield parameters of six cereals: wheat, triticale, chickpea, lentil, pigeon pea and summer moong	• Treated effluent vs a control of lake water for 8 years • Same dose of fertiliser is used • Impact on soil and yield of crops	• Treated effluent followed the standards so it is suitable for irrigation • No significant accumulation of metals in soil and grains • Metals levels in grain were below standards and suitable for human consumption • Irrigation with wastewater increases the yield for all crops except moong	• Risk of soil contamination with metals affecting food chain in the future is expected • Policies for industrial pollution and food security are required
Howe and Wagner (1996)	Impact of irrigation with paper mill wastewater with application of gypsum on soil and cottonwood growth rate and sodium uptake	• Untreated wastewater • Four gypsum application rates w/w of 100, 175, 325 and 625 mg on wastewater base • Wastewater pH and rate of gypsum application • Cotton biomass, stem sodium and leaves calcium, potassium and sodium	• Cottonwood biomass production is affected by gypsum application and not pH • Stem biomass production is dependent on pH • High cottonwood growth with application of gypsum at low pH values • Stem and leaves sodium levels were affected by gypsum application rate and not pH • Gypsum application and wastewater pH affected infiltration rate	• Problem of sodium accumulation in the irrigated soil, thus application of gypsum was the management action • Management of calcium as amendment when irrigation with sodic wastewater is required • Long-term evaluation is required

Table 2.20 (cont.)

El Hamouri et al., (1996)	Impact of irrigation with domestic wastewater on soil and yield microbiological quality, the hygienic quality of salt sensitive crops such as cucumber and turnips as well as salt tolerant crops such as alfalfa, corn, zucchini, beans, and tomato	• Raw wastewater • Wastewater treated by stabilisation pond • Ground water • Irrigation with different methods: surface, drip and sprinklers • Assessing soil and crops faecal coliform and helminth eggs contents	• Wastewater stabilisation pond produced effluent meeting WHO guidelines for irrigation • Cucumber was highly affected by salinity with lower yield when using raw wastewater compared to that of treated wastewater • For salinity tolerant crops there were no significant differences in yield of wastewater and ground water • No helminth eggs were detected in soil and crops irrigated with treated wastewater, while highly detected in those irrigated with raw wastewater • Raw wastewater was not suitable for irrigation • High crop performance and yield was recorded for drip irrigation system	• Using of treated wastewater for irrigation crops of high salt sensitivity in salt areas will be highly advantageous in terms of reduced salt impact on crop yield and growth, reduced aquifer salinisation with saving of fertiliser cost • Technology for wastewater treatment is required • Policy development for arid and saline areas is required

Table 2.20 (cont.)

Shahalam et al., (1998)	Impact of irrigation with wastewater on soil and crops such as alfalfa, tomato and radish and risk to health and of ground water pollution	• Treated wastewater vs standard fresh water with and without fertiliser • Impact on growth and yield of crops • Impact on soil porosity, pH, salinity, alkalinity and drainage • Levels of faecal coliform on irrigated crops and environments	• Trends of yield: Alfalfa: fresh water with fertiliser > wastewater with fertiliser, Radish: no significant differences when using wastewater, Tomato: wastewater only > wastewater with fertiliser • Irrigation with wastewater mixed with fertiliser was comparable with fresh water mixed with fertiliser • Increasing soil porosity and salinity with lowering pH when irrigating with wastewater • No contamination was detected through subsurface drainage analysis • No faecal coliforms were detected on tomatoes after 24 hr with no odour or synthetic impact in terms of hygienic quality	• Irrigation with wastewater below the standards did not show any risk to human, soil or environment, however, chlorination is required • Using wastewater for irrigation in Jordan is a valuable solution for the water scarcity problem in such regions • Policy for national water security is highly required

Table 2.20 (cont.)

Parames-waran (1999	Irrigation with urban wastewater agro-economic feasibility for Jerusalem artichoke in Australia	• Irrigation using furrow method • Several artichoke cultivars were used • Plant biomass (top and tuber yield) and nutrient analysis • Soil salinity and pH • Impact of long-term irrigation with wastewater	• Artichoke requires a lot of fertiliser supplied by wastewater • No deficiency in nutrients were detected • No symptoms of growth toxicity or damage in growth due to high level of nutrients in the wastewater • Nutrients levels were higher in the top than tuber parts • High yield of artichoke irrigated with wastewater compared with the others • Channel water units cost more than irrigation with wastewater • No change in pH of irrigated soil • Salinity was increased slightly • No significant change in nutrient levels of irrigated soil • Long term application of wastewater will increase nutrients and accumulation of iron	• Wastewater is viable source for irrigation • Production of artichoke as an alternative to urban wastewater land disposal • Biomass of artichoke can be used for producing ethanol • Production costs investigation is required

Table 2.20 (cont.)

Reboll et al., (1999)	Impact of irrigation with wastewater on citrus growth, yield and leaves metal accumulation	• Flood irrigation with treated wastewater vs ground water as a control • Growth parameters: height, canopy and trunk diameter • Yield quality parameter: fruit weight, diameter, colour, acidity, ripeness index and juice • Soil nitrogen, sodium, chlorine, and boron	• Irrigation with wastewater did not affect plant height and diameter, while canopy diameter was increased • Irrigation with wastewater may result in increasing forage growth and delay in ripening due to high nutrients • Wastewater irrigation did not affect fruit yield • High boron concentrations in the wastewater did not affect the fruit quality, soil boron with no plant boron toxicity • Overall fruit production not affected by irrigation with wastewater • No significant impact of irrigation with wastewater on citrus plants after duration of 3 years • Soil nitrogen and chlorine levels not affected by wastewater irrigation • Increasing soil sodium due to wastewater irrigation	• Treated wastewater is a suitable source for irrigation of citrus • Irrigation with wastewater reduced required fertiliser for citrus • Citrus is a valuable crop in Spain and irrigation with wastewater will be of high implication

2.14.3 Impacts on soil resources

Impacts on soil are of specific importance since they may reduce soil quality in terms of productivity, fertility and yield. Soil should remain at a good level of chemical and physical characteristics in order to enable long-term sustainable use and profitable agriculture. However, the expected soil problems associated with wastewater use for irrigation are: salinisation, alkalinity and reduced soil permeability, accumulation of nutrients and potential toxic elements, as well as microbes in soil irrigated with wastewater (FAO, 2003).

For example, sodium in irrigation water may affect soil structure and reduce the rate of water moving into the soil in addition to reducing soil aeration. This will reduce the infiltration rate causing problems in providing the crops and landscape plants with adequate water for good growth.

Moreover, the accumulation of wastewater salts in the root zone will harm the soil health and affect the yield and crop productivity. According to Bond (1999), soil and ground water pollution may result from leaching of such salts below the roots zone. Frequent use of saline wastewater will destroy soil structure and consequently impact on productivity resulting in land-use unsustainability. This observation was confirmed by Travis, Wiel-Shafran, Weisbrod, Adar, and Gross (2010) who undertook a controlled experiment to study the effect of using raw and treated artificial grey water for irrigation purposes. Findings indicated that raw artificial grey water considerably increased the development of hydrophobicity in the sand and loam soils, and subsequently affected plant growth. In comparison, treated artificial grey water was successfully used for irrigation without any detrimental effects on soil or plant growth. These results agreed with those obtained by Gross, Shmueli, Ronen, and Raveh (2007) who investigated the impacts of treated artificial grey water on soil and plant parameters over time. The authors' results showed that the recycled water had no significant negative impact on soil and plants, meeting standards for unlimited irrigation, except for the complete removal of pathogens. Moreover, Al-Hamaiedeh and Bino (2010) stated that salinity, sodium adsorption ratio (SAR) and organic content increase if soil is irrigated with grey water. However, the chemical characteristics of the irrigated crops were not affected while the biological quality of some of them was negatively affected.

The permeability is another problem which may occur in the surface of the soil due to high sodium or very low calcium concentrations in this soil zone or in the used irrigation water (Westcot & Ayers, 1985). The potential for permeability problems could be evaluated by measuring the sodium adsorption ration (SAR) in combination with electrical conductivity, since there is a direct relationship between infiltration rate and salinity. High sodium content will result in high SAR values causing a major concern in reusing treated wastewater for irrigation projects.

However, since irrigation with sodic water will cause the problem of degradation of soil structure, then chemical and biological modifications should be undertaken over time to amend the soil structure. For example, in calcareous soils using acid formers will improve the dissolution of calcite in the root zone, also the actions of plant roots will increase the level of carbon dioxide providing soluble calcium to balance sodium effects (Qadir, Noble, Oster, Schubert, & Ghafoor, 2005).

Although soil salinity problems can be resolved by application of either natural of artificial amendments, the measures for reclamation of soil will be very costly adding extra economic limitation and resulting in productivity losses. Also, maintaining the efficiency level of the soil will be difficult when using soil amendments. As a result, saline issues associated with application of wastewater will impact economically on soil in terms of prices and land value due to soil salinity and water logging.

Suppression of crop growth, mainly in the early stages, is the main problem associated with salinity due to imbalances in nutrients and toxic ions (Kijne et al., 1998) leading to yield reduction and loss of farmers' profits.

Accumulation of heavy metals in the soil is another issue associated with application of wastewater that will affect soil fauna and flora in addition to the contamination of crops. According to Kruse and Barrett (1985), theses heavy metals either bio-accumulate in the soil or may be reallocated via certain fauna, such as earthworm, in the case of cadmium and copper. Assadin et al. (1998) studied the impact of irrigation with wastewater mixed with river water for irrigation of crops in Mexico. The authors' results showed that using such waters for irrigation resulted in more than 31% of heavy metal accumulation in soil surfaces leading to up-taking of those metals by alfalfa plants. However, their results stated that the contamination of alfalfa with heavy metals did not present any risk to animal or human health.

Moreover, McBride (1995) stated that long-term application of sewage of high heavy metal content will affect sensitive plants with high potential to reduce soil productivity. However, the concerns regarding heavy metals pollution are considered to be higher in sewage than in wastewater application, as the sewage produced during the treatment process contains most of the heavy metals. Chary, Kamala, and Raj (2008) assessed the heavy metal pollution of soil irrigated by sewage and wastewater. The results showed that the partitioning pattern of the soil showed high levels of zinc, chromium and copper associated with labile fractions making them more mobile and plant available.

Furthermore, wastewater sources, properties of the soil and characteristics of plants are the main factors which influence the soil impacts from irrigation with wastewater.

Moreover, the impacts from irrigation with various wastewaters such as commercial, domestic, industrial and dairy wastewaters are recorded to be widely different. Degens et al. (2000) stated that effluent from dairy factors application in New Zealand for 22 years resulted in the accumulation of most of the phosphorus in the soil and was responsible for leaching of most of the nitrogen from the soil, resulting in nitrate ground water pollution .

Table 2.21 lists some guidelines regarding the recommended levels of heavy metals in agricultural soils. For example, FAO/WHO (2001) recommended the following thresholds for metals in soils: arsenic (20 mg/kg), cadmium (3 mg/kg), cobalt (50 mg/kg), chromium (100 mg/kg), copper (100 mg/kg), iron (50000 mg/kg), manganese (2000 mg/kg), nickel (50 mg/kg), lead (100 mg/kg) and zinc (300 mg/kg).

According to Kabata-Pendias (2011), there are maximum allowable concentrations for lead (20 to 300 mg/kg), cadmium (1 to 5 mg/kg), nickel (20 to 60 mg/kg), chromium (50 to 200 mg/kg) and manganese (1500 to 3000 mg/kg) in agricultural soils. However, Sattar (1996) reported typical value ranges in uncontaminated soil for lead (0 to 500 mg /kg), cadmium (0 to 1 mg /kg), nickel (20 to50 mg /kg), chromium (0 to100 mg /kg) and manganese (0 to 500 mg /kg). Alloway (1990) classified the normal ranges of zinc, chromium, copper, nickel, cobalt and lead in soil to be from 1 to 100 mg/kg, 0.03 to 14 mg/kg, 5 to 20 mg/kg, 0.02 to 5.2 mg/kg, 5 to 20 mg/kg and 5 to 15 mg/kg, respectively.

In comparison, Kabata-Pendias and Pendias (1992) classified the tolerable levels of zinc, chromium, copper, nickel, cobalt and lead in soil to be 300, 50, 100, 60, 40, and 100 mg/kg, respectively. However, the total amounts of trace elements in soil naturally have an essential influence on the soluble or plant-available amounts, and this is affected by several factors such as pH, texture, organic matter, clay minerals, moisture content, redox potential, interrelations of trace elements, weather and climate conditions, microbiological activity in soils and soil drainage (FAO, 1972) and soil drainage (FAO, 1972).

Furthermore, accumulation of various microbes in soils is another issue associated with the application of wastewater for irrigation. For example, Aiello et al. (2007) assessed the effects of reclaimed urban wastewater for irrigation on vegetable quality and hydrological soil behaviour. Wastewater application resulted in increased microbial contamination (*E. coli*: 3000 most probable number (MPN)/100 ml; Faecal Streptococci: 1200 MPN/100 ml) on the soil surface. A disturbed layer of soil was observed to be characterised by reduced soil porosity and a corresponding decrease in water retention and hydraulic conductivity.

Table 2.21: Recommended levels of metals content in vegetables.

Metals (mg/kg)	**Guidelines**							
	FAO/WHO (2001)	**Kabata-Pendias (2011)**	**Sattar (1996)**	**Alloway (1990)**	**Kabata-Pendias & Pendias (1992)**	**Bideli & Seilsepour (2008)**	**MEPPRC (1995)**	**Zhuang et al. (2009)**
Arsenic	20	-	-	-	-	20	-	-
Cadmium	3	1.5	0–1	-	-	3	0.3	0.2
Cobalt	50	-	-	5–20	40	50	-	-
Chromium	100	50–200	0–100	0.03–14	50	100	-	-
Copper	100	-	-	5–20	100	100	50	35
Iron	50000	-	-	-	-	50	-	-
Manganese	2000	1500–3000	0–500	-	-	2000	-	-
Nickel	50	20–60	20–50	0.02–5.2	60	50	-	-
Lead	100	20–300	0–500	5–15	100	100	250	35
Zinc	300	-	-	1–100	300	300	200	100

2.14.4 Impacts on ground water resources

Another considerable impact associated with wastewater long-term application is the quality of ground water due to the leaching of salts and nutrients from wastewater below the root zone of plants. However, this impact may depend on several factors such as water table depth, and ground water quality as well as the drainage of the soil and wastewater scale for irrigation. For example, the impact of leaching nitrate will be determined from the ground water quality and in the case of brackish groundwater then the leaching nitrate will be of less concern as the water will be invaluable for use. Based on this, evaluation of ground water for the possibility of contamination should be undertaken before application of the programme of irrigation with wastewater (FAO, 2003).

Moreover, using wastewater for irrigation will cause pathogenic bacteria and viruses translocation to the ground water (NRC, 1996; WHO, 1989). For example, in Gabal el Asfar farm (Cairo) where primary treated wastewater has been used for irrigation of crops since 1915, Farid et al. (1993) stated that there was a considerable increase in ground water salinity in addition to the contamination by coliform in the ground water, which is similar to the observations reported by Downs et al. (1999) and Gallegos et al. (1999) in Mexico. However, the same tendency was reported in ground water for chloride, sulphate and total dissolved solids in Gabal el Asfar farm which were recorded in greater amounts in ground water than in the sewage effluent (Rashed et al., 1995).

Irrigation water percolation to ground water has a significant impact on ground water recharge and quality as more than 70% of irrigation water worldwide is infiltrated to aquifers (Rashed et al., 1995). Based on this, recharging of ground water via application of wastewater is economically substantial in spite of its poor quality. This is especially valid in areas of limited sources for fresh water and where the ground water is removed at a rate greater than it is replaced.

From this point of view, there should be an adjustment between cost of ground water pollution and benefits of ground water recharge when wastewater is applied.

2.14.5 Impacts on property values

There are two main issues which should be considered when discussing the impact of irrigation with wastewater on property values. The first one is the pollution source discomforts related to noise, irritation, odour and hazards which have been investigated widely. In this case the cost will include both health and clean-up costs in addition to legal responsibility, as reported by Page and Rabinowitz (1993). Moreover, several studies have been undertaken to study the impact of polluted streams on close property values. For example, Epp and Al-Ani (1979) reported that properties located near a clean stream were significantly more expensive than those along polluted streams. This agreed with Polhemus et al. (1985) who reported the considerable reduction of about 23% in property values which were close to a polluted beach in New Jersey.

The second issue of consideration when applying wastewater is the eventual use which may be considered as a pollution source for the related property.

For example, property values in areas of residential, industrial or commercial buildings in which polluted ground water is used as a water resource will be substantially lower than those using clean ground water, since the resources could not be used for the purpose of design. However, if there are water resources available in such areas then the value impact will only be associated with the first issue.

Moreover, irrigation with wastewater of high salinity will impact on soil productivity and consequently affect the land values and tenancy profits. On the other hand, land irrigated with wastewater may have appreciated values as well. From this point of view, irrigation with wastewater may affect the property values either positively or negatively depending on the circumstances. In this case, both costs and benefits analysis should be considered when using wastewater for irrigation.

2.14.6 Impacts on ecology

Eutrophication is one of the min problems which may occur at water surfaces and in water bodies when draining wastewater used for irrigation, due to the abundancy of nutrients mainly nitrogen and phosphorous. According to Smith et al. (1999), eutrophication will lead to imbalance in water body plant and microbe communities. As a result, the aquatic life will be affected in ways such as reductions in number of water birds and biodiversity. However, this ecological impact will indirectly affect the economics of local communities where their needs are met from those water bodies. For example, death of fishes may result from draining of wastewater containing high organic materials which will reduce the dissolved oxygen levels and subsequently affect aquatic life composition. Moreover, this ecological potential impact of wastewater for irrigation can be assessed using biomarkers or indices for counting the monetary values using specific economic techniques (Hussain et al., 2002).

2.14.7 Impacts on society

The potential social impacts of using wastewater for irrigation may include general impacts, such as environment poor quality, poor sanitation, and odour with high accident possibility. Food safety, health, well-being, and property value losses with reduction in land use sustainability are other social concerns associated with using wastewater for irrigation. Furthermore, water resources pollution with loss of wildlife are natural concerns linked to the reuse of wastewater. Business risk can be created due to concerns of irrigation with wastewater that should be addressed properly to avoid lobby group abuse. Appropriate insurance levels should be obtained in order to cover business risk and legal responsibility. However, the structure of such insurance will be considerably different among different regions and crops (Hussain et al., 2002).

2.15 Summary

This chapter discusses the use of constructed wetlands technology to treat wastewater and the possibility for irrigation reuse. The compositions of wetlands and factors which may affect their behaviour are described. Furthermore, this chapter talks about the standards required for wastewater irrigation, the suitable irrigation methods for wastewater reuse and techniques of crop selection for irrigation with wastewater. Lastly, the potential impacts of wastewater reuse for irrigation are discussed in detail in this chapter.

CHAPTER 3

METHODOLOGY

3.1 Overview

This chapter describes the materials and methods used in the conducted research. In this study, selected vegetables were irrigated with different water types from various sources under laboratory conditions, aiming to investigate their impacts on soil and vegetable growth, quality and safety for human consumption (Figure 3.1). The chapter is divided into several sections. Sections 3.1 and 3.2 introduce the chapter and irrigation water resources, respectively, while irrigation water quality analysis is discussed in section 3.3. Monitoring of laboratory boundary conditions, selection and growing of plants are presented in sections 3.4, 3.5 and 3.6, respectively. Moreover, soil quality analysis with fruit assessment and quality analysis are discussed in sections 3.7 and 3.8, in that order. Risk assessment associated with laboratory work is stated in section 3.9, while statistical analysis used to interpret the data is described in section 3.10. Lastly, limitations of this research work and a summary of the chapter are presented in sections 3.11 and 3.12, respectively.

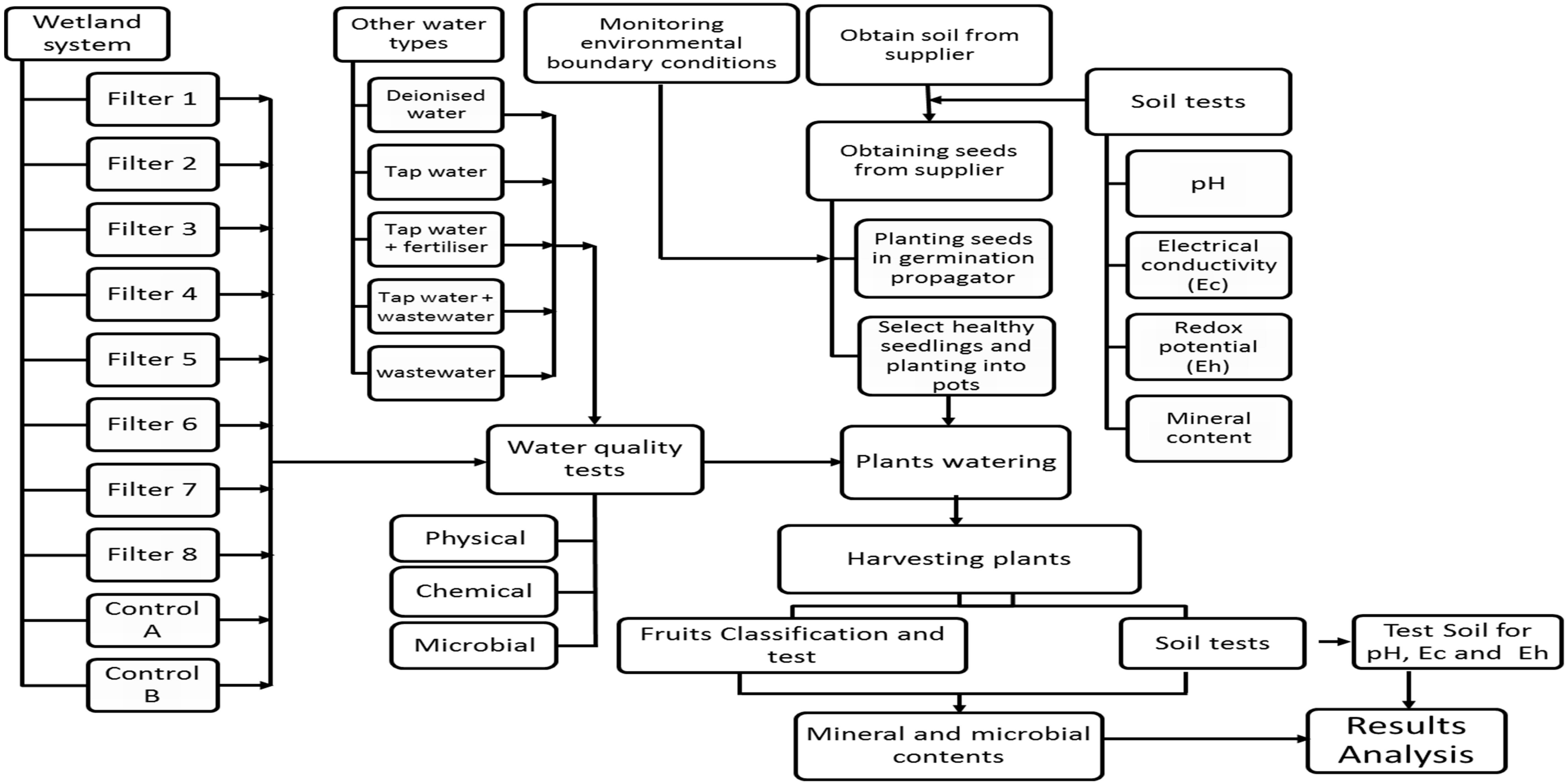

Figure 3.1: Flow chart of experimental work methodology.

3.2 Irrigation water sources

In this study, various water types were used for irrigating plants (Figure 3.1). However, the main source is the treated wastewater effluent from the vertical-flow constructed wetlands system. This system is located within a greenhouse (door left open) on the open top door of the Newton Building (Figure 3.2 a) of The University of Salford, Greater Manchester, UK. It was operated between 27 June 2011 and 01 October 2016. The set-up includes two filters that are essentially controls receiving clean de-chlorinated water. Table 3.1 presents an overview of the statistical experimental set-up (completely randomised design) used to test the impact of four variables: aggregate size and inflow loading rate, as well as contact and resting times. Filters 1 and 2 compared to Filters 3 and 4 test the influence of a larger aggregate diameter. Filters 5 and 6 compared to Filters 3 and 4 check the impact of a higher loading rate. The application of a lower contact rate is tested if Filter 7 is compared with Filters 3 and 4. Finally, a lower resting time is the difference between Filters 7 and 8. The ten laboratory-scale vertical-flow constructed wetlands are constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm (Figure 3.2 b). The filters were filled with siliceous (minimum of 30%) pea gravel up to a depth of 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). Dead macrophyte plant material was harvested in winter and returned to the corresponding wetland filters by placing it on top of the litter zone (Sani et al., 2013). The main outlet valve is located at the bottom of each constructed wetland system. Eight further valves (used to test for clogging) are located on the sidewall of each wetland column. The sidewall valves are located at heights of 10, 20, 30, 40, 45, 50, 55 and 60 cm from the bottom of each column (Figure 3.2 b).

The wetland systems were operated using a batch flow mode. Two water types were used as wetland system inflow: raw wastewater (preliminary treated wastewater) and raw wastewater diluted with de-chlorinated tap water. Raw wastewater was used to feed wetland filters of high inflow loading rate (Filters 5 and 6); while wastewater diluted with tap water was used to feed the other system filters except the controls which were fed with only tap water (Table 3.1). The batch flow mode was applied intermittently as the inflow was fed through the filter surface and then gradually percolated toward the bottom via the gravel substrate (Sani et al., 2013).

Wetland columns received 6.5 l of inflow water during the feeding mode, which was different across several filters (Table 3.1). Columns 1 to 6 were sampled after 72 hours contact time and then left to rest for 48 hours, while columns 7 and 8 were sampled after 36 hours contact time and left to rest for 48 hours and 24 hours, respectively. These resting times allow air to enter the system substrate enhancing oxygen transfer available for the next feeding dose (Sani et al., 2013).

This treatment technology is dependent on processes equivalent to those used widely in gravel "filter beds" improved by *Phragmites australis* of an extensive root system. Macrophytes can transfer the oxygen from the atmosphere and release it into the system substrate via their rhizomatous root system, enhancing bacterial communities.

All water quality parameters discussed in this work were usually determined during or directly after sampling. The preliminary (screens) treated urban wastewater used for the inflow water was obtained from the Davyhulme Sewage Works, one of the largest wastewater treatment plants in Europe, operated by the water company United Utilities in Greater Manchester (www.unitedutilities.com). Fresh wastewater was collected approximately once per week, and was stored and aerated by standard aquarium air pumps in a cold room before use. The wastewater quality was highly variable, and comprised domestic and industrial wastewater as well as surface water run-off.

In order to simulate diesel fuel (100% pure; no additives) spills, 130 grams (equivalent to an inflow concentration of 20 g/l) of diesel were poured into Filters 1, 3 and 5, and into Control A on 26 September 2013 (Table 3.1). The fuel was obtained from a petrol station operated by Tesco Extra (Pendleton Way, Salford, UK).

Diesel was used as the petroleum hydrocarbons model to investigate the suitability of related contaminated wetlands outflow for irrigation purposes when compared with the standards. Diesel was selected as it is the most popular fuel used worldwide mainly in industrial and technological developed countries (Al-Baldawi et al., 2013). Moreover, diesel is a harmful organic compound which may impact human health due its toxicity and carcinogenicity (Moreira et al., 2011) in addition to its highly negative effect on the ecosystem even in small concentrations as stated by Benmaamar and Bengueddach (2007). Furthermore, compared to other types of fuel such as kerosene and gasoline, diesel has the lowest volatilisation rate affecting the microbial communities in the soil, as reported by Truax, Britto, and Sherrard (1996).

Aqua Medic Titan chillers (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were used to maintain the root system and debris layer of all wetland systems at semi-natural below-surface temperatures of about 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located (Sani et al., 2013).

The chemical oxygen demand (COD) was used as the criterion to differentiate between low and high loads (Table 3.1). An inflow target COD of about 244.7 mg/l (usually between 122.0 and 360.0 mg/l) was set for wetlands with a high loading rate (Filters 5 and 6). The remaining Filters 1, 2, 3, 4, 7 and 8 received wastewater diluted with de-chlorinated tap water. The target inflow COD for these filters was approximately 123.3 mg/l (usually between 43.0 and 350.0 mg/l).

In addition to the irrigation waters obtained from the wetland systems (Table 3.1), some plants were irrigated with other water types for comparison. For example, deionised water (DW) and tap water (TW) were used to monitor the depletion of nutrients and trace elements supplied by the organic media. Tap water spiked with fertiliser (TW+F) was used to assess the impact of artificial fertiliser on growth. Furthermore, real and diluted wastewaters (WW and WW+TW) were used to study the impact of high nutrients and trace elements on plant growth and production (Figure 3.1).

However, deionised water was produced in the university laboratory via the ELGA PURELAB Option water purification system supplied by ELGA LabWater (Windsor Court, Kingsmead Business Park, High Wycombe, UK, www.elgalabwater.com), while tap water was collected from laboratory taps. Fruit and vegetable fertiliser was obtained from the B&Q plc verve range (Product code: 5397007068245). The fertiliser had a nitrogen to phosphorus to potassium ratio of 4:4:4 according to the EC fertiliser solution for the UK. The total nitrogen component was 4%. Nitric nitrogen and ureic nitrogen parts were 1.1 and 2.1%, respectively. Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) made up 4% each of the solution, but the corresponding P and K content were only 1.7 and 3.3%, respectively. Moreover, the fertiliser also contained trace elements (names not listed) of unspecified quantities. Liquid fertiliser was added to the inflow water as specified.

The plants were irrigated using the surface irrigation method as it’s the most popular way of irrigating with wastewater around the world in addition to its advantages such as low cost, being easy to understand and apply, as well as there being no problems associated with clogging the irrigation system unlike other methods as mentioned in chapter 2 (section 2.10 and 2.11). The plants were visually monitored to check the soil moisture content empirically on a daily basis. If the top soil was dry, sufficient irrigation water was carefully added (without splashing) to the pots using a Graduated Cylinder supplied by Fisher Scientific (Fisher Scientific UK Ltd, Loughborough, UK, www.fishersci.co.uk) until a few drops of water drained out of the pot into a saucer, which was located directly below the pot to capture drainage (Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 b). The volume of irrigation water required was recorded.

Table 3.1: Comparison of the experimental vertical-flow wetland set-ups.

Filters	Design variables					
	Aggregate diameter (mm)	Contact time (h)	Resting time (h)	Chemical oxygen demand (mg/l)	Annually treated volumes of wastewater (l/a)	Diesel spill*
Filter 1	20	72	48	123.3	470	Yes
Filter 2	20	72	48	123.3	470	No
Filter 3	10	72	48	123.3	470	Yes
Filter 4	10	72	48	123.3	470	No
Filter 5	10	72	48	244.7	470	Yes
Filter 6	10	72	48	244.7	470	No
Filter 7	10	36	48	123.3	624	No
Filter 8	10	36	24	123.3	858	No
Control A	10	72	48	2.3	470	Yes
Control B	10	72	48	2.3	470	No

Note: *On 26 September 2013, 130 g of diesel (equivalent to an inflow concentration of 20 g/l) were added.

Figure 3.2: (a) Overview of experimental set-up of wetland filters in the greenhouse on15 June 2014.

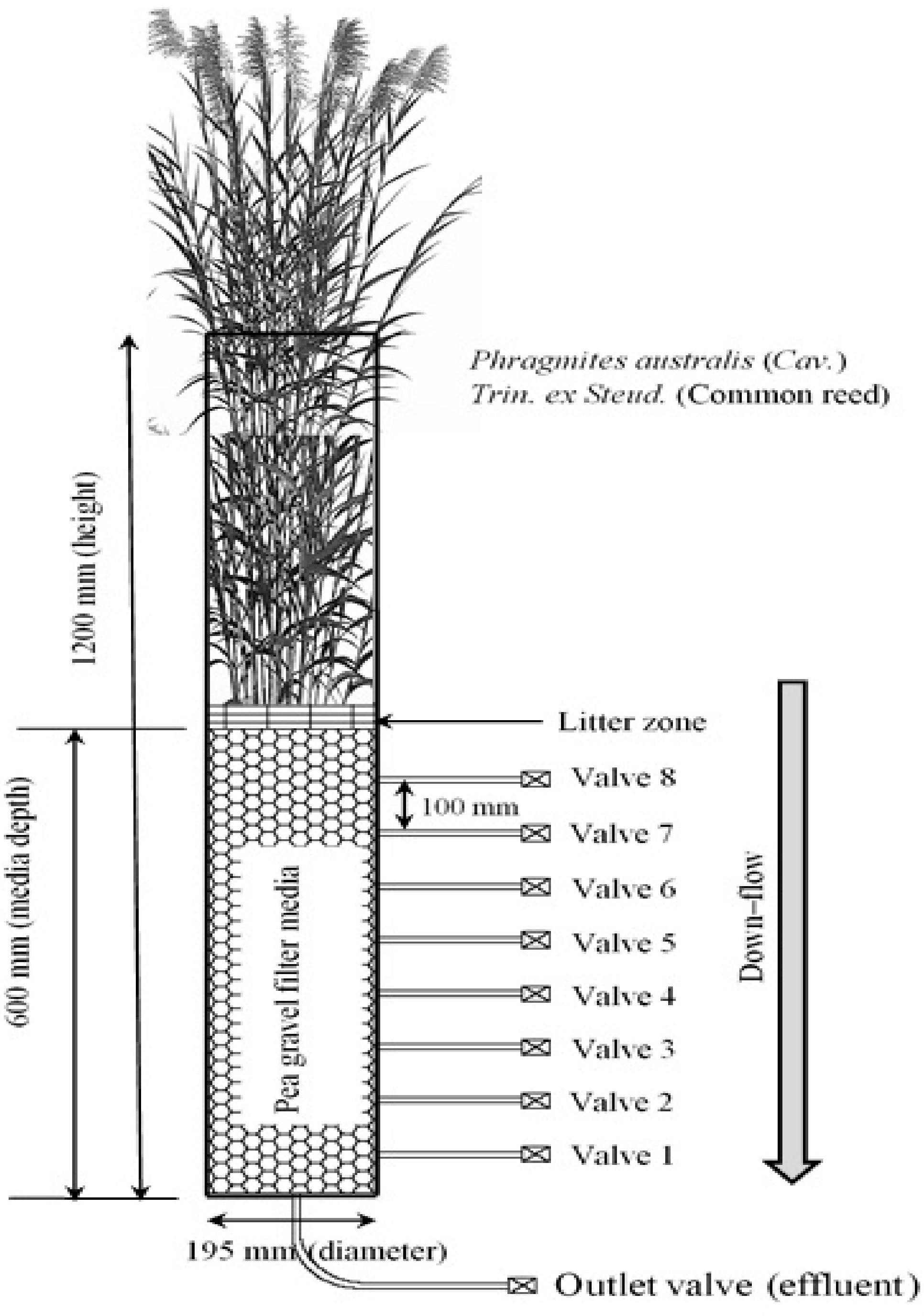

Figure 3.2: (b) Schematic diagram of a vertical-flow constructed wetland.

3.3 Irrigation water quality analysis

3.3.1 Water chemical and physical quality analysis

Routine water quality sampling was carried out according to the standard methods for examination of water and wastewater of the American Public Health Association (APHA, 2005). The spectrophotometer DR 2800 Hach Lange (www.hach.com) was used for standard water quality analysis for variables including chemical oxygen demand (COD), ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), ortho-phosphate-phosphorus (PO_4-P) and suspended solids (SS). Standards methods and laboratory procedures were used to test all of these parameters within 24 h of collection the samples.

Chemical oxygen demand (COD) of water samples was measured using the dichromate method by Lange Tubetest (product code: LCK 314). In this method, the sample is heated for 2 h with sulphuric acid and a strong oxidising agent, potassium dichromate. Oxidisable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. The absorbance of the chemical oxygen demand value for the tested water sample was read using a wavelength of 620 nm with the spectrophotometer DR 2800 Hach Lange (www.hach.com).

Automated precision colorimetry methods were used to measure nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N) and ortho-phosphate-phosphorus (PO_4-P) using Lange Tubetest (product codes: LCK 339, LCK 303 and LCK 049, respectively). The chemical 2,6-dimethylphenol was used to measure nitrate ions in the solutions which contain sulphuric and phosphoric acids which react with 2,6 dimethylphenol to form 4-

nitro-2,6-dimethylphenol, the measurement was read using a wavelength of 345 nm by the spectrophotometer DR 2800 Hach Lange (www.hach.com).

Moreover, the indophenol blue method was used to measure ammonia ions in water samples in which hypochlorite was added which combines with free ammonia to form more monochloramine. In the presence of a cyanoferrate catalyst, monochloramine in the sample reacts with a substituted phenol to form an intermediate monoamine compound. The intermediate couples with excess substituted phenol to form a green indophenol, which is proportional to the amount of monochloramine present in the sample. Free ammonia was determined by comparing the colour intensities, with and without added hypochlorite. The measurement was read using a wavelength of 655 nm or 610 nm for colorimeters by the spectrophotometer DR 2800 Hach Lange (www.hach.com). Furthermore, the vanadate-molybdate method was used to measure ortho-phosphate ions in water samples which reacts with vanadate-molybdate reagent to form a yellow dye then the measurement was read using a wavelength of 435 nm by the spectrophotometer DR 2800 Hach Lange (www.hach.com).

The five-day BOD in this study was determined in all water samples using the incubation method with the OxiTop IS 12-6 system, a manometric measurement device, supplied by the Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany. The five-day BOD measurement is based on the principles of pressure differences determination by electronic pressure sensors (piezo-resistive). Nitrification was suppressed by adding 0.05 ml of 5 g/l N-Allylthiourea (WTW chemical solution No. NTH600) solution per 50 ml of sample water.

Turbidity (NTU) was measured with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, Dortmund, Germany; www.lovibond.com).

Total petroleum hydrocarbons were determined by gas chromatography and flame ionisation externally by Exova Health Sciences (70 Montrose Ave, Hillington Park, Glasgow G52 4LA) according to their own TPH in Waters (with Aliphatic/Aromatic Splitting) Method (Exova, 2014), which is accredited to the British Standard (BS) method BS EN ISO IEC 17025 by the United Kingdom Accreditation Service and compatible with the International Organization for Standardization (ISO) standards (e.g., ISO17025), BS method BS DD 220 1994, and American Standard methods (United States Environmental Protection Agency (USEPA) Method 3510C and US EPA SW846 Method 8015).

The analysis of water samples for heavy metals and trace elements was performed using a Varian 720-ES Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK Ltd, Wharfedale Road, Wokingham, Berkshire, UK). According to EPA (1994), water samples of 50 ml were preserved in glassware bottles at 4 °C. The samples were then acidified, if appropriate, by adding 1 ml of 70% concentrated nitric acid (HNO_3) to dissolve any suspended material in order to extract heavy metals and to reduce the pH to below 2.0, which was required for analysis. The samples were then filtered through a filter paper with a diameter of 0.45 µm before analyses by the ICP–OES technique.

The pH and redox potential were measured with a sensION+Benchtop Multi-Parameter Meter (Hach Lange, Düsseldorf, Germany). The electrical conductivity (EC) was determined using the conductivity meter METTLER TOLEDO Five Go™ (Keison Products, Chelmsford, Essex, UK). The dissolved oxygen was measured with a HQ30d flexi meter (Hach Lange, Düsseldorf, Germany). These meters were known to be handy, robust, and easily used as well as low cost waterproof instruments used to monitor the most important parameters of wastewater.

Moreover, these meters were provided with sensors and required solution for meter maintenance and measurement calibration. Turbidity, pH, redox potential, electrical conductivity and dissolved oxygen were measured for water samples directly by placing samples in the corresponding instrument. In addition, each equipment calibration was undertaken when necessary according to the instructions provided by the equipment user manual. For instance, the spectrophotometer DR 2800 Hach Lange was calibrated before measuring suspended solids (SS) for water samples according to instructions described in the Hach Lange user manual (www.hach.com). Moreover, turbidity and pH meters were calibrated according to their user manual books (www.lovibond.com and www.hach.com, respectively) when the indication for calibration appeared in the meter digital screens. However, more details on measuring irrigation water qualities are available in Appendix A.

3.3.2 Water microbial quality analysis

Total coliforms, *Escherichia coli*, *Streptococcus* spp. and *Salmonella* spp. were estimated by using aseptic pour plate techniques according to standard methods (APHA, 1998). The agars used for growing bacteria colonies are characterised below.

Chromocult Coliform Agar (Central of Merck Group, Darmstadt, Germany) was used for estimating coliforms and *Escherichia coli* (Manafi & Kneifel, 1989; Ossmer, Schmidt, & Mende, 1999). Following the manufacturer's instructions, the agar was prepared by dispersing 26.5 g of powder in 1 litre of deionised water. The mixture was allowed to soak for 10 min, swirled to mix and then sterilised during boiling for 30 min. The agar was subsequently cooled to between 45 and 50 °C, and subsequently thoroughly mixed. About 12.5 ml of the prepared agar was dispensed into Petri dishes with a diameter of 90 mm and a height of 16.2 mm (Fisher Scientific UK Ltd, Loughborough, UK).

Chromocult Coliform Agar is composed of peptone (3 g/l), sodium chloride (5 g/l), sodium hydrogen phosphate (2.7 g/l), sodium dihydrogen phosphate (2.2 g/l), sodium pyruvate (1 g/l), tryptophane (1 g/l), tergitol-7 (0.15 g/l), sorbitol (1 g/l), chromogenic mixture (0.4 g/l) and agar-agar (10 g/l). The pH of the agar was 6.8±0.1. According to the manufacturer's instructions, typical *Escherichia coli* colonies may appear as dark blue to violet convex colonies, which could be entirely glossy with a size between 0.1 and 2.0 mm. Coliform colonies are coloured rose-pink. The convex may be entirely glossy with a size of between 1.5 and 2.5 mm in the agar plates after the incubation period.

Kanamycin Aesculin Azide Agar (LABM limited, Lancashire, UK) is a selective isolation and enumeration medium for *Streptococcus* spp. Sodium azide and kanamycin provide the selective inhibition required, whilst iron salts and aesculin form an indicator system for the presumptive identification of *Enterococci* (Mossel, Bijken, Eelderink, & Van Spreekens, 1978). This agar was prepared by dispersing 43 g of powder in 1 litre of deionised water, allowing it to soak for 10 min, mixing it and subsequently sterilising it by autoclaving at 121 °C for 15 min. The agar was then allowed to cool to 47 °C before dispensing it into Petri dishes, following the manufacturer's instructions. Kanamycin Aesculin Azide Agar is composed of tryptone (20 g/l), yeast extract (5 g/l), sodium chloride (5 g/l), sodium citrate (1 g/l), aesculin (1 g/l), ferric ammonium citrate (0.5 g/l), sodium azide (0.15 g/l) and kanamycin sulphate (0.02 g/l). According to manufacturer's instructions, white and/or grey colonies with a diameter of approximately 2 mm surrounded by a black halo can be expected in the agar plates after the incubation period.

Harlequin™ *Salmonella* ABC (LABM limited, Heywood, Lancashire, UK) medium has been developed for the isolation of *Salmonella* spp. The agar utilises a dual chromogenic system to visualise enzymatic activities. Sodium desoxycholate and sodium citrate function as inhibitors. The ABC medium reduces the requirement for "false positive" screening, which saves labour and reduces consumable costs (Perry et al., 1999). The agar was prepared by dispersing 36.5 g of powder in 1 litre of deionised water. The mixture was allowed to soak for 10 min, and was subsequently mixed and then sterilised by boiling. The medium was allowed to cool to 47 °C, mixed well and dispensed into Petri dishes, following the manufacturer's instructions. Harlequin™ *Salmonella* ABC is composed of beef extract (5 g/l), peptone (5 g/l), sodium citrate (8.5 g/l), sodium desoxycholate (5 g/l), agar (12 g/l), ferric ammonium citrate (0.5 g/l), X-a-Gal (0.08 g/l), CHE-ß-Gal (0.3 g/l), isopropyl β-D-1-thiogalactopyranoside (IPTG; 0.03 g/l). The pH was 7±0.2. According to manufacturer's instructions, black colonies with a diameter of 1 to 2 mm diameter are formed on the agar plates after the incubation period, if *Salmonellae* are present.

Buffered Peptone Water (LABM limited, Heywood, Lancashire, UK) was applied as a pre-enrichment medium designed to support sub-lethally damaged *Salmonellae* to recovery before introducing the bacteria into a selective medium (Poemla & Silliker, 1976). This nutrient medium is free from inhibitors and is well buffered to maintain the pH at 7.2 during the incubation period. Following manufacturer's guidelines, this medium was prepared by weighing 20 g of powder and dispersing it in 1 litre of deionised water. The solution was subsequently distributed into tubes and bottles, and sterilised by autoclaving at 121 °C for 15 min. Buffered Peptone Water comprises peptone (10 g/l), sodium chloride (5 g/l), disodium hydrogen phosphate (3.7 g/l) and potassium dihydrogen phosphate (1.5 g/l). The pH is 7±0.2.

The initial water samples were prepared as a 1:10 dilution using buffered peptone water. Subsequently, ten-fold dilutions were conducted with the same medium (APHA, 1998). The prepared agar media were poured into Petri dishes with a diameter of 90 mm and a depth of 16.2 mm (Fisher Scientific UK Ltd, Loughborough, UK, www.fishersci.co.uk). Each dish contained about 20 ml of agar. According to standard methods, 100 µl from each dilution was plated into a Petri dish in duplicate by gently swirling clockwise and anti-clockwise on the surface of the media using a sterilised spreader (Fisher Scientific UK Ltd, Loughborough, UK, www.fishersci.co.uk). The prepared dishes were incubated at 37° C for 24 h. Following the incubation period, bacteria colonies were counted on dishes containing between 30 and 300 colonies.

3.4 Monitoring of laboratory environment conditions

In this laboratory experimental work, simulation of natural climate conditions was performed in terms of the most important factors affecting vegetable growth, such as light intensity (simulating sun light), temperature and relative humidity of the laboratory environment. Sun light was simulated using OSRAM HQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights provided by OSRAM (North Industrial Road, Foshan, Guangdong, China) and supported by a H4000 Gear Unit, which was supplied by Philips (London Road, Croydon CR9 3QR). The bulbs were comparable to those used by Boyden and Rababah (1996). The lights were set on timers, simulating the sunrise and sunset times in Salford (http://www.timeanddate.com). Light measurement readings were undertaken using the LUX meter ATP-DT-1300 for the range between 200 LUX and 50,000 LUX (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, Cheshire, UK). Values for full day light under optimal conditions are often between approximately 10,000 and 20,000 lux.

The humidity and temperature were controlled with the support of a combined Thermometer-Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The humidity measuring range was between 20 and 99%. The corresponding precision was ±4% between 35 and 75%. The temperature was controlled by the electrical heater Rhino H029400 TQ3 2.8kW Thermo Quartz Infrared Heater 230V supplied by Express Tools Ltd (Alton Road, Bournemouth, UK). The humidity was artificially elevated by a varying number of humidity meters (Challenge 3.0L Ultrasonic Humidifier; Argos, Avebury Boulevard, Central Milton Keynes, England, UK) to create more realistic growing conditions.

3.5 Selection of plants

As mentioned in chapter 2 section 2.13, many vegetables have the potential to grow well on recycled wastewater. However, according to the basic points considered when selecting plants to be irrigated with wastewater, Chilli and Sweet Peppers were used as plant examples for this experimental work. This is because their edible fruit are located far away from the ground, reducing the risk of becoming contaminated with microbes accumulated on the surface of the irrigated soil. Moreover, they are relatively cost-effective plants (see Table 2.15) with high nutritional value (see Table 2.16). Chilli and Sweet Peppers are classified as easy to grow vegetables as they require a relatively short period for maturation (see Table 2.17) and are able to be grown in environmental conditions which can be simulated in either greenhouse (Jones, 2013; Nickels, 2012) or laboratory conditions, mainly in terms of climate temperature and relative humidity, as well as their ability to grow in all soil types (see also Table 2.17). Both vegetable types were selected from a large supplier as outlined below:

Sweet Pepper (California Wonder) and Chilli (De Cayenne) were supplied by B&Q plc (Chandlers Ford, Hants SO53 3LE; www.diy.com) as part of their verve brand. The verve product codes were 311137 and 362387, respectively. All seeds were bought on 14 September 2013, and should normally be sown in the UK before June 2014 (i.e. sow by date) according to the supplier. Sowing months for Sweet Pepper and Chilli are usually March and April. Planting out dates for Sweet Pepper are from 15 May to 15 June in the UK according to the supplier. In comparison, Chilli should be planted out between May and June. Based on supplier information, Sweet Pepper and Chilli can be harvested between August and October in the UK.

Sweet Pepper (California Wonder; cultivar of Capsicum annuum Linnaeus Grossum Group) is described by the supplier, B&Q plc, as a high cropping large fruit growing from green to red that can be picked at either stage. The vegetable is usually used for salads and cooking. Sweet Pepper is also known as Bell Pepper or Traffic Light Pepper, and is often sold in packs of three with different colours (red, yellow and green; decrease in sweetness in that order). Sweet Peppers prefer to grow in warm soil (21 to 29 °C), which should be kept moist but not waterlogged (Nickels, 2012).

According to Chemicals (2014), the optimal temperature for Sweet Pepper during the germination stage is between 20 and 25 °C, and for the vegetative growth stage, the corresponding range is between 20 and 25 °C through the day, and between 16 and 18 °C through the night. Furthermore, for the flowering and fruiting stage, the recommended temperature should vary between 26 and 28 °C and between 18 and 20 °C during the day and night, respectively. Sweet Peppers are sensitive to an abundance of moisture and excessive temperatures. They are known to be rich in vitamin C (Nickels, 2012) and sensitive to high levels of salinity, requiring salinity conditions below 1280 mg/l (FAO, 2003).

Furthermore, the electronic conductivity for irrigation water should be less than 2000 µs/cm (Chemicals, 2014) and sodium adsorption ratio, SAR, should be between 0 me/l and 15 me/l (FAO, 1994).

Chilli (De Cayenne; Capsicum annuum (Linnaeus) Longum Group "De Cayenne") is described by B&Q as a good crop of slender and hot fruits ideal for growing in pots on the patio, balcony or in a greenhouse. It is also described as a perfect Chilli for general cooking. This type of easy-to-grow Chilli is also known as Guinea spice, cow-horn pepper, aleva, bird pepper and red pepper. This type of pepper needs approximately 100 days to mature.

Chillies prefer warm, moist and nutrient-rich soil in a warm climate. The germination time is 5 to 14 days. The plants grow to about 45 cm in height and should be planted about 40 cm apart from each other. The sowing to cropping time is approximately 18 weeks. Chillies are mostly perennial (often more than three years) in sub-tropical and tropical regions (Nickels, 2012). However, they are usually grown as annuals in temperate climates such as the UK.

Peppers prefer to grow in light and well-drained soil that should be rich in organic matter such as sandy loam or loams with a pH value between 6.5 and 7.5 (Chemicals, 2014) while Chillies prefer a loamy soil with a pH of between 7.0 and 8.5 (i.e. neutral to weakly alkaline soil) (Nickels, 2012).

3.6 Growing of plants

According to the supplier instructions, Sweet Peppers and Chillies were grown through three main stages (Table 3.2) as shown below:

- First planting or germination stage,
- Second planting or first replanting stage, and
- Third (final) planting or second replanting stage.

In the first planting stage, the seeds were sown thinly in a propagator (verve; B&Q plc) into seed and cutting compost (verve; B&Q plc: product code 03717644) and covered with a 6 mm compost layer for about one week. Each propagator contained 72 planting cells with an average depth of 5 cm (only planted up to about 4 cm; measured before initial watering) and square sides of approximately 3.5 cm.

According to the supplier, the compost comprised 58% sustainably sourced (in terms of ecology, archaeology and conservation) *Sphagnum* moss peat and unspecified amounts of composted bark, green compost, wood fibre and coir (natural fibre extracted from the husk (outer shell) of coconuts), and oyster shells (optional), vermiculture (optional), perlite (optional), loam (optional), charcoal (optional), alcosorb (optional), sand (optional), grit (optional), wetting agent (to retain moisture better; between 200 and 400 ml/m^3) and essential nutrients and trace minerals lasting for approximately six weeks.

The remaining 42% comprised among other components more than 48% non-peat composted organic material such as a mixture of composted green waste and spent brewery grains. The fertiliser content was between 0 and 3 kg/m^3. The dolomitic limestone content was between 0 and 7 kg/m^3. However, the exact combination of ingredients is commercially sensitive, and was therefore not communicated by the supplier.

The propagators were placed within a dark incubation room and the transparent covers of the propagators were kept on top of the propagator bases. The temperature was maintained between 19.5 and 22.5 °C (mean of 20.8 °C).

The recommended temperature range according to the supplier is between 18 and 21 °C. The compost was kept moist until the seeds germinated. After germination of some seeds all seeds were relocated to a lab characterised in section 3.4. The transparent covers of the propagators were kept on top of the propagator bases (gap of approximately 6.0 cm) until the first seedlings reached them.

In the second planting stage, the strongest plants took place when most seedlings had at least two true leaves and were large enough to handle. Seedlings were transplanted into 60-mm diameter nursery pots of moist multipurpose compost and grown on for three to four weeks. Each tray contained 40 pots of depth 60 mm each. Multipurpose compost was filled up to a depth of 4.5 cm and covered with a 1.0 cm layer of bark (B&Q verve range: product code 5397007188110). The topping contained small chipped bark from mixed wood (responsibly sourced), which was described by B&Q as ideal for pots, beds and borders to control weeds, retain moisture and insulate soil.

Some vegetables were planted in pure sand to assess the impact of the organic growth substrate on plant growth. The product Play Pit Sand (silica: product code 5060096123309), which is described by the supplier Deko-Pak Limited (Deco House, Halifax Road, Hipperholme, Brighouse HX3 8BW) as non-staining, non-toxic, safe and clean, was used. Sand was filled up to a depth of 5.5 cm. All vegetables were kept indoors in the same heated laboratory fixed with grow lights.

The third planting (final) took place 28 days after the second planting. In this stage, the strongest plants were planted individually into 10 litre plastic and round plant pots provided by Scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK). The plant pot dimensions were as follows: height of 22.0 cm, bottom diameter of 22.0 cm and top diameter of 28.5 cm. The top 2 cm were left unplanted.

Sand-based plants were planted to a depth of 20.0 cm. In comparison, soil-based plants were planted to a depth of 17.5 cm. and covered by a further 2.5 cm of bark. Sufficient space between plants was always allowed for the plants which remained indoors in the laboratory characterised above. All plants were initially supported by small bamboo canes (diameter of approximately 0.3 cm; length of up to 30 cm) and later on by bigger bamboo canes (diameter average of 0.8 cm; range between 0.6 and 1.2 cm; length of up to 150 cm) if and when necessary.

Furthermore, plants were supported using a string, which was loosely tied to the main stem against the cane when required. Domestic cultivars were selected to maximise self-fertility. This is not the same as self-pollination. In an outside setting, wind or insects provide sufficient motion to produce commercially viable crops (Jones, 2013). Therefore, mechanical movement of the plants and manual pollen transfer between plants was practised in this study.

Cross-pollination between Sweet Peppers and Chillies was prevented by separating the growing space with the help of temporary walls. Figure 3.3 shows an overview of vegetable growth stages and plant development. Table 3.3 shows the overview and outlines of the experimental set-up for both Sweet Peppers and Chillies (experiment No.s 1, 2 and 3, see Table 3.2).

In this study, the experimental set-up was performed according to complete randomised design principles (Almuktar et al, 2015 a, b); for example, in experiment No.s 1 and 2, plants irrigated with Filters 1 and 2 outflow waters are different by only one variable (presence or absence of diesel spill; Table 3.1). Three out of ninety plants were randomly chosen for each of the two treatments.

Regarding chilli new generation plants (experiment No. 3, Table 3.2), this experiment was undertaken in order to obtain a new cultivar adapted to urban wastewater.

The plants were grown in the compost using seeds randomly chosen from the original chilli plants in experiment No.2 (Table 3.2), which were irrigated with wetlands system outflow water linked to standard filters (not contaminated with hydrocarbons) as indicated in Table 3.2 and Table 3.3. This is because of the best results in terms of growth rate and yield quality obtained from plants associated with those filters (Almuktar et al., 2015 a, b).

Table 3.2: Plant growing details.

Experiment No.	Plant	Planting periods			Soils detail		Seed source	Irrigation water types
		First	Second	Third	Status	Type		
1	Sweet Pepper	16/9/2013 to 10/10/2013 (90 seeds)	11/10/2013 to 7/11/2013 (76 seedlings)	8/11/2013 to 25/9/2014 (50 plants)	New	Organic and inorganic	B&Q plc	Filters 1 to 8 Controls A and B DW, TW, TW+F, TW+WW and WW
2	Chilli	16/9/2013 to 10/10/2013 (90 seeds)	11/10/2013 to 7/11/2013 (70 seedlings)	8/11/2013 to 25/9/2014 (50 plants)	New	Organic and inorganic	B&Q plc	Filters 1 to 8 Controls A and B DW, TW, TW+F, TW+WW and WW
3	Chilli new generation	23/9/2014 to 9/11/2014 (360 seeds)	10/11/2014 to 18/12/2014 (150 seedlings)	19/12/2014 to 25/9/2015 (50 plants)	New	Organic	Experiment No. 2	Filters 2, 4, 6, 7 and 8

Note:
Some plants either did not germinate or died before the second planting, seedlings were assessed visually to choose the strongest ones for the third planting. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%) and WW, raw wastewater (100%).

First planting stage

Second planting stage

Third planting stage

Sweet Pepper plants (experiment No. 1)

Chilli plants (experiment No. 2)

Chilli new generation plants (experiment No. 3)

Figure 3.3: Vegetables growth stages and set-up in the laboratory.

Table 3.3: Experiment design set-up in terms of plant number allocations after the final planting stage.

Inflow source	Growth media	Sweet Pepper [b]	Chilli (original) [b]	Chilli (generation) [c]
Filter 1 outflow [a]	Compost with bark	P1; P2	C3; C4	-
Filter 2 outflow	Compost with bark	P5; P6	C6; C8: C9	C6; C7; C9; C10; C11; C12; C13; C15; C27; C28
Filter 3 outflow [a]	Compost with bark	P8; P9; P10	C10; C11; C12	-
Filter 4 outflow	Compost with bark	P12; P16	C16:C17	C3; C7; C8; C9; C10; C11; C12; C14; C21; C22
Filter 5 outflow [a]	Compost with bark	P18; P19; P20	C18; C19; C20	-
Filter 6 outflow	Compost with bark	P22; P23	C21	C2; C7; C8; C10; C11; C14; C16; C17; C18; C23
Filter 7 outflow	Compost with bark	P26; P28	C25:C26	C9; C11; C12, C13; C14; C15; C16; C20; C21; C22
Filter 8 outflow	Compost with bark	P31; P32; P33	C27; C28; C29	C2; C3; C6; C7; C11; C12; C13; C17; C26; C27
Control A outflow [a]	Compost with bark	P35	C31; C33	-
Control B outflow	Compost with bark	P39	C37;C38	-
Deionised water	Compost with bark	P41	C41	-
Tap water (100%)	Compost with bark	P44	C42; C43	-
Tap water with fertiliser (0.7 ml/l)	Compost with bark	P45; P46	C45; C46	-
Wastewater (20%); tap water (80%)	Compost with bark	P47	C49	-
Wastewater (100%)	Compost with bark	P51; P54	C52; C54	-
Filter 1 outflow	Silica sand	P55; P56	C56	-
Filter 2 outflow	Silica sand	P57	C58	-
Filter 3 outflow	Silica sand	P59	C61	-
Filter 4 outflow	Silica sand	P61	C63; C64	-
Filter 5 outflow	Silica sand	P65	C66	-
Filter 6 outflow	Silica sand	P66; P67	C68	-
Filter 7 outflow	Silica sand	P17; P69	C71	-
Filter 8 outflow	Silica sand	P70; P71	C72; C73	-
Control A outflow	Silica sand	P73	C74	-
Control B outflow	Silica sand	P74	C76; C77	-
Deionised water	Silica sand	P80	C80	-
Tap water (100%)	Silica sand	P81	C82	-
Tap water with fertiliser (0.7 ml/l)	Silica sand	P83; P84	C84; C85	-
Wastewater (20%); tap water (80%)	Silica sand	P86; P87	C87	-
Wastewater (100%)	Silica sand	P89; P90	C90	-

Note: [a] Filters contaminated with hydrocarbons.

[b] Original seed planting reference numbers during the first planting stage; Sweet Pepper (P1–P90) and Chilli (C1–C90). Three plants were allocated at random to each treatment. Note that 40 plants did not survive the first and second planting stage.

[c] Original seedling reference numbers during the second planting stage (C1-C30) per each treatment

3.7 Soil quality analysis

3.7.1 Soil chemical quality analysis

At the end of the experiment, soil samples were randomly taken from treatment pots for analysing heavy metal and trace elements content using the Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES) technique. According to Chary et al. (2008) and Kapungwe (2013), soil samples were taken from the surface, until a depth of 20 cm was reached, by a soil auger. In the laboratory, the soil samples were spread on glass plates and then dried in a GallenKamp Hotbox Oven Size 1 (Wargrave Road, Twyford, Berkshire, UK) at 105 °C for six hours (Chiroma, Ebewele, & Hymore, 2014). The dried soil samples were sieved to obtain a fraction of less than 2 mm (Chary et al., 2008; Kapungwe, 2013). A dried weight of > 0.2 g was digested in 10 ml of 70% concentrated HNO_3 by using microwave Teflon tubes (UOW, 2005). A Mars 5 microwave (Buckingham Industrial Estate, Buckingham, UK) digester was used. After digestion, the samples were diluted with deionised water up to 25 ml (Kapungwe, 2013) in a volumetric flask and transferred into 15-ml polystyrene tubes to be analysed by the ICP–OES technique using a Varian 720-ES Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK Ltd, Wharfedale Road, Wokingham, Berkshire, UK).

Soil phosphorus content can be measured using the Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES) technique. However, the Salford university ICP–OES machine (Varian 720-ES) did not provide this option. Because of this, phosphorus content in the soil samples was measured in the ALS Environmental laboratory (Torrington Avenue, Coventry, www.alsenvironmental.co.uk) using the inductively coupled plasma optical emission spectroscopy technique (Aqua Regia Extractable Metal).

Phosphorus was extracted from air-dried soil samples (ground to pass through a 2-mm sieve) by microwave extraction using a 3:1 mixture of concentrated hydrochloric and nitric acids, by volume, (Aqua Regia). The concentrations of the phosphorus were determined by the technique of inductively coupled plasma optical emission spectroscopy. An ICP source consists of a flowing stream of argon gas ionised by an applied radio frequency field. This field is inductively coupled to ionised gas by a water-cooled coil surrounding a quartz “torch” that supports and confines the plasma. A sample aerosol is generated in a nebuliser and spray chamber and is carried into the plasma by an injector tube. The sample aerosol is injected directly into the ICP subjecting the constituent atoms to temperatures of between 6000 and 8000 K.

In addition to phosphorus, Total Kjeldahl Nitrogen was measured in the ALS Environmental laboratory as well. In this method, the digestion converts free ammonia and organic nitrogen compounds to ammonia sulphate, which can then be read using an ammonia-selective electrode.

The soil pH and redox potential were measured by soaking 5.0 g of soil in 2.5 ml of distilled water and mixing it well until dissolved. The solution was left for 16 hours; then the pH and redox potential were determined by using pH and redox meters (sensION+Benchtop Multi-Parameter Meter, Hach Lange, Düsseldorf, Germany) after calibration (Al-Jaboobi et al., 2014). The electrical conductivity of soil was measured by adding distilled water to 20 g of soil, stirring it well until saturation was reached. The solution was left for 16 h; it was then centrifuged at 1500 rpm for 5 min using a MSE MISTRAL 1000 centrifuge (Fisher Scientific UK Ltd, Loughborough, UK, www.fishersci.co.uk). The electrical conductivity for the supernatant was measured using an electrical conductivity meter (METTLER TOLEDO Five Go™, Keison Products, Chelmsford, Essex, UK) at 25 °C (Al-Jaboobi et al., 2014).

3.7.2 Soil microbial quality analysis

Undisturbed soil samples were collected with sterile equipment and consumables for subsequent bacterial testing (Lopez et al., 2006). Samples were collected from the top 10 cm of soil, and 1 g of soil sample was added to 9 ml of buffered peptone water for subsequent bacteria extraction in a sterile blender jar. The mixture was then blended for 2 min at low speed (8000 rpm) according to APHA (1998). The appropriate decimal dilutions of the homogenised slurry were prepared quickly to minimise settling. The solution was subsequently poured into the prepared agar located within the Petri dishes. Counting of the developed colonies was undertaken according to APHA (1998).

3.8 Fruit quality and analysis

3.8.1 Fruit classification and brief cost-benefit analysis

Harvested red Chilli fruits from each plant were categorised according to the novel harvest classification scheme for Chillies shown in Table 3.4, which has been adapted from Almuktar et al. (2015 a, b). The variables length, width, weight and bending were used for classifying the harvested Chilli fruits. In comparison, Table 3.5 shows the novel harvest classification scheme for Sweet Peppers which has been adapted from Almuktar and Scholz (2016 b). For the purpose of this study, only the following numerical and objective variables were used to classify Sweet Pepper fruits: length, diameter and weight (more details are available in Appendix A). Only the higher classes are of great commercial interest.

However, the lowest variable class entry for any individual fruit assessment determined the final class (Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 b). For example, if a fruit is categorised as class A in terms of length, class B in terms of diameter (or width) and E in terms of weight, then the final class for this fruit is class E, and accordingly the corresponding price for this fruit will be zero pence. Moreover, the monetary value of the harvest was only calculated for the example vegetables according to estimated prices in the UK market in 2014.

Furthermore, Sweet Pepper and Chilli seed packets were purchased from B&Q plc for £1.48 or 148 pence each. The corresponding seed numbers were 45 and 70, respectively. One seed of Sweet Pepper or Chilli costs therefore 3.29 and 2.11 pence, respectively. Considering the germination success seedlings of 78 and 74 for Sweet Pepper and Chilli, respectively, each seedling costs 257 and 156 pence in that order. However, only 50 seedlings of Sweet Pepper and Chilli each reached maturity. This corresponds to 165 and 106 pence, respectively. Sweet Pepper and Chilli can be purchased in the UK for approximately 56 and 16 pence each or 362 and 1040 pence per kilogram. However, reality is more complex. Taking into account the costs of watering, fertiliser and maintenance, the calculation becomes more complex.

Moreover, the potential fear and disgust from consumers of eating microbially contaminated vegetables decreases considerably if vegetables are cooked for a long time at considerable heat. Menegaki et al. (2009) assessed the fear and disgust factors by comparing the effects of descriptive terms on farmers' willingness to use and willingness to pay for recycled water for irrigation and consumers' willingness to use and willingness to pay for products irrigated with recycled water. Treated effluent from wastewater treatment plants was described as "recycled water" for one experimental group and as "treated wastewater" for another. Although the two terms describe the same commodity, willingness to use the water was reliably higher with the "recycled water" descriptor for both farmers and consumers.

However, the descriptor affected willingness to pay only in the consumer sample. Both farmers and consumers who were unwilling to use recycled water commodities cited feelings of disgust (32%) as the main cause of their rejection (Menegaki et al., 2009).

Sweet Peppers are often eaten both raw and cooked. Chillies are usually cooked, and the risk of microbial contamination is therefore very low. Considering that Sweet Peppers in comparison to Chillies are more likely to be used in a salad than in a cooked dish, it is more difficult to sell these when recycled water has been used, because of the fear and disgust factors discussed above. Therefore, the likelihood of selling the selected plants at a fair price taking the fear of contamination factor into account is likely to be less for Sweet Peppers compared to Chillies.

Table 3.4: Chilli (C) harvest classification scheme (adapted from Almuktar et al. (2015a, b)).

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
Approximate Codex Standard (2013) mapping	"Extra" Class	Class I	Class II	Not applicable	Not applicable
Mean price estimate; pence (Sterling)/gram	C: 2.00	C: 1.00	C: 0.50	C: 0.25	C: 0.00
Target market	Top restaurant	National supermarket	Independent retailer or market	Vegetable industry	Waste company
Product	Fresh vegetable	Fresh vegetable	Fresh vegetable	Powder or canned	Waste
Contamination	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Contaminated
Illnesses	None	None	None	Likely; no harm	Likely; harmful (rotten)
Aesthetics	Fully characteristic; virtually no flaws (≤0.5% of surface area)	Fully characteristic; minor flaws (≤2.0% of surface area)	Essential characteristics only; flaws (≤3.0% of surface area)	Major flaws (>3.0% of surface area); potentially broken, pests and damaged	Too many major flaws including broken, pests and damaged
Length (L, mm)	Very long (L≥80)	Long (60≤ L<80)	Medium (40≤ L<60)	Short (20≤L<40)	Very short (L<20)
Width (W, mm)	Very wide (W≥20)	Wide (16≤W<20)	Medium (12≤W<16)	Slim (8≤W<12)	Very slim (W<8)
Weight (w, g)	Very Large (w≥9)	Large (7≤w<9)	Medium (5≤w≤7)	Small (3≤w<5)	Very Small (w<3)
Tolerance by weight per plant (%)	5	10	10	10	10
Bending	Character-istically bent; L/W≥3.5	Character-istically bent; L/W≥3.5	Character-istically bent; L/W≥3.5	Uncharacteristically bent; L/W<3.5	Uncharacteristically bent; L/W<3.5
Colour	Characteristically red	Characteristically red	Characteristically red	Not fully red or unripe	Not fully red or unripe
Pungency (flavour) in Scoville (SHU) units	Strongly characteristic; SHU≥18,000	Characteristic; 8,000≤SHU< 18,000	Characteristic; 8,000≤SHU<18,000	Characteristic; 8,000≤SHU<18,000	Poor; SHU<8,000
Pungency (flavour) as total capsaicinoids (C; µg/g dry weight)	Strongly characteristic; C≥1,200	Characteristic; 533≤C<1,200	Characteristic; 533≤C<1,200	Characteristic; 533≤C<1,200	Poor; C<533

Table 3.5: Sweet Pepper (P) harvest classification scheme (adapted from Almuktar and Scholz (2016 b)).

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
European Union classification equivalent	"Extra" Class	Class I	Class II	Not applicable	Not applicable
Mean price estimate; pence (Sterling)/gram	P: 0.28	P: 0.22	P: 0.16	P: 0.10	P: 0.00
Target market	Top restaurant	National supermarket	Independent retailer or market	Vegetable industry	Waste company
Product	Fresh vegetable	Fresh vegetable	Fresh vegetable	Frozen or canned	Waste
Contamination	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Contaminated
Illnesses	None	None	None	Likely; no harm	Likely; harmful (rotten)
Length (L, mm)	Jumbo ($L \geq 110$)	Extra-large ($90 \leq L < 110$)	Large ($70 \leq L < 90$)	Medium ($40 \leq L < 70$)	Small ($L < 40$)
Diameter (D, mm)	Jumbo ($D \geq 90$)	Extra-large ($70 \leq D < 90$)	Large ($50 \leq D < 70$)	Medium ($30 \leq D < 50$)	Small ($D < 30$)
Weight (w, g)	Very Large ($w \geq 190$)	Large ($120 \leq w < 190$)	Medium ($70 \leq w \leq 120$)	Small ($20 \leq w < 70$)	Very Small ($w < 20$)
Tolerance by weight or number per plant (%)	5	10	10	10	10
Defect in shape (Damage (%) of surface area)	Damage≤10	10≤Damage<20	20≤Damage<30	30≤Damage<60	Too much damage (>60)
Defect of the skin (Damage (%) of surface area)	Damage≤3	3≤Damage<4	4≤Damage<5	5≤Damage<20	Too much damage (>20)

3.8.2 Fruit mineral content

After finishing the harvest, fruits were randomly selected from each treatment and analysed for heavy metal and trace elements content using the Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES) technique. The analysis was undertaken according to (Plank, 1992). The fruit samples were placed within an envelope to be subsequently dried in a GallenKamp Hotbox Oven Size 1 (Wargrave Road, Twyford, Berkshire, UK) at 80 °C for 24 h. A dried weight of > 0.3 g was required for the digestion procedure (Bressy, Brito, Barbosa, Teixeira, & Korn, 2013).

The drying process removed water from the plant tissue to stop enzymatic reactions and to stabilise the sample (Plank, 1992). After the drying process, the samples were ground to a fine powder in a James Martin ZX809X Spice and Coffee Grinder (WAHL Global, Herne Bay Trade Park, Sea Street, Herne Bay, Kent, UK) to facilitate organic matter destruction. Samples of 0.3 g each were placed in crucibles to be subsequently turned into complete white ash in a Carbolite muffle furnace for complete decomposition of organic matter at 550 °C for 4 h (Almuktar & Scholz, 2016 a).

The ash samples were subsequently transferred into Teflon tubes to be dissolved in 7 ml of 70% concentrated nitric acid (Plank, 1992; Bressy et al., 2013). A Mars 5 microwave (Buckingham Industrial Estate, Buckingham, UK) digester was used. After digestion, the samples were diluted with deionised water up to 25 ml (Bressy et al., 2013) in a volumetric flask and transferred into 15-ml polystyrene tubes to be analysed by the ICP–OES technique using a Varian 720-ES Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK Ltd, Wharfedale Road, Wokingham, Berkshire, UK).

The bioavailability of each element was indicated by calculating the Concentration Factor (CF) value. This factor is defined as the relationship, as a percentage, between element concentration in the plant organ and its concentration in the soil (Mohamed et al., 2003; Q.Li et al., 2012) as follows:

$$\mathbf{CF=100^* (C_{fruit}/C_{soil})}$$

Where,

CF: concentration factor (%)

C_{fruit}: element concentration in the fruit (mg/kg)

C_{soil}: element concentration in the soil (mg/kg)

3.8.3 Fruit microbial content

Chilli fruits were harvested at different distances from the soil: 0 to 50 cm, 50 to 100 cm and more than 100 cm. All fruits were washed with 50 ml of distilled water using an ultrasonic Sonicor Table Top Cleaner machine (Sonicor Inc., New York, NY, USA). Collected washing solutions were analysed for bacterial contamination according to standard methods (APHA, 1998). For fruits, which were directly harvested from any position of the plant, skin was manually separated from the fruit flesh using a scalpel (Lustig, Bernstein, & Gophen, 2014). The two proportions of the fruits were analysed for their microbiological contamination. One gram of fruit skin or flesh was homogenised with 9 ml of buffered peptone water into a Stomacher Lab-Blender 80 (Gemini BV, Apeldoorn, The Netherlands), and then mixed for 1 min. The mixture was allowed to settle for 2–3 min. A series of dilution was subsequently carried out for the agar plates according to APHA (1998).

3.9 Risk assessment

In this research, there are various chemical and biological materials which need to be used while doing experimental work and analysis. As these materials are usually harmful and carcinogenic, a risk assessment before starting this research was required to evaluate the associated potential risk. Control of Substances Hazardous to Health Regulations (COSHH) and Product Safety Data Sheet (PSDS) forms of Salford University were used for risk assessment. In these documents, the risk assessment and safe work systems information associated with application of hazardous substances were explained to minimise the potential health risk to the students and other people who may be affected. Furthermore, instructions about materials which need to be used while undertaking the experimental work such as laboratory coats, glasses, masks and gloves were explained to ensure safety during each step as well as training about possible methods of exposure such as inhalation, oral ingestion and skin absorption when working with hazardous materials. The risk assessment forms were finalised after reading of Control of Substances Hazardous to Health Regulations then delivered to the hazardous substances users who accepted them and reported it in the declaration section.

The risk assessment was approved in the Public Health Department of the Environmental and Life Sciences school in Salford University. Moreover, the instructions provided in the safety sheets of chemicals and biological materials regarding use and disposal of such substances were carefully followed.

3.10 Data analysis

IBM SPSS Statistics Version 20 (www.ibm.com) was applied to compute all data statistical analysis with 5% significance level. After collection of the data, a normality test was carried out before data analysis. Two main methods were used for assessing data normality; the graphical method using Normal Q-Q Plots, and the numerical method using the Shapiro-Wilk test as it is the well-known and more appropriate test for small sample sizes (< 50 samples) and can also handle sample sizes as large as 2000 (Field, 2009). Moreover, homogeneity of variances was assessed using Levene's test.

Comparisons between two independent variables of two categorical, independent groups were performed using the Independent T-Test when data were normally distributed, while the Mann-Whitney U-test was used instead for non-normally distributed data (Field, 2009).

One-way analysis of variance (ANOVA) was used to determine whether there were any significant differences (p value of less than 0.05) between the means of three or more independent (unrelated) groups which were normally distributed, while the Kruskal-Wallis H test was used alternatively for non-normally distributed data sets. However, ANOVA and Kruskal-Wallis were used as an extension of the Independent T-Test and Mann-Whitney U-test, respectively, to allow the comparison of more than two independent groups (Field, 2009).

ANOVA could not display which specific groups were significantly different from each other. For that reason, a post-hoc test was run to confirm where the differences occurred between groups. Assumption of variances homogeneity decided which post-hoc test was run. When the data met the assumption of variances homogeneity, two possible tests could be used, either Tukey's honestly significant difference (HSD) or Scheffé post-hoc tests. However, in this study Tukey's HSD was used as it is highly recommended by statisticians because it is not as conservative as the Scheffé test (Field, 2009). Moreover, when the data did not meet the homogeneity of variances assumption, either the Games Howell or Dunnett's C post-hoc test was used. However, in this study, the Games Howell test was used as it is highly recommended by statisticians (Field, 2009).

The one-sample t-test was used to determine whether a sample came from a population with a specific mean for a normally distributed data set, while the one-sample Wilcoxon signed-rank test was used instead for non-normally distributed data sets.

Moreover, investigations of the correlation relationships between variables were performed by using the Pearson product-moment correlation coefficient (Pearson's correlation) which is used to measure the strength and direction of association that exists between normally distributed variables, while the non-parametric Spearman's rank-order correlation coefficient (Spearman's correlation) was used with non-normally distributed variables (Field, 2009). A linear regression analysis was performed to establish the relationships between variables.

Lastly, Microsoft Excel (www.microsoft.com) was used for general data analysis such as mean, minimum, maximum and standard deviation values. Figure 3.4 summarises the general statistical analysis performed in this study.

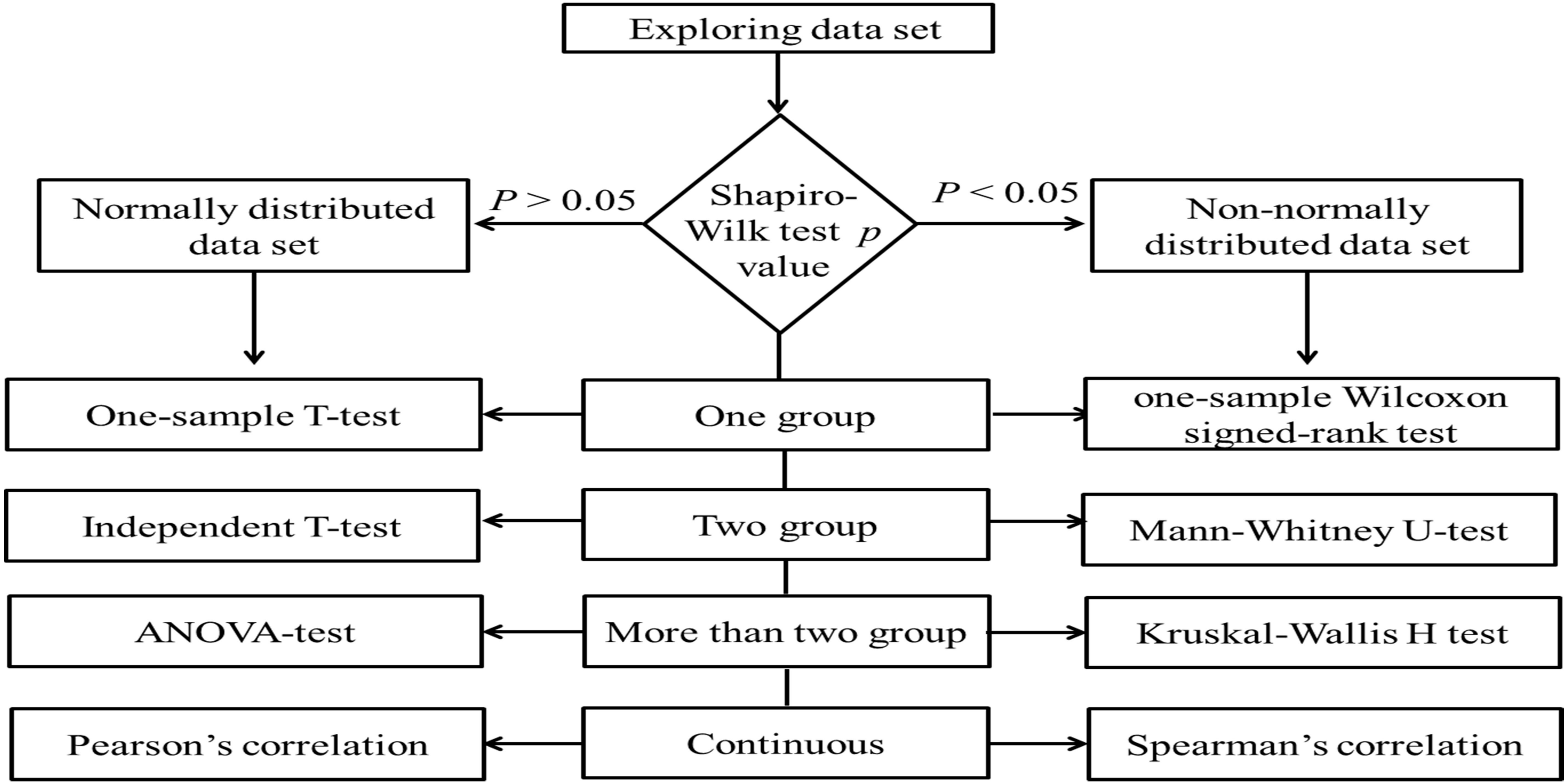

Figure 3.4: Flow chart of the statistical tests used in this study.

3.11 Limitations of the experimental study

In this study, the experimental constructed wetlands system is not similar to that of large-scale ones used in industries. However, several studies have been performed based on comparable systems such as those undertaken by Babatunde et al. (2011), Sani et al. (2013), Al-Isawi et al. (2015), Almuktar et al. (2015 a, b), Almuktar and Scholz (2015) and Almuktar and Scholz (2016 a, b) who reported the acceptance of their system by the scientific community after validation of their results to be applicable to field scale. Moreover, the constructed wetlands in this study were operated under semi-real (controlled) conditions in a greenhouse and therefore, cannot be compared to those in the real field. However, the wetlands system in this study can be considered as a model of new wetlands design that can be operated under various climate conditions.

Furthermore, real wetlands utilise a large land area with high natural energy resulting in a suitable environment for biodiversity, mainly in terms of various microorganism types as well as different types of animals which have a significant impact on the wetland processes. As a result this is considered as another limitation of the constructed wetlands used in this study as they will not provide the requirements available in natural systems of huge land area.

In addition, the constructed wetland filters in this study did not have sufficient replicates due to insufficient resource, mainly in terms of space availability to accommodate the essential replicates number, adding another limitation of studied wetlands to simulate actual ones in spite of various conducted studies on the same wetland system (Sani et al., 2013; Almuktar et al, 2015 a, b; Almuktar& Scholz, 2015; Almuktar & Scholz, 2016 a, b) which were accepted by the scientific community.

A significant limitation associated with the studied constructed wetland filters was the unavailability of enough replicate numbers leading to an insufficient outflow water amount available for plant irrigation, which subsequently limited the number of plant pots in all experimental set-ups. Furthermore, limitation of space in the controlled conditions laboratory where the plant pots were accommodated, also constricted the number of plant pots replicates for each treatment.

Moreover, the various operation modes of the studied constructed wetlands, mainly in terms of contact and resting time, resulted in various water amounts available for plant pot irrigation (Table 3.1). This meant the irrigated plants were not subjected to the same amount of irrigation water volume, and subsequently different nutrients and trace elements applied load on plants via irrigation water, affecting the plant growth rates, yield amounts and quality.

However, in this recycling project the results priority was on the harvested fruits per plant associated with each treatment. For example, in Sweet pepper and Chilli experiments, regular harvested fruits per treatment were collected during the whole period of the experiment life then total harvest per treatment was subjected to the required analysis (statistics). Moreover, soil quality analysis per treatment was performed by taking samples randomly from each replicate, which were mixed together then the required number of replicates were selected for subsequent analysis (Almuktar & Scholz, 2016 a).

3.12 Summary

This chapter describes various irrigation water resources used for plant irrigation, mainly those associated with constructed wetland system effluents. It also explains the set-up, design and operation of wetlands filters as well as the experimental design set-up associated with plant pots. Moreover, analyses for irrigation waters, soils and harvest quality are detailed in this chapter. Furthermore, monitoring of laboratory environmental conditions for running the experiments under controlled conditions is explained. Finally, this chapter talks about the risk assessment and methods used for data analysis to interpret the results as well as the limitations associated with this research study.

CHAPTER 4
RECYCLING OF DOMESTIC WASTEWATER TREATED BY VERTICAL-FLOW WETLANDS FOR IRRIGATION OF SWEET PEPPERS AND CHILLIES

Almuktar, S. A. A. A. N., Scholz, M., Al-Isawi, R. H. K., & Sani, A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for irrigating Chillies and Sweet Peppers. *Agricultural Water Management, 149*, 1-22.

Almuktar, S. A. A. A. N., Scholz, M., Al-Isawi, R. H. K., & Sani, A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for watering of vegetables. *Water Practice and Technology, 10*(3), 445-464.

Almuktar, S. A., & Scholz, M. (2016). Experimental assessment of recycled diesel spill-contaminated domestic wastewater treated by reed beds for irrigation of Sweet Peppers. *International Journal of Environmental Research and Public Health, 13*(2), 208.

4.1 Overview

This chapter discusses the impact of recycling urban wastewater treated by vertical-flow wetlands on growth of two crop types, namely Sweet Peppers and Chillies (experiment No. 1 and 2). Section 4.1 introduces this chapter, while section 4.2 discusses the comparisons of irrigation water qualities in terms of different variables. Environmental conditions available for growing these crops are explained in section 4.3, while the growth and yield production comparisons are provided in section 4.4. Lastly, the summary of this chapter is provided in section 4.5.

4.2 Comparison of irrigation water qualities

Tables 4.1 to 4.22 show the inflow water quality received by the plants and corresponding analysis. Note that the wetland effluent was used as the influent for the vegetable pots. Figure 4.1 explains the comparison strategy of irrigation water qualities according to wetland design variables.

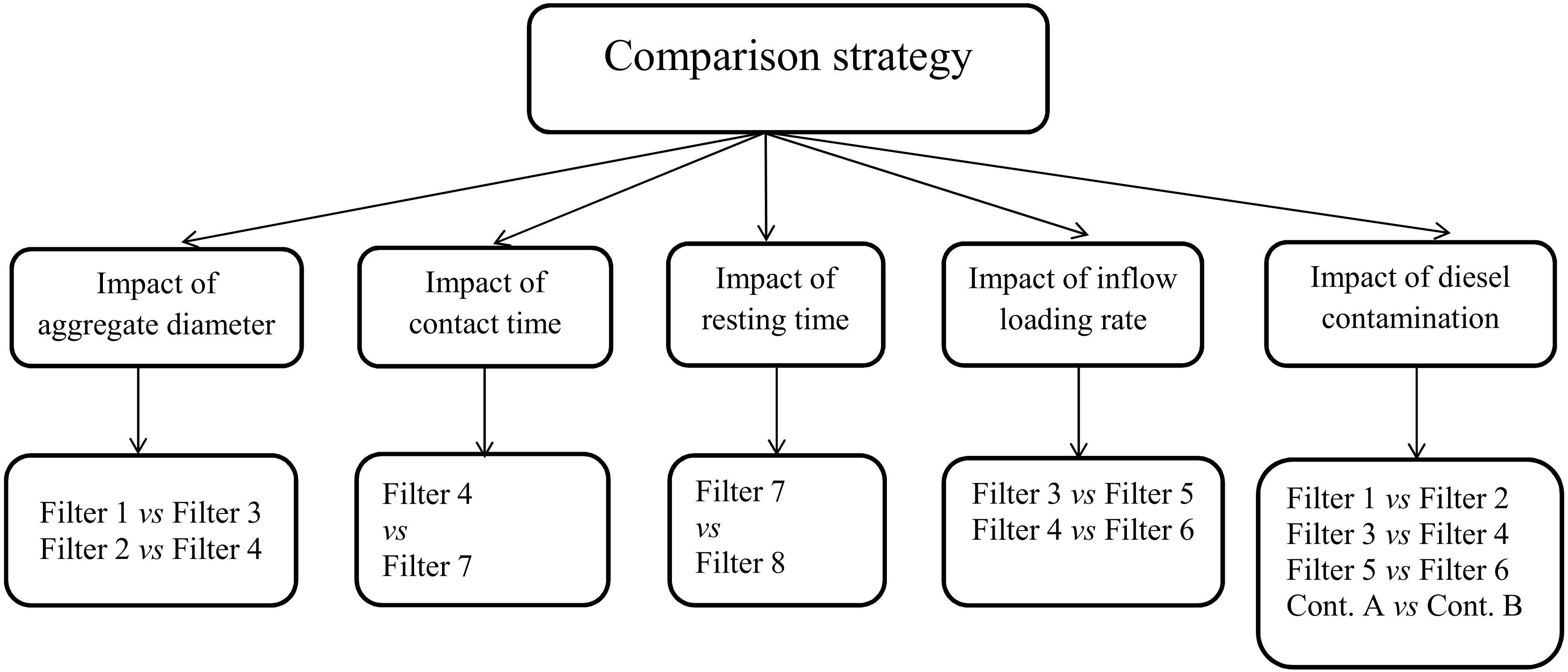

Figure 4.1: Comparison strategy of irrigation water qualities.

4.2.1 Comparison of hydrocarbon values

Highly fluctuating values for total petroleum hydrocarbon were observed in the irrigation water obtained from Filters 1, 3, 5, 8 and Control A outflows (Table 4.1). The total petroleum hydrocarbon values followed this order: Control A > Filter 8 > Filter 1 > Filter 3 > Filter 5. Regarding filters contaminated by diesel (Filters 1, 3, 5 and Control A), the total petroleum hydrocarbon concentrations in the effluent were 100 µg/l, 69 µg/l, 14 µg/l and 346 µg/l, respectively (Table 4.1). These concentrations are in compliance with the Chinese standards for irrigation water quality (SEPA, 2005) highlighting a maximum allowable threshold value of 1000 µg/l. Note that Chinese standards were used, considering that China produces about 54% (estimated in 2008) of peppers (including Chilli) in the world (ERS/USDA, 2008). Control A, which lacks mature biomass, showed high total petroleum hydrocarbon concentration values compared with those values for the other filters. This can be explained by diesel toxicity to microorganisms due to the absence of sufficient nutrients in tap water. Although Filter 8 lacked diesel spill contamination, the total petroleum hydrocarbon concentration was 116 µg/l. This can be explained by the elevated loading rate for this filter, resulting in the accumulation of hydrocarbons originating from the petroleum background concentration in wastewater. Moreover, total petroleum hydrocarbon values for Filter 1 outflow water were higher than those for Filter 3, explaining the impact of aggregate size on the diesel removal process. However, substrate size has an important role in the system mechanism as it may affect the surface area for growing the biofilm in addition to the system pores blockage probability. For instance, Meng et al. (2014) reported that excessively large aggregate size will reduce the surface area for microorganisms to grow, while Brix and Arias (2005) indicated that the small-sized-grain media will support the growth of biofilm by increasing the available surface area and subsequently enhancing the degradation bacteria activity. Moreover, the diesel concentration in Filter 5 outflow water was lower than those concentrations in Filters 1 and 3 due to the strong and mature biomass available in Filter 5 as a result of a high inflow loading rate (Table 3.1) supporting the growth of microorganisms and subsequently enhancing the hydrocarbon biodegradation. However, correlation analysis results (Table 4.4) show that total petroleum hydrocarbon was significantly positively ($p < 0.05$) correlated with the chemical oxygen demand variable in the system due to high COD values in petroleum hydrocarbons (Scholz, 2010) resulting in high COD values in the corresponding contaminated filters outflows (Chavan & Mukherji, 2008; Lohi et al. 2008). Moreover, total petroleum hydrocarbon was significantly ($p < 0.05$) positively correlated with selected microorganisms (total coliforms: R = 0.860, P = 0.001; *E. coli*: R = 0.724, P = 0.018; Salmonella spp.: R = 0.782, P = 0.007) due to high organic matter available in the petroleum hydrocarbon, supporting microorganism growth and population by providing a sustainable carbon and nutrients source (Scholz, 2010).

4.2.2 Comparison of oxygen demand variables

Table 4.1 shows that chemical oxygen demand values were the highest for raw wastewater (domestic wastewater) followed by Filters contaminated with diesel following the order of F5 > F3 > F1 > Control A. In contrast, the lowest mean values were noted for Control B. Filters 2 and 4 had relatively similar chemical oxygen demand concentrations to those of Filters 4 and 7, respectively, indicating that aggregate size and contact time may not matter. Filter 8 outflow water had chemical oxygen demand values which were higher than those for Filter 7, indicating the impact of long resting time on outflow water chemical oxygen demand. However, Table 4.2

shows that different wetland designs did not show any statistical significant differences in outflow chemical oxygen values. Song et al. (2006) indicated that organic matter can be removed via the development of microbes in the system media and/or by adhering to wetland plant roots. Moreover, Table 4.2 shows that COD values for Filters 1, 3, 5 and Control A were statistically significantly higher than those for Filters 2, 4, 6 and Control B, respectively, indicating the impact of diesel contamination on COD outflow values. This is due to the high organic matter available in the petroleum hydrocarbon resulting in high COD values in the corresponding contaminated filters outflows (Scholz, 2010). Furthermore, COD values for different filter outflows were different during various vegetable growth periods. This can be explained by the seasonal variation in wetland systems confirming the results observed by Song et al. (2006) and Sani et al. (2013) which indicated a clear seasonal trend with high COD values in autumn and low COD values in summer. Correlation analysis results (Table 4.3) show that COD values were significantly ($p < 0.05$) positively correlated with BOD and total petroleum hydrocarbons values in the system confirming the results reported previously (Chavan & Mukherji, 2008; Lohi et al. 2008).

Table 4.1 shows that the five-day BOD was the highest for raw urban wastewater followed by filters contaminated with diesel (Filters 1, 3 and 5), while tap water had the lowest five-day BOD values. Filters 1 and 2 had biochemical oxygen demand values which were higher than those for Filters 3 and 4, respectively, indicating the impact of aggregate size (Figure 4.1). Moreover, Filters 4 and 8 showed biochemical oxygen demand levels which were higher than those for Filter 7 explaining the impact of contact and resting times, respectively. Furthermore, the five-day BOD for Filter 6 outflow water of high inflow load was greater than that for Filter 4 of low inflow loading rate confirming the results reported by Sani et al. (2013) indicating that high load filters tend to be overloaded. However, statistical analysis results (Table 4.2) did not show any significant differences ($p > 0.05$) in biochemical oxygens demands values associated with the different filters outflows which had various designs in terms of aggregate size, contact time, resting time and inflow loading rate. Nevertheless, hydrocarbon contamination had a significant ($p < 0.05$) impact on biochemical oxygen demand values, as shown in Table 4.2 when comparing values of Filters 1, 3, 5 and control A with those of Filters 2, 4, 6 and Control B. This is due to the high organic matter available in the petroleum hydrocarbon resulting in high five-day BOD values in the corresponding contaminated filter outflows (Scholz, 2010). Furthermore, the five-day BOD values for different filter outflows were different during various vegetable growth periods confirming the results from previous studies (Scholz, 2011; Sani et al., 2013) which reported a seasonal trend with high BOD values in summer and low BOD values in winter. Correlation analysis results (Table 4.3) show that BOD values were significantly ($p < 0.05$) positively correlated with those of COD as reported by Scholz (2010).

Table 4.1 shows that dissolved oxygen mean values were higher for those filters without diesel. However, statistical analysis results (Table 4.2) did not show any significant differences in dissolved oxygen values for the various filter outflows. Moreover, correlation analysis results (Table 4.3) indicate that dissolved oxygen was negatively correlated with total petroleum hydrocarbons, chemical oxygen demand and biochemical oxygen demand in the system. Furthermore, dissolved oxygen values were significantly ($p < 0.05$) negatively correlated with microorganisms (e.g., total coliforms; $R = -0.726$; $P = 0.017$ and salmonella; $R = -0.751$, $p = 0.012$). This negative correlation can be explained by an improvement of the chemical oxygen demand, biochemical oxygen demand and total petroleum hydrocarbon removal efficiencies as microorganisms responsible for biodegradation acclimatised, resulting in a reduction of the amount of available dissolved oxygen in the system (Scholz, 2010).

Table 4.1: Comparison of the total petroleum hydrocarbons, chemical oxygen demand, biochemical oxygen demand and dissolved oxygen for irrigation water received by the vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Total petroleum hydrocarbons (µg/l)				
Filter 1	100.0	nm	nm	100.0
Filter 2	< 10.0	nm	nm	< 10.0
Filter 3	69.0	nm	nm	69.0
Filter 4	< 10.0	nm	nm	< 10.0
Filter 5	14.0	nm	nm	14.0
Filter 6	< 10.0	nm	nm	< 10.0
Filter 7	< 10.0	nm	nm	< 10.0
Filter 8	116.0	nm	nm	116.0
Control A	346.0	nm	nm	346.0
Control B	< 10.0	nm	nm	< 10.0
Chemical oxygen demand (mg/l)				
Filter 1	77.7±23.35 (18)	nm	87.5±41.13 (4)	75.0±16.86 (14)
Filter 2	34.9±19.21 (15)	nm	74.8±6.72 (2)	28.8±11.03 (13)
Filter 3	87.5±26.00 (18)	nm	86.2±34.17 (4)	87.8±24.78 (14)
Filter 4	34.9±23.77 (15)	nm	70.6±2.26 (2)	29.4±20.33 (13)
Filter 5	100.8±67.90 (18)	nm	169.4±125.43 (4)	81.2±23.24 (14)
Filter 6	35.6±22.46 (14)	nm	76.1±n.a (1)	32.5±19.98 (13)
Filter 7	32.5±20.40 (14)	nm	69.5±14.92 (2)	26.3±13.49 (12)
Filter 8	55.9±86.05 (15)	nm	163.7±170.06 (3)	28.9±14.21 (12)
Control A	66.4±44.32 (17)	nm	59.7±1.56 (3)	67.9±49.03 (14)
Control B	16.0±15.12 (15)	nm	47.6±10.68 (2)	11.1±8.10 (13)
DW	3.5±0.08 (3)	nm	3.6±0.06 (2)	3.4±n.a (1)
TW	6.2±0.33 (3)	nm	6.4±n.a (1)	6.3±n.a (1)
TW+F	8.6±0.22 (3)	nm	8.4±n.a (1)	8.8±n.a (1)
TW+WW	47.6±15.39 (17)	nm	72.0±n.a (1)	46.1±14.51 (16)
WW	237.9±76.96 (17)	nm	360.0±n.a (1)	230.3±72.54 (16)
Biochemical oxygen demand (mg/l)				
Filter 1	25.8±16.74 (53)	50.7±23.86 (3)	22.2±17.02 (12)	25.0±14.89 (38)
Filter 2	13.6±8.11(51)	24.7±10.26 (3)	13.1±8.73 (11)	12.8±7.32 (37)
Filter 3	22.8 ±16.42(51)	67.3±38.59 (3)	25.0±14.88 (10)	18.7±7.11 (38)
Filter 4	12.8 ±8.86 (50)	22.7±1.15 (3)	11.8±8.21 (9)	12.3±9.00 (38)
Filter 5	22.5 ±16.36 (51)	26.0±19.80 (2)	39.4±23.32 (10)	18.1±10.78 (39)
Filter 6	15.9 ±12.68 (52)	44.0±0.00 (3)	16.4±10.07 (11)	13.6±11.16 (38)
Filter 7	11.9 ±8.01 (61)	28.0±9.93 (4)	10.3±9.32 (15)	11.0±5.47 (42)
Filter 8	13.9 ±7.50 (69)	26.0±7.07 (5)	13.1±7.42 (17)	12.9±6.53 (47)
Control A	12.0 ±7.58 (51)	20.0±8.72 (3)	12.5±10.00 (11)	11.2±6.47 (37)
Control B	8.8 ±7.58 (52)	15.0±11.79 (3)	11.4±11.55 (10)	7.7±5.67 (39)
DW	7.3±1.84 (3)	8.6±n.a (1)	8.6±n.a (1)	6.0±n.a (1)
TW	4.9 ±1.13 (3)	5.7±n.a (1)	5.7±n.a (1)	4.1±n.a (1)
TW+F	8.0 ±2.62 (3)	9.8±n.a (1)	9.8±n.a (1)	6.1±n.a (1)
TW+WW	21.8 ±15.99 (55)	38.3±19.44 (5)	21.0±12.23 (10)	19.9±15.55 (40)
WW	105.3±75.98 (55)	187.2±95.54 (5)	105.0±61.15 (10)	95.2±72.32 (40)

Table 4.1 (cont.)

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
		Dissolved oxygen (mg/l)		
Filter 1	1.5±1.03 (15)	nm	nm	1.5±1.03 (15)
Filter 2	1.7±1.10 (15)	nm	nm	1.7±1.10 (15)
Filter 3	1.7±1.18 (15)	nm	nm	1.7±1.18 (15)
Filter 4	2.0±1.08 (15)	nm	nm	2.0±1.08 (15)
Filter 5	1.5±0.82 (15)	nm	nm	1.5±0.82 (15)
Filter 6	1.6±0.86 (15)	nm	nm	1.6±0.86 (15)
Filter 7	1.7±0.86 (25)	nm	nm	1.7±0.86 (25)
Filter 8	1.9±1.15 (22)	nm	nm	1.9±1.15 (22)
Control A	1.4±0.93 (15)	nm	nm	1.4±0.93 (15)
Control B	1.8±1.04 (15)	nm	nm	1.8±1.04 (15)
DW	8.6±0.30 (3)	nm	nm	8.6± 0.30(3)
TW	8.1±0.11 (3)	nm	nm	8.1±0.11 (3)
TW+F	8.5±0.25 (3)	nm	nm	8.5±0.25 (3)
TW+WW	7.6±0.74 (16)	nm	nm	7.6±0.74 (16)
WW	5.2±3.72 (16)	nm	nm	5.2±3.72 (16)

Note: nm, not measured. [a] 11/10/13 to 25/09/14. [b] Second planting period: 11/10/13 to 07/11/13. [c] Final planting period before fruiting: 08/11/13 to 19/01/14. [d] Final planting period after fruiting: 20/01/14 to 25/09/14. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). n.a, not applicable.

Table 4.2: Statistical assessment of chemical oxygen demand, biochemical oxygen demand and dissolved oxygen variables for irrigation waters linked to wetland filters during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b] for different system combinations
		Chemical oxygen demand (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.032	M-W-U	0.304
Filter 2 & Filter 4	0.004	M-W-U	0.468
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.727
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.663
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.924
Filter 4 & Filter 6	0.004	M-W-U	0.445
Hydrocarbon influence			
Filter 1 & Filter 2	0.080	I-T	< 0.001
Filter 3 & Filter 4	0.063	I-T	< 0.001
Filter 5 & Filter 6	< 0.001	M-W-U	< 0.001
Control A & Control B	< 0.001	M-W-U	< 0.001

Table 4.2 (cont.)

Design variables	Shapiro-Wilk test (p-value)[a]	Statistical test	P-values[b] for different system combinations
Biochemical oxygen demand (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.220
Filter 2 & Filter 4	< 0.001	M-W-U	0.502
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.723
Resting time			
Filter 7 & Filter 8	0.002	M-W-U	0.054
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.786
Filter 4 & Filter 6	< 0.001	M-W-U	0.405
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	< 0.001
Filter 3 & Filter 4	< 0.001	M-W-U	< 0.001
Filter 5 & Filter 6	< 0.001	M-W-U	0.024
Control A & Control B	< 0.001	M-W-U	0.020
Dissolved oxygen (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	0.003	M-W-U	0.755
Filter 2 & Filter 4	0.196	I-T	1.000
Contact time			
Filter 4 & Filter 7	0.397	I-T	0.961
Resting time			
Filter 7 & Filter 8	0.036	M-W-U	0.669
Inflow loading rate			
Filter 3 & Filter 5	0.028	M-W-U	0.787
Filter 4 & Filter 6	0.028	M-W-U	0.520
Hydrocarbon influence			
Filter 1 & Filter 2	0.006	M-W-U	0.349
Filter 3 & Filter 4	0.034	M-W-U	0.917
Filter 5 & Filter 6	0.003	M-W-U	0.771
Control A & Control B	0.009	M-W-U	0.253

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test. Filters are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test. I-T, the parametric Independent samples T-test.

Table 4.3: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	Variable			
		TPH	COD	BOD	DO
TPH	R	1.000	0.694*	0.381	-0.427
	p	n.a	0.026	0.277	0.219
COD	R	0.694*	1.000	0.815**	-0.574
	p	0.026	n.a	0.004	0.083
BOD	R	0.381	0.815**	1.000	-0.351
	p	0.277	0.004	n.a	0.320
DO	R	-0.427	-0.574	-0.351	1.000
	p	0.219	0.083	0.320	n.a

Note: TPH, total petroleum hydrocarbon (µg/l); COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); DO, dissolved oxygen (mg/l); R, correlation coefficient; *p,* probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level.

4.2.3 Comparison of nutrient variables

Table 4.4 shows an overview of ammonia-nitrogen, nitrate-nitrogen and ortho-phosphate-phosphorus for irrigation water received by the vegetable pots. Results indicate that the highest ammonia-nitrogen values were observed for the raw urban wastewater followed by those for Filters 5 and 6 outflow waters which were fed with high inflow loading rate, while the lowest values were recorded for deionised and tap waters. Filter 1 outflow water had ammonia-nitrogen greater than that of Filter 3.

Statistical analysis results (Table 4.5) show significant ($p < 0.05$) differences in ammonia-nitrogen values between Filters 2 and 4 outflow waters indicating the impact of aggregate size on the ammonia-nitrogen removal process. Filters 4 and 7 outflow waters had ammonia-nitrogen values greater than those for Filters 7 and 8, respectively, indicating the impact of contact and resting time wetland variable design on ammonia nitrogen-removal processes.

The inflow water was high in chemical oxygen demand, resulting in statistically significant ($p < 0.05$) differences between the overall mean daily ammonia-nitrogen values of Filters 3 and 4 compared to those of Filters 5 and 6, respectively (Table 4.5). This could be explained by the fact that, it is likely that high rate filters are overloaded (Sani et al., 2013). Table 4.4 indicates that ammonia-nitrogen had various values during different periods of crop growth due to seasonal behaviour differences in the wetland system as indicated by Sani et al. (2013).

Compared to the standards of 5 mg/l (Pescod, 1992; FAO, 2003), statistical analysis results (Table 4.6) show that irrigation waters linked to Filters 3, 4, 8, Control A and Control B had ammonia-nitrogen values which were significantly ($p < 0.05$) lower than the thresholds, while higher ammonia-nitrogen values which significantly ($p < 0.05$) exceeded the standards were observed in outflow from Filters 5 and 6, which were fed with high inflow loads as well as in raw wastewater and wastewater samples diluted with tap water. However, ammonia-nitrogen has a negative effect on plant fruit, leaf and stem development as discussed by Bar-Tal, Aloni, Karni, and Rosenberg, (2001). Moreover, correlation analysis results (Table 4.7) show that ammonia-nitrogen values were negatively correlated with total petroleum hydrocarbons and dissolved oxygen and positively correlated with chemical oxygen demand, biochemical oxygen demand and nitrate-nitrogen in the system. This can be explained by the increasing of nitrification processes by oxidation of ammonia-nitrogen to nitrate-nitrogen via microorganisms with the abundancy of organic sources in terms of hydrocarbons (Scholz, 2010), which will reduce the oxygen availability in the system (Cooper et al., 1996; Scholz, 2010; Fan et al., 2013; Vymazal, 2014). Table 4.4 shows that the highest nitrate-nitrogen values were observed in outflow water from Filter 6 followed by that from Filters 7 and 8, while the lowest values were recorded in Control B outflow water.

Filter 2 outflow water had nitrate-nitrogen values which were greater than those for Filter 4, indicating the impact of aggregate diameter. Statistical analysis results (Table 4.5) indicate significant differences in nitrate-nitrogen values in the outflow of Filters 4 and 7, explaining the impact of contact time on the nitrate-nitrogen removal process. Moreover, filters of high inflow loading rate showed nitrate-nitrogen concentrations which were significantly greater than those of diluted inflow (Filters 3 and 4 compared to Filters 5 and 6, respectively) confirming the results reported previously by Sani et al. (2013). Furthermore, filters contaminated with hydrocarbons showed nitrate-nitrogen levels which were lower than those associated with standard filters. This can be explained by the fact that increasing the diesel spills biodegradation processes in the corresponding filters supported microorganism growth by providing a rich source for carbon and energy (Chavan & Mukherji, 2008; Tang et al., 2010) resulting in nitrogen decreasing (Scholz, 2010; Al-Isawi et al., 2015).

Table 4.4 shows that nitrate-nitrogen values were different during various periods of crop growth due to seasonal changes in wetlands system behaviour as discussed by Sani et al. (2013) indicating high nitrate-nitrogen values in winter and low values in summer, confirming previous results (Werker et al., 2002; Kuschk et al., 2003; Gikas et al., 2007). Compared to the standards of 30 mg/l (Pescod, 1992; FAO, 2003), nitrate-nitrogen for all filter outflow waters was significantly ($p < 0.05$) less than the maximum threshold value.

However, the total yield increases as the nitrate-nitrogen to ammonia-nitrogen ratio increases. This is due to a reduction in fruit physiological disorders, which usually reduce fruit mean weight (Bar-Tal et al., 2001). Correlation analysis results (Table 4.7) show that nitrate-nitrogen values were negatively correlated with total petroleum hydrocarbons in the system. This can be explained by the consuming of nitrogen by microorganisms during hydrocarbons degradation simulating nitrogen removal via additional carbon supplied via diesel spills as reported by Scholz (2010).

Furthermore, Table 4.7 shows that nitrate-nitrogen correlated positively with ammonia-nitrogen and dissolved oxygen in the system. This is because with high availability of ammonia-nitrogen and oxygen in the treatment plant, more oxidation of ammonia to nitrate will occur (Cooper et al., 1996, Scholz, 2010; Fan et al., 2013; Vymazal, 2014).

Table 4.4 shows that the highest ortho-phosphate-phosphorus values were observed for raw domestic wastewater followed by those of outflow waters from Filters 5 and 6 which were fed with high inflow loads, while the lowest ortho-phosphate-phosphorus values were recorded for deionised and tap waters. Outflow waters from Filters 1 and 2 had ortho-phosphate-phosphorus values greater than those for Filters 3 and 4, respectively, indicating the impact of aggregate diameter. Filter 4 outflow water showed lower ortho-phosphate-phosphorus values compared to that of Filter 7, while Filter 7 had values higher than those for Filter 8, explaining the impact of wetlands contact and resting times on ortho-phosphate-phosphorus removal. Moreover, irrigation waters linked to Filters 5 and 6 of high inflow COD values showed ortho-phosphate-phosphorus values greater than those of Filters 3 and 4, respectively, which were fed with diluted domestic wastewater.

However, statistical analysis (Table 4.5) did not show any statistical differences (*p* values of greater than 0.05) in ortho-phosphate-phosphorus values of the outflow waters indicating that wetland aggregate diameter, contact and resting times as well as inflow loading rate have little effect on ortho-phosphate-phosphorus treatment. In general, phosphorus is one of the most difficult pollutants to remove by mature constructed wetlands (Pant, Reddy, & Lemon, 2001). This is due to the fact that phosphorus is usually present in particulate form, and does not dissolve well in filters that are not yet saturated by phosphorus or other compounds competing for adsorption sites (Scholz, 2006, 2010). Compared to standards of 2 mg/l (FAO, 1994, 2003), all irrigation waters associated with wetland filters were reported with ortho-phosphate-phosphorus values which significantly ($p < 0.05$) exceeded the thresholds except those harvested from Controls A and B. However, phosphorus deficiency has been identified to limit crop yields. Little research has been undertaken concerning the effects of high phosphorus on plants. High phosphorus levels are known to interfere with plant normal metabolisms. Also, it is known to promote manganese uptake by plants (FAO, 1972; McCauly et al., 2011).

Table 4.4: Comparison of the ammonia-nitrogen, nitrate-nitrogen and ortho-phosphate-phosphorus for irrigation water received by the vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
		Ammonia-nitrogen (mg/l)		
Filter 1	4.8±2.77 (20)	6.6±n.a (1)	8.0±1.51 (3)	4.1±2.57 (16)
Filter 2	5.8±5.81 (19)	18.6±n.a (1)	2.2±2.72 (3)	5.7±5.28 (15)
Filter 3	3.7±2.54 (20)	7.8±n.a (1)	4.9±0.10 (3)	3.2±2.55 (16)
Filter 4	2.7±2.87 (19)	11.0±n.a (1)	1.8±2.17 (3)	2.3±2.16 (15)
Filter 5	10.2±2.57 (20)	9.8±n.a (1)	8.3±1.07 (3)	10.5±2.72 (16)
Filter 6	9.3±7.38 (19)	10.4±n.a (1)	8.3±7.56 (3)	9.4±7.84 (15)
Filter 7	3.8±5.42 (22)	0.3±n.a (1)	0.8±0.18 (3)	4.5±5.77 (18)
Filter 8	1.4±1.26 (19)	1.2±n.a (1)	0.7±0.37 (3)	1.5±1.38 (15)
Control A	1.1±1.53 (20)	0.0±n.a (1)	4.3±0.20 (3)	0.6±0.72 (16)
Control B	1.2±1.70 (19)	0.1±n.a (1)	1.2±0.23 (3)	1.2±1.90 (15)
DW	0.1±0.00 (3)	0.0±n.a (1)	0.0±n.a (1)	0.2±n.a (1)
TW	0.1±0.00 (3)	0.1±n.a (1)	0.1±n.a (1)	0.1±n.a (1)
TW+F	16.0±0.01 (3)	16.0±n.a (1)	16.0±n.a (1)	16.0±n.a (1)
TW+WW	6.7±3.69 (22)	2.4±n.a (1)	7.6±5.54 (3)	6.8±3.46 (18)
WW	33.6±18.46 (22)	12.1±n.a (1)	38.1±27.68 (3)	34.1±17.31 (18)

Table 4.4 (cont.)

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
		Nitrate-nitrogen (mg/l)		
Filter 1	0.4±0.22 (19)	0.3±n.a (1)	0.5±0.15 (2)	0.4±0.23 (16)
Filter 2	2.2±2.72 (18)	7.0±n.a (1)	1.3±1.18 (2)	2.0±2.66 (15)
Filter 3	0.4±0.28 (19)	0.4±n.a (1)	0.7±0.53 (2)	0.3±0.23 (16)
Filter 4	1.8±3.27 (18)	8.3±n.a (1)	1.4±0.89 (2)	1.4±3.11 (15)
Filter 5	0.9±0.86 (19)	0.7±n.a (1)	1.5±0.72 (2)	0.9±0.90 (16)
Filter 6	3.6±4.68 (18)	17.9±n.a (1)	1.5±0.72 (2)	2.9±3.29 (15)
Filter 7	2.8±2.98 (18)	4.6±n.a (1)	1.4±0.69 (2)	2.9±3.20 (15)
Filter 8	2.8±3.51 (16)	10.7±n.a (1)	1.5±0.86 (2)	2.4±3.12 (13)
Control A	0.4±0.44 (19)	0.0±n.a (1)	1.3±0.95 (2)	0.3±0.19 (16)
Control B	0.3±0.35 (18)	0.1±n.a (1)	0.9±0.08 (2)	0.2±0.29 (15)
DW	0.0±0.00 (3)	0.0±n.a (1)	0.0±n.a (1)	0.0±n.a (1)
TW	0.2±0.00 (3)	0.2±n.a (1)	0.2±n.a (1)	0.2±n.a (1)
TW+F	8.9±0.38 (3)	8.6±n.a (1)	8.8± n.a (1)	9.3±n.a (1)
TW+WW	0.5±0.64 (21)	0.1±n.a (1)	0.8±0.87 (2)	0.5±0.65 (18)
WW	2.4±3.22 (21)	0.7±n.a (1)	4.2±4.37 (2)	2.3±3.24 (18)
		Ortho-phosphate-phosphorus (mg/l)		
Filter 1	4.0±2.48 (18)	3.1±n.a (1)	1.1±n.a (1)	4.2±2.51 (16)
Filter 2	3.3±1.33 (18)	3.1±n.a (1)	5.2±n.a (1)	3.2±1.32 (16)
Filter 3	3.3±2.04 (18)	3.8±n.a (1)	0.9±n.a (1)	3.5±2.07 (16)
Filter 4	2.9±1.06 (18)	2.7±n.a (1)	5.7±n.a (1)	2.8±0.86 (16)
Filter 5	4.4±2.07 (18)	7.7±n.a (1)	1.0±n.a (1)	4.4±1.83 (16)
Filter 6	4.6±3.16 (18)	2.7±n.a (1)	4.9±n.a (1)	4.7±3.33 (16)
Filter 7	3.6±2.23 (17)	2.7±n.a (1)	4.6±n.a (1)	3.6±2.36 (17)
Filter 8	3.3±1.90 (16)	1.9±n.a (1)	6.0±n.a (1)	3.2±1.86 (14)
Control A	1.8±0.56 (18)	1.5±n.a (1)	0.9±n.a (1)	1.9±0.52 (16)
Control B	1.9±0.33 (18)	1.7±n.a (1)	1.0±n.a (1)	1.9±0.26 (16)
DW	0.0±0.00 (3)	0.0±n.a (1)	0.0±n.a (1)	0.0±n.a (1)
TW	0.8±0.00 (3)	0.9±n.a (1)	0.8±n.a (1)	0.8±n.a (1)
TW+F	14.9±0.07 (3)	14.8±n.a (1)	14.9±n.a (1)	14.8±n.a (1)
TW+WW	3.0±1.43 (21)	2.4±n.a (1)	4.0±n.a (1)	3.0±1.48 (19)
WW	14.9±7.15 (21)	11.8±n.a (1)	20.0±n.a (1)	14.8±7.40 (19)

Note: [a] 11/10/13 to 25/09/14. [b] Second planting period: 11/10/13 to 07/11/13.
[c] Final planting period before fruiting: 08/11/13 to 19/01/14. [d] Final planting period after fruiting: 20/01/14 to 25/09/14. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). n.a, not applicable.

Table 4.5: Statistical assessment of ammonia-nitrogen, nitrate-nitrogen and ortho-phosphate-phosphorus variables for irrigation waters linked to wetland filters during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b] for different system combinations
Ammonia-nitrogen (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	0.013	M-W-U	0.213
Filter 2 & Filter 4	< 0.001	M-W-U	0.045
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.834
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.302
Inflow loading rate			
Filter 3 & Filter 5	0.141	I-T	< 0.001
Filter 4 & Filter 6	< 0.001	M-W-U	0.003
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.779
Filter 3 & Filter 4	0.003	M-W-U	0.177
Filter 5 & Filter 6	0.146	I-T	0.636
Control A & Control B	< 0.001	M-W-U	0.527
Nitrate-nitrogen (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.770
Filter 2 & Filter 4	< 0.001	M-W-U	0.129
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.024
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.972
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.019
Filter 4 & Filter 6	< 0.001	M-W-U	0.012
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.118
Filter 3 & Filter 4	< 0.001	M-W-U	0.456
Filter 5 & Filter 6	< 0.001	M-W-U	0.040
Control A & Control B	< 0.001	M-W-U	0.023
Ortho-phosphate-phosphorus (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.613
Filter 2 & Filter 4	0.002	M-W-U	0.569
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.680
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.746
Inflow loading rate			
Filter 3 & Filter 5	0.040	M-W-U	0.100
Filter 4 & Filter 6	< 0.001	M-W-U	0.178
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.527
Filter 3 & Filter 4	< 0.001	M-W-U	0.658
Filter 5 & Filter 6	0.003	M-W-U	0.728
Control A & Control B	0.008	M-W-U	0.669

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test. Filters are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test. I-T, the parametric Independent samples T-test.

Table 4.6: Statistical assessment of ammonia-nitrogen, nitrate-nitrogen and ortho-phosphate-phosphorus variables for irrigation waters compared to standards.

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value (mg/l)	Standard (mg/l)	Statistic (p-value)[b]
		Ammonia-nitrogen			
Filter 1 outflow	0.075	O-S-T	4.8	5.0	0.717
Filter 2 outflow	0.001	O-S-W	5.8	5.0	0.717
Filter 3 outflow	0.054	O-S-T	3.7	5.0	0.032
Filter 4 outflow	0.004	O-S-W	2.7	5.0	0.007
Filter 5 outflow	0.239	O-S-T	10.2	5.0	< 0.001
Filter 6 outflow	0.116	O-S-T	9.3	5.0	0.020
Filter 7 outflow	< 0.001	O-S-W	3.8	5.0	0.149
Filter 8 outflow	0.008	O-S-W	1.4	5.0	< 0.001
Control A outflow	< 0.001	O-S-W	1.1	5.0	< 0.001
Control B outflow	< 0.001	O-S-W	1.2	5.0	< 0.001
Wastewater (20%); tap water (80%)	0.331	O-S-T	6.7	5.0	0.040
Wastewater (100%)	0.331	O-S-T	33.6	5.0	< 0.001
		Nitrate-nitrogen			
Filter 1 outflow	0.021	O-S-W	0.4	30.0	< 0.001
Filter 2 outflow	0.002	O-S-W	2.2	30.0	< 0.001
Filter 3 outflow	< 0.001	O-S-W	0.4	30.0	< 0.001
Filter 4 outflow	< 0.001	O-S-W	1.8	30.0	< 0.001
Filter 5 outflow	0.007	O-S-W	0.9	30.0	< 0.001
Filter 6 outflow	0.001	O-S-W	3.6	30.0	< 0.001
Filter 7 outflow	0.017	O-S-W	2.8	30.0	< 0.001
Filter 8 outflow	< 0.001	O-S-W	2.8	30.0	< 0.001
Control A outflow	< 0.001	O-S-W	0.4	30.0	< 0.001
Control B outflow	< 0.001	O-S-W	0.3	30.0	< 0.001
Wastewater (20%); tap water (80%)	< 0.001	O-S-W	0.5	30.0	< 0.001
Wastewater (100%)	< 0.001	O-S-W	2.4	30.0	< 0.001

Table 4.6 (cont.)

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value (mg/l)	Standard (mg/l)	Statistic (p-value)[a]
		Ortho-phosphate-phosphorus			
Filter 1 outflow	0.001	O-S-W	4.0	2.0	0.001
Filter 2 outflow	0.011	O-S-W	3.3	2.0	0.001
Filter 3 outflow	0.570	O-S-T	3.3	2.0	0.013
Filter 4 outflow	0.006	O-S-W	2.9	2.0	0.002
Filter 5 outflow	0.346	O-S-T	4.4	2.0	< 0.001
Filter 6 outflow	0.000	O-S-W	4.6	2.0	< 0.001
Filter 7 outflow	0.000	O-S-W	3.6	2.0	0.001
Filter 8 outflow	0.000	O-S-W	3.3	2.0	0.002
Control A outflow	0.078	O-S-T	1.8	2.0	0.207
Control B outflow	0.011	O-S-W	1.9	2.0	0.306
Wastewater (20%); tap water (80%)	0.566	O-S-T	3.0	2.0	0.005
Wastewater (100%)	0.566	O-S-T	14.9	2.0	< 0.001

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter).
O-S-T, the parametric one sample t-test, O-S-W, the non-parametric Wilcoxon signed rank test.

Table 4.7: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	Variable						
		TPH	COD	BOD	DO	NH_4-N	NO_3-N	PO_4-P
TPH	R	1.000	0.694*	0.381	-0.427	-0.356	-0.304	-0.200
	p	n.a	0.026	0.277	0.219	0.313	0.393	0.579
COD	R	0.694*	1.000	0.815**	-0.574	0.298	-0.243	0.371
	p	0.026	n.a	0.004	0.083	0.403	0.498	0.291
BOD	R	0.381	0.815**	1.000	-0.351	0.564	-0.079	0.648*
	p	0.277	0.004	n.a	0.320	0.090	0.829	0.043
DO	R	-0.427	-0.574	-0.351	1.000	-0.320	0.117	-0.357
	p	0.219	0.083	0.320	n.a	0.367	0.748	0.311
NH_4-N	R	-0.356	0.298	0.564	-0.320	1.000	0.394	0.903**
	p	0.313	0.403	0.090	0.367	n.a	0.260	0.000
NO_3-N	R	-0.304	-0.243	-0.079	0.117	0.394	1.000	0.394
	p	0.393	0.498	0.829	0.748	0.260	n.a	0.260
PO_4-P	R	-0.200	0.371	0.648*	-0.357	0.903**	0.394	1.000
	p	0.579	0.291	0.043	0.311	0.000	0.260	n.a

Note: TPH, total petroleum hydrocarbon (µg/l); COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); DO, dissolved oxygen (mg/l); NH_4-N, ammonia-nitrogen (mg/l); NO_3-N, nitrate-nitrogen (mg/l); PO_4-P, ortho-phosphate-phosphorus (mg/l); R, correlation coefficient; *p,* probability of the statistical test (if p-value > 0.05, the variables are not statistically significantly correlated, if p-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level.

4.2.4 Comparison of trace elements

Table 4.8 provides an overview of the ICP–OES analysis for selected elements determined in the irrigation waters. Results show that iron concentrations were observed with highest values in raw wastewater followed by those associated with filters contaminated with hydrocarbons (Filters 1, 3 and 5), while the lowest values were recorded for outflow water from Control B followed by those for deionised water. Filters 3 and 4 outflow waters had iron concentrations greater than those for Filters 1 and 2, respectively, indicating the impact of wetland aggregate diameter on iron removal processes. Filter 4 outflow water showed iron levels which were lower than those for Filter 7, while outflow water from Filter 7 was observed with iron concentrations greater than those for Filter 8 outflow explaining the impact of both contact and resting time on iron removal processes.

Moreover, hydrocarbon contamination showed a significant ($p < 0.05$) impact on outflow waters iron levels when comparing Filters 1, 3, 5 and Control A outflow waters with those associated with Filters 2, 4, 6 and Control B (Table 4.9). Compared to the standards of 5.0 mg/l (FAO, 1994, 2003), all irrigation water types were reported with iron concentrations which were significantly lower than the threshold (Table 4.10). Moreover, correlation analysis (Table 4.11) shows that iron concentration values were significantly positively ($p < 0.05$) correlated with chemical and biochemical oxygen demands, ortho-phosphate-phosphorus, manganese, potassium, boron, calcium and magnesium, but were negatively correlated with the dissolved oxygen values in the treatment system (Almuktar & Scholz, 2016 a, b). According to the previous studies (Wiseman & Edwards, 2004; Lesley et al., 2008), iron can be removed from the wastewater treated by wetland systems mainly through oxidative processes and iron hydroxides formation. Moreover, biotic processes can be significantly considered in iron removal processes in treatment system as reported by Lesley et al. (2008). Oxygen availability and water pH can be also considered as crucial factors in iron removal processes as discussed by Goulet and Pick (2001) contributing to the impact of plant photosynthesis which may considerably control the oxygen levels in the wetland systems and subsequent impact on iron removal rate mainly for concentrations of less than 2 mg/l as confirmed by Batty and Younger (2002). Furthermore, bacterial communities also can mediate the iron oxidation in the system as reported by Lesley et al. (2008).

Table 4.8 shows that manganese concentrations were the highest for outflow waters from filters which were contaminated with hydrocarbons (Filters 1, 3 and 5), while the lowest values were observed for Control B outflow water. The outflow waters of filters of large aggregate size (Filters 1 and 2) showed manganese concentrations which were lower than those for filters of small aggregate diameter (Filters 3 and 4, respectively). Filter 4 outflow water had manganese levels which were lower than those for Filter 7 outflow, while the latter showed values which were greater than those for Filter 8 drain water, explaining the impact of contact and resting times of the wetlands system on manganese removal processes. Statistical analysis results (Table 4.9) show that irrigation waters harvested from filters contaminated with diesel spills (Filters 1, 3, 5 and Control A) showed manganese levels which were significantly ($p < 0.05$) greater than those for standard filters (uncontaminated with diesel) as reported by other studies (Almuktar & Scholz, 2016 a, b). Compared to the standards of 0.2 mg/l for manganese (Pescod, 1992; FAO, 2003), most of the irrigation water types had manganese concentrations which were significantly ($p < 0.05$) lower than the thresholds (Table 4.10). However, results for Filters 3 and 5, which were contaminated with diesel, show relatively high manganese concentrations, which exceeded the threshold. Correlation analysis results (Table 4.11) showed that manganese values were significantly (p <0.05) positively correlated with chemical and biochemical oxygen demands, ortho-phosphate-phosphorus, iron, potassium and magnesium values as well as with the monitored microorganisms in the system such as total coliforms, *Escherichia coli and Salmonella* spp. confirming the results reported by another study (Burdige, Dhakar, & Nealson, 1992) indicating that there is a significantly positively correlation (R = 0.61; $p < 0.01$) between manganese and heterotrophic bacteria recovered on different strengths of nutrient agar. Manganese removal in wetland systems was reported to be related to oxygenic photosynthesis resulting in high removal rates during the summer season (Hallberg & Johnson, 2005). Lesley et al. (2008) indicated that manganese can be removed successfully using wetland systems referring that iron need to be removed before manganese removal is able to take place.

High manganese concentrations resulted in low growth rates of plants (Rahimi et al., 2013), particularly for peppers grown in sand. However, manganese is an essential trace element for most plants, intervening in several metabolic processes, mainly in photosynthesis. Nevertheless, an excess of this micronutrient is often toxic for plants. Manganese phyto-toxicity is exhibited in a reduction of biomass and photosynthesis, and biochemical disorders including oxidative stress (Millaleo, Reyes-Díaz, Ivanov, Mora, & Alberdi, 2010).

Table 4.8 shows that zinc concentrations in irrigation waters were highest for raw wastewater followed by wastewater samples diluted with tap water, while the lowest value was observed for deionised water. Outflow waters from Filters 1 and 2 of large aggregate diameter showed zinc concentrations which were lower than those for Filters 3 and 4, respectively, which had small aggregate size. Filters 4 and 7 outflow waters had zinc concentrations which were different from those of Filters 7 and 8, respectively, due to differences in both contact and resting time in the design of the wetland system. Moreover, irrigation water harvested from Filter 6 had zinc concentrations which were higher than those for Filter 4 due to differences in inflow loading rate. However, statistical analysis results (Table 4.9) do not show any significant differences in irrigation water zinc concentrations associated with wetland filters of different designs, indicating that aggregate diameter, contact time, resting time and inflow loading rate may not matter in zinc removal processes (Almuktar & Scholz, 2016 a, b). This agrees with the results reported by Ying et al (2001) who investigated the efficiency of eight laboratory-scale constructed wetlands to treat heavy metals in synthetic mine water which indicated that hydraulic loading, and substrate composition did not usually affect the treatment efficiency. Compared to the standard of 2.0 mg/l for zinc (FAO, 1994, 2003), statistical analysis (Table 4.10) shows that all irrigation water types were significantly ($p < 0.05$) lower than the threshold. According to Sheoran and Sheoran (2006), heavy metals can be removed in wetland systems via physical, chemical and biological processes including sedimentation, settling, filtration, adsorption, precipitation, co-precipitation into insoluble compounds.

Table 4.8 shows that the highest potassium concentration values were recorded for samples of tap water spiked with fertiliser followed by those for raw wastewater and outflow waters from filters of high inflow loading rate (Filters 5 and 6), while the lowest potassium values were observed for deionised water. Statistical analysis results (Table 4.9) show that Filter 2 outflow water had potassium concentrations which were significantly ($p < 0.05$) greater than those for Filter 4, while the latter outflow had potassium levels which were significantly ($p < 0.05$) lower than those for Filter 7, explaining the impact of aggregate diameter and contact time variables, respectively, of wetland design on potassium treatment (Almuktar & Scholz, 2016 a, b).

Wetland filters which were fed with undiluted wastewater had outflow water with potassium concentrations which were significantly ($p < 0.05$) greater than those of diluted inflow wastewater as shown when comparing Filter 6 with Filter 4. This agrees with the results reported by Sani et al. (2013) indicating that filters of high inflow loading rate tend to be overloaded. Moreover, Filters 1, 3, 5 and Control A outflow waters showed potassium concentrations which were significantly ($p < 0.05$) higher than those for Filters 2, 4, 6 and Control B, respectively, explaining the impact of hydrocarbon contamination on potassium treatment. Compared to the standards of 2.0 mg/l of potassium (FAO,1994, 2003), most of the irrigation water types were observed with high potassium concentrations which significantly ($p < 0.05$) exceeded the thresholds (Table 4.10).

Correlation analysis results (Table 4.11) show that potassium concentration values were significantly ($p < 0.05$) positively correlated with most other variables in the system, such as biochemical oxygen demand, ammonia-nitrogen, ortho-phosphate-phosphorus, iron, manganese, boron, sodium, calcium and magnesium (Almuktar & Scholz, 2016 a, b). This result confirmed findings from other studies (Choi, Yu, Lee, & Yu, 2011), explaining that there are linear correlation coefficients between the pairs potassium and ortho-phosphate-phosphorus, and magnesium and ortho-phosphate-phosphorus, while assessing the role of potassium, magnesium and calcium ions in enhanced biological phosphorus removal from wastewaters using membrane bioreactors. Cakmak (2005) reported that increasing potassium concentration in irrigation water provided important protection against stem damage from low night temperatures in plants. Furthermore, decreases in yield and increases in leaf damage induced by frost under field conditions could be alleviated by high application of potassium fertiliser. Hakerlerler, Oktay, Eryüce, and Yagmur (1997) indicated that improvement in low-temperature-stress tolerance of plants by increasing potassium supply was also shown in tomato, pepper, and eggplant seedlings growing outside, with temperatures ranging from 4 to 16 °C. Depending on the source of potassium fertilisers, potassium supply enhanced total plant yield by 2.4-fold, 1.9-fold, and 1.7-fold in tomato, pepper, and eggplant, respectively. Moreover, potassium supply also reduced the rate of seedling death due to low temperature (Cakmak, 2005).

Table 4.8 shows that the highest boron concentration values were observed in raw wastewater followed by those for Filters 6 and 1 of high inflow loading rate and large aggregate diameter, respectively, while the lowest boron values were observed in deionised and tap waters. Filters 1 and 2 outflow waters had boron concentrations higher than those for Filters 3 and 4, respectively due to differences in aggregate diameter. Filters 4 and 7 harvested waters had boron concentrations which were relatively close to those for Filters 7 and 8, respectively. Moreover, irrigation waters harvested from Filters 5 and 6 of high inflow loading rate had boron concentrations greater than those for Filters 3 and 4 of diluted inflow waters, respectively. Furthermore, Filters 1, 3 and Control A drained waters had boron values greater than those of Filters 2, 4 and Control B, respectively, due to hydrocarbon contamination. However, statistical results (Table 4.9) do not show any significant ($p > 0.05$) differences among boron concentrations associated with wetland filters of different design indicating that aggregate diameter, contact time, resting time and inflow loading rate may not matter in boron treatment (Almuktar &Scholz, 2016 a, b). Compared to the standard of 0.75 mg/l for boron (FAO, 1994, 2003), statistical analysis (Table 4.10) shows that all irrigation water types had boron concentration values which were significantly ($p < 0.05$) lower than the threshold. Correlation analysis results (Table 4.11) show than boron concentrations were significantly ($p < 0.05$) positively correlated with most other elements in the system, such as biochemical oxygen demand, ammonia-nitrogen, ortho-phosphate-phosphorus, iron, potassium, sodium and magnesium (Almuktar et al., 2015 a, b).

Table 4.8 shows that sodium concentrations were observed with the highest values in raw wastewater followed by those for the outflow waters obtained from Filters 6 and 5 of high inflow loading rate in wetland system, while the lowest sodium concentrations were recorded for the deionised water followed by tap water. Drain waters from Filters 1 and 2, of large aggregate diameter, showed sodium concentrations similar to those of Filters 3 and 4 of small aggregate size, respectively. Irrigation waters harvested from Filters 4 and 7 had sodium concentrations higher than those for Filters 7 and 8, respectively due to contact time and resting time differences, in that order.

Moreover, statistical analysis results (Table 4.9) showed that waters harvested from Filters 3 and 4 had sodium concentrations which were significantly ($p < 0.05$) lower than those of Filters 5 and 6, respectively, explaining the impact of high inflow loading rate of wetlands on outflow water sodium concentrations resulting in filter overloading (Sani et al., 2013). Compared to the standard of 920 mg/l for sodium (FAO, 2003), statistical analysis results (Table 4.10) show that all irrigation water types had sodium concentrations which were significantly ($p < 0.05$) lower than the threshold. Moreover, correlation analysis results (Table 4.11) show that sodium concentrations were significantly ($p < 0.05$) positively correlated with other elements in the system such as ammonia-nitrogen, ortho-phosphate-phosphorus, potassium, boron, calcium and magnesium (Essington, 2015; Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 a, b).

Calcium concentrations were observed with highest values in raw wastewater followed by irrigation waters harvested from Filter 3 then Filters 5 and 6, while the lowest values were recorded for deionised and tap waters (Table 4.8). Statistical analysis results (Table 4.9) show that irrigation water obtained from Filters 1 and 2 had calcium concentrations which were significantly ($p < 0.05$) lower than those for Filters 3 and 4, respectively due to differences in aggregate diameter. Moreover, Filters 4 and 7 had calcium concentrations different from those of Filters 7 and 8, respectively, due to differences in contact and resting times, in that order, while water harvested from Filter 4 which was fed with diluted wastewater had calcium concentrations lower than those for Filter 6 of high inflow loading rate. Furthermore, filters contaminated with hydrocarbons showed calcium concentrations higher than those for uncontaminated ones (Table 4.8). Compared to the standards of 400 mg/l for calcium (FAO, 2003), statistical analysis results (Table 4.10) show that all irrigation water types had calcium concentrations which were significantly ($p < 0.05$) lower than the threshold. Moreover, correlation analysis results (Table 4.11) show that calcium concentrations were significantly ($p < 0.05$) positively correlated with iron, zinc, potassium, sodium, and magnesium values in the system (Essington, 2015; Almuktar & Scholz, 2016 a, b).

The highest magnesium concentrations were observed in raw wastewater followed by those for irrigation waters harvested from wetland filters of high inflow loading rate, while the lowest magnesium concentrations were recorded for deionised and tap waters (Table 4.8). Filters 1 and 2 drain waters had magnesium concentrations lower than those for Filters 3 and 4, respectively, due to differences in aggregate diameter, while magnesium values in irrigation waters obtained from Filters 4 and 7 were higher than those harvested from Filters 7 and 8, in that order, explaining the impact of contact and resting times, respectively, on magnesium treatment by the wetland system. Statistical analysis results (Table 4.9) show that outflow waters from wetland filters of high inflow loading rate had magnesium levels which were significantly ($p < 0.05$) greater than those from filters which were fed with diluted wastewater. Moreover, filters which were contaminated with hydrocarbons showed magnesium concentrations which were relatively higher than those for uncontaminated filters. Compared to the standard of 60 mg/l for magnesium (FAO, 2003), statistical analysis results (Table 4.10) show that all irrigation water types had magnesium levels which were significantly ($p < 0.05$) lower than the threshold. Moreover, correlation analysis results (Table 4.11) show that magnesium concentrations were significantly ($p < 0.05$) positively correlated with most other variables in the treatment system (Essington, 2015). Generally, the irrigation waters linked to the wetland system showed highly fluctuating quality during different crop growth periods (Table 4.8) due to the seasonal variation of wetland system behaviour (Scholz, 2010, 2011; Sani et al., 2013).

However, in constructed wetlands, heavy metals and trace elements can be removed by various mechanisms. For example, Denga, Yea, and Wonga (2004); Galletti, Verlicchi, and Ranieri (2010) and Guittonny-Philippe et al. (2014) reported that these elements can be removed via different physical, chemical, and biological processes performed in the wetland systems, such as settling, sedimentation sorption, adsorption, complexation, cation and anion exchange, oxidation and reduction, chemical precipitation and co-precipitation as insoluble salts, photo-degradation, phyto-accumulation, biodegradation, microbial activity, and plant uptake. In vertical flow wetlands, these elements are most likely to accumulate in the litter layer at the top of the system, while in horizontal flow wetlands, heavy metals and trace elements tend to accumulate near the system inlet regardless of elimination pathways (Cheng et al., 2002).

Moreover, most of those elements available in the wastewater are removed in wetlands through the interaction with system media after treatment by wetland plants which is considered as a polishing system, as stated by Matagi, Swai, and Muganbe (1998) and

Guittonny-Philippe et al. (2014). Moreover, in wetland systems, the heavy metals can be removed effectively by settling and sedimentation processes after a series of dynamic transformations performed in the system (Matagi et al., 1998; Prestes et al., 2006; Terzakis et al., 2008). However, the sedimentation of those heavy metals will occur after their agglomeration to bigger particles that can be trapped by wetland sediment, as reported by Walker and Hurl (2002).

According to Scholz (2006, 2010), wetlands macrophytes can also be considered as a trapper to the metal solids available in the wastewater while it passes through the surface of system plants. Moreover, the accumulation of heavy metals in wetland biomass can be considered as a predominant way to eliminate those metals in the wetland system as reported by Madera-Parra et al. (2015) who agreed with the observation reported by Guittonny-Philippe et al. (2014) showing that the heavy metals in the wetland system can be removed by accumulation in the system sediment as well as in different parts of macrophyte tissue such as roots, stems, leaves and shoots. Furthermore, sorption process in wetland systems which include adsorption, absorption and precipitation reactions can be considered as the main chemical means of heavy metal removal (Marchand et al., 2010). However, wetland macrophytes can uptake heavy metals with different capacities depending on several factors, such as plants species, heavy metal levels, sediment chemistry and pH, in addition to the temperature and organic matter content as reported by Sheoran (2004); Sheoran and Sheoran (2006); Liu et al. (2007) and Marchand et al. (2010).

Table 4.8: Overview of the Inductively Coupled Plasma (ICP) Optical Emission Spectrometer analysis for the detected trace elements in the irrigation waters received by vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	FPPBF[b]	FPPAF[c]
Iron (mg/l)			
Filter 1	1.053±1.6347 (13)	0.995±n.a (1)	1.057±1.7073 (12)
Filter 2	0.186±0.2341 (13)	0.056±n.a (1)	0.197±0.2411 (12)
Filter 3	1.468±1.6413 (9)	3.145±n.a (1)	1.258±1.6207 (8)
Filter 4	0.190±0.1561 (11)	0.173±n.a (1)	0.191±0.1644 (10)
Filter 5	1.317±1.1867 (13)	0.722±n.a (1)	1.366±1.2253 (12)
Filter 6	0.552±0.6591 (13)	1.407±n.a (1)	0.481±0.6340 (12)
Filter 7	0.480±1.0679 (12)	0.195±n.a (1)	0.506±1.1161 (11)
Filter 8	0.304±0.5748 (10)	0.122±n.a (1)	0.325±0.6058 (9)
Control A	0.123±0.1268 (15)	0.182±n.a (1)	0.119±0.1304 (14)
Control B	0.041±0.0319 (14)	0.045±n.a (1)	0.041±0.0332 (13)
DW	0.053±0.0031 (4)	0.053±n.a (1)	0.053±0.0031 (3)
TW	0.743±2.1115 (10)	6.752±n.a (1)	0.075±0.0026 (9)
TW+F	0.833±2.1141 (9)	6.764±n.a (1)	0.092±0.0055 (8)
TW+WW	0.531±0.7463 (11)	0.992±n.a (1)	0.485±0.7701 (10)
WW	2.330±4.1918 (12)	10.072±n.a (1)	1.627±3.5763 (11)
Manganese (mg/l)			
Filter 1	0.131±0.1420 (13)	0.099±n.a (1)	0.133±0.1479 (12)
Filter 2	0.042±0.0585 (13)	0.000±n.a (1)	0.045±0.0596 (12)
Filter 3	0.263±0.2041 (9)	0.539±n.a (1)	0.229±0.1881 (8)
Filter 4	0.052±0.0678 (11)	0.000±n.a (1)	0.057±0.0691 (10)
Filter 5	0.258±0.1888 (13)	0.237±n.a (1)	0.259±0.1971 (12)
Filter 6	0.078±0.1110 (13)	0.000±n.a (1)	0.085±0.1134 (12)
Filter 7	0.087±0.2021 (12)	0.020±n.a (1)	0.093±0.2108 (11)
Filter 8	0.054±0.0999 (10)	0.000±n.a (1)	0.060±0.1040 (9)
Control A	0.063±0.0563 (15)	0.064±n.a (1)	0.062±0.0584 (14)
Control B	0.019±0.0339 (14)	0.000±n.a (1)	0.020±0.0349 (13)
DW	0.078±0.0011 (4)	0.078±n.a (1)	0.078±0.0011 (3)
TW	0.081±0.0025 (8)	0.082±n.a (1)	0.081±0.0027 (7)
TW+F	0.087±0.0018 (8)	0.086±n.a (1)	0.087± 0.0018(7)
TW+WW	0.061±0.0498 (11)	0.023±n.a (1)	0.065±0.0508 (10)
WW	0.129±0.12224 (12)	0.036±n.a (1)	0.137±0.1246 (11)
Zinc (mg/l)			
Filter 1	0.042±0.0407 (13)	0.100±n.a (1)	0.037±0.0384 (12)
Filter 2	0.053±0.0469 (13)	0.170±n.a (1)	0.044±0.0325 (12)
Filter 3	0.086±0.1324 (9)	0.412±n.a (1)	0.046±0.0549 (8)
Filter 4	0.060±0.0659 (11)	0.145±n.a (1)	0.051±0.0627 (10)
Filter 5	0.085±0.1033 (13)	0.118±n.a (1)	0.082±0.1074 (12)
Filter 6	0.095±0.0927 (13)	0.194±n.a (1)	0.086±0.0917 (12)
Filter 7	0.056±0.0435 (12)	0.163±n.a (1)	0.046±0.0287 (11)
Filter 8	0.105±0.1305 (10)	0.243±n.a (1)	0.089±0.1285 (9)
Control A	0.045±0.0747 (15)	0.239±n.a (1)	0.031±0.0539 (14)
Control B	0.076±0.1427 (14)	0.125±n.a (1)	0.073±0.1478 (13)
DW	0.043±0.0013 (4)	0.043±n.a (1)	0.043±0.0013 (3)
TW	0.118± 0.1510(9)	0.520±n.a (1)	0.068±0.0094 (8)
TW+F	0.127±0.1486 (9)	0.522±n.a (1)	0.077±0.0111 (8)
TW+WW	0.149±0.1632 (11)	0.324±n.a (1)	0.131±0.1608 (10)
WW	0.296±0.5506 (12)	0.241±n.a (1)	0.301±0.5772 (11)

Table 4.8 (cont.)

Parameter	Overall[a]	FPPBF[b]	FPPAF[c]
Potassium (mg/l)			
Filter 1	7.741±4.2855 (13)	8.395±n.a (1)	7.687±4.4714 (12)
Filter 2	7.073±2.8315 (13)	7.344±n.a (1)	7.050±2.9562 (12)
Filter 3	8.545±3.3270 (9)	10.659±n.a (1)	8.280±3.4542 (8)
Filter 4	3.471±2.5491 (11)	5.115±n.a (1)	3.307± 2.6248(10)
Filter 5	11.635±4.3243 (13)	11.684±n.a (1)	11.631±4.5165 (12)
Filter 6	10.909±4.8546 (13)	16.780±n.a (1)	10.419±4.7238 (12)
Filter 7	6.498±2.5958 (12)	7.605±n.a (1)	6.397±2.6979 (11)
Filter 8	4.929±3.7894 (10)	5.935±n.a (1)	4.818±4.0017 (9)
Control A	1.550±1.5399 (15)	1.184±n.a (1)	1.576±1.5946 (14)
Control B	0.635±0.6803 (14)	0.705±n.a (1)	0.630±0.7078 (13)
DW	< 0.0003±n.a (4)	< 0.0003±n.a (1)	< 0.0003±n.a (3)
TW	0.416±0.1095 (6)	0.636±n.a (1)	0.372±0.0205 (5)
TW+F	18.898±0.2275 (4)	18.898±n.a (1)	18.898± 0.2275(3)
TW+WW	3.583±0.7465 (10)	4.286±n.a (1)	3.505±0.7472 (9)
WW	15.931±7.2402 (12)	11.144±n.a (1)	16.366±7.4272 (11)
Boron (mg/l)			
Filter 1	0.057±0.0541 (13)	0.131±n.a (1)	0.051±0.0516 (12)
Filter 2	0.046±0.0418 (13)	0.093± n.a (1)	0.042±0.0411 (12)
Filter 3	0.041±0.0486 (9)	0.127± n.a (1)	0.031±0.0390 (8)
Filter 4	0.035±0.0313 (11)	0.081± n.a (1)	0.030±0.0288 (10)
Filter 5	0.054±0.0435 (13)	0.104± n.a (1)	0.050±0.0426 (12)
Filter 6	0.060±0.0475 (13)	0.082± n.a (1)	0.058±0.0491 (12)
Filter 7	0.036±0.0339 (12)	0.061± n.a (1)	0.033±0.0346 (11)
Filter 8	0.033±0.0342 (10)	0.049± n.a (1)	0.032±0.0358 (9)
Control A	0.007±0.0097 (15)	0.006± n.a (1)	0.007±0.0101 (14)
Control B	0.004±0.0063 (14)	0.008± n.a (1)	0.003±0.0064 (13)
DW	< 0.0001±n.a (3)	< 0.0001±n.a (1)	< 0.0001±n.a (2)
TW	< 0.0001±n.a (5)	< 0.0001±n.a (1)	< 0.0001±n.a (4)
TW+F	0.001±00017 (5)	0.003± n.a (1)	0.001±0.0016 (4)
TW+WW	0.030±0.0602 (10)	0.024± n.a (1)	0.031±0.0638 (9)
WW	0.083±0.0686 (12)	0.118± n.a (1)	0.080±0.0711 (11)
Sodium (mg/l)			
Filter 1	35.106±16.0134 (13)	32.069±n.a (1)	35.359±16.6982 (12)
Filter 2	36.433±7.8479 (13)	29.666±n.a (1)	36.997±7.9169 (12)
Filter 3	36.301±10.6442 (9)	41.742±n.a (1)	35.621±11.1681 (8)
Filter 4	38.005±6.0328 (11)	37.240±n.a (1)	38.081±6.3535 (10)
Filter 5	52.153±15.5837 (13)	47.268±n.a (1)	52.560±16.2043 (12)
Filter 6	58.893±22.8294 (13)	53.288±n.a (1)	59.360±23.7795 (12)
Filter 7	36.774±12.2925 (12)	50.727±n.a (1)	35.505±12.0406 (11)
Filter 8	34.160±12.4370 (10)	29.928±n.a (1)	34.631±13.0968 (9)
Control A	8.760±7.2991 (15)	5.857±n.a (1)	8.967±7.5287 (14)
Control B	5.939±2.3780 (14)	6.647±n.a (1)	5.885±2.4660 (13)
DW	0.058±0.1150 (4)	0.230±n.a (1)	0.000±0.0000 (3)
TW	4.648±1.3643 (6)	7.381±n.a (1)	4.101±0.2926 (5)
TW+F	5.555±1.1779 (6)	7.424±n.a (1)	5.181±0.8287 (5)
TW+WW	17.823±3.0869 (9)	20.559±n.a (1)	17.481±3.1125 (8)
WW	60.393±19.9412 (11)	48.685±n.a (1)	61.563±20.1675 (10)

Table 4.8 (cont.)

Parameter	Overall[a]	FPPBF[b]	FPPAF[c]
Calcium (mg/l)			
Filter 1	31.613±12.3804 (10)	36.815±n.a (1)	31.035±12.9875 (9)
Filter 2	30.467±3.5511 (10)	32.382±n.a (1)	30.255±3.6983 (9)
Filter 3	60.349±31.4825 (6)	119.167±n.a (1)	48.586±14.1804 (5)
Filter 4	43.895±7.2237 (8)	49.940±n.a (1)	43.031±7.3428 (7)
Filter 5	57.189±15.2902 (10)	72.675±n.a (1)	55.468±15.1560 (9)
Filter 6	53.687±13.9916 (10)	76.426±n.a (1)	51.160±12.1872 (9)
Filter 7	38.780±8.4831 (9)	54.201±n.a (1)	36.852±6.6351 (8)
Filter 8	39.464±7.3106 (7)	44.283±n.a (1)	38.661±7.6626 (6)
Control A	24.560±5.4283 (12)	23.791±n.a (1)	24.630±5.6876 (11)
Control B	25.805±6.1408 (11)	29.693±n.a (1)	25.416±6.3287 (10)
DW	0.000±0.0000 (4)	0.000±n.a (1)	0.000±0.0000 (3)
TW	10.372±0.2162 (7)	10.372±n.a (1)	10.318±0.1774 (6)
TW+F	10.400±0.2162 (7)	10.400±n.a (1)	10.346±0.1774 (6)
TW+WW	22.577±1.7522 (7)	23.285±n.a (1)	22.460±1.8888 (6)
WW	62.574±15.5820 (8)	67.864±n.a (1)	61.819±16.6714 (7)
Magnesium (mg/l)			
Filter 1	5.252±2.3837 (13)	4.757±n.a (1)	5.294±2.4849 (12)
Filter 2	5.014±0.7438 (13)	4.355±n.a (1)	5.069±0.7489 (12)
Filter 3	5.963±1.3861 (9)	7.156±n.a (1)	5.814±1.4024 (8)
Filter 4	5.264±0.6378 (11)	5.776±n.a (1)	5.213±0.6480 (10)
Filter 5	8.628±2.4527 (13)	7.837±n.a (1)	8.694±2.5497 (12)
Filter 6	8.726±2.6745 (13)	9.351±n.a (1)	8.674± 2.7869(12)
Filter 7	5.118±1.1393 (12)	5.582±n.a (1)	5.075±1.1850 (11)
Filter 8	4.843±1.4353 (10)	4.998±n.a (1)	4.825±1.5212 (9)
Control A	1.587±0.9720 (15)	1.100±n.a (1)	1.622±0.9989 (14)
Control B	1.275±0.3935 (14)	1.348±n.a (1)	1.269±0.4090 (13)
DW	0.098± 0.0325(5)	0.050±n.a (1)	0.110±0.0207 (4)
TW	0.965±0.1169 (13)	1.241±n.a (1)	0.942±0.0861 (12)
TW+F	1.083±0.1544 (13)	1.269±n.a (1)	1.067±0.1502 (12)
TW+WW	3.033±0.4183 (11)	3.214±n.a (1)	3.015±0.4364 (10)
WW	10.527±3.9020 (12)	10.030±n.a (1)	10.572±4.0892 (11)

Note: [a] 11/10/13 to 25/09/14. [b] Final planting period before fruiting: 08/11/13 to 19/01/14. [c] Final planting period after fruiting: 20/01/14 to 25/09/14. Detection limits (mg/l) are: 0.10×10^{-3}, 0.03×10^{-3}, 0.20×10^{-3}, 0.30×10^{-3}, 0.10×10^{-3}, 0.15×10^{-3}, 0.01×10^{-3} and 0.01×10^{-3} for iron, manganese, zinc, potassium, boron, sodium, calcium and magnesium, respectively. Elements not listed in this table (i.e., arsenic, barium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, strontium and titanium) were either below (or close to) the detection limits or could not be measured via the ICP–OES technology. No data were available for the second planting period (11/10/13 to 07/11/13). DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). n.a, not applicable.

Table 4.9: Statistical assessment of detected trace elements for irrigation waters linked to wetland filters during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b] for different system combinations
Iron (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.664
Filter 2 & Filter 4	< 0.001	M-W-U	0.401
Contact time			
Filter 4 & Filter 7	0.001	M-W-U	0.712
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.947
Inflow loading rate			
Filter 3 & Filter 5	0.003	M-W-U	0.867
Filter 4 & Filter 6	< 0.001	M-W-U	0.068
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.029
Filter 3 & Filter 4	< 0.001	M-W-U	0.119
Filter 5 & Filter 6	0.001	M-W-U	0.038
Control A & Control B	< 0.001	M-W-U	0.023
Manganese (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	0.002	M-W-U	0.124
Filter 2 & Filter 4	< 0.001	M-W-U	0.683
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.902
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.596
Inflow loading rate			
Filter 3 & Filter 5	0.066	M-W-U	0.867
Filter 4 & Filter 6	0.001	M-W-U	0.399
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.027
Filter 3 & Filter 4	0.001	M-W-U	0.022
Filter 5 & Filter 6	0.002	M-W-U	0.002
Control A & Control B	< 0.001	M-W-U	0.007
Zinc (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.570
Filter 2 & Filter 4	< 0.001	M-W-U	0.602
Contact time			
Filter 4 & Filter 7	0.001	M-W-U	0.460
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.843
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.593
Filter 4 & Filter 6	< 0.001	M-W-U	0.132
Hydrocarbon influence			
Filter 1 & Filter 2	0.002	M-W-U	0.317
Filter 3 & Filter 4	< 0.001	M-W-U	0.970
Filter 5 & Filter 6	< 0.001	M-W-U	0.369
Control A & Control B	< 0.001	M-W-U	0.337

Table 4.9 (cont.)

Design variables	Shapiro-Wilk test (p-value)[a]	Statistical test	P-values[b] for different system combinations
		Potassium (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.482	I-T	0.642
Filter 2 & Filter 4	0.097	I-T	0.004
Contact time			
Filter 4 & Filter 7	0.095	I-T	0.010
Resting time			
Filter 7 & Filter 8	0.495	I-T	0.264
Inflow loading rate			
Filter 3 & Filter 5	0.320	I-T	0.157
Filter 4 & Filter 6	0.052	I-T	< 0.001
Hydrocarbon influence			
Filter 1 & Filter 2	0.654	I-T	0.643
Filter 3 & Filter 4	0.044	M-W-U	0.004
Filter 5 & Filter 6	0.873	I-T	0.882
Control A & Control B	< 0.001	M-W-U	0.018
		Boron (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.013	M-W-U	0.384
Filter 2 & Filter 4	0.013	M-W-U	0.321
Contact time			
Filter 4 & Filter 7	0.033	M-W-U	0.951
Resting time			
Filter 7 & Filter 8	0.014	M-W-U	0.691
Inflow loading rate			
Filter 3 & Filter 5	0.042	M-W-U	0.364
Filter 4 & Filter 6	0.052	I-T	0.152
Hydrocarbon influence			
Filter 1 & Filter 2	0.012	M-W-U	0.589
Filter 3 & Filter 4	0.010	M-W-U	0.939
Filter 5 & Filter 6	0.083	I-T	0.750
Control A & Control B	0.000	M-W-U	0.305
		Sodium (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.040	M-W-U	0.404
Filter 2 & Filter 4	0.164	I-T	0.593
Contact time			
Filter 4 & Filter 7	0.678	I-T	0.767
Resting time			
Filter 7 & Filter 8	0.763	I-T	0.627
Inflow loading rate			
Filter 3 & Filter 5	0.241	I-T	0.016
Filter 4 & Filter 6	0.112	I-T	0.007
Hydrocarbon influence			
Filter 1 & Filter 2	0.019	M-W-U	0.778
Filter 3 & Filter 4	0.010	M-W-U	0.909
Filter 5 & Filter 6	0.104	I-T	0.388
Control A & Control B	< 0.001	M-W-U	0.631

Table 4.9 (cont.)

Design variables	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b] for different system combinations
		Calcium (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.006	M-W-U	0.023
Filter 2 & Filter 4	0.024	M-W-U	0.001
Contact time			
Filter 4 & Filter 7	0.571	I-T	0.204
Resting time			
Filter 7 & Filter 8	0.233	I-T	0.868
Inflow loading rate			
Filter 3 & Filter 5	0.048	M-W-U	0.745
Filter 4 & Filter 6	0.877	I-T	0.092
Hydrocarbon influence			
Filter 1 & Filter 2	0.314	I-T	0.784
Filter 3 & Filter 4	< 0.001	M-W-U	0.245
Filter 5 & Filter 6	0.990	I-T	0.600
Control A & Control B	0.034	M-W-U	0.498
		Magnesium (mg/l)	
Aggregate diameter			
Filter 1 & Filter 3	0.052	I-T	0.432
Filter 2 & Filter 4	0.568	I-T	0.391
Contact time			
Filter 4 & Filter 7	0.017	M-W-U	0.806
Resting time			
Filter 7 & Filter 8	0.088	I-T	0.622
Inflow loading rate			
Filter 3 & Filter 5	0.685	I-T	0.008
Filter 4 & Filter 6	0.040	M-W-U	0.001
Hydrocarbon influence			
Filter 1 & Filter 2	0.003	M-W-U	0.626
Filter 3 & Filter 4	0.125	I-T	0.152
Filter 5 & Filter 6	0.002	M-W-U	0.898
Control A & Control B	< 0.001	M-W-U	0.663

Note: [a] Test of normality (if *p*-value > 0.05, data are normally distributed; if *p*-value < 0.05, data are not normally distributed. [b] *p*-value, probability of the statistical test. Filters are statistically significantly different only if the *p*-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test. I-T, the parametric Independent samples T-test.

Table 4.10: Statistical assessment of detected trace elements for irrigation waters compared to standards.

Irrigation water type	Shapiro-Wilk test (*p*-value)[a]	Statistical test	Mean value (mg/l)	Standard (mg/l)	Statistic (*p*-value)[a]
		Iron			
Filter 1 outflow	0.007	O-S-W	1.053	5.0	0.002
Filter 2 outflow	0.022	O-S-W	0.186	5.0	0.001
Filter 3 outflow	0.105	O-S-T	1.468	5.0	< 0.001
Filter 4 outflow	0.057	O-S-T	0.190	5.0	< 0.001
Filter 5 outflow	0.040	O-S-W	1.317	5.0	0.001
Filter 6 outflow	0.001	O-S-W	0.552	5.0	0.001
Filter 7 outflow	0.003	O-S-W	0.480	5.0	0.002
Filter 8 outflow	< 0.001	O-S-W	0.304	5.0	0.005
Control A outflow	0.057	O-S-T	0.123	5.0	< 0.001
Control B outflow	0.094	O-S-T	0.041	5.0	< 0.001
TW+WW	0.005	O-S-W	0.531	5.0	0.003
WW	< 0.001	O-S-W	2.330	5.0	0.209
		Manganese			
Filter 1 outflow	0.006	O-S-W	0.131	0.2	0.196
Filter 2 outflow	0.007	O-S-W	0.042	0.2	0.002
Filter 3 outflow	0.314	O-S-T	0.263	0.2	0.382
Filter 4 outflow	0.002	O-S-W	0.052	0.2	0.004
Filter 5 outflow	0.354	O-S-T	0.258	0.2	0.294
Filter 6 outflow	0.102	O-S-T	0.078	0.2	0.002
Filter 7 outflow	0.010	O-S-W	0.087	0.2	0.034
Filter 8 outflow	0.000	O-S-W	0.054	0.2	0.009
Control A outflow	0.012	O-S-W	0.063	0.2	0.001
Control B outflow	0.000	O-S-W	0.019	0.2	0.001
TW+WW	0.114	O-S-T	0.061	0.2	< 0.001
WW	0.474	O-S-T	0.129	0.2	0.069
		Zinc			
Filter 1 outflow	0.036	O-S-W	0.042	2.0	0.01
Filter 2 outflow	0.068	O-S-T	0.053	2.0	< 0.001
Filter 3 outflow	< 0.001	O-S-W	0.086	2.0	0.008
Filter 4 outflow	0.025	O-S-W	0.060	2.0	0.003
Filter 5 outflow	0.025	O-S-W	0.085	2.0	0.001
Filter 6 outflow	0.071	O-S-T	0.095	2.0	< 0.001
Filter 7 outflow	0.213	O-S-T	0.056	2.0	< 0.001
Filter 8 outflow	0.010	O-S-W	0.105	2.0	0.005
Control A outflow	0.005	O-S-W	0.045	2.0	0.001
Control B outflow	0.001	O-S-W	0.076	2.0	0.001
TW+WW	0.007	O-S-W	0.149	2.0	0.003
WW	< 0.001	O-S-W	0.301	2.0	0.003

Table 4.10 (cont.)

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value (mg/l)	Standard (mg/l)	Statistic (p-value)[a]
		Potassium			
Filter 1 outflow	0.874	O-S-T	7.741	2.0	< 0.001
Filter 2 outflow	0.025	O-S-W	7.073	2.0	0.001
Filter 3 outflow	0.294	O-S-T	8.545	2.0	< 0.001
Filter 4 outflow	0.022	O-S-W	3.471	2.0	0.248
Filter 5 outflow	0.474	O-S-T	11.197	2.0	< 0.001
Filter 6 outflow	0.640	O-S-T	10.909	2.0	< 0.001
Filter 7 outflow	0.438	O-S-T	6.498	2.0	< 0.001
Filter 8 outflow	0.111	O-S-T	4.929	2.0	0.037
Control A outflow	0.080	O-S-T	1.550	2.0	0.277
Control B outflow	0.009	O-S-W	0.635	2.0	0.002
TW+WW	0.890	O-S-T	3.583	2.0	< 0.001
WW	0.349	O-S-T	15.931	2.0	< 0.001
		Boron			
Filter 1 outflow	0.757	O-S-T	0.057	0.75	< 0.001
Filter 2 outflow	0.109	O-S-T	0.046	0.75	< 0.001
Filter 3 outflow	0.036	O-S-W	0.041	0.75	0.007
Filter 4 outflow	0.154	O-S-T	0.035	0.75	< 0.001
Filter 5 outflow	0.753	O-S-T	0.054	0.75	< 0.001
Filter 6 outflow	0.620	O-S-T	0.060	0.75	< 0.001
Filter 7 outflow	0.400	O-S-T	0.036	0.75	< 0.001
Filter 8 outflow	0.150	O-S-T	0.033	0.75	< 0.001
Control A outflow	0.067	O-S-T	0.007	0.75	< 0.001
Control B outflow	< 0.001	O-S-W	0.001	0.75	0.003
TW+WW	0.195	O-S-T	0.030	0.75	< 0.001
WW	0.197	O-S-T	0.083	0.75	< 0.001
		Sodium			
Filter 1 outflow	0.120	O-S-T	35.106	920	< 0.001
Filter 2 outflow	0.526	O-S-T	36.433	920	< 0.001
Filter 3 outflow	0.030	O-S-W	36.301	920	0.008
Filter 4 outflow	0.806	O-S-T	38.005	920	< 0.001
Filter 5 outflow	0.247	O-S-T	52.153	920	< 0.001
Filter 6 outflow	0.431	O-S-T	58.893	920	< 0.001
Filter 7 outflow	0.586	O-S-T	36.774	920	< 0.001
Filter 8 outflow	0.413	O-S-T	34.160	920	< 0.001
Control A outflow	0.015	O-S-W	8.760	920	0.001
Control B outflow	0.179	O-S-T	5.939	920	< 0.001
TW+WW	0.824	O-S-T	17.823	920	< 0.001
WW	0.947	O-S-T	60.393	920	< 0.001

Table 4.10 (cont.)

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value (mg/l)	Standard (mg/l)	Statistic (p-value)[a]
		Calcium			
Filter 1 outflow	0.701	O-S-T	31.613	400	< 0.001
Filter 2 outflow	0.412	O-S-T	30.467	400	< 0.001
Filter 3 outflow	0.135	O-S-T	60.349	400	< 0.001
Filter 4 outflow	0.748	O-S-T	43.895	400	< 0.001
Filter 5 outflow	0.975	O-S-T	57.189	400	< 0.001
Filter 6 outflow	0.958	O-S-T	53.687	400	< 0.001
Filter 7 outflow	0.590	O-S-T	38.780	400	< 0.001
Filter 8 outflow	0.186	O-S-T	39.464	400	< 0.001
Control A outflow	0.828	O-S-T	24.560	400	< 0.001
Control B outflow	0.153	O-S-T	25.805	400	< 0.001
TW+WW	0.468	O-S-T	22.577	400	< 0.001
WW	0.213	O-S-T	62.574	400	< 0.001
		Magnesium			
Filter 1 outflow	0.100	O-S-T	5.252	60	< 0.001
Filter 2 outflow	0.090	O-S-T	5.014	60	< 0.001
Filter 3 outflow	0.005	O-S-W	5.963	60	0.008
Filter 4 outflow	0.659	O-S-T	5.264	60	< 0.001
Filter 5 outflow	0.356	O-S-T	8.628	60	< 0.001
Filter 6 outflow	0.025	O-S-W	8.726	60	0.001
Filter 7 outflow	0.008	O-S-W	5.118	60	0.002
Filter 8 outflow	0.866	O-S-T	4.843	60	< 0.001
Control A outflow	0.093	O-S-T	1.587	60	< 0.001
Control B outflow	0.908	O-S-T	1.275	60	< 0.001
TW+WW	0.762	O-S-T	3.033	60	< 0.001
WW	0.087	O-S-T	10.527	60	< 0.001

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). O-S-T, the parametric one sample t-test, O-S-W, the non-parametric Wilcoxon signed rank test. TW+WW, tap water (80%) spiked with wastewater (20%) and WW, raw wastewater (100%).

Table 4.11: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	TPH	COD	BOD	DO	NH_4-N	NO_3-N	PO_4-P	Fe	Mn	Zn	K	B	Na	Ca	Mg
TPH	R	1.000	0.694*	0.381	-0.427	-0.356	-0.304	-0.200	0.175	0.369	-0.071	-0.032	-0.162	-0.498	-0.097	-0.213
	p	n.a	0.026	0.277	0.219	0.313	0.393	0.579	0.630	0.295	0.845	0.929	0.656	0.143	0.790	0.554
COD	R	0.694*	1.000	0.815**	-0.574	0.298	-0.243	0.371	0.705*	0.766**	0.103	0.620	0.426	0.128	0.480	0.474
	p	0.026	n.a	0.004	0.083	0.403	0.498	0.291	0.023	0.010	0.776	0.056	0.220	0.725	0.160	0.166
BOD	R	0.381	0.815**	1.000	-0.351	0.564	-0.079	0.648*	0.830**	0.697*	0.164	0.794**	0.745*	0.309	0.600	0.661*
	p	0.277	0.004	n.a	0.320	0.090	0.829	0.043	0.003	0.025	0.651	0.006	0.013	0.385	0.067	0.038
DO	R	-0.427	-0.574	-0.351	1.000	-0.320	0.117	-0.357	-0.283	-0.529	0.412	-0.419	-0.431	-0.062	0.135	-0.197
	p	0.219	0.083	0.320	n.a	0.367	0.748	0.311	0.428	0.116	0.236	0.229	0.214	0.866	0.709	0.586
NH_4-N	R	-0.356	0.298	0.564	-0.320	1.000	0.394	0.903**	0.624	0.442	0.091	0.891**	0.915**	0.794**	0.515	0.758*
	p	0.313	0.403	0.090	0.367	n.a	0.260	0.000	0.054	0.200	0.803	0.001	0.000	0.006	0.128	0.011
NO_3-N	R	-0.304	-0.243	-0.079	0.117	0.394	1.000	0.394	0.055	-0.067	0.273	0.248	0.333	0.612	0.200	0.297
	p	0.393	0.498	0.829	0.748	0.260	n.a	0.260	0.881	0.855	0.446	0.489	0.347	0.060	0.580	0.405
PO_4-P	R	-0.200	0.371	0.648*	-0.357	0.903**	0.394	1.000	0.794**	0.648*	0.224	0.915**	0.915**	0.721*	0.612	0.806**
	p	0.579	0.291	0.043	0.311	0.000	0.260	n.a	0.006	0.043	0.533	0.000	0.000	0.019	0.060	0.005
Fe	R	0.175	0.705*	0.830**	-0.283	0.624	0.055	0.794**	1.000	0.915**	0.297	0.867**	0.709*	0.515	0.818**	0.806**
	p	0.630	0.023	0.003	0.428	0.054	0.881	0.006	n.a	0.000	0.405	0.001	0.022	0.128	0.004	0.005
Mn	R	0.369	0.766**	0.697*	-0.529	0.442	-0.067	0.648*	0.915**	1.000	0.091	0.733*	0.552	0.345	0.624	0.648*
	p	0.295	0.010	0.025	0.116	0.200	0.855	0.043	0.000	n.a	0.803	0.016	0.098	0.328	0.054	0.043
Zn	R	-0.071	0.103	0.164	0.412	0.091	0.273	0.224	0.297	0.091	1.000	0.273	0.018	0.248	0.661*	0.333
	p	0.845	0.776	0.651	0.236	0.803	0.446	0.533	0.405	0.803	n.a	0.446	0.960	0.489	0.038	0.347
K	R	-0.032	0.620	0.794**	-0.419	0.891**	0.248	0.915**	0.867**	0.733*	0.273	1.000	0.903**	0.697*	0.721*	0.855**
	p	0.929	0.056	0.006	0.229	0.001	0.489	0.000	0.001	0.016	0.446	n.a	0.000	0.025	0.019	0.002

Table 4.11 (cont.)

Variable	Statistic	TPH	COD	BOD	DO	NH_4-N	NO_3-N	PO_4-P	Fe	Mn	Zn	K	B	Na	Ca	Mg
B	R	-0.162	0.426	0.745*	-0.431	0.915**	0.333	0.915**	0.709*	0.552	0.018	0.903**	1.000	0.709*	0.503	0.806**
	p	0.656	0.220	0.013	0.214	0.000	0.347	0.000	0.022	0.098	0.960	0.000	n.a	0.022	0.138	0.005
Na	R	-0.498	0.128	0.309	-0.062	0.794**	0.612	0.721*	0.515	0.345	0.248	0.697*	0.709*	1.000	0.673*	0.867**
	p	0.143	0.725	0.385	0.866	0.006	0.060	0.019	0.128	0.328	0.489	0.025	0.022	n.a	0.033	0.001
Ca	R	-0.097	0.480	0.600	0.135	0.515	0.200	0.612	.818**	0.624	0.661*	0.721*	0.503	0.673*	1.000	0.855**
	p	0.790	0.160	0.067	0.709	0.128	0.580	0.060	0.004	0.054	0.038	0.019	0.138	0.033	n.a	0.002
Mg	R	-0.213	0.474	0.661*	-0.197	0.758*	0.297	0.806**	0.806**	0.648*	0.333	0.855**	0.806**	0.867**	0.855**	1.000
	p	0.554	0.166	0.038	0.586	0.011	0.405	0.005	0.005	0.043	0.347	0.002	0.005	0.001	0.002	n.a

Note: TPH, total petroleum hydrocarbon (µg/l); COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); DO, dissolved oxygen (mg/l); NH_4-N, ammonia-nitrogen (mg/l); NO_3-N, nitrate-nitrogen (mg/l); PO_4-P, ortho-phosphate-phosphorus (mg/l); Fe, Iron (mg/l); Mn, manganese (mg/l); Zn, zinc (mg/l); k, potassium (mg/l); B, boron (mg/l); Na, sodium (mg/l); Ca, calcium (mg/l); Mg, magnesium (mg/l); R, correlation coefficient; *p,* probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level.

4.2.5 Comparison of particles

Table 4.12 shows that the maximum suspended solids values were observed for raw wastewater followed by those for wastewater samples diluted with 80% tap water and outflow waters from filters which were contaminated with hydrocarbons (Filters 1, 3, 5 and Control A), while the minimum suspended solids values were recorded for deionised and tap waters. Irrigation water harvested from Filters 1 and 2 had suspended solids values lower than those for Filters 3 and 4, respectively due to the difference in aggregate diameter. Filter 4 drain water had suspended solids which were significantly ($p < 0.05$) greater than those for Filter 7 (Table 4.13) explaining the impact of the contact time variable of wetland design on outflow suspended solid values (Sani et al., 2013). Moreover, Filters 7 and 8 outflow waters showed suspended solids values which were relatively similar, indicating that the resting time variable may not matter in terms of suspended solids treatment. Furthermore, irrigation waters harvested from hydrocarbon contaminated filters (Filters 1, 3, 5 and Control A) had suspended solid values which were significantly ($p < 0.05$) greater than those for uncontaminated filters (Filters 2, 4, 6 and Control B) due to hydrocarbon biodegradation processes (Scholz, 2010).

Similarly to the suspended solids, turbidity values were observed with the highest values in raw wastewater followed by those for wastewater samples diluted with 80% tap water and outflow waters from filters which were contaminated with hydrocarbons (Filters 1, 3, 5 and Control A), while the minimum suspended solids values were recorded for deionised and tap waters (Table 4.12). Filter 4 had outflow water with turbidity values which were significantly ($p < 0.05$) greater than those for Filter 7 (Table 4.15) explaining the impact of the contact time variable on outflow water turbidity values. In contrast, resting time may not matter in terms of turbidity treatment, as Filters 7 and 8 had outflow waters of relatively similar turbidity values. Furthermore, irrigation waters harvested from hydrocarbon contaminated filters (Filters 1, 3, 5 and Control A) had turbidity values which were significantly ($p < 0.05$) greater than those for uncontaminated filters (Filters 2, 4, 6 and Control B) as shown in Table 4.13 due to hydrocarbon biodegradation processes (Scholz, 2010). Correlation analysis results (Table 4.14) show that suspended solids, turbidity, chemical oxygen demand, biochemical oxygen demand and monitored microorganisms in the system (total coliforms, *Escherichia coli, Streptococci* spp. and *Salmonella* spp.) were significantly ($p < 0.05$) positively correlated with each other in the treatment system. This can be explained by the fact that increasing the microorganisms in the treatment system will increase the biodegradation process for the organic matter resulting in high suspended solids and turbidity concentrations in the outflow waters. This indicates a good relationship between suspended solids, turbidity and indicator microorganism activity due to the degradation of organic matter and a subsequent increase in particles (Sani et al., 2013; Almuktar& Scholz, 2015).

Suspended solids and turbidity values for irrigation waters obtained from the wetland system highly fluctuated during different crop growth periods due to seasonal behaviour change in the wetland system. For example, as above-ground *p.australis* plant parts decay in winter and early spring, more particles are created as by-products of the biodegradation process (Scholz, 2010, 2011; Sani et al., 2013; Almuktar & Scholz, 2016 b). However, high values of suspended solids and turbidity associated with irrigation water will considerably increase the development of hydrophobicity in the soil, and subsequently affect plant growth (Travis et al., 2010; Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 a, b). Previous studies (Kadlec & Knight, 1996; Green et al., 1997; Garcia et al., 2010; Hua et al., 2013) have reported that most solids can be removed using constructed wetlands (CWs) technology through sedimentation, settling, adsorption and biological degradation processes performed in the CWs system. Moreover, in surface-flow constructed wetlands, solids removal will occur through flocculation, sedimentation and filtration processes undertaken in the system as reported by Kadlec and Wallace (2009). In addition, interaction and adhering of suspended solids with other constituents available in the wetlands system, such as heavy metals and nutrients, pathogens and organic matter, will improve their removal from wastewater (Sundaravadivel & Vigneswaran, 2001). In subsurface vertical-flow constructed wetlands, the removal of solids will depend on characteristics of the substrate, hydraulic load and microorganisms available in the system (Manios, Stentiford, & Millner, 2003).

Table 4.12: Comparison of the suspended solids and turbidity for irrigation water received by the vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Suspended solids (mg/l)				
Filter 1	11.3±10.42 (56)	25.3±6.66 (3)	16.5±16.65 (12)	8.8±6.44 (41)
Filter 2	6.7±9.49 (56)	14.3±12.10 (3)	11.6±17.58 (12)	4.7±3.97 (41)
Filter 3	11.7±10.79 (56)	25.0±11.27 (3)	16.6±16.28 (12)	9.3±7.39 (41)
Filter 4	7.4±10.57 (56)	20.0±21.63 (3)	11.8±17.07 (12)	5.1±5.20 (41)
Filter 5	11.3±12.76 (57)	43.0±24.52 (3)	17.8±15.35 (13)	6.9±4.33 (41)
Filter 6	6.9±8,68 (57)	18.7±10.07 (3)	11.1±13.33 (13)	4.7±5.13 (41)
Filter 7	2.6±3.86 (66)	3.0±3.46 (4)	2.5±2.58 (11)	2.6±4.16 (51)
Filter 8	2.9±4.31 (76)	9.7±7.77 (3)	2.0±2.22 (18)	2.8±4.38 (55)
Control A	9.0±10.25 (56)	12.7±11.24 (3)	11.7±14.14 (12)	8.0±8.88 (41)
Control B	3.6±8.18 (56)	7.0±7.81 (3)	9.4±15.80 (12)	1.6±2.32 (41)
DW	2.0±2.28 (4)	0.0±n.a (1)	0.0±n.a (1)	4.0±n.a (1)
TW	2.0±2.83 (4)	0.0±n.a (1)	0.0±n.a (1)	4.0±n.a (1)
TW+F	1.6±0.57 (4)	1.2±n.a (1)	1.2±n.a (1)	2.0±n.a (1)
TW+WW	26.4±18.48 (63)	38.0±1.86 (4)	15.1±11.83 (12)	28.3±19.44 (47)
WW	131.9±92.64 (63)	189.8±9.32 (4)	75.4±59.17 (12)	141.4±97.52 (47)
Turbidity (NTU)				
Filter 1	9.0±5.65 (54)	18.6±2.91 (3)	10.0±7.58 (12)	7.9±4.32 (39)
Filter 2	5.4±5.75 (53)	11.4±12.76 (3)	8.2±8.97 (12)	4.1±2.56 (38)
Filter 3	8.7±6.09 (53)	17.0±11.94 (3)	11.0±8.09 (12)	7.3±3.94(38)
Filter 4	5.7±5.46 (53)	10.0±6.99 (3)	7.8±8.36 (12)	4.7±3.82 (38)
Filter 5	8.6±6.22 (53)	22.1±11.94 (3)	12.5±7.60 (12)	6.3±2.05 (38)
Filter 6	5.4±4.41 (53)	8.1±0.63 (3)	8.4±7.32 (12)	4.3±2.57 (38)
Filter 7	3.4±2.24 (62)	4.2± 1.11(4)	2.9±0.39 (11)	3.4±2.53 (47)
Filter 8	3.6±2.48 (76)	4.5±2.08 (4)	3.6±1.22 (19)	3.5±2.83 (53)
Control A	5.7±4.31 (53)	4.1±1.84 (3)	7.5±6.43 (12)	5.3±3.50 (38)
Control B	4.1±4.54 (53)	5.7±4.92 (3)	7.4±7.99 (12)	2.9±1.87 (38)

Table 4.12 (cont.)

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
DW	1.4±0.21 (3)	1.5±n.a (1)	1.5±n.a (1)	1.2±n.a (1)
TW	3.0±0.49 (3)	2.6±n.a (1)	2.6±n.a (1)	3.3±n.a (1)
TW+F	2.8±0.64 (3)	3.2±n.a (1)	3.2±n.a (1)	2.3±n.a (1)
TW+WW	16.2±15.18 (56)	17.5±3.56 (4)	8.6±5.04 (13)	18.5±17.29 (39)
WW	80.4±75.97 (56)	87.5±17.80 (4)	42.4±25.76 (13)	92.3±86.45 (39)

Note: NTU, Nephelometric turbidity unit. [a] 11/10/13 to 25/09/14. [b] Second planting period: 11/10/13 to 07/11/13. [c] Final planting period before fruiting: 08/11/13 to 19/01/14. [d] Final planting period after fruiting: 20/01/14 to 25/09/14. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). n.a, not applicable.

Table 4.13: Statistical assessment of suspended solids and turbidity for irrigation waters linked to wetland filters during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (p-value)[a]	Statistical test	P-values[b] for different system combinations
Suspended solids (mg/l)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.575
Filter 2 & Filter 4	< 0.001	M-W-U	0.958
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	< 0.001
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.325
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.511
Filter 4 & Filter 6	< 0.001	M-W-U	0.998
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	< 0.001
Filter 3 & Filter 4	< 0.001	M-W-U	< 0.001
Filter 5 & Filter 6	< 0.001	M-W-U	0.002
Control A & Control B	< 0.001	M-W-U	< 0.001
Turbidity (NTU)			
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.643
Filter 2 & Filter 4	< 0.001	M-W-U	0.593
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.001
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.220
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.927
Filter 4 & Filter 6	< 0.001	M-W-U	0.850
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	< 0.001
Filter 3 & Filter 4	< 0.001	M-W-U	< 0.001
Filter 5 & Filter 6	< 0.001	M-W-U	< 0.001
Control A & Control B	< 0.001	M-W-U	< 0.001

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test. Filters are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test.

Table 4.14: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	Variable			
		SS	NTU	COD	BOD
SS	R	1.000	0.976**	0.796**	0.709*
	p	n.a	0.000	0.006	0.022
NTU	R	0.976**	1.000	0.766**	0.697*
	p	0.000	n.a	0.010	0.025
COD	R	0.796**	0.766**	1.000	0.815**
	p	0.006	0.010	n.a	0.004
BOD	R	0.709*	0.697*	0.815**	1.000
	p	0.022	0.025	0.004	n.a

Note: SS, suspended solids (mg/l); NTU, turbidity in Nephelometric turbidity unit; COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); R, correlation coefficient; *p,* probability of the statistical test (if p-value > 0.05, the variables are not statistically significantly correlated, if p-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level.

4.2.6 Comparison of pH and salinity

Table 4.15 shows pH and salinity values for irrigation water received by the vegetable pots. Results show raw wastewater had the highest pH values followed by those for wastewater samples diluted with 80% tap water, while the lowest values were recorded for deionised water. Table 4.16 shows that there is a statistically significant ($p < 0.05$) difference in pH values of outflow waters from filters compared in terms of aggregate diameter, resting time and inflow loading rate. However, the pH values for all irrigation water types were within the normal range of between 6.0 and 8.5 (Pescod, 1992; FAO, 2003). Moreover, pH values varied throughout the day due to respiration (after sunset) and photosynthesis (after sunrise) of plants in the wetland systems. This directly affected the dissolved oxygen and carbon dioxide concentrations in the systems leading to fluctuations in pH values. For example, after sunset, dissolved oxygen concentrations decline as photosynthesis stops and all plants and animals in the system consume oxygen (respiration) resulting in increasing of carbon dioxide concentrations. The latter will react with the water producing carbonic acid leading to reduce pH values.

On the other hand, during day time the dissolved oxygen will increase and carbon dioxide will decrease due to photosynthesis leading to an increase in the pH values of water in the system (Zang et al., 2011). Wastewater pH is an important factor that may affect the performance of wetlands mainly in terms of nitrogen and organic matter removal. For example, consumption of most of the alkalinity during the nitrification process will lead to a significant drop in pH values in the system, subsequently affecting the denitrification rate as discussed by Kadlec and Knight (1996). However, the optimum pH value for the denitrification process can range between 6.0 and 8.0, while the highest rate can occur at a pH value of 7.0 to 7.5, as reported by Saeed and Sun (2012). Moreover, Vymazal (2007) noted that a slower rate of denitrification process can occur at a pH value of 5.0, while insignificant denitrification rates can be observed at pH values below 4. Wastewater pH value is also important for organic matter, mainly for anaerobic degradation processes (Saeed & Sun, 2012). This is because of the high sensitivity of the bacteria responsible for formation of methane gas in the system to the narrow ranges of pH values; they can survive only in pH values of between 6.5 and 7.5. As a result, the anaerobic degradation process will not complete if the pH value is not in this range leading to volatile fatty acid accumulation in the system and a subsequent drop in the pH value which will kill all methanogens available in the wetland system, as reported by Copper et al. (1996) and Vymazal (1999).

Electrical conductivity is the most important indirect measure of salinity, which poses a great hazard to crops and determines the suitability of water for irrigation use (FAO, 1994, 2003). Table 4.15 shows that the highest electrical conductivity values were recorded for the raw wastewater followed by those for irrigation waters harvested from wetland filters which were fed with high inflow loading rate (Filters 5 and 6). Statistical analysis results (Table 4.16) show that outflow water from Filter 1 had electrical conductivity values which were significantly ($p < 0.05$) lower than those for Filter 3 explaining the impact of aggregate diameter of wetlands on outflow water salinity concentrations (Almuktar & Scholz, 2016 a, b). Filter 7 drain water had electrical conductivity values greater than those for Filters 4 and 8, explaining the impact of contact time and resting time, respectively, of the wetland system on outflow water salinity concentrations (Almuktar et al., 2015 a, b). Wetland filters which were fed with undiluted wastewater (Filters 5 and 6) had outflow waters of salinity values which were significantly ($p < 0.05$) greater than those for filters fed with diluted wastewater (Filters 3 and 4), confirming the results obtained by other researchers indicating that filters of high inflow loading rate tend to be overloaded (Sani et al., 2013). Moreover, salinity values for waters harvested from hydrocarbon contaminated filters (Filters 1, 3 and 5) showed values which were relatively higher than those of standard filters (Filters 2, 4 and 6) as a result of hydrocarbon biodegradation processes (Scholz, 2010, 2011). Compared to the standards of 3000 µS/cm for electrical conductivity (FAO, 1994, 2003), all irrigation water types had salinity values which were significantly ($p < 0.05$) lower than the thresholds (Table 4.17). High levels of electrical conductivity in irrigation water create saline soil. Salts negatively impact on the growth of plants, soil structure and soil permeability which indirectly affect plant growth as well (Maas & Grattan, 1999).

Correlation analysis results (Table 4.18) show that salinity values in the treatment system were significantly ($p < 0.05$) positively correlated with the values of other elements such as ammonia–nitrogen, ortho-phosphate–phosphorus, iron, manganese, potassium, sodium, calcium and magnesium (Almuktar & Scholz, 2016 a, b).

Table 4.15: Comparison of the pH and salinity values for irrigation water received by the vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
pH (-)				
Filter 1	6.4±0.26 (54)	6.5±0.33 (3)	6.5±0.18 (11)	6.3±0.27 (40)
Filter 2	6.5±0.21 (54)	6.6±0.06 (3)	6.4±0.11 (11)	6.5±0.23 (40)
Filter 3	6.5±0.18 (54)	6.6±0.05 (3)	6.6±0.12 (11)	6.5±0.20 (40)
Filter 4	6.5±0.19 (54)	6.7±0.08 (3)	6.6±0.10 (11)	6.5±0.20 (40)
Filter 5	6.6±0.19 (54)	6.8±0.06 (3)	6.8±0.21 (12)	6.6±0.17 (39)
Filter 6	6.8±0.19 (55)	6.9±0.27 (3)	6.8±0.13 (12)	6.8±0.20 (40)
Filter 7	6.6±0.18 (62)	6.8±0.23 (3)	6.5±0.19 (11)	6.6±0.17 (48)
Filter 8	6.5±0.20 (78)	6.6±0.08 (3)	6.6±0.28 (18)	6.5±0.16 (57)
Control A	6.7±0.17 (55)	6.9±0.10 (3)	6.7±0.13 (11)	6.7±0.18 (41)
Control B	6.5±0.20 (54)	6.9±0.06 (3)	6.6±0.16 (11)	6.5±0.17 (40)
DW	5.1±0.92 (4)	5.7±n.a (1)	5.7±n.a (1)	4.4±n.a (1)
TW	6.1±1.06 (4)	5.3±n.a (1)	5.3±n.a (1)	6.8±n.a (1)
TW+F	6.0±0.28 (4)	6.2±n.a (1)	6.2±n.a (1)	5.8±n.a (1)
TW+WW	7.3±0.07 (55)	7.0±0.06 (4)	7.5±0.03 (9)	7.3±0.05 (42)
WW	7.5±0.42 (55)	7.8±0.45 (4)	7.6±0.28 (9)	7.5±0.44 (42)
Electrical conductivity (µS/cm)				
Filter 1	336.5±50.82 (22)	nm	nm	336.5±50.82 (22)
Filter 2	328.6±53.37 (22)	nm	nm	328.6±53.37 (22)
Filter 3	396.7±76.59 (22)	nm	nm	396.7±76.59 (22)
Filter 4	352.6±67.56 (22)	nm	nm	352.6±67.56 (22)
Filter 5	564.1±163.66 (22)	nm	nm	564.1±163.66 (22)
Filter 6	524.3±152.66 (22)	nm	nm	524.3±152.66 (22)
Filter 7	355.0±83.11 (28)	nm	nm	355.0±83.11 (28)
Filter 8	339.7±104.74 (25)	nm	nm	339.7±104.74 (25)
Control A	149.2±32.47 (22)	nm	nm	149.2±32.47 (22)
Control B	153.9±29.87 (22)	nm	nm	153.9±29.87 (22)
DW	1.5±0.72 (4)	nm	nm	1.5±0.72 (4)
TW	95.8±15.20 (4)	nm	nm	95.8±15.20 (4)
TW+F	204.0±5.66 (4)	nm	nm	204.0±5.66 (4)
TW+WW	122.1±55.98 (22)	nm	nm	122.1±55.98 (22)
WW	575.5±181.66 (22)	nm	nm	575.5±181.66 (22)

Note: nm, not measured. [a] 11/10/13 to 25/09/14. [b] Second planting period: 11/10/13 to 07/11/13. [c] Final planting period before fruiting: 08/11/13 to 19/01/14. [d] Final planting period after fruiting: 20/01/14 to 25/09/14. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). n.a, not applicable.

Table 4.16: Statistical assessment of pH and salinity values for irrigation waters linked to wetland filters during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (p-value)[a]	Statistical test	P-values[b] for different system combinations
	pH (-)		
Aggregate diameter			
Filter 1 & Filter 3	0.052	I-T	< 0.001
Filter 2 & Filter 4	< 0.001	M-W-U	0.832
Contact time			
Filter 4 & Filter 7	0.019	M-W-U	0.135
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.022
Inflow loading rate			
Filter 3 & Filter 5	0.003	M-W-U	0.014
Filter 4 & Filter 6	< 0.001	M-W-U	< 0.001
Hydrocarbon influence			
Filter 1 & Filter 2	0.003	M-W-U	< 0.001
Filter 3 & Filter 4	0.013	M-W-U	0.799
Filter 5 & Filter 6	< 0.001	M-W-U	< 0.001
Control A & Control B	0.542	I-T	< 0.001
	Electrical conductivity (μS/cm)		
Aggregate diameter			
Filter 1 & Filter 3	0.022	M-W-U	0.005
Filter 2 & Filter 4	0.004	M-W-U	0.098
Contact time			
Filter 4 & Filter 7	0.001	M-W-U	0.428
Resting time			
Filter 7 & Filter 8	< 0.001	M-W-U	0.412
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	< 0.001
Filter 4 & Filter 6	< 0.001	M-W-U	< 0.001
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.231
Filter 3 & Filter 4	0.003	M-W-U	0.013
Filter 5 & Filter 6	< 0.001	M-W-U	0.385
Control A & Control B	0.128	I-T	0.619

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test. Filters are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test, I-T, the parametric Independent samples T-test.

Table 4.17: Statistical assessment of salinity for irrigation waters compared to standards.

Irrigation water type	Shapiro-Wilk test (*p*-value)[a]	Statistical test	Mean value (µg/l)	Standard (µg/l)	Statistic (*p*-value)[b]
Electrical conductivity					
Filter 1 outflow	0.001	O-S-W	336.5	3000	< 0.001
Filter 2 outflow	0.002	O-S-W	328.6	3000	< 0.001
Filter 3 outflow	0.010	O-S-W	396.7	3000	< 0.001
Filter 4 outflow	0.017	O-S-W	352.6	3000	< 0.001
Filter 5 outflow	0.011	O-S-W	564.1	3000	< 0.001
Filter 6 outflow	0.006	O-S-W	524.3	3000	< 0.001
Filter 7 outflow	0.008	O-S-W	355.0	3000	< 0.001
Filter 8 outflow	0.010	O-S-W	339.7	3000	< 0.001
Control A outflow	0.102	O-S-T	149.2	3000	< 0.001
Control B outflow	0.566	O-S-T	153.9	3000	< 0.001
TW+WW	< 0.001	O-S-W	122.1	3000	< 0.001
WW	0.006	O-S-W	575.5	3000	< 0.001

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). O-S-T, the parametric one sample t-test, O-S-W, the non-parametric Wilcoxon signed rank test. TW+WW, tap water (80%) spiked with wastewater (20%) and WW, raw wastewater (100%).

Table 4.18: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	Variable								
		NH_4-N	PO_4-P	Fe	Mn	K	Na	Ca	Mg	Ec
NH_4-N	R	1.000	0.903**	0.624	0.442	0.891**	0.794**	0.515	0.758*	0.685*
	p	n.a	0.000	0.054	0.200	0.001	0.006	0.128	0.011	0.029
PO_4-P	R	0.903**	1.000	0.794**	0.648*	0.915**	0.721*	0.612	0.806**	0.782**
	p	0.000	n.a	0.006	0.043	0.000	0.019	0.060	0.005	0.008
Fe	R	0.624	0.794**	1.000	0.915**	0.867**	0.515	0.818**	0.806**	0.806**
	p	0.054	0.006	n.a	0.000	0.001	0.128	0.004	0.005	0.005
Mn	R	0.442	0.648*	0.915**	1.000	0.733*	0.345	0.624	0.648*	0.648*
	p	0.200	0.043	0.000	n.a	0.016	0.328	0.054	0.043	0.043
K	R	0.891**	0.915**	0.867**	0.733*	1.000	0.697*	0.721*	0.855**	0.794**
	p	0.001	0.000	0.001	0.016	n.a	0.025	0.019	0.002	0.006
Na	R	0.794**	0.721*	0.515	0.345	0.697*	1.000	0.673*	0.867**	0.818**
	p	0.006	0.019	0.128	0.328	0.025	n.a	0.033	0.001	0.004
Ca	R	0.515	0.612	0.818**	0.624	0.721*	0.673*	1.000	0.855**	0.927**
	p	0.128	0.060	0.004	0.054	0.019	0.033	n.a	0.002	0.000
Mg	R	0.758*	0.806**	0.806**	0.648*	0.855**	0.867**	0.855**	1.000	0.891**
	p	0.011	0.005	0.005	0.043	0.002	0.001	0.002	n.a	0.001
Ec	R	0.685*	0.782**	0.806**	0.648*	0.794**	0.818**	0.927**	0.891**	1.000
	p	0.029	0.008	0.005	0.043	0.006	0.004	0.000	0.001	n.a

Note: NH_4-N, ammonia-nitrogen (mg/l); PO_4-P, ortho-phosphate-phosphorous (mg/l); Fe, iron (mg/l); Mn, manganese; K, potassium (mg/l); Na, sodium (mg/l); Ca, calcium (mg/l); Mg, magnesium (mg/l); Ec, electrical conductivity (µS/cm); R, correlation coefficient; *p,* probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level.

4.2.7 Comparison of microbial content

Microbial characteristics of irrigation waters are summarised in Table 4.19. The results show that the highest total coliforms values were recorded for the outflow from filters which were contaminated by hydrocarbons (F5 > F1 > F3), followed by those for raw wastewater and wastewater samples which were diluted with up to 80% tap water, while the lowest values were recorded for outflow water from Filter 4. Irrigation waters harvested from Filter 2 had total coliforms which were significantly ($p < 0.05$) greater than those for Filter 4 (Table 4.20) indicating the impact of aggregate diameter on total coliforms removal processes. Harvested water from Filter 4 had total coliforms values which were significantly ($p<0.05$) lower than those from Filter 7, while the latter had values lower than those of Filter 8 due to differences in contact and resting times variables on pathogen removal processes. Wetland filters which were fed with high inflow loading rate had outflow waters with total coliforms which were significantly ($p < 0.05$) greater than those from filters which fed with low inflow loading rate as shown when comparing Filters 3 and 4 with Filters 5 and 6, respectively (Table 4.20). Hydrocarbon contamination filters had outflow waters with total coliforms which were significantly ($p < 0.05$) greater than those from uncontaminated ones. Compared to the standards of 1000 CFU/100 ml (WHO, 1989; FAO, 2003; USEPA, 2004) for total coliforms to irrigate crops, which are often eaten uncooked, the outflow waters from all wetland filters were too highly contaminated by total coliforms which significantly ($p < 0.05$) exceeded the threshold (Table 4.21). Correlation analysis results (Table 4.22) show that total coliforms in the treatment system were significantly ($p < 0.05$) positively correlated with other variables such as chemical oxygen demand, biochemical oxygen demand, iron, suspended solids, turbidity, *Escherichia coli and Salmonella* spp., while total coliforms were significantly ($p < 0.05$) negatively correlated with dissolved oxygen in the system, as discussed previously (Almuktar & Scholz, 2015, 2016 a). However, total coliforms were treated well by the standard wetland filters which were not contaminated with hydrocarbons. This finding confirmed research undertaken by Cui et al. (2003), reporting that the removal rates for total heterotrophic bacteria and total coliforms when using vertical-flow bed systems were between 80 and 90%, and between 85 and 96%, respectively.

Table 4.19 shows that *Escherichia coli* was detected only in raw wastewater, wastewater diluted with tap water and in the outflow waters from wetland filters contaminated with hydrocarbons. However, *Escherichia coli* values followed the order of: raw wastewater (8000 CFU/100 ml) > Filter 5 (5667 CFU/100 ml) > wastewater spiked with tap water (2000 CFU/100 ml) > F1 (1833 CFU/100 ml) > F3 (1167 CFU/100 ml). No contamination by *Escherichia coli* was detected for outflow waters from other wetland filters. However, this result contradicted findings reported by Cirelli et al. (2012), who presented results of a reuse scenario where municipal wastewater was treated by constructed wetlands (tertiary treatment step), and reused for the supply of irrigation water for vegetables in Eastern Sicily, Italy. They found increased numbers of *Escherichia coli* in the irrigation water, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 ml for secondary treated urban wastewater effluents. Moreover, correlation analysis results (Table 4.22) show that *Escherichia coli* values in the treatment system were significantly ($p < 0.05$) positively correlated with other variables such as chemical and biochemical oxygen demands, iron, manganese, suspended solids and turbidity as well as with other microbes in the system such as total coliforms, *Streptococcus* spp. and *Salmonella* spp., but were significantly ($p < 0.05$) negatively correlated with dissolved oxygen in the system as reported by Almuktar and Scholz (2015, 2016 a).

The highest contamination by *Streptococcus* spp. was associated with Filter 1 outflow water (114833 CFU/100 ml) followed by that for Filter 3 (70333 CFU/100 ml) and Filter 5 (24667 CFU/100 ml), as shown in Table 4.19.

Raw wastewater was observed to have higher contamination by *Streptococcus* spp. than wastewater samples, which were diluted with up to 80% tap water. Irrigation water harvested from Filter 1 had *Streptococcus* spp. levels which were significantly ($p < 0.05$) greater than those for Filter 3 (Table 4.20) indicating the impact of wetland aggregate diameter on outflow microbe values (Almuktar & Scholz, 2015). Although Filters 3 and 4 were fed with diluted wastewater, the statistical analysis results (Table 4.20) show that they had outflow waters of *Streptococcus* spp. levels which were significantly ($p < 0.05$) greater than those for Filters 5 and 6, which had high inflow loading rate. These results contradict those reported by Sani et al. (2013) indicating that filters of high inflow loading rates tend to be overloaded. Moreover, hydrocarbon contaminated filters had outflow waters with *Streptococcus* spp. colonies which were significantly ($p < 0.05$) greater than those for uncontaminated filters due to the abundancy of organic matters provided by diesel spill contamination supporting a greater microbe population (Almuktar & Scholz, 2015; 2016 a, 2016 b).

Similarly to other microbes, *Streptococcus* spp. values in the treatment system were observed to be significantly ($p < 0.05$) positively correlated with other variables (Table 4.22) such as chemical and biochemical oxygen demands, suspended solids, turbidity and *Escherichia coli,* while being correlated significantly ($p < 0.05$) negatively with dissolved oxygen in the system as discussed previously by Almuktar and Scholz (2015).

Table 4.19 shows that the highest *Salmonella* spp. count was observed in the outflow water from Filter 5 (232500 CFU/100 ml) followed by raw wastewater (202167 CFU/100 ml), while the lowest values were recorded for Filter 2 outflow (2000

CFU/100 ml). Filter 1 outflow water was associated with higher *Salmonella* spp. contamination than the water from Filter 3 due to differences in aggregate diameter. Outflow waters from Filter 7 had *Salmonella* spp. colonies which were significantly ($p < 0.05$) greater than those for Filters 4 and 8, indicating the impact of contact and resting times of wetland design on outflow microbe levels. Moreover, filters of high inflow loading rate (Filters 5 and 6) had *Salmonella* spp. contamination which was significantly ($p < 0.05$) greater than that of filters of low inflow loading rate (Filters 3 and 4) as shown in Table 4.20, confirming the results reported previously (Sani et al., 2013; Almuktar & Scholz, 2015).

Wetland filters which were contaminated with hydrocarbons had outflows with *Salmonella* spp. colonies which were significantly ($p < 0.05$) greater than those for standard filters as shown when comparing Filters 1, 3, 5 and Control A outflow values with those of Filters 2, 4, 6 and Control B (Table 4.20). Correlation analysis results (Table 4.22) show that *Salmonella* spp. values in the treatment system were significantly ($p < 0.05$) positively correlated with chemical and biochemical oxygen demands, iron, manganese, suspended solids, and turbidity as well as with other microbes such as total coliforms and *Escherichia coli*, confirming the results reported previously (Almuktar & Scholz, 2015, 2016 a, b). However, *Salmonellae* were treated well in the wetland systems confirming the results obtained by Cui et al. (2003) indicating good bacteria removal efficiency when using vertical-flow bed systems.

Generally, results show that the microbial contamination of outflow water from wetland filters contaminated with hydrocarbons was higher than that from standard filter (uncontaminated) outflow water. This confirms findings by Benedek et al. (2013), who studied the impact of long-term TPH, volatile petroleum hydrocarbons, total alkyl benzenes and polycyclic aromatic hydrocarbons on the structure of bacterial communities. Their results indicated that a very high concentration of TPH positively affected the diversity of hydrocarbon-degrading bacteria. Furthermore, wetland filters fed with undiluted inflow water showed higher microbial contamination levels than those fed with diluted inflow. This confirms results by Sani et al. (2013) that high rate filters tend to be overloaded.

Filters of large aggregate diameter showed microbial contamination levels higher than those of small aggregate diameter. This is because a large aggregate size allows for more microorganisms to colonise the empty spaces between the filter media (Almuktar & Scholz 2015, 2016 a, b)

Constructed wetland systems have been reported to remove various types of pathogens effectively (Scholz, 2006, 2010). Arias et al. (2003), Hansen et al. (2004) and Molleda et al. (2008) demonstrated that in subsurface flow constructed wetlands, pathogens can be removed through different mechanisms, such as antibiotics excretion (Garcia et al., 2013). However, this mechanism cannot be evidenced, as reported by Stottmeister et al., (2003). Moreover, in constructed wetlands, pathogens can be removed directly or indirectly via different processes such as filtration, sedimentation, adsorption, and predation by protozoa and bacteriophages (Kadlec & Wallace, 2008).

The investigation of the sedimentation role in pathogen removal in the wetland systems was performed by Karim et al. (2004). The authors' results showed that statistically, there are no significant differences in faecal coliform and coliphage numbers in effluent water compared to those in the sediment indicating that macrophyte roots of the wetland system played an important role in pathogen removal. These results agreed with those obtained by Garcia et al., (2013) who reported that *E-Coli* were removed well by wetland plants.

Table 4.19: Microbiological examination of the irrigation waters (colony forming units (CFU)/100 ml).

Microbes	Mean	Minimum	Maximum	Standard deviation
Total coliforms				
Filter 1	130 500	19 000	284 000	119 582.2
Filter 2	3833	2000	6000	1472.0
Filter 3	118 167	0	243 000	108 147.0
Filter 4	1333	0	2000	816.5
Filter 5	194 833	21 000	387 000	131 099.1
Filter 6	19 667	4000	48 000	16 033.3
Filter 7	4667	0	12 000	4366.5
Filter 8	5167	0	15 000	5492.4
Control A	47 167	0	148 000	60 832.3
Control B	5000	0	26 000	10 353.7
TW+WW	80 833	0	147 000	64 126.2
WW	113 167	3000	205 000	84 383.5
Escherichia coli				
Filter 1	1833	0	7000	2994.4
Filter 2	0	0	0	0.0
Filter 3	1167	0	4000	1834.8
Filter 4	0	0	0	0.0
Filter 5	5667	0	18 000	8140.4
Filter 6	0	0	0	0.0
Filter 7	0	0	0	0.0
Filter 8	0	0	0	0.0
Control A	0	0	0	0.0
Control B	0	0	0	0.0
TW+WW	2000	0	6000	2529.8
WW	8000	0	22 000	10 526.2
***Streptococci* spp.**				
Filter 1	114 833	0	370 000	178 628.6
Filter 2	1333	0	8000	3266.0
Filter 3	70 333	0	290 000	119 869.4
Filter 4	4833	0	15 000	7139.1
Filter 5	24 667	0	105 000	42 949.6
Filter 6	0	0	0	0.0
Filter 7	0	0	0	0.0
Filter 8	0	0	0	0.0
Control A	0	0	0	0.0
Control B	0	0	0	0.0
TW+WW	500	0	1000	547.7
WW	2667	0	7000	2658.3

Table 4.19 (cont.)

Microbes	Mean	Minimum	Maximum	Standard deviation
***Salmonella* spp.**				
Filter 1	161 500	25 000	366 000	137 004.7
Filter 2	3000	0	5000	1673.3
Filter 3	114 167	0	266 000	113 520.8
Filter 4	3167	2000	7000	1940.8
Filter 5	232 500	123 000	317 000	78 025.0
Filter 6	25 167	6000	58 000	17 971.3
Filter 7	12 000	1000	32 000	15 126.1
Filter 8	6833	1000	16 000	5419.1
Control A	71 833	0	181 000	74 831.6
Control B	11 500	0	38 000	17 952.7
TW+WW	87 333	0	173 000	76 288.1
WW	202 167	18 000	467 000	171 352.8

Note: 0 entries indicate absolutely no growth on the plate after incubation, TW+WW, tap water (80%) spiked with wastewater (20%), and WW, raw wastewater (100%). Twenty water samples were tested.

Table 4.20: Statistical assessment of irrigation water microbes (colony forming units (CFU)/100 ml) during overall experiment period (11/10/13 to 25/09/14).

Design variables	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b] for different system combinations
	Total coliforms		
Aggregate diameter			
Filter 1 & Filter 3	0.073	I-T	0.855
Filter 2 & Filter 4	0.531	I-T	0.005
Contact time			
Filter 4 & Filter 7	0.006	M-W-U	0.016
Resting time			
Filter 7 & Filter 8	0.093	I-T	0.865
Inflow loading rate			
Filter 3 & Filter 5	0.460	I-T	0.029
Filter 4 & Filter 6	0.002	M-W-U	0.004
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	0.004
Filter 3 & Filter 4	< 0.001	M-W-U	0.025
Filter 5 & Filter 6	0.008	M-W-U	0.016
Control A & Control B	< 0.001	M-W-U	0.028

Table 4.20 (cont.)

Design variables	Shapiro-Wilk test (p-value)[a]	Statistical test	P-values[b] for different system combinations
	***Streptococci* spp.**		
Aggregate diameter			
Filter 1 & Filter 3	< 0.001	M-W-U	0.025
Filter 2 & Filter 4	< 0.001	M-W-U	0.216
Contact time			
Filter 4 & Filter 7	n.a	n.a	n.a
Resting time			
Filter 7 & Filter 8	n.a	n.a	n.a
Inflow loading rate			
Filter 3 & Filter 5	< 0.001	M-W-U	0.032
Filter 4 & Filter 6	n.a	n.a	n.a
Hydrocarbon influence			
Filter 1 & Filter 2	< 0.001	M-W-U	< 0.001
Filter 3 & Filter 4	< 0.001	M-W-U	< 0.001
Filter 5 & Filter 6	n.a	n.a	n.a
Control A & Control B	n.a	n.a	n.a
	***Salmonella* spp.**		
Aggregate diameter			
Filter 1 & Filter 3	0.130	I-T	0.529
Filter 2 & Filter 4	0.184	I-T	0.877
Contact time			
Filter 4 & Filter 7	< 0.001	M-W-U	0.041
Resting time			
Filter 7 & Filter 8	0.002	M-W-U	0.038
Inflow loading rate			
Filter 3 & Filter 5	0.396	I-T	0.032
Filter 4 & Filter 6	0.004	M-W-U	0.006
Hydrocarbon influence			
Filter 1 & Filter 2	0.001	M-W-U	0.004
Filter 3 & Filter 4	< 0.001	M-W-U	0.033
Filter 5 & Filter 6	0.038	M-W-U	0.004
Control A & Control B	0.002	M-W-U	0.042

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test. Filters are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test, I-T, the parametric Independent samples T-test; n.a, not applicable as the corresponding bacteria are not detected.

Table 4.21: Statistical assessment of total coliforms for irrigation waters compared to standards.

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value	Standard	Statistic (p-value)[b]
		Total coliforms (CFU)/100 ml			
Filter 1 outflow	0.087	O-S-T	130 500	1000	0.045
Filter 2 outflow	0.804	O-S-T	3833	1000	0.005
Filter 3 outflow	0.200	O-S-T	118 167	1000	0.045
Filter 4 outflow	0.091	O-S-T	1333	1000	0.363
Filter 5 outflow	0.857	O-S-T	194 833	1000	0.015
Filter 6 outflow	0.273	O-S-T	19 667	1000	0.036
Filter 7 outflow	0.532	O-S-T	4667	1000	0.045
Filter 8 outflow	0.160	O-S-T	5167	1000	0.012
Control A outflow	0.110	O-S-T	47 167	1000	0.011
Control B outflow	< 0.001	O-S-W	5000	1000	0.046
TW+WW	0.060	O-S-T	80 833	1000	0.028
WW	0.089	O-S-T	113 167	1000	0.023

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). O-S-T, the parametric one sample t-test, O-S-W, the non-parametric Wilcoxon signed rank test. CFU, colony forming units. TW+WW, tap water (80%) spiked with wastewater (20%), and WW, raw wastewater (100%)

Table 4.22: Overview of correlation coefficients and associated significances between variables using the non-parametric Spearman correlation test.

Variable	Statistic	Variable											
		COD	BOD	DO	Fe	Mn	K	SS	NTU	TC	*E- coli*	*Strep.*	*Salm.*
COD	R	1.000	0.815**	-0.574	0.705*	0.766**	0.620	0.796**	0.766**	0.863**	0.800**	0.610*	0.766**
	p	n.a	0.004	0.083	0.023	0.010	0.056	0.006	0.010	0.001	0.005	0.041	0.010
BOD	R	0.815**	1.000	-0.351	0.830**	0.697*	0.794**	0.709*	0.697*	0.685*	0.768**	0.743*	0.057*
	p	0.004	n.a	0.320	0.003	0.025	0.006	0.022	0.025	0.029	0.009	0.014	0.032
DO	R	-0.574	-0.351	1.000	-0.283	-0.529	-0.419	-0.505	-0.523	-0.726*	-0.469	-0.177	-0.751*
	p	0.083	0.320	n.a	0.428	0.116	0.229	0.137	0.121	0.017	0.171	0.624	0.012
Fe	R	0.705*	0.830**	-0.283	1.000	0.915**	0.867**	0.552	0.479	0.636*	0.768**	0.627	0.673*
	p	0.023	0.003	0.428	n.a	0.000	0.001	0.098	0.162	0.048	0.009	0.052	0.033
Mn	R	0.766**	0.697*	-0.529	0.915**	1.000	0.733*	0.612	0.539	0.758*	0.768**	0.537	0.842**
	p	0.010	0.025	0.116	0.000	n.a	0.016	0.060	0.108	0.011	0.009	0.110	0.002
K	R	0.620	0.794**	-0.419	0.867**	0.733*	1.000	0.455	0.406	0.564	0.664*	0.511	0.552
	p	0.056	0.006	0.229	0.001	0.016	n.a	0.187	0.244	0.090	0.036	0.131	0.098
SS	R	0.796**	0.709*	-0.505	0.552	0.612	0.455	1.000	0.976**	0.685*	0.753*	0.795**	0.685*
	p	0.006	0.022	0.137	0.098	0.060	0.187	n.a	0.000	0.029	0.012	0.006	0.029
NTU	R	0.766**	0.697*	-0.523	0.479	0.539	0.406	0.976**	1.000	0.648*	0.768**	0.847**	0.636*
	p	0.010	0.025	0.121	0.162	0.108	0.244	0.000	n.a	0.043	0.009	0.002	0.048
TC	R	0.863**	0.685*	-0.726*	0.636*	0.758*	0.564	0.685*	0.648*	1.000	0.813**	0.433	0.939**
	p	0.001	0.029	0.017	0.048	0.011	0.090	0.029	0.043	n.a	0.004	0.211	0.000
E- coli	R	0.800**	0.768**	-0.469	0.768**	0.768**	0.664*	0.753*	0.768**	0.813**	1.000	0.819**	0.813**
	p	0.005	0.009	0.171	0.009	0.009	0.036	0.012	0.009	0.004	n.a	0.004	0.004
Strep.	R	0.610*	0.743*	-0.177	0.627	0.537	0.511	0.795**	0.847**	0.433	0.819**	1.000	0.446
	p	0.041	0.014	0.624	0.052	0.110	0.131	0.006	0.002	0.211	0.004	n.a	0.196
Salm.	R	0.766**	0.0576*	-0.751*	0.673*	0.842**	0.552	0.685*	0.636*	0.939**	0.813**	0.446	1.000
	p	0.010	0.032	0.012	0.033	0.002	0.098	0.029	0.048	0.000	0.004	0.196	n.a

Note: COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); DO, dissolved oxygen (mg/l); Fe, Iron (mg/l); Mn, manganese (mg/l); k, potassium (mg/l); SS, suspended solids (mg/l); NTU, turbidity in Nephelometric turbidity units; *TC,* Total coliforms (colony forming unit (CFU)/100 ml); *E- coli, Escherichia coli* (CFU/100 ml); *Strep.*, *Streptococci* spp. (CFU/100 ml); *Salm., Salmonella* spp. (CFU/100 ml); R, correlation coefficient; *p,* probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level, and *, correlation is significant at the 0.05 level.

4.3 Environmental boundary conditions

Table 4.23 shows an overview of the environmental boundary conditions associated with the vegetable pots. The light intensity records for this experiment during the flowering and fruiting stage were below the proposed range from about 8600 and 17200 lux (Deli & Tiessen, 1969). Low light intensity may lead to flower inhibition or cause flower abscission (Wein & Zhang, 1991). Moreover, low light intensity applied to plants will produce leggy plants growing toward light, which is necessary for photosynthesis (Ciju, 2013). For the germination stage (Table 4.23), the temperature records complied with the optimal temperature for peppers during this stage (Chemicals, 2014). Concerning the vegetative growth stage, the temperature records for this experiment were higher than the recommended optimum values of between 21 and 23 °C (Bakker & Ufflen, 1988). However, temperature records for this stage complied with the values associated with the highest photosynthesis rate, which can be achieved at temperatures between 24 and 29 °C (Bhatt & Srinivasa Rao, 1989; Nilwik, 1980). Table 4.23 shows that the relative humidity before and after fruiting was low (37±7.6% and 57±7.8%, respectively). Humidity values below 50% could have a negative impact on the fruit development as a humid atmosphere is necessary for flowers to successfully pollinate, otherwise, the unfertilised flowers will drop off, as reported elsewhere (Nilwik, 1980).

Table 4.23: Overview of environmental boundary conditions associated with the vegetable pots (mean±standard deviation (number of records)).

Parameter	Unit	A[a]	B[b]	C[c]	D[d]	E[e]	F[f]
Illuminance (one-off record during lab visit)	lux	5587±5501.1 (918)	nm	4208± 2560.5(36)	12316±1823.3 (102)	3682±3246.1 (513)	5877±9262.2 (267)
Temperature (one-off record during lab visit)	°C	25.4±2.12 (603)	20.5±1.25 (13)	24.8±1.17 (48)	25.0±1.89 (102)	26.3±2.32 (204)	25.0±1.83 (236)
Temperature (minimum within a 24-hour period)	°C	20.8±1.97 (75)	nm	nm	20.3±1.87 (8)	21.2±2.02 (33)	20.6±2.05 (34)
Temperature (maximum within a 24-hour period)	°C	26.8±2.59 (75)	nm	nm	25.3±1.98 (8)	27.0±2.83 (33)	26.6±2.26 (34)
Relative humidity (one-off record during lab visit)	%	49±11.7 (488)	nm	nm	42±5.4 (96)	37±7.6 (156)	57±7.8 (236)
Relative humidity (minimum within a 24-hour period)	%	35±7.1 (75)	nm	nm	36±3.7 (8)	30±3.5 (33)	38±8.5 (34)
Relative humidity (maximum within a 24-hour period)	%	55±12.5 (75)	nm	nm	46±5.6 (8)	48±10.5 (33)	63±9.8 (34)

Note: [a] A: Overall period (11/10/13 to 25/09/14); [b] B: Germination period (17/09/13 to 22/09/13); [c] C: First Planting period (23/09/13 to 10/10/13); [d] D: Second planting period (11/10/13 to 07/11/13); [e] E: Final planting period before fruiting (08/11/13 to 19/01/14); [f] F: Final planting period after fruiting (20/01/14 to 25/09/14); nm: not measured.

4.4 Crop growth comparisons

4.4.1 Visual growth problems

Table 4.24a–g shows visual growth problems observed in Sweet Pepper and Chilli plants at final planting stage. The reference numbers indicating individual plants, which had a visual problem of a particular nature, are highlighted in bold. Table 4.24a indicates deficiencies for both Sweet Peppers and Chillies for old plant parts regarding magnesium and potassium. Table 4.24a also highlights phosphorus deficiencies for old Sweet Pepper plant parts. Deficiencies for both Sweet Peppers and Chillies for young plant parts were recorded for calcium and sulphur (Table 4.24b). A sulphur deficiency was noted for new Chilli plant parts only (Table 4.24b). Table 4.24c summarises molybdenum deficiencies. Boron and zinc deficiencies were noted for new Sweet Pepper plant parts (Table 4.24d). Copper deficiencies were observed for new plant parts for both Sweet Peppers and Chillies (Table 4.24d).

A surplus of nitrogen was noted for new plant parts of both Sweet Peppers and Chillies (Table 4.24e). A surplus of molybdenum was noted for the old plant parts for both Sweet Peppers and Chillies (Table 4.24f). However, this observation is ambiguous, because symptoms for some plants also indicate deficiencies. Table 4.24g is concerned with surpluses of zinc, manganese, copper and boron.

Table 4.24a: Overview of visual growth problems associated with macronutrient deficiency in old plant parts observed during the final planting phase on 11 March 2014. (see Table 2.8 for possible reasons for nutritional disorders), corresponding numbers of plants highlighted in **bold**; O, organic media; IO, inorganic media.

Inflow source and growth media	Stunted growth		Few flowers with poor and deformed fruits		Chlorosis		Burning of leaf margins with midrib remaining green		Interveinal chlorosis	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	P1;P2	C3;C4	**P1;P2**	C3;C4	**P1;P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	**C3;C4**
Filter 2 /O	P5;P6	C6;C8;C9	**P5;P6**	C6;C8;C9	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	**C6;C8;C9**
Filter 3 /O	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	P8;**P9**;P10	C10;C11;C12	**P8;P9;P10**	**C10;C11;C12**
Filter 4 /O	P12;P16	C16;C17	**P12;P16**	C16;C17	**P12;P16**	C16;C17	P12;P16	C16;C17	**P12;P16**	**C16;C17**
Filter 5 /O	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	**P18;P19;P20**	**C18**;C19;**C20**	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	**C18;C19;C20**
Filter 6 /O	P22;P23	C21	**P22;P23**	C21	**P22;P23**	**C21**	P22;P23	C21	**P22;P23**	**C21**
Filter 7 /O	P26;P28	C25;C26	**P26;P28**	C25;C26	**P26;P28**	C25;**C26**	P26;P28	C25;C26	**P26;P28**	**C25;C26**
Filter 8 /O	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	**P31;P32;P33**	**C27;C28;C29**	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	**C27;C28;C29**
Control A /O	P35	C31;C33	**P35**	C31;C33	**P35**	C31;C33	P35	C31;C33	**P35**	**C31;C33**
Control B /O	P39	C37;C38	**P39**	C37;C38	**P39**	**C37**;C38	P39	C37;C38	**P39**	**C37;C38**
Deionised water /O	P41	C41	**P41**	C41	**P41**	C41	P41	C41	**P41**	**C41**
Tap water /O	P44	C42;C43	**P44**	C42;C43	**P44**	**C42;C43**	P44	C42;C43	**P44**	**C42;C43**
Tap water/ fertiliser/O	P45;P46	C45;C46	**P45;P46**	C45;C46	**P45;P46**	**C45**;C46	P45;P46	C45;C46	**P45;P46**	**C45;C46**
Wastewater/ tap water /O	P47	C49	**P47**	C49	**P47**	**C49**	P47	C49	**P47**	**C49**
Wastewater /O	P51;P54	C52;C54	**P51;P54**	C52;C54	**P51;P54**	**C52;C54**	P51;**P54**	C52;C54	**P51;P54**	**C52;C54**
Filter 1 /IO	**P55;P56**	**C56**	**P55;P56**	**C56**	**P55;P56**	**C56**	P55;P56	C56	**P55;P56**	**C56**
Filter 2 /IO	**P57**	**C58**	**P57**	C58	**P57**	**C58**	P57	C58	**P57**	**C58**
Filter 3 /IO	**P59**	**C61**	**P59**	**C61**	**P59**	**C61**	P59	C61	**P59**	**C61**
Filter 4 /IO	**P61**	**C63;C64**	**P61**	C63;C64	**P61**	**C63;C64**	P61	C63;C64	**P61**	**C63;C64**
Filter 5 /IO	**P65**	**C66**	**P65**	C66	**P65**	**C66**	P65	C66	**P65**	**C66**

Table 4.24a (cont.)

Filter 6 /IO	**P66;P67**	**C68**	**P66;P67**	C68	**P66;P67**	**C68**	P66;P67	C68	**P66;P67**	**C68**
Filter 7 /IO	**P17;P69**	**C71**	**P17;P69**	C71	**P17;P69**	C71	P17;P69	C71	**P17;P69**	**C71**
Filter 8 /IO	**P70;P71**	**C72;C73**	**P70;P71**	C72;C73	**P70;P71**	**C72**;C73	P70;P71	C72;C73	**P70;P71**	**C72;C73**
Control A /IO	**P73**	**C74**	**P73**	C74	**P73**	**C74**	P73	C74	**P73**	**C74**
Control B /IO	**P74**	**C76;C77**	**P74**	C76;C77	**P74**	**C76;C77**	P74	C76;C77	**P74**	**C76;C77**
Deionised water /IO	**P80**	**C80**	**P80**	C80	**P80**	**C80**	P80	C80	**P80**	**C80**
Tap water /IO	**P81**	**C82**	**P81**	C82	**P81**	**C82**	P81	C82	**P81**	**C82**
Tap water/ fertiliser/IO	P83;P84	C84;C85	**P83;P84**	C84;C85	**P83;P84**	C84;**C85**	P83;P84	C84;C85	**P83;P84**	**C84;C85**
Wastewater/ tap water /IO	**P86;P87**	**C87**	**P86;P87**	C87	**P86;P87**	**C87**	P86;P87	C87	**P86;P87**	**C87**
Wastewater /IO	P89;P90	**C90**	**P89;P90**	C90	**P89;P90**	C90	P89;P90	C90	**P89;P90**	**C90**

Inflow source and growth media	Curly small leaves		Bending of petioles and hang downwards; parallel to stem		Necrosis		Leaf tips brown and necrotic	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	**P1;P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;**C4**	P1;P2	C3;C4
Filter 2 /O	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5**;P6	C6;C8;C9	P5;P6	C6;C8;C9
Filter 3 /O	P8;P9;**P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	**C10;C11; C12**	P8;P9;P10	C10;C11;C12
Filter 4 /O	P12;**P16**	C16;**C17**	P12;P16	C16;C17	**P12;P16**	**C16**;C17	P12;P16	C16;C17
Filter 5 /O	P18;P19;P20	**C18**;C19;**C20**	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C 20	P18;P19;P20	C18;C19;C20
Filter 6 /O	**P22;P23**	C21	P22;P23	C21	**P22;P23**	**C21**	P22;P23	C21
Filter 7 /O	P26;P28	C25;C26	P26;P28	C25;C26	P26;**P28**	**C25;C26**	P26;P28	**C25;C26**
Filter 8 /O	**P31**;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C 29	P31;P32;P33	**C27;C28;C29**
Control A /O	P35	C31;**C33**	P35	C31;C33	P35	**C31**;C33	P35	C31;C33
Control B /O	**P39**	C37;C38	P39	C37;C38	**P39**	C37;**C38**	P39	C37;C38
Deionised water /O	P41	C41	P41	C41	**P41**	C41	P41	C41
Tap water /O	**P44**	C42;C43	P44	C42;C43	**P44**	C42;C43	P44	C42;C43

Table 4.24a (cont.)

Inflow source and growth media	Curly small leaves		Bending of petioles and hang downwards; parallel to stem		Necrosis		Leaf tips brown and necrotic	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Tap water/fertiliser/O	P45;P46	C45;**C46**	P45;P46	C45;C46	**P45;P46**	C45;C46	P45;P46	C45;C46
Wastewater/tap water /O	**P47**	**C49**	P47	C49	P47	**C49**	P47	C49
Wastewater /O	P51;P54	C52; **C54**	P51;P54	C52; C54	P51;**P54**	**C52; C54**	P51;**P54**	C52; C54
Filter 1 /IO	**P55;P56**	C56	P55;P56	**C56**	**P55**;P56	C56	P55;P56	C56
Filter 2 /IO	**P57**	C58	P57	C58	P57	C58	P57	C58
Filter 3 /IO	P59	C61	P59	**C61**	**P59**	C61	P59	C61
Filter 4 /IO	**P61**	C63;C64	P61	C63;C64	P61	C63;C64	P61	C63;C64
Filter 5 /IO	P65	C66	P65	C66	P65	C66	P65	C66
Filter 6 /IO	**P66**;P67	C68	P66;P67	**C68**	**P66;P67**	C68	P66;P67	C68
Filter 7 /IO	**P17;P69**	C71	P17;P69	C71	**P17;P69**	C71	P17;P69	C71
Filter 8 /IO	**P70**;P71	C72;C73	P70;P71	C72;C73	P70;**P71**	C72;C73	P70;P71	C72;C73
Control A /IO	**P73**	C74	P73	C74	P73	C74	P73	C74
Control B /IO	**P74**	C76;C77	P74	C76;C77	P74	C76;C77	P74	C76;C77
Deionised water /IO	P80	C80	P80	C80	P80	C80	P80	C80
Tap water /IO	P81	C82	P81	C82	**P81**	C82	P81	C82
Tap water/fertiliser /IO	**P83;P84**	C84;C85	P83;P84	C84;C85	**P83;P84**	C84;C85	P83;P84	C84;C85
Wastewater/tap water /IO	**P86;P87**	C87	**P86;P87**	C87	**P86;P87**	C87	P86;P87	C87
Wastewater /IO	**P89;P90**	C90	**P89;P90**	C90	**P89;P90**	C90	P89;P90	C90

Table 4.24b: Overview of visual growth problems associated with macronutrient deficiency in new plant parts observed during the final planting phase on 11 March 2014.

Inflow source and growth media	Stunted growth		Spindly small plant with thin stem		Premature falling of buds and blossoms		Necrosis	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	P1;P2	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;C4	P1;P2	C3;C4
Filter 2 /O	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	C6;C8;C9	P5;**P6**	C6;C8;C9
Filter 3 /O	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12
Filter 4 /O	P12;P16	C16;C17	P12;P16	C16;C17	**P12;P16**	C16;C17	P12;P16	C16;C17
Filter 5 /O	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	P18;**P19;P20**	C18;C19;C20
Filter 6 /O	P22;P23	C21	P22;P23	C21	**P22;P23**	C21	P22;P23	C21
Filter 7 /O	P26;P28	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;C26	P26;**P28**	C25;**C26**
Filter 8 /O	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29
Control A /O	P35	C31;C33	P35	C31;C33	**P35**	C31;C33	P35	C31;C33
Control B /O	P39	C37;C38	P39	C37;C38	**P39**	C37;C38	P39	C37;C38

Table 4.24b (cont.)

Inflow source and growth media	Stunted growth		Spindly small plant with thin stem		Premature falling of buds and blossoms		Necrosis	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Deionised water /O	P41	C41	P41	C41	**P41**	C41	P41	C41
Tap water /O	P44	C42;C43	P44	C42;C43	**P44**	C42;C43	P44	**C42;C43**
Tap water/ fertiliser/O	P45;P46	C45;C46	P45;P46	C45;C46	**P45;P46**	C45;C46	P45;P46	C45;C46
Wastewater/ tap water /O	P47	C49	P47	C49	**P47**	C49	P47	C49
Wastewater /O	P51;P54	C52; C54	P51;P54	C52;C54	**P51;P54**	C52;C54	P51;P54	C52;C54
Filter 1 /IO	**P55;P56**	**C56**	P55;P56	C56	**P55;P56**	C56	P55;P56	C56
Filter 2 /IO	**P57**	**C58**	P57	**C58**	**P57**	C58	P57	C58
Filter 3 /IO	**P59**	**C61**	P59	C61	**P59**	C61	P59	C61
Filter 4 /IO	**P61**	**C63;C64**	P61	**C63;C64**	**P61**	C63;C64	P61	C63;C64
Filter 5 /IO	**P65**	**C66**	P65	C66	**P65**	C66	P65	C66
Filter 6 /IO	**P66;P67**	**C68**	P66;P67	C68	**P66;P67**	C68	P66;P67	C68
Filter 7 /IO	**P17;P69**	**C71**	P17;P69	C71	**P17;P69**	C71	P17;P69	C71
Filter 8 /IO	**P70;P71**	**C72;C73**	P70;P71	**C72;C73**	**P70;P71**	C72;C73	P70;P71	C72;C73
Control A /IO	**P73**	**C74**	P73	C74	**P73**	C74	P73	C74
Control B /IO	**P74**	**C76;C77**	P74	C76;C77	**P74**	C76;C77	P74	C76;C77
Deionised water /IO	**P80**	**C80**	P80	C80	**P80**	C80	P80	C80
Tap water /IO	**P81**	**C82**	P81	C82	**P81**	C82	P81	C82
Tap water/ fertiliser /IO	P83;P84	C84;C85	P83;P84	C84;C85	**P83;P84**	C84;C85	P83;P84	C84;C85
Wastewater /tap water /IO	**P86;P87**	**C87**	P86;P87	C87	**P86;P87**	C87	P86;P87	C87
Wastewater /IO	**P89;P90**	**C90**	P89;P90	**C90**	**P89;P90**	C90	P89;P90	C90

Table 4.24c: Overview of visual growth problems associated with micronutrient deficiency in old plant parts observed during the final planting phase on 11 March 2014.

Inflow source and growth media	Stunted growth		Light green to yellowish young leaves	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	P1;P2	C3;C4	P1;P2	C3;C4
Filter 2 /O	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9
Filter 3 /O	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12
Filter 4 /O	P12;P16	C16;C17	P12;P16	C16;C17
Filter 5 /O	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20
Filter 6 /O	P22;P23	C21	P22;P23	C21
Filter 7 /O	P26;P28	C25;C26	P26;P28	C25;C26
Filter 8 /O	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29
Control A /O	P35	C31;C33	P35	C31;C33
Control B /O	P39	C37;C38	P39	C37;C38
Deionised water /O	P41	C41	P41	C41
Tap water /O	P44	C42;C43	P44	C42;C43
Tap water/ fertiliser/O	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/ tap water /O	P47	C49	P47	C49
Wastewater /O	P51;P54	C52; C54	P51;P54	C52; C54
Filter 1 /IO	**P55;P56**	**C56**	P55;P56	C56
Filter 2 /IO	**P57**	**C58**	P57	C58
Filter 3 /IO	**P59**	**C61**	P59	C61
Filter 4 /IO	**P61**	**C63;C64**	P61	C63;C64
Filter 5 /IO	**P65**	**C66**	P65	C66
Filter 6 /IO	**P66;P67**	**C68**	P66;P67	C68
Filter 7 /IO	**P17;P69**	**C71**	P17;P69	C71
Filter 8 /IO	**P70;P71**	**C72;C73**	P70;P71	C72;C73
Control A /IO	**P73**	**C74**	P73	C74
Control B /IO	**P74**	**C76;C77**	P74	C76;C77
Deionised water /IO	**P80**	**C80**	P80	C80
Tap water /IO	**P81**	**C82**	P81	C82
Tap water/ fertiliser/IO	**P83;P84**	**C84;C85**	P83;P84	C84;C85
Wastewater/tap /IO	**P86;P87**	**C87**	P86;P87	C87
Wastewater /IO	**P89;P90**	**C90**	P89;P90	C90

Table 4.24d: Overview of visual growth problems associated with micronutrient deficiency in new plant parts observed during the final planting phase on 11 March 2014.

Inflow source and growth media	Stunted growth		Death of terminal buds		Thick and curly leaf tips		Necrosis		Poor flowering and seeds	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	P1;P2	C3;C4	**P1;P2**	C3;C4	**P1**;P2	C3;C4	P1;P2	C3;C4	Sweet Pepper	Chilli
Filter 2 /O	P5;P6	C6;C8;C9	**P5;P6**	C6;C8;C9	P5;**P6**	C6;C8;C9	P5;**P6**	C6;C8;C9	**P1;P2**	C3;C4
Filter 3 /O	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;**C11;C12**	P8;P9;P10	C10;C11;C12	**P5;P6**	C6;C8;C9
Filter 4 /O	P12;P16	C16;C17	**P12;P16**	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	**P8;P9;P10**	C10;C11;C12
Filter 5 /O	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	P18;P19;**P20**	**C18**;C19;**C20**	P18;**P19;P20**	C18;C19;C20	**P12;P16**	C16;C17
Filter 6 /O	P22;P23	C21	**P22;P23**	C21	P22;**P23**	C21	P22;P23	C21	**P18;P19;P20**	C18;C19;C20
Filter 7 /O	P26;P28	C25;C26	**P26;P28**	C25;C26	P26;P28	C25;**C26**	P26;**P28**	C25;**C26**	**P22;P23**	C21
Filter 8 /O	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	**P31**;P32;P33	C27;**C28;C29**	**P31**;P32;P33	C27;C28;C29	**P26;P28**	C25;C26
Control A /O	P35	C31;C33	**P35**	C31;C33	P35	C31;**C33**	P35	C31;C33	**P31;P32;P33**	C27;C28;C29
Control B /O	P39	C37;C38	**P39**	C37;C38	P39	**C37;C38**	P39	C37;C38	**P35**	C31;C33
Deionised water /O	P41	C41	**P41**	C41	**P41**	C41	P41	C41	**P39**	C37;C38
Tap water /O	P44	C42;C43	**P44**	C42;C43	**P44**	C42;**C43**	P44	**C42;C43**	**P41**	C41
Tap water/ fertiliser/O	P45;P46	C45;C46	**P45;P46**	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	**P44**	C42;C43
Wastewater/ tap water /O	P47	C49	**P47**	C49	**P47**	**C49**	P47	C49	**P45;P46**	C45;C46
Wastewater /O	P51;P54	C52;C54	**P51;P54**	C52;C54	P51;P54	C52;C54	P51;P54	C52;C54	**P47**	C49
Filter 1 /IO	**P55;P56**	**C56**	**P55;P56**	C56	**P55**;P56	C56	P55;P56	C56	**P51;P54**	C52;C54
Filter 2 /IO	**P57**	**C58**	**P57**	C58	P57	C58	P57	C58	**P55;P56**	C56
Filter 3 /IO	**P59**	**C61**	**P59**	C61	P59	C61	P59	C61	**P57**	C58
Filter 4 /IO	**P61**	**C63;C64**	**P61**	C63;C64	**P61**	C63;C64	P61	C63;C64	**P59**	C61
Filter 5 /IO	**P65**	**C66**	**P65**	C66	**P65**	C66	P65	C66	**P61**	C63;C64
Filter 6 /IO	**P66;P67**	**C68**	**P66;P67**	C68	P66;P67	C68	P66;P67	C68	**P65**	C66
Filter 7 /IO	**P17;P69**	**C71**	**P17;P69**	C71	P17;P69	C71	P17;P69	C71	**P66;P67**	C68
Filter 8 /IO	**P70;P71**	**C72;C73**	**P70;P71**	C72;C73	P70;P71	C72;C73	P70;P71	C72;C73	**P17;P69**	C71
Control A /IO	**P73**	**C74**	**P73**	C74	**P73**	C74	P73	C74	**P70;P71**	C72;C73
Control B /IO	**P74**	**C76;C77**	**P74**	C76;C77	P74	C76;C77	P74	C76;C77	**P73**	C74
Deionised water /IO	**P80**	**C80**	**P80**	C80	P80	C80	P80	C80	**P74**	C76;C77
Tap water /IO	**P81**	**C82**	**P81**	C82	P81	C82	P81	C82	**P80**	C80
Tap water/ fertiliser/IO	P83;P84	C84;C85	**P83;P84**	C84;C85	P83;**P84**	C84;**C85**	P83;P84	C84;C85	**P81**	C82
Wastewater/tap /IO	**P86;P87**	**C87**	**P86;P87**	C87	P86;P87	C87	P86;P87	C87	**P86;P87**	C87
Wastewater /IO	**P89;P90**	**C90**	**P89;P90**	C90	P89;**P90**	**C90**	P89;P90	C90	**P89;P90**	C90

Table 4.24e: Overview of visual growth problems associated with macronutrient surplus in old plant parts observed during the final planting phase on 11 March 2014.

Inflow source and growth media	Dark green and abundant foliage		Stunting and reducing in branches	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	**P1;P2**	**C3;C4**	P1;P2	C3;C4
Filter 2 /O	**P5;P6**	**C6;C8;C9**	P5;P6	C6;C8;C9
Filter 3 /O	**P8;P9;P10**	**C10;C11;C12**	P8;P9;P10	C10;C11;C12
Filter 4 /O	**P12;P16**	**C16;C17**	P12;P16	C16;C17
Filter 5 /O	**P18;P19;P20**	**C18;C19;C20**	P18;P19;P20	C18;C19;C20
Filter 6 /O	**P22;P23**	**C21**	P22;P23	C21
Filter 7 /O	**P26;P28**	**C25;C26**	P26;P28	C25;C26
Filter 8 /O	P31;**P32;P33**	**C27;C28;C29**	P31;P32;P33	C27;C28;C29
Control A /O	**P35**	**C31;C33**	P35	C31;C33
Control B /O	**P39**	**C37;C38**	P39	C37;C38
Deionised water /O	**P41**	**C41**	P41	C41
Tap water /O	**P44**	**C42;C43**	P44	C42;C43
Tap water/ fertiliser/O	**P45;P46**	C45;C46	P45;P46	C45;C46
Wastewater/ tap water /O	**P47**	C49	P47	C49
Wastewater /O	**P51;**P54	**C52;C54**	P51;P54	C52;C54
Filter 1 /IO	P55;P56	C56	**P55;P56**	**C56**
Filter 2 /IO	P57	**C58**	**P57**	**C58**
Filter 3 /IO	P59	C61	**P59**	**C61**
Filter 4 /IO	P61	**C63;C64**	**P61**	**C63;C64**
Filter 5 /IO	P65	C66	**P65**	**C66**
Filter 6 /IO	P66;P67	**C68**	**P66;P67**	**C68**
Filter 7 /IO	P17;P69	**C71**	**P17;P69**	**C71**
Filter 8 /IO	P70;P71	C72;**C73**	**P70;P71**	**C72;C73**
Control A /IO	P73	C74	**P73**	**C74**
Control B /IO	P74	C76;C77	**P74**	**C76;C77**
Deionised water /IO	P80	C80	**P80**	**C80**
Tap water /IO	P81	C82	**P81**	**C82**
Tap water/ fertiliser/IO	**P83;P84**	C84;C85	P83;P84	C84;C85
Wastewater/tap /IO	P86;P87	C87	**P86;P87**	**C87**
Wastewater /IO	**P89**;P90	**C90**	**P89;P90**	**C90**

Table 4.24f: Overview of visual growth problems associated with micronutrient surplus in old plant parts observed during the final planting phase on 11March 2014.

Inflow source and growth media	Stunting and reducing in branches		Golden yellowish leaves	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	P1;P2	C3;C4	P1;P2	C3;C4
Filter 2 /O	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9
Filter 3 /O	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12
Filter 4 /O	P12;P16	C16;C17	P12;P16	C16;C17
Filter 5 /O	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20
Filter 6 /O	P22;P23	C21	P22;P23	C21
Filter 7 /O	P26;P28	C25;C26	P26;P28	C25;C26
Filter 8 /O	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29
Control A /O	P35	C31;C33	P35	C31;C33
Control B /O	P39	C37;C38	P39	C37;C38
Deionised water /O	P41	C41	P41	C41
Tap water /O	P44	C42;C43	P44	C42;C43
Tap water/ fertiliser/O	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/ tap water /O	P47	C49	P47	C49
Wastewater /O	P51;P54	C52;C54	P51;P54	C52;C54
Filter 1 /IO	**P55;P56**	**C56**	P55;P56	C56
Filter 2 /IO	**P57**	**C58**	P57	**C58**
Filter 3 /IO	**P59**	**C61**	**P59**	C61
Filter 4 /IO	**P61**	**C63;C64**	P61	C63;C64
Filter 5 /IO	**P65**	**C66**	P65	**C66**
Filter 6 /IO	**P66;P67**	**C68**	P66;P67	C68
Filter 7 /IO	**P17;P69**	**C71**	P17;P69	C71
Filter 8 /IO	**P70;P71**	**C72;C73**	P70;P71	**C72;C73**
Control A /IO	**P73**	**C74**	P73	C74
Control B /IO	**P74**	**C76;C77**	P74	**C76;C77**
Deionised water /IO	**P80**	**C80**	**P80**	C80
Tap water /IO	**P81**	**C82**	**P81**	**C82**
Tap water/ fertiliser/IO	P83;P84	C84;C85	P83;P84	C84;C85
Wastewater/tap water /IO	**P86;P87**	**C87**	P86;P87	C87
Wastewater /IO	**P89;P90**	**C90**	P89;P90	C90

Table 4.24g: Overview of visual growth problems associated with micronutrient surplus in new plant parts observed during the final planting phase on 11 March 2014.

Inflow source and growth media	Dark green and abundant foliage		Low growth rate		Yellowing and necrosis of leaf tip or margins toward midrib		Stunting and reducing in branches		Necrotic lesions on leaves	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 /O	**P1;P2**	C3;C4	P1;P2	C3;C4	P1;**P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;C4
Filter 2 /O	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	P5;**P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	C6;**C8**;C9
Filter 3 /O	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12
Filter 4 /O	**P12;P16**	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;**P16**	C16;C17
Filter 5 /O	**P18;P19;P20**	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19**;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19;P20**	C18;C19;C20
Filter 6 /O	**P22;P23**	C21	P22;P23	C21	P22;**P23**	C21	P22;P23	C21	**P22;P23**	C21
Filter 7 /O	**P26;P28**	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;**C26**
Filter 8 /O	**P31;P32;P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29
Control A /O	**P35**	C31;C33	P35	C31;C33	**P35**	C31;C33	P35	C31;C33	**P35**	C31;C33
Control B /O	**P39**	C37;C38	P39	C37;C38	P39	C37;C38	P39	C37;C38	**P39**	C37;C38
Deionised water /O	**P41**	C41	P41	C41	P41	C41	P41	C41	**P41**	C41
Tap water /O	**P44**	**C42;C43**	P44	C42;C43	**P44**	C42;C43	P44	C42;C43	**P44**	C42;**C43**
Tap water/ fertiliser/O	**P45;P46**	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/ tap water /O	**P47**	C49	P47	C49	**P47**	C49	P47	C49	**P47**	C49
Wastewater /O	**P51;P54**	**C52;C54**	P51;P54	C52;C54	**P51**;P54	C52;C54	P51;P54	C52;C54	P51;P54	C52;**C54**
Filter 1 /IO	P55;P56	C56	**P55;P56**	**C56**	P55;P56	C56	**P55;P56**	**C56**	P55;P56	C56
Filter 2 /IO	P57	C58	**P57**	**C58**	P57	C58	**P57**	**C58**	P57	C58
Filter 3 /IO	P59	C61	**P59**	**C61**	P59	C61	**P59**	**C61**	P59	C61
Filter 4 /IO	P61	C63;C64	**P61**	**C63;C64**	P61	C63;C64	**P61**	**C63;C64**	P61	C63;C64
Filter 5 /IO	P65	C66	**P65**	**C66**	P65	C66	**P65**	**C66**	P65	C66
Filter 6 /IO	P66;P67	C68	**P66;P67**	**C68**	P66;P67	C68	**P66;P67**	**C68**	P66;P67	C68
Filter 7 /IO	P17;P69	C71	**P17;P69**	**C71**	P17;P69	C71	**P17;P69**	**C71**	P17;P69	C71
Filter 8 /IO	P70;P71	C72;C73	**P70;P71**	**C72;C73**	P70;P71	C72;C73	**P70;P71**	**C72;C73**	P70;P71	C72;C73
Control A /IO	P73	C74	**P73**	**C74**	P73	C74	**P73**	**C74**	P73	C74
Control B /IO	P74	C76;C77	**P74**	**C76;C77**	P74	C76;C77	**P74**	**C76;C77**	P74	C76;C77

Table 4.24g (cont.)

Inflow source and growth media	Dark green and abundant foliage		Low growth rate		Yellowing and necrosis of leaf tip or margins toward midrib		Stunting and reducing in branches		Necrotic lesions on leaves	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Deionised water /IO	P80	C80	**P80**	**C80**	P80	C80	**P80**	**C80**	P80	C80
Tap water /IO	P81	C82	**P81**	**C82**	P81	C82	**P81**	**C82**	P81	C82
Tap water/ fertiliser/IO	P83;P84	C84;C85	**P83;P84**	**C84;C85**	P83;**P84**	C84;C85	**P83;P84**	**C84;C85**	P83;P84	C84;C85
Wastewater/ Tap water /IO	P86;P87	C87	**P86;P87**	**C87**	P86;P87	C87	**P86;P87**	**C87**	P86;P87	C87
Wastewater /IO	P89;P90	C90	**P89;P90**	**C90**	P89;P90	C90	**P89;P90**	**C90**	P89;P90	C90

4.4.2 Sweet Pepper growth comparisons and marketable yield assessment

Figure 4.2a–f shows Sweet Pepper growth details in terms of plant overall height, number of leaves, buds, flowers, fruits and total weight of fruits harvested from each treatment when using organic growth media and subjected to different irrigation water types. Regarding the overall height of plants growing in organic media, Figure 4.2a shows that the maximum height was associated with plants irrigated with raw wastewater followed by those irrigated with tap water spiked with fertiliser. This can be explained by the high nutrient load (Table 4.25) applied via irrigation water (Table 4.26), as discussed by FAO (2003) and Almuktar and Scholz (2016 b).

Regarding the total number of leaves (Figure 4.2b) linked to peppers grown in organic media, findings indicated that peppers irrigated with tap water spiked by fertiliser produced the highest number of leaves, followed by those plants irrigated with water harvested from Filter 4 and raw wastewater, while the lowest leaf numbers were recorded for plants irrigated with deionised water followed by tap water, and Controls A and B due to lack of nutrients available in the water sources (FAO, 2003). Figure 4.2c–f provides summaries of plant developments. Very high numbers of buds were recorded for peppers grown in organic media. However, most of the buds associated with the compost fell down before reaching the flowering stage. Also, most flowers died before producing any fruits. This can be explained by the elevated nutrient concentrations, mainly ammonia-nitrogen, supplied to those plants grown in rich organic media (Table 4.27) and irrigated by wastewater (Table 4.4 and Figure 4.3) as indicated by Chemicals (2014) and Almuktar and Scholz (2016 b). Moreover, falling of most buds and flowers before reaching the fruiting stage can be explained as well by the adverse environmental conditions in the laboratory in terms of light intensity provided by the grow lights and the relative humidity which was elevated artificially by using humidifiers, as explained in section 3.4. These may cause flower inhibition or cause flower abscission, as reported by Wein and Zhang (1991).

Figure 4.4 shows that the plants started to produce buds and flowers in January 2014. However, fruit production took place between June 2014 and September 2014. This is possibly due to the depletion of organic media nutrients causing the plants to start to depend mainly on nutrients supplied by the irrigation water resulting in a better balance in supplied nutrients, confirming the results obtained by Almuktar and Scholz (2016b) reporting that the combination of fresh compost and treated wastewater is usually too high in a particular nutrient to produce a good pepper harvest. Results showed that the highest number of fruits were harvested from plants irrigated with raw wastewater followed by those irrigated with Control A outflow water and tap water. Based on that, plants irrigated with the raw wastewater produced the maximum total weight of harvested fruits as shown in Figure 4.2f. However, the potential of a rather moderate diesel spill to function as stimulation for plant growth in clean water becomes apparent when comparing both controls with each other.

Figure 4.5 summarises differences in fruit characteristics. Statistical analysis using one-way analysis of variance (ANOVA) showed that there is no statistical significant difference ($p > 0.05$) in diameters of peppers harvested from plants irrigated with different water types. However, findings show that fruits harvested from plants irrigated with Filter 1 outflow water had diameter, which were greater than those obtained from peppers irrigated with waters from Filter 2 due to high element loads applied to plants associated with Filter 2 compared to Filter 1 (Table 4.25). Moreover, fruits belonging to Filter 1 had diameters, which were greater than others indicating the impact of nutrient (mainly nitrogen) and trace element loads provided by irrigation water obtained from Filter 1 compared to the other filters (Table 4.25).

One-way analysis of variance (ANOVA) test results showed that there is no statistically significant difference ($p > 0.05$) in mean fruit length harvested from plants irrigated with different irrigation water types. However, fruits harvested from plants irrigated with Filter 7 (low contact time) outflow water were the longest followed by those irrigated with water obtained from Filters 1 (large aggregate size) and 3 (small aggregate size), which were contaminated with hydrocarbons. The shortest fruit lengths were observed for those harvested from plants irrigated with Filter 6 (high inflow rate) outflow water.

Regarding mean fruit weight, Kruskal-Wallis test results (Table 4.28) showed that fruits harvested from plants irrigated with water harvested from Filter 1 had mean weights which were statistically significantly ($p < 0.05$) greater than those associated with plants irrigated with Filters 3, 8, and Control A outflows, and raw wastewater. Moreover, irrigation of plants with Filter 7 outflow water produced fruits of mean weights which were statistically significantly ($p <0.05$) greater than those of Filters 2, 3 and Control A (Table 4.28 and Figure 4.5).

Furthermore, plants irrigated with tap water spiked with fertiliser produced fruits of mean weights which were significantly ($p < 0.05$) greater than those associated with plants irrigated with water harvested from Filter 8 and Control A. This can be explained by the impact of different nutrients (mainly nitrogen) and trace elements loads (Table 4.25) applied on plants through different irrigation water types. Figure 4.5 indicates that Sweet Peppers irrigated with water harvested from Filter 1 produced fruits of the highest mean weight (54 g) followed by those harvested from plants irrigated with Filter 7 outflow water, which produced fruits of 52 g mean weight, while the lowest mean fruit weight was recorded for those plants irrigated with Filter 6 outflow water (16 g), explaining the negative impact of high nutrients and trace elements applied to plant fruit weight (Table 4.25), as discussed by Almuktar and Scholz (2016 b).

Ammonia-nitrogen has a negative effect on plant fruit, leaf and stem developments (Bar-Tal et al., 2001; Bar-Tal, Aloni, Karni, & Rosenberg, 2001). However, the total yield increases as the nitrate-nitrogen to ammonia-nitrogen ratio increases. This can be explained by a reduction in fruit physiological disorders, which usually reduce fruit mean weight, as discussed by Bar-Tal et al (2001). Moreover, high phosphorus levels are known to interfere with the normal metabolism of peppers. Also, it is known to promote manganese uptake by plants (FAO, 1972). However, Cakmak (2005) reported that high potassium concentration in irrigation water provides protection against stem damage from low night temperatures. Manganese is an essential trace element for most plants, intervening in several metabolic processes (mainly in photosynthesis). Nevertheless, an excess of this micronutrient is often toxic for plants. Manganese phyto-toxicity is exhibited in a reduction of biomass and photosynthesis, and biochemical disorders including oxidative stress (FAO, 1972).

Correlation analysis findings indicate that fruit weights were significantly positively correlated with total water volumes used for irrigation ($R = 0.821$, $p < 0.001$). Since the peppers irrigated with raw wastewater and grown in organic media had the highest number of fruits (Figure 4.2e), this helps to explain why the total weight of harvested fruits was associated with plants irrigated with raw urban wastewater (Figure 4.2g). The provision of plants with high nutrient and trace element loads leads to increases in the quantity at the expense of quality of yield (Almuktar &Scholz, 2016 b).

Table 3.5 proposes a novel but conservative harvest classification scheme for Sweet Peppers. Only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, diameter and weight. The lowest variable class entry for any individual pepper fruit assessment determined the final class. If a fruit is categorised, for example, as Class A with respect to length, Class B in terms of diameter and E regarding weight, then the final class for this fruit is class E. It follows that the corresponding price for this pepper sample will be zero pence (Table 3.6).

Figure 4.6 indicates the monetary value of the pepper harvest. No fruits from any plant were categorised as Class A, B or C. The highest number of fruits categorised as Class D was harvested from peppers grown in organic media and irrigated with raw wastewater followed by those irrigated with tap water, Control A and Filter 1 outflow waters. The highest number of fruits categorised as Class E was also harvested from plants grown in organic media and watered with raw wastewater.

This low marketable yield can be explained by the low unit fresh weight due to the surplus of nutrients (particularly nitrogen), leading to physiological plant disorders that are often associated with small and deformed fruits of low weight (Bar-Tal, Aloni, Karni, & Rosenberg, 2001). It follows that rather low yields in terms of financial return can be expected as long as there is no smart system to control nutrient input. Another reason for the poor harvest could be the relatively low humidity in the laboratory. Humidity values below 50% could have a negative impact on the fruit development (Bakker, 1989).

However, considering that the monetary value of the harvest was low, Sweet Pepper are unlikely to be chosen as fruiting vegetables to be grown on recycled wastewater streams in the future. No microbial contamination was detected in fruits (skin, flesh and washing solution) harvested from any treatments. However, microbial contamination of peppers is rather unlikely due to the relatively long distance between the fruits and the contaminated soil (Almuktar & Scholz, 2016 b).

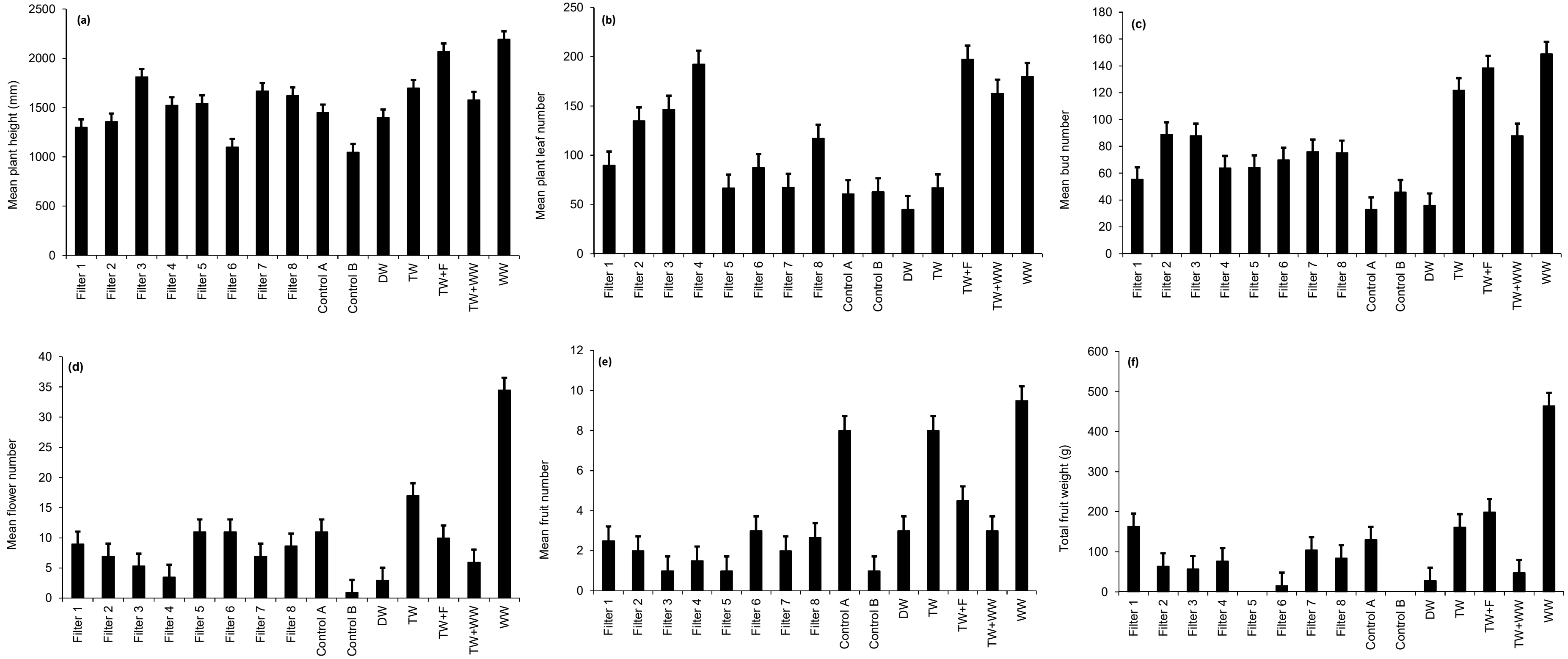

Figure 4.2: Growth of Sweet Pepper plants in organic media and subjected to different irrigation water types: (a) mean plant height; (b) mean leaf number; (c) mean bud number; (d) mean flower number; (e) mean fruit number; and (f) total fruit weight. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.25: Overview of total element mass applied to Sweet Pepper plants grown in organic media subjected to different irrigation water types.

Irrigation water type	Total applied mass (mg)									
	NH_4-N[a]	NO_3-N[b]	PO_4-P[c]	Ca[d]	Fe[e]	K[f]	Mg[g]	Mn[h]	Zn[i]	B[j]
Filter 1 outflow	164.3±42.77	13.7±3.56	136.9±35.64	1082.1±281.66	36.0±9.38	265.0±68.97	179.8±46.79	4.5±1.17	1.4±0.37	2.0±0.51
Filter 2 outflow	178.7±7.18	67.8±2.72	101.7±4.08	938.5±37.70	36.5±1.47	217.9±8.75	154.5±6.20	1.3±0.05	1.6±0.07	1.4±0.06
Filter 3 outflow	93.4±8.42	10.1± 0.91	83.3±7.51	1524.0±137.30	37.1±3.34	215.8±19.44	150.6±13.57	6.6±0.60	2.2±0.20	1.0±0.09
Filter 4 outflow	81.6±1.15	54.4±0.76	87.6±1.23	1326.5±18.62	5.7±0.08	104.9±1.47	159.1±2.23	1.6±0.02	1.8±0.03	1.1±0.01
Filter 5 outflow	246.9±15.30	21.8±1.35	106.5±6.60	1384.2±85.80	31.9±1.98	281.6±17.46	208.8±12.94	6.2±0.39	2.1±0.13	1.3±0.08
Filter 6 outflow	246.2±38.14	95.3±14.76	121.8±18.87	1421.1±220.18	14.6±2.26	288.8±44.74	231.0±35.79	2.1±0.32	2.5±0.39	1.6±0.25
Filter 7 outflow	133.6±13.03	98.4±9.60	126.5±12.35	1362.9±132.99	16.9±1.65	228.4±22.28	179.9±17.55	3.1±0.30	2.0±0.19	1.3±0.12
Filter 8 outflow	38.3±4.84	76.6±9.69	90.3±11.42	1080.1±136.54	8.3±1.05	134.9±17.05	132.6±16.76	1.5±0.19	2.9±0.36	0.9±0.11
Control A outflow	39.1±0.00	14.2±0.00	64.0±0.00	909.2±0.00	4.4±0.00	55.1±0.00	56.4±0.00	2.2±0.00	1.6±0.00	0.2±0.00
Control B outflow	35.7±0.00	8.9±0.00	56.6±0.00	768.2±0.00	1.2±0.00	18.9±0.00	38.0±0.00	0.6±0.00	2.3±0.00	0.1±0.00
Deionised water	2.9±0.00	0.0±0.00	0.0±0.00	0.0±0.00	1.6±0.00	0.0±0.00	2.9±0.00	2.3±0.00	1.3±0.00	0.0±0.00
Tap water (100%)	4.1±0.00	8.3±0.00	33.1±0.00	428.6±0.00	30.7±0.00	17.2±0.00	39.9±0.00	3.3±0.00	4.9±0.00	0.0±0.00
Tap water with fertiliser (0.7 ml/l)	775.1±84.29	431.2± 46.88	721.8±78.49	503.8±54.79	40.4±4.39	915.5±99.55	52.5±5.71	4.2±0.46	6.2±0.67	0.0±0.01
Wastewater (20%); tap water (80%)	281.5±0.00	21.0±0.00	126.1±0.00	948.7±0.00	22.3±0.00	150.6±0.00	127.4±0.00	2.6±0.00	6.3±0.00	1.3±0.00
Wastewater (100%)	1679.0± 116.42	119.9± 8.32	744.6±51.63	3126.8±216.81	116.4±8.07	796.1±55.20	526.0±36.47	6.4±0.45	14.8± 1.03	4.1±0.29

Note: [a] NH_4-N: ammonia-nitrogen; [b] NO_3-N: nitrate-nitrogen; [c] PO_4-P: ortho-phosphate-phosphorus; [d] Ca: calcium; [e] Fe: iron; [f] K: potassium; [g] Mg: magnesium; [h] Mn: manganese; [i] Zn: zinc; [j] B: boron. Total applied mass of each element calculated from irrigation water volume and average concentration.

Table 4.26: Overview of total water volume for Sweet Pepper plants grown in organic media during different planting periods.

Irrigation water type	Total irrigation water volume (l)			
	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Filter 1 outflow	34.230±8.9095	0.330±0.0000	4.900±0.1414	29.000±8.7681
Filter 2 outflow	30.805±1.2374	0.330±0.0000	4.800±0.2121	25.675±1.4496
Filter 3 outflow	25.253±2.2750	0.320±0.0000	4.467±0.3215	20.467±2.2496
Filter 4 outflow	30.220±0.4243	0.320±0.0000	4.650±0.0707	25.250±0.3536
Filter 5 outflow	24.203±1.5003	0.320±0.0000	4.250±0.3464	19.633±1.8113
Filter 6 outflow	26.470±4.1012	0.320±0.0000	4.400±0.0707	21.750±4.0305
Filter 7 outflow	35.145±3.4295	0.320±0.0000	5.400±0.7778	29.425±2.6517
Filter 8 outflow	27.370±3.4598	0.320±0.0000	5.100±0.8544	21.950±2.6963
Control A outflow	35.570±0.0000	0.320±0.0000	4.900±0.0000	30.350±0.0000
Control B outflow	29.770±0.0000	0.320±0.0000	5.250±0.0000	24.200±0.0000
Deionised water	29.270±0.0000	0.320±0.0000	4.750±0.0000	24.200±0.0000
Tap water (100%)	41.320±0.0000	0.320±0.0000	4.800±0.0000	36.200±0.0000
Tap water with fertiliser (0.7 ml/l)	48.445±5.2679	0.320±0.0000	5.475±0.0354	42.650±5.3033
Wastewater (20%); tap water (80%)	42.020±0.0000	0.320±0.0000	6.100±0.0000	35.600±0.0000
Wastewater (100%)	49.970±3.4648	0.320±0.0000	6.100±0.0000	43.550±3.4648

[a] 11/10/13 to 25/09/14; [b] Second planting period: 11/10/13 to 07/11/13; [c] Final planting period before fruiting: 08/11/13 to 19/01/14; [d] Final planting period after fruiting: 20/01/14 to 25/09/14.

Table 4.27: Basic soil properties based on three replicates each (14 /09/2013).

Parameter	Soil type		Total per pot (mg)	
	Compost	Sand	Compost	Sand
pH	6.43	9.40	-	-
Redox potential (mV)	62.60	−79.20	-	-
Electrical conductivity (µS/cm)	2438.50	116.00	-	-
Total nitrogen (mg/kg)	998.75	7.60	3495.63	114.00
Total phosphor (mg/kg)	367.50	0.85	1286.25	12.75
Aluminium (mg/kg)	1118.38	1180.43	3914.33	17,706.45
Calcium (mg/kg)	18,421.96	174.16	64,476.86	2612.40
Iron (mg/kg)	6233.15	1196.48	21,816.03	17,947.20
Potassium (mg/kg)	2776.02	168.57	9716.07	2528.55
Magnesium (mg/kg)	5287.67	279.53	18,506.85	4192.95
Manganese (mg/kg)	201.59	8.09	705.57	121.35
Zinc (mg/kg)	26.59	1.95	93.07	29.25
Boron (mg/kg)	12.29	< 0.0001	43.02	0.0015
Organic matter (%)	89.00	0.03	-	-
Bulk density (g/l)	350	1522	-	-

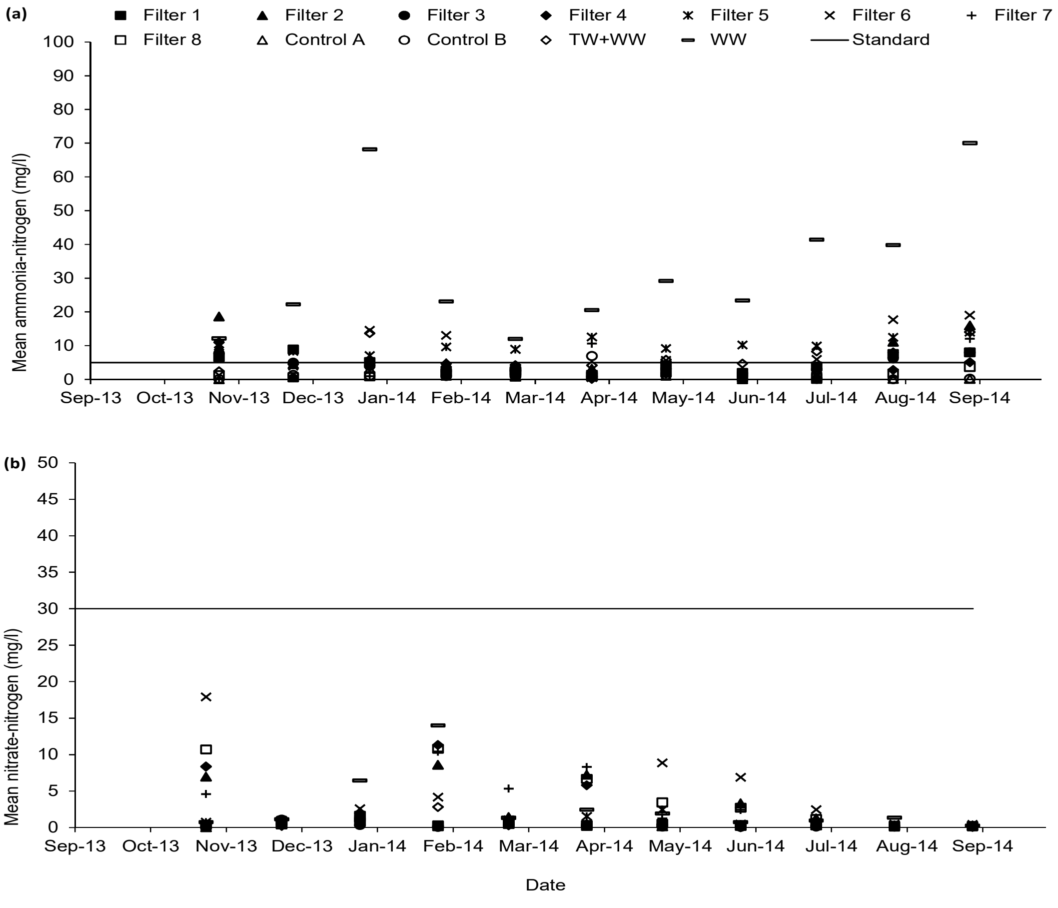

Figure 4.3: Variation of nitrogen in different irrigation water types in terms of: (a) ammonia-nitrogen and (b) nitrate-nitrogen.

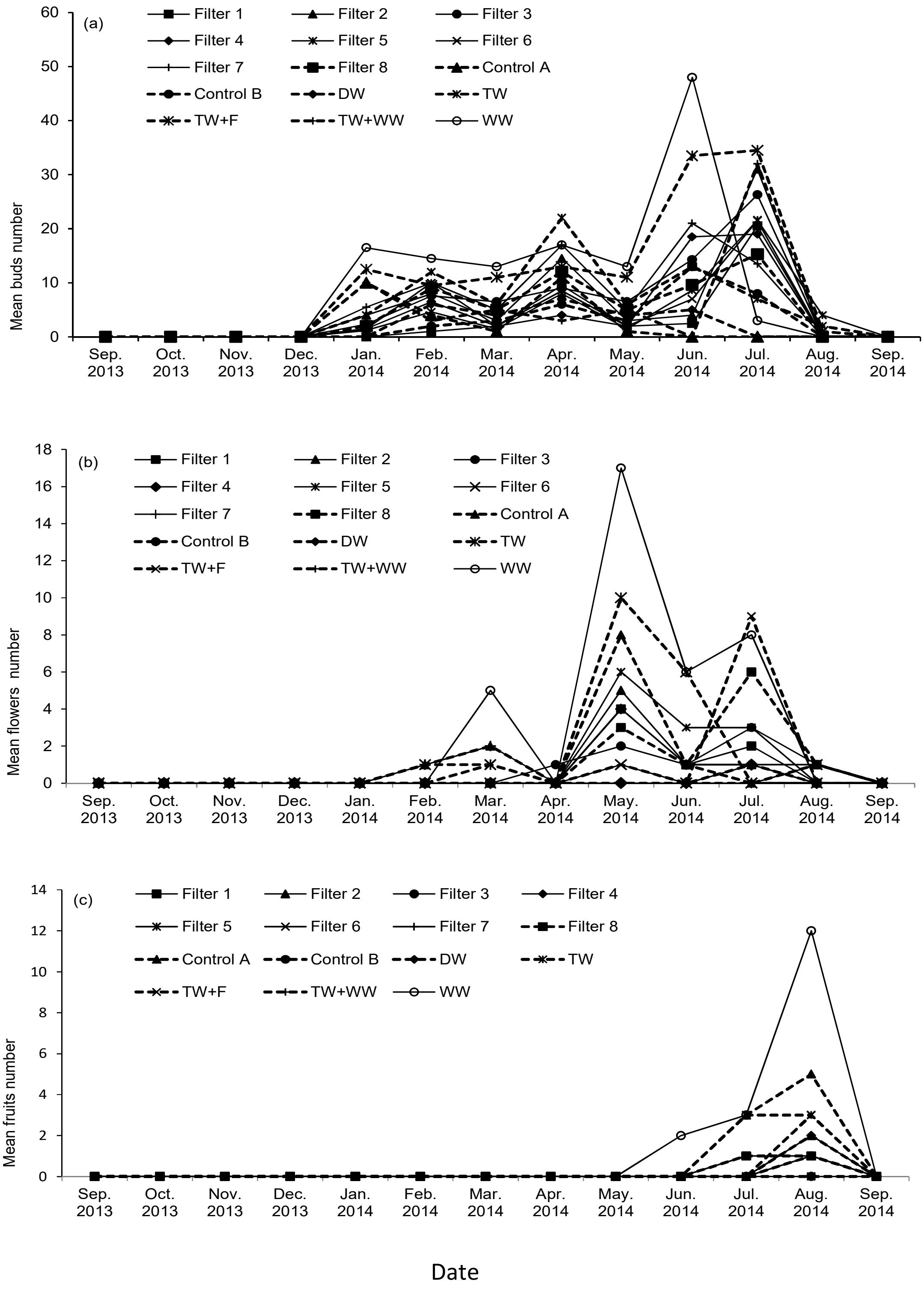

Figure 4.4: Overview of Sweet Pepper plant developments grown in organic media during whole experiment duration. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

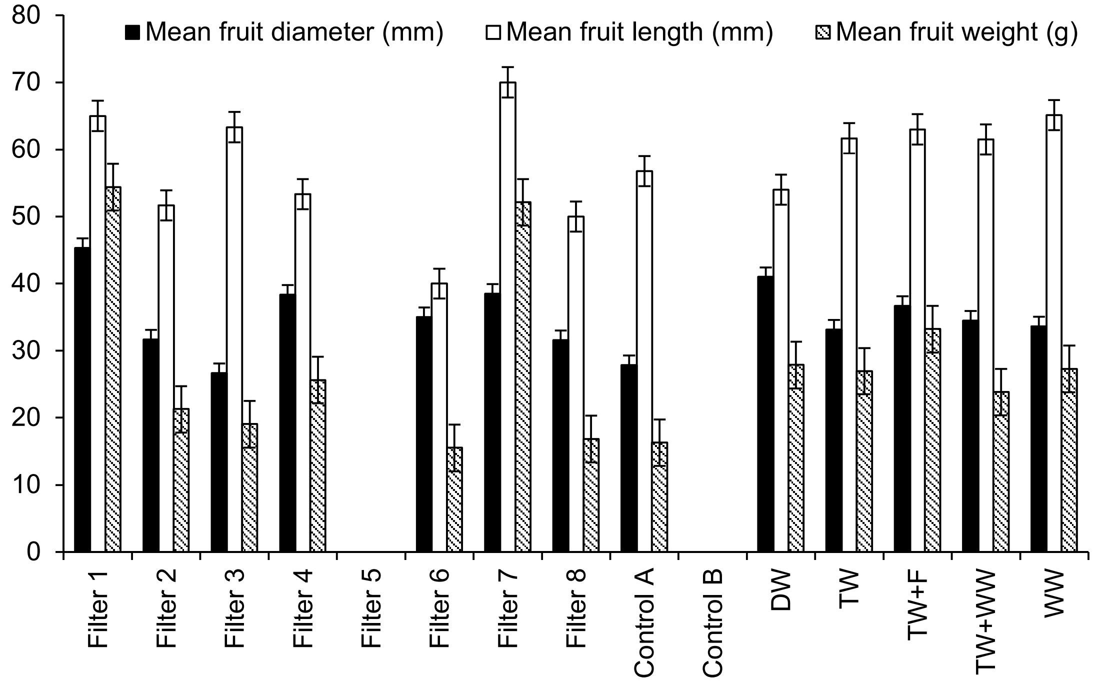

Figure 4.5: Differences in mean fruit diameter, mean fruit length and mean fruit weight linked to harvested plants irrigated with different water types and grown in organic media. Notes: No fruit harvest has been noted for plants associated with Filter 5 and Control B. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

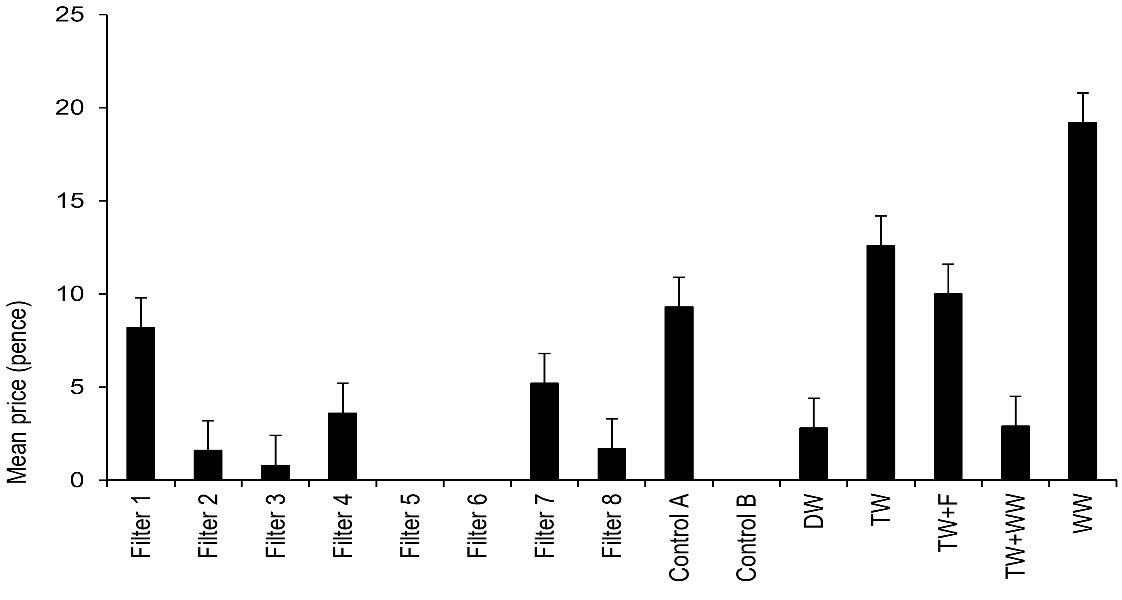

Figure 4.6: Sweet Pepper harvest outcome linked to plants grown in organic media (after classification scheme (Table 3.5) application). DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.28: Overview of the statistically significant differences among Sweet Pepper fruit weights harvested from plants grown in organic media and subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

Treatment	Statistic (*p*-value)[a]														
	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5	Filter 6	Filter 7	Filter 8	Control A	Control B	DW[b]	TW[c]	TW+F[d]	TW+WW[e]	WW[f]
Filter 1	n.a	0.029	**0.013**	0.055	n.a	n.a	0.991	**0.005**	**0.002**	n.a	n.a	0.042	0.300	0.071	**0.035**
Filter 2	0.029	n.a	0.768	0.787	n.a	n.a	**0.049**	0.627	0.662	n.a	n.a	0.619	0.136	0.878	0.453
Filter 3	**0.013**	0.768	n.a	0.572	n.a	n.a	**0.026**	0.864	0.935	n.a	n.a	0.402	0.067	0.676	0.256
Filter 4	0.055	0.787	0.572	n.a	n.a	n.a	0.084	0.438	0.445	n.a	n.a	0.854	0.239	0.930	0.691
Filter 5	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Filter 6	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Filter 7	0.991	**0.049**	**0.026**	0.084	n.a	n.a	n.a	0.012	**0.008**	n.a	n.a	0.077	0.363	0.098	0.076
Filter 8	**0.005**	0.627	0.864	0.438	n.a	n.a	0.012	n.a	0.902	n.a	n.a	0.263	**0.027**	0.554	0.130
Control A	**0.002**	0.662	0.935	0.445	n.a	n.a	**0.008**	0.902	n.a	n.a	n.a	0.230	**0.012**	0.584	0.074
Control B	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
DW[b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
TW[c]	0.042	0.619	0.402	0.854	n.a	n.a	0.077	0.263	0.230	n.a	n.a	n.a	0.223	0.796	0.804
TW+F[d]	0.300	0.136	0.067	0.239	n.a	n.a	0.363	**0.027**	**0.012**	n.a	n.a	0.223	n.a	0.263	0.219
TW+WW[e]	0.071	0.878	0.676	0.930	n.a	n.a	0.098	0.554	0.584	n.a	n.a	0.796	0.263	n.a	0.659
WW[f]	**0.035**	0.453	0.256	0.691	n.a	n.a	0.076	0.130	0.074	n.a	n.a	0.804	0.219	0.659	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); [b], Deionised water; [c], Tap water; [d], Tap water with fertiliser (0.7 ml/l); [e], Wastewater (20%); tap water (80%); [f], Wastewater (100%) and n.a, not applicable either because the treatment compared with itself or due to lack of data for statistical analysis. Bold values are significant (*p* <0.05).

Figure 4.7a–f shows Sweet Pepper growth details in terms of plant overall height, number of leaves, buds, flowers, fruits and total weight of fruits harvested from each treatment when using inorganic growth media and subjected to different irrigation water types. Regarding the overall height of plants growing in sand soil, Figure 4.7a shows that the maximum height was associated with plants irrigated with raw wastewater followed by those irrigated with tap water spiked with fertiliser, while the minimum plant height was observed for those plants irrigated with deionised water and water harvested from Control A. This can be explained by the high nutrient load (Table 4.29) applied via irrigation water (Table 4.30) as discussed by FAO (2003) and Almuktar and Scholz (2016 b). Regarding the total number of leaves (Figure 4.7b) linked to peppers grown in inorganic media; findings indicated that peppers irrigated with tap water spiked by fertiliser produced the highest leaf numbers followed by those plants irrigated with water harvested from Filter 6 of high inflow loading rate (Table 3.1). Figure 4.7c–f provides summaries of plant developments. The highest numbers of buds were recorded for peppers irrigated with tap water spiked by fertiliser followed by those irrigated with water harvested from Filter 6 of high inflow loading rate and Filter 2 of large aggregate size, while plants irrigated with deionised water showed the minimum buds number due to lack of nutrients available for plant development (FAO, 1979,2003). Moreover, peppers irrigated with water harvested from Filter 2 showed the highest number of flowers followed by those irrigated with raw wastewater and tap water mixed with fertiliser. However, plants provided with a suitable nutrient load from tap water with fertiliser, outflow water from Filter 7 of low contact time and from Filter 6 of undiluted wastewater inflow produced the highest number of fruits, resulting in maximum total weight of harvested fruits (Figure 4.7f).

Moreover, most buds and flowers fell down before reaching the fruiting stage. This can be explained by the adverse environmental conditions in the laboratory in terms of light intensity provided by the grow lights and the relative humidity which was elevated artificially by using humidifiers as explained in section 3.4. These may cause flower inhibition or cause flower abscission as reported by Wein and Zhang (1991).

Figure 4.8 shows that the plants started to produce buds and flowers in January 2014. However, fruit production took place between May 2014 and September 2014. This is possibly due to lack of nutrients available for plants from the poor inorganic soil (Table 4.27) and dependency mainly on fertiliser provided by irrigation water resulting in deprived plant development including fruit production (FAO, 1972. 1991, 1994; Almuktar & Scholz, 2016b). Figure 4.9 summarises differences in fruit characteristics associated with sandy soil. Results show that the best fruit quality was linked to those peppers irrigated with outflow water from Filter 2 of large aggregate diameter and tap water spiked with fertiliser.

Figure 4.10 indicates the monetary value of the pepper harvest linked to inorganic media. No fruits from any plant were categorised as Class A, B or C. The highest number of fruits categorised as Class D was harvested from peppers irrigated with water harvested from Filter 7 followed by those irrigated with outflow from Filter 6 and raw wastewater, while the highest number of fruits categorised as Class E was harvested from plants irrigated with tap water mixed with fertiliser followed by those irrigated with raw wastewater. However, plants irrigated with tap water spiked with fertiliser produced the highest number of fruits resulting in maximum weight and subsequently the highest price as shown in Figure 4.10.

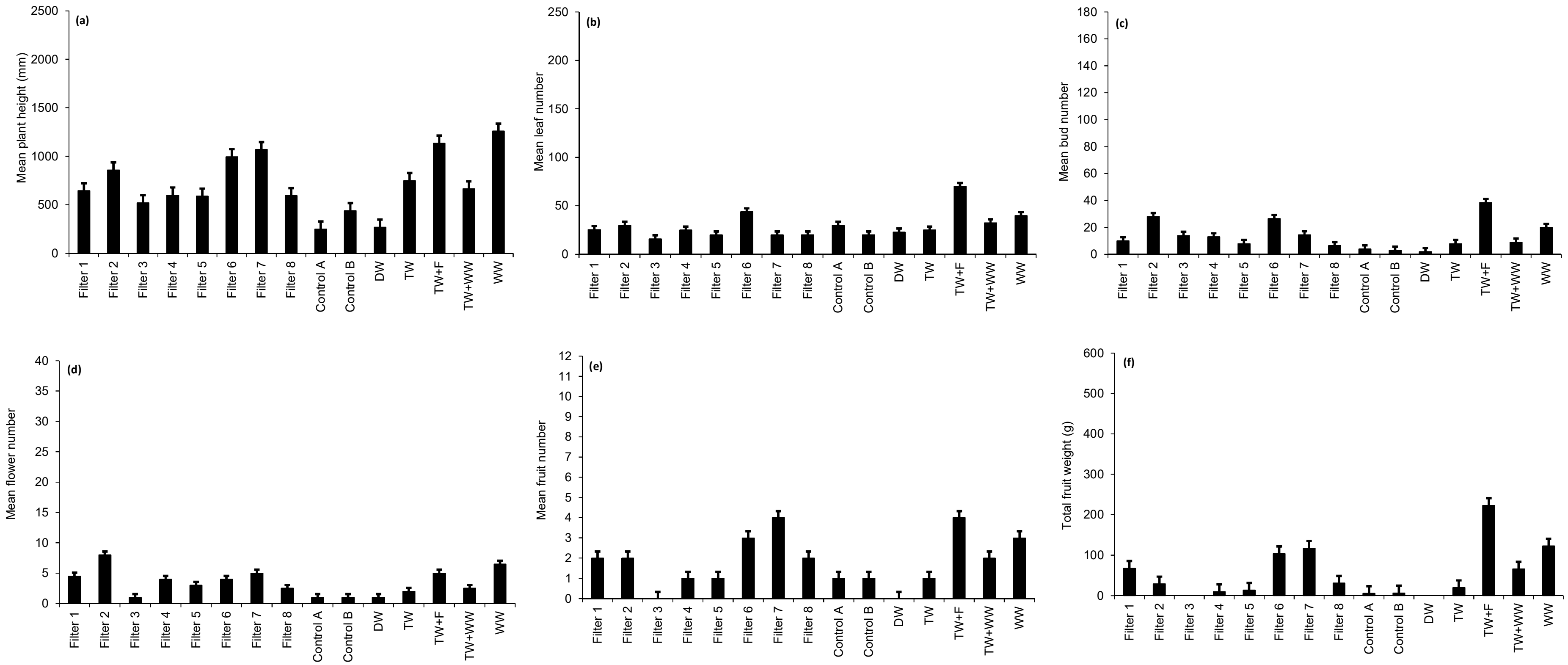

Figure 4.7: Growth of Sweet Pepper plants in inorganic media and subjected to different irrigation water types: (a) mean plant height; (b) mean leaf number; (c) mean bud number; (d) mean flower number; (e) mean fruit number; and (f) total fruit weight. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.29: Overview of total element mass applied to Sweet Pepper plants grown in inorganic media subjected to different irrigation water types.

Irrigation water type	Total applied mass (mg)									
	NH_4-N[a]	NO_3-N[b]	PO_4-P[c]	Ca[d]	Fe[e]	K[f]	Mg[g]	Mn[h]	Zn[i]	B[j]
Filter 1 outflow	111.7±5.26	9.3±0.44	93.1±4.38	735.8±34.65	24.5±1.15	180.2±8.48	122.2±5.76	3.0±0.14	1.0±0.05	1.3±0.06
Filter 2 outflow	116.9±0.00	44.3±0.00	66.5±0.00	613.9±0.00	23.9±0.00	142.5±0.00	101.0±0.00	0.8±0.00	1.1±0.00	0.9±0.00
Filter 3 outflow	54.6±0.00	5.9±0.00	48.7±0.00	890.1±0.00	21.7±0.00	126.0±0.00	88.0±0.00	3.9±0.00	1.3±0.00	0.6±0.00
Filter 4 outflow	53.9±0.00	35.9±0.00	57.9±0.00	875.7±0.00	3.8±0.00	69.2±0.00	105.0±0.00	1.0±0.00	1.2±0.00	0.7±0.00
Filter 5 outflow	138.7±0.00	12.2±0.00	59.8±0.00	777.8±0.00	17.9±0.00	158.2±0.00	117.3±0.00	3.5±0.00	1.2±0.00	0.7±0.00
Filter 6 outflow	200.4±4.60	77.6±1.78	99.1±2.28	1157.0±26.57	11.9±0.27	235.1±5.40	188.0±4.32	1.7±0.04	2.0±0.05	1.3±0.03
Filter 7 outflow	88.9±0.08	65.5±0.06	84.2±0.08	906.9±0.82	11.2±0.01	152.0±0.14	119.7±0.11	2.0±0.00	1.3±0.00	0.8±0.00
Filter 8 outflow	26.1±1.48	52.2±2.97	61.5±3.50	736.0±41.86	5.7±0.32	91.9±5.23	90.3±5.14	1.0±0.06	2.0±0.11	0.6±0.04
Control A outflow	24.2±0.00	8.8±0.00	39.6±0.00	540.3±0.00	2.7±0.00	34.1±0.00	34.9±0.00	1.4±0.00	1.0±0.00	0.2±0.00
Control B outflow	23.8±0.00	6.0±0.00	37.7±0.00	512.2±0.00	0.8±0.00	12.6±0.00	25.3±0.00	0.4±0.00	1.5±0.00	0.1±0.00
Deionised water	2.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	1.1±0.00	0.0±0.00	2.0±0.00	1.6±0.00	0.9±0.00	0.0±0.00
Tap water (100%)	2.3±0.00	4.6±0.00	18.2±0.00	236.0±0.00	16.9±0.00	9.5±0.00	22.0±0.00	1.8±0.00	2.7±0.00	0.0±0.00
Tap water with fertiliser (0.7 ml/l)	530.0±7.35	294.8±4.09	493.6±6.85	344.5±4.78	27.6±0.38	626.0±8.69	35.9±0.50	2.9±0.04	4.2±0.06	0.0±0.00
Wastewater (20%); tap water (80%)	186.3±5.69	13.9±0.42	83.4±2.55	627.6±19.16	14.8±0.45	99.6±3.04	84.3±2.57	1.7±0.05	4.1±0.13	0.8±0.03
Wastewater (100%)	1013.0±2.38	72.4±0.17	449.2±1.05	1886.6±4.42	70.2±0.16	480.3±1.13	317.4±0.74	3.9±0.01	8.9±0.02	2.5±0.01

Note: [a] NH_4-N: ammonia-nitrogen; [b] NO_3-N: nitrate-nitrogen; [c] PO_4-P: ortho-phosphate-phosphorus; [d] Ca: calcium; [e] Fe: iron; [f] K: potassium; [g] Mg: magnesium; [h] Mn: manganese; [i] Zn: zinc; [j] B: boron. Total applied mass of each element calculated from irrigation water volume and average concentration.

Table 4.30: Overview of total water volume for Sweet Pepper plants grown in inorganic media during different planting periods.

Irrigation water type	Total irrigation water volume (l)			
	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Filter 1 outflow	23.275±1.0960	0.300±0.0000	5.050±0.0000	17.925±1.0960
Filter 2 outflow	20.150±0.0000	0.300±0.0000	5.150±0.0000	14.700±0.0000
Filter 3 outflow	14.750±0.0000	0.300±0.0000	5.200±0.0000	9.250±0.0000
Filter 4 outflow	19.950±0.0000	0.300±0.0000	5.450±0.0000	14.200±0.0000
Filter 5 outflow	13.600±0.0000	0.300±0.0000	5.050±0.0000	8.250±0.0000
Filter 6 outflow	21.550±0.4950	0.300±0.0000	4.525±0.1061	16.725±0.6010
Filter 7 outflow	23.385±0.0210	0.310±0.0141	5.850±0.0707	17.225±0.1061
Filter 8 outflow	18.650±1.0610	0.300±0.0000	4.300±0.0707	14.050±0.9899
Control A outflow	22.000±0.0000	0.300±0.0000	4.350±0.0000	17.350±0.0000
Control B outflow	19.850±0.0000	0.300±0.0000	5.450±0.0000	14.100±0.0000
Deionised water	20.300±0.0000	0.300±0.0000	6.000±0.0000	14.000±0.0000
Tap water (100%)	22.750±0.0000	0.300±0.0000	5.450±0.0000	17.000±0.0000
Tap water with fertiliser (0.7 ml/l)	33.125±0.4600	0.300±0.0000	6.000±0.0707	26.825±0.3889
Wastewater (20%); tap water (80%)	27.800±0.8490	0.300±0.0000	5.600±0.2828	21.900±0.5657
Wastewater (100%)	30.200±0.0710	0.300±0.0000	5.450±0.0707	24.400±0.0000

[a] 11/10/13 to 25/09/14; [b] Second planting period: 11/10/13 to 07/11/13; [c] Final planting period before fruiting: 08/11/13 to 19/01/14; [d] Final planting period after fruiting: 20/01/14 to 25/09/14.

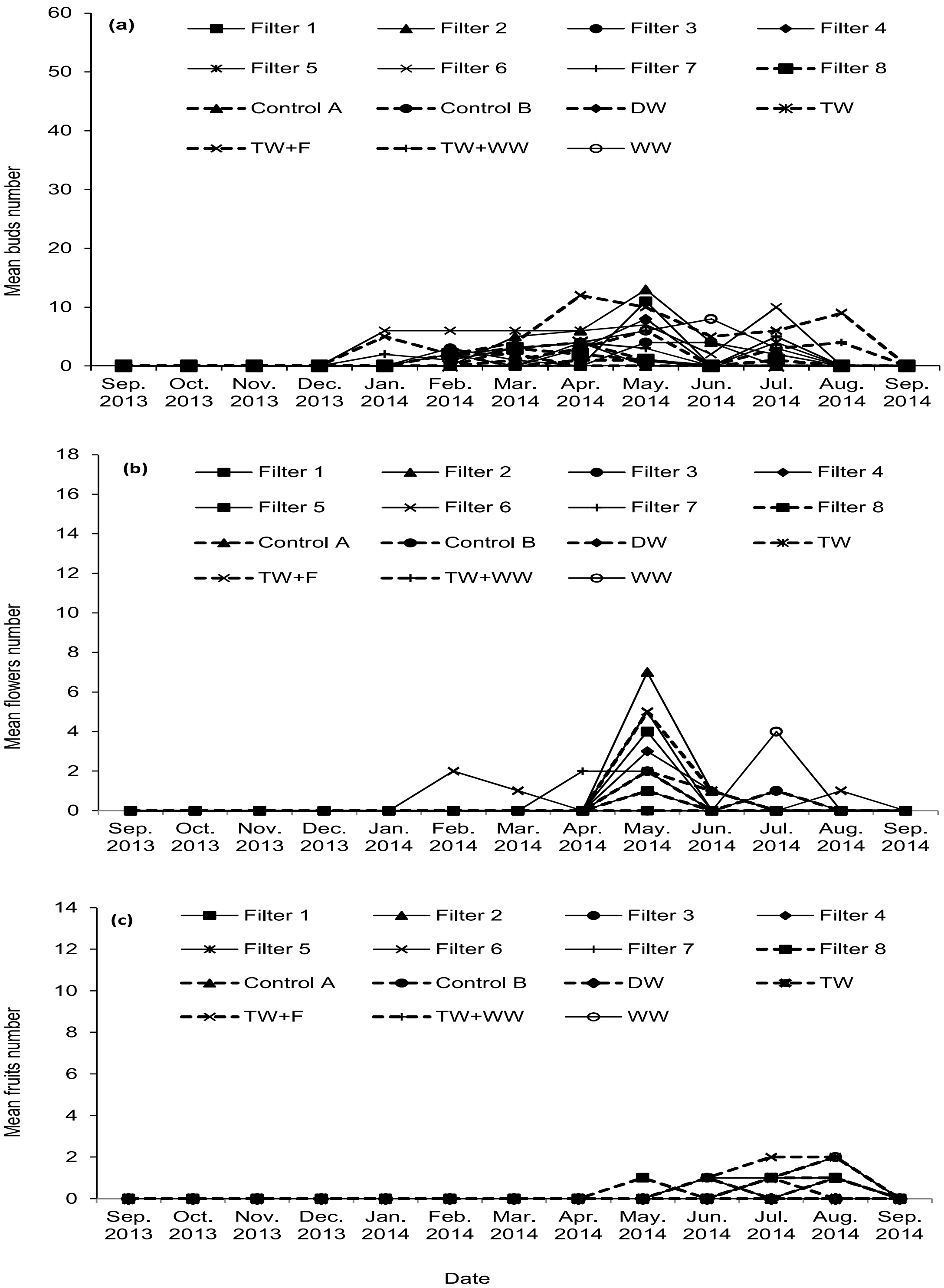

Figure 4.8: Overview of Sweet Pepper plant developments grown in inorganic media during whole experiment duration. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

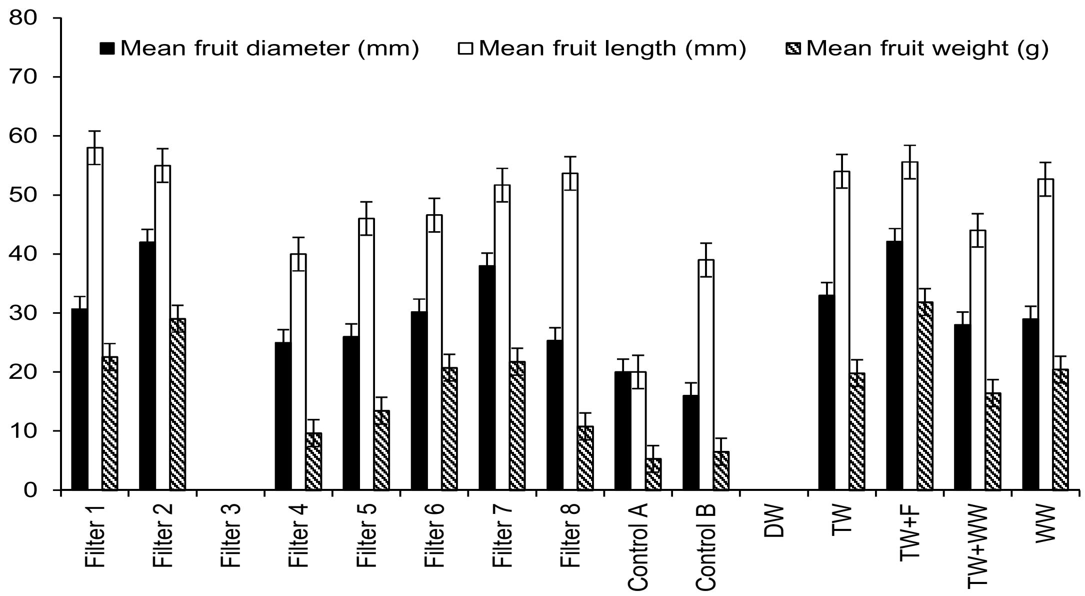

Figure 4.9: Differences in mean fruit diameter, mean fruit length and mean fruit weight linked to harvested pepper plants irrigated with different water types and grown in inorganic media. Notes: No fruit harvest has been noted for plants associated with Filter 3 and deionised water. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

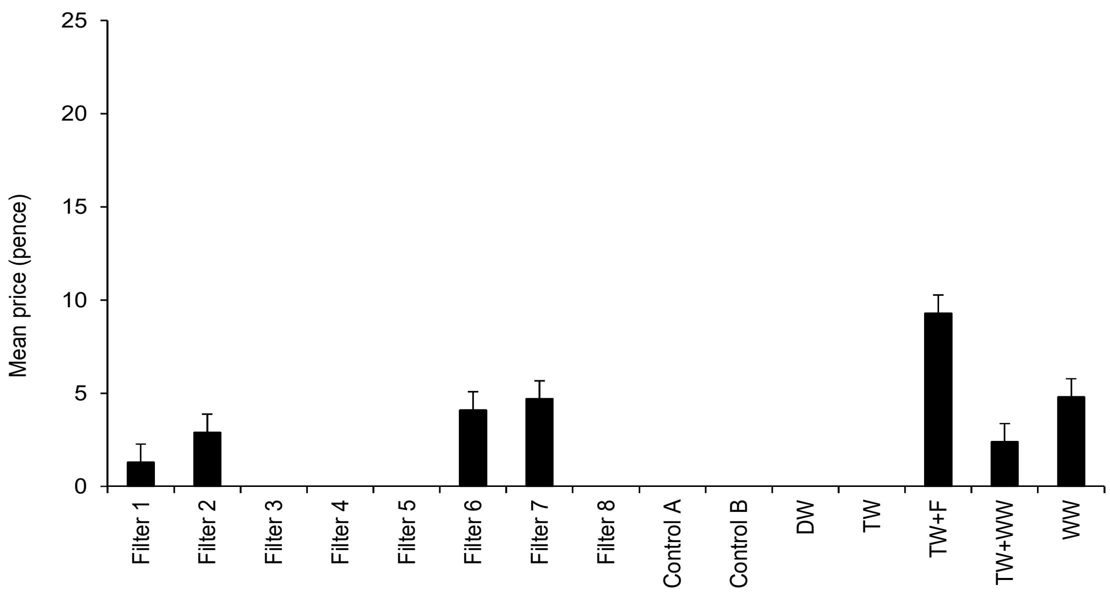

Figure 4.10: Sweet Pepper harvest outcome linked to plants grown in inorganic media (after classification scheme (Table 3.5) application). DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Findings indicate that compost compared to sand is associated with considerably greater plant growth and productivity. This is due to the elevated nutrient availability in basic compost (Rahimi et al., 2013) compared to sand (Table 4.27). Moreover, organic substrates decompose over time, and subsequently release nutrients. The rate of decomposition and the physical conditions of the media vary with the parent material. That in turn will enhance crop growth and development. Moreover, better aeration of peat promotes vigorous root growth, which allows rapid development of foliage and therefore increases the whole plant yield. In contrast, for inorganic media such as sand, nutrient provision to the crops is limited to the nutrients that are part of the irrigation water resulting in a delay of plant foliage growth with a subsequent poor yield (Olle et al., 2012). Generally, fruits harvested from plants grown in organic media had diameters, lengths and weights greater than those from plants raised in inorganic media. These results, in addition to findings based on other research studies undertaken in greenhouse conditions to assess the effect of different growth media on Sweet Pepper growth rates and yields, indicate that seedlings benefited from peat moss media (Rahimi et al., 2013). Another study was undertaken, to determine the effects of peat and sand on variables such as fruit length, diameter and weight, as well as the total fruit number per plant and yield, by Gungor and Yildirim (2013) which showed that peat significantly increased length, diameter and weight of fruits in all cultivars grown in comparison to sand. Sweet Pepper prefers light and well-drained soil, which is rich in organic substances with a pH value from 6.5 to 7.5 (Table 4.27), as reported by Chemicals (2014). However, under acid soil conditions (soil pH < 7), heavy metals could be a challenge to Sweet Pepper (Baudoin et al., 2013). Moreover, plants grown in compost consume more water than those grown in sand and subsequently increase the nutrient load applied to plants via irrigation water, leading to higher foliage and yield production.

4.4.3 Chilli growth comparisons and marketable yield assessment

Figure 4.11a–f show Chilli growth details in terms of plant overall height, number of leaves, buds, flowers, fruits and total weight of fruits harvested from each treatment when using organic growth media and subjected to different irrigation water types. Regarding the overall height of plants growing in organic media, Figure 4.11a shows that the maximum height was associated with plants irrigated with raw wastewater followed by those irrigated with tap water spiked with fertiliser and outflow from Filter 6 of high inflow loading rate. This can be explained by the high nutrient load (Table 4.31) applied via irrigation water (Table 4.32) as discussed by FAO (2003) and Almuktar and Scholz (2016 b). However, all plants showed total heights which were considerably higher than those reported by Nickels (2012) of 45 cm.

Regarding the total number of leaves (Figure 4.11b) linked to Chillies grown in organic media, findings indicate that plants irrigated with raw wastewater and tap water spiked by fertiliser produced the highest leaf numbers followed by those irrigated with water harvested from Filter 2 of large aggregate diameter, while the lowest leaf numbers were recorded for plants irrigated with deionised water and tap water, due to lack of nutrients available in the water sources (FAO, 2003). Figure 4.11c–f provides summaries of plant developments. Very high numbers of buds were recorded for Chillies grown in organic media. However, most of the buds associated with the compost fell down before reaching the flowering stage. Also, most flowers died before producing any fruits. This can be explained by the elevated nutrient concentrations, mainly ammonia-nitrogen, supplied to those plants grown in rich organic media (Table 4.27) and irrigated by wastewater (Table 4.4 and Figure 4.3) as indicated by FAO (1994, 2003) and Almuktar et al. (2016 a, b). Moreover, falling of most buds and flowers before reaching the fruiting stage can be explained as well by the adverse environmental conditions in the laboratory in terms of light intensity provided by the grow lights and the relative humidity which was elevated artificially by using humidifiers as explained in section 3.4. These may cause flower inhibition or cause flower abscission as reported by Wein and Zhang (1991).

Figure 4.12 shows that the plants started to produce buds and flowers in January 2014. However, most treatments start producing fruits in April 2014 with the exception of plants irrigated with tap water which start fruiting in March 2014. This can be explained by the balance of nutrients supplied to plants irrigated with only tap water rather than other plants which were irrigated with treated wastewater of high nutrient, mainly nitrogen, in combination with those supplied by the rich organic soil (Table 4.27) leading to most buds and flowers falling before the fruiting stage (Almuktar et al., 2015a, b), compared to those depending mainly on soil nutrients when irrigated with tap water.

Moreover, fruiting of plants irrigated with treated wastewater began relatively late, possibly due to the depletion of organic media nutrients, and the plants started to depend mainly on nutrients supplied by the irrigation water resulting in a better balance in supplied nutrients confirming the results obtained by Almuktar and Scholz (2016 b) reporting that the combination of fresh compost and treated wastewater is usually too high in a particular nutrient to produce a good Chilli harvest. However, as the compost is depleted of nutrients after about 8 months, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost explaining that a high yield is rather related with the most suitable provision of nutrients and trace elements (Almuktar et al., 2015a, b).

Results show that the highest number of fruits was harvested from plants irrigated with tap water mixed with fertiliser followed by those irrigated with tap water and outflow water from Filter 7, of small aggregate diameter, short contact time and low inflow loading rate, supplying more suitable nutrient levels for plants than other treatments. Based on that, plants irrigated with tap water spiked with fertiliser, tap water and outflow water from Filter 7 produced the maximum total weight of harvested fruits as shown in Figure 4.11f. Figure 4.13 and Table 4.33 summarise differences in fruit characteristics. Statistical analysis (Table 4.33a) using the Kruskal-Wallis test showed that fruits harvested from plants irrigated with Filter 3 outflow water were statistically significantly ($p < 0.05$) different from those irrigated with Filter 1 outflow water, indicating the impact of the aggregate diameter of the wetland system (Table 3.1) on outflow water qualities and subsequently the nutrients load applied to plants (Table 4.31) by irrigation water (Table 4.32). Moreover, irrigation with water harvested from Filter 3 which was contaminated with hydrocarbons produced fruit widths which were statistically significantly ($p < 0.05$) different from those linked to fresh tap water. Furthermore, plants irrigated with raw wastewater of high nutrient and trace element concentrations (Table 4.31) produced fruits of widths which were statistically significantly ($p < 0.05$) greater than the others, while irrigation with deionised water produced fruits of widths which were significantly ($p < 0.05$) smaller than the others due to the lack of nutrients and trace elements supplied to the plants (FAO, 2003).

Regarding Chilli fruit lengths harvested from plants grown in organic media, one-way analysis of variance (ANOVA) test results (Table 4.33b) showed that irrigation with raw wastewater of high nutrient and trace element concentrations produced fruits which were statistically significantly ($p < 0.05$) longer than the others while the fruits irrigated with Filter 3 outflow water were reported to be significantly ($p < 0.05$) the shortest confirming the impact of the nutrient and trace element load supplied to the plants on the fruits dimensions (Gungor & Yildirim, 2013). Figure 4.13 shows that fruit lengths associated with filters contaminated with hydrocarbons (Filters 1, 3, 5 and Control A) were shorter than those linked to the standard (uncontaminated) filters (Filters 2, 4, 6 and Control B, respectively) confirming the results reported previously (Wyszkowska et al., 2001, 2002) which indicated that soil fertility can be changed with the presence of hydrocarbons resulting in imbalance in nutrient levels in the plants which will lead to low growth rate and low biomass production due to hydrocarbon degradation (Hester & Mendelssohn, 2000).

Statistical analysis results using the Kruskal-Wallis test (Table 4.33c) showed that the weights of Chillies harvested from plants irrigated with raw wastewater were statistically significantly ($p < 0.05$) greater than the others while those irrigated with water harvested from Filter 3 produced fruit weights which were statistically significantly ($p < 0.05$) the lowest. However, Filter 3 (small aggregate diameter and contaminated with hydrocarbons) fruit weights were relatively similar to those linked to deionised water plants which were mainly dependent on the nutrients supplied by the soil only. Moreover, fruits harvested from plants irrigated with deionised water had weights which were statistically significantly ($p < 0.05$) lower than those of Filter 2, Control B, tap water and wastewater diluted with tap water. Furthermore, fruits linked to Filter 8 had weights which were statistically significantly ($p < 0.05$) different from those of Filter 2 and tap water. These results can be explained by differences in mean nutrient and trace element loads supplied to the plants by different irrigation water types and those available from the soil. This could be directly affected by soil conditions in addition to the types of irrigation water. For example, presence of hydrocarbons in the irrigation water and subsequent accumulation in the irrigated soil will highly affect the soil fertility due to hydrocarbon degradation leading to an imbalance in nutrients available for plants as discussed by Hester and Mendelssohn (2000). However, correlation analysis findings indicate that fruit weights were significantly positively correlated with total water volumes used for irrigation (R = 0.821, $p < 0.001$). Potential water stress might have reduced cell division and caused cell enlargement to cease. This could have led to a slowdown of the growth rate and might have been the reason for relatively low weight, width and length of fruits (Tedesse, 1997). Table 3.4 shows a novel harvest classification scheme for Chillies. Only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, bending and weight. The lowest variable class entry for any individual Chilli fruit assessment determined the final class. If a fruit is categorised, for example, as Class A with respect to length, Class B in terms of diameter and E regarding weight, then the final class for this fruit is Class E. It follows that the corresponding price for this Chilli sample will be zero pence (Table 3.4). However, the estimated prices are dependent on global commodity market developments. Figure 4.14 shows the number of Chilli fruits categorised as Class A, B, C, D or E per treatment. The highest number of fruits categorised as Class A was recorded for tap water plants (7 fruits) followed by those irrigated with tap water spiked with fertiliser (4 fruits) and outflow water from Filter 7 (3 fruits). Plants irrigated with water harvested from Control B showed the highest number of Class B fruits (11 fruits) followed by those irrigated with tap water spiked with fertiliser and outflow waters from Filters 7 and 5 which all produced 10 fruits categorised as Class B. Plants irrigated with tap water spiked with fertiliser produced the highest number of Class C fruits (17 fruits) followed by those plants irrigated with waters harvested from Filters 5 and 8 (12 fruits). The highest number of Class D fruits were recorded for plants irrigated with Filter 8 outflow water (30 fruits) followed by those irrigated with Filter 3 outflow (26 fruits) and tap water mixed with fertiliser (24 fruits). Finally, 9 fruits from the harvest of Filter 3 plants were categorised as class E followed by that of tap water plants (8 fruits). Figure 4.15 indicates the monetary value of the Chilli harvest. The highest mean price of harvested fruits is associated with tap water followed by tap water with fertiliser, Filter 7 (small aggregate diameter, short contact time and low inflow loading rate) and Filter 6 (small aggregate diameter, long contact time and high inflow loading rate), as these treatments produced high numbers of fruits which received high category classifications, while the lowest price of Chilli harvest was recorded for Filter 3 as it was associated with the highest fruit number of low category classification (i.e. C = 10, D = 26, and E = 9). Generally, Figure 4.15 shows that Filters 1, 3, 5 and Control A, which were contaminated with hydrocarbons, had lower economic value than Filters 2, 4, 6 and Control B, respectively, which were uncontaminated with hydrocarbons, explaining the negative impact of hydrocarbon contamination on marketable yield production, as it could result in nutrient and trace element imbalance available for plant growth and development as discussed by other researchers (Hester & Mendelssohn, 2000; Wyszkowska et al., 2001, 2002). However, findings showed that the productivity of Chillies in terms of marketable yield was independent of wastewater consumption volume, but may depend on the water quality (e.g., nutrient and trace mineral availability).

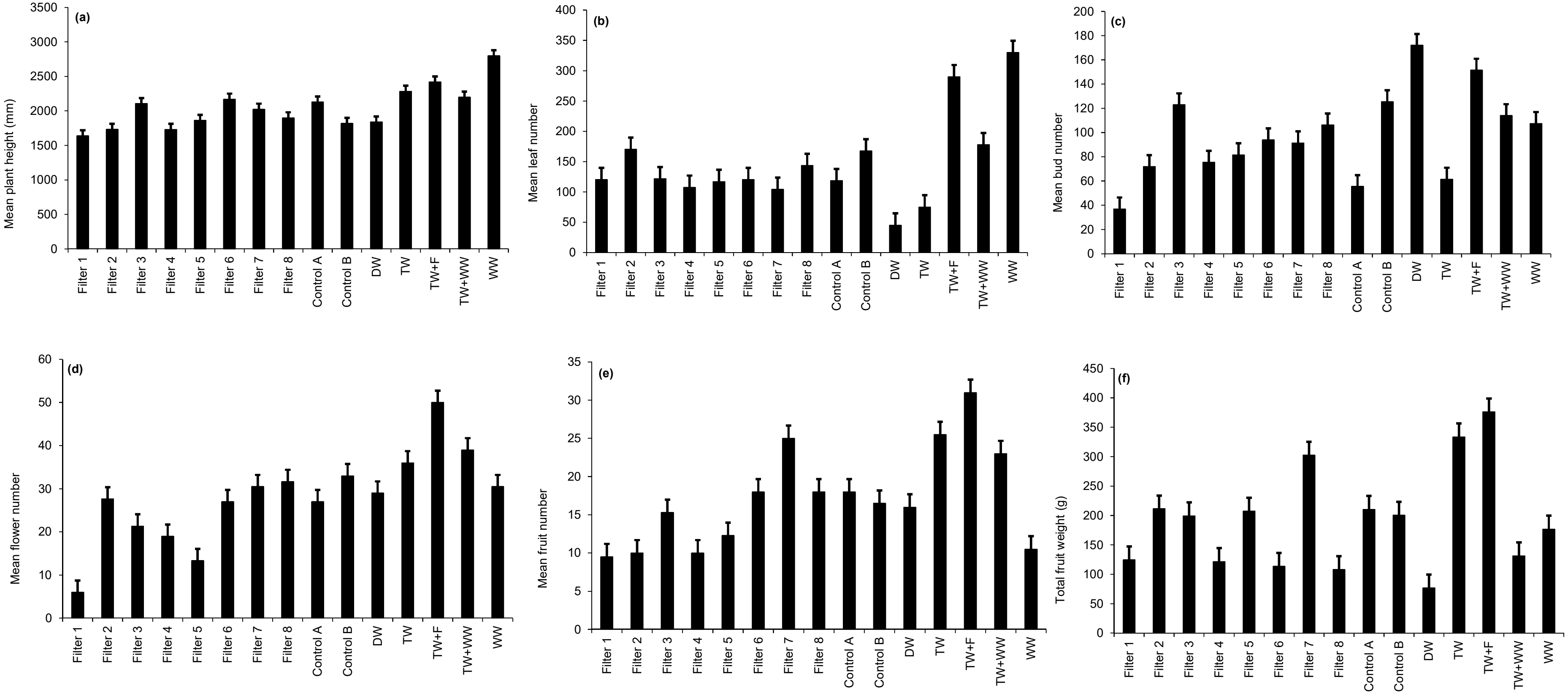

Figure 4.11: Growth of Chilli plants in organic media and subjected to different irrigation water types: (a) mean plant height; (b) mean leaf number; (c) mean bud number; (d) mean flower number; (e) mean fruit number; and (f) total fruit weight. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.31: Overview of total element mass applied to Chilli plants grown in organic media subjected to different irrigation water types.

Irrigation water type	Total applied mass (mg)									
	NH_4-N[a]	NO_3-N[b]	PO_4-P[c]	Ca[d]	Fe[e]	K[f]	Mg[g]	Mn[h]	Zn[i]	B[j]
Filter 1 outflow	179.0±13.58	14.9±1.13	149.2±11.31	1178.8±89.42	39.3±2.98	288.7±21.89	195.8±14.85	4.9±0.37	1.6±0.12	2.1±0.16
Filter 2 outflow	194.2±21.20	73.7±8.04	110.5±12.06	1020.5±111.39	39.7±4.33	236.9±25.85	167.9±18.33	1.4±0.15	1.8±0.19	1.5±0.17
Filter 3 outflow	106.7±8.28	11.5±0.89	95.2±7.38	1740.9±134.99	42.3±3.28	246.5±19.11	172.0±13.34	7.6±0.59	2.5±0.19	1.2±0.09
Filter 4 outflow	98.7±29.21	65.8±19.47	106.0±31.37	1604.4±474.89	6.9±2.06	126.9±37.55	192.4±56.95	1.9±0.56	2.2±0.65	1.3±0.38
Filter 5 outflow	293.7±20.30	25.9±1.79	126.7±8.76	1646.9±113.82	37.9±2.62	335.0±23.16	248.5±17.17	7.4±0.51	2.4±0.17	1.6±0.11
Filter 6 outflow	430.9±0.00	166.8±0.00	213.1±0.00	2487.3±0.00	25.6±0.00	505.4±0.00	404.3±0.00	3.6±0.00	4.4±0.00	2.8±0.00
Filter 7 outflow	154.7±3.90	114.0±2.87	146.5±3.69	1578.5±39.76	19.5±0.49	264.5±6.66	208.3±5.25	3.5±0.09	2.3±0.06	1.5±0.04
Filter 8 outflow	49.9±3.96	99.9±7.92	117.7±9.34	1407.4±111.66	10.8±0.86	175.8±13.95	172.7±13.70	1.9±0.15	3.7±0.30	1.2±0.09
Control A outflow	54.2±0.08	19.7±0.03	88.7±0.13	1210.3±1.74	6.1±0.01	76.4±0.11	78.2±0.11	3.1±0.00	2.2±0.00	0.3±0.00
Control B outflow	49.7±5.60	12.4±1.40	78.6±8.87	1067.8±120.43	1.7±0.19	26.3±2.96	52.8±5.95	0.8±0.09	3.1±0.35	0.2±0.02
Deionised water	3.6±0.00	0.0±0.00	0.0±0.00	0.0±0.00	1.9±0.00	0.0±0.00	3.5±0.00	2.8±0.00	1.5±0.00	0.0±0.00
Tap water (100%)	5.1±0.21	10.2±0.41	40.9±1.64	530.3±21.27	38.0±1.52	21.3±0.85	49.3±1.98	4.1±0.17	6.0±0.24	0.0±0.00
Tap water with fertiliser (0.7 ml/l)	925.7±17.54	514.9±9.75	862.0±16.33	601.7±11.40	48.2±0.91	1093.3± 20.71	62.7±1.19	5.0±0.10	7.3±0.14	0.1±0.00
Wastewater (20%); tap water (80%)	305.7±0.00	22.8±0.00	136.9±0.00	1030.2±0.00	24.2±0.00	163.5±0.00	138.4±0.00	2.8±0.00	6.8±0.00	1.4±0.00
Wastewater (100%)	1408.8±83.16	100.6±5.94	624.8±36.88	2623.7±154.86	97.7±5.77	668.0±39.43	441.4±26.05	5.4±0.32	12.4± 0.73	3.5±0.21

Note: [a] NH_4-N: ammonia-nitrogen; [b] NO_3-N: nitrate-nitrogen; [c] PO_4-P: ortho-phosphate-phosphorus; [d] Ca: calcium; [e] Fe: iron; [f] K: potassium; [g] Mg: magnesium; [h] Mn: manganese; [i] Zn: zinc; [j] B: boron. Total applied mass of each element calculated from irrigation water volume and average concentration.

Table 4.32: Overview of total water volume for Chilli plants grown in organic media during different planting periods.

Irrigation water type	Total irrigation water volume (l)			
	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Filter 1 outflow	37.290±2.8284	0.290±0.0000	6.175±0.1061	30.825±2.7224
Filter 2 outflow	33.487±3.6551	0.287±0.0058	5.767±0.3253	27.433±3.5642
Filter 3 outflow	28.847±2.2368	0.280±0.0000	4.800±0.0000	23.767±2.2368
Filter 4 outflow	36.550±10.8187	0.280±0.0000	5.400±1.0607	30.870±9.7581
Filter 5 outflow	28.797±1.9902	0.280±0.0000	5.133±0.2021	23.383±2.0251
Filter 6 outflow	46.330±0.0000	0.280±0.0000	4.800±0.0000	41.250±0.0000
Filter 7 outflow	40.705±1.0253	0.280±0.0000	6.550±0.7778	33.875±1.8031
Filter 8 outflow	35.663±2.8295	0.280±0.0000	4.900±0.1732	30.483±2.6760
Control A outflow	49.280±0.0707	0.280±0.0000	4.900±0.0000	44.100±0.0707
Control B outflow	41.380±4.6669	0.280±0.0000	5.700±0.1414	35.400±4.8083
Deionised water	36.030±0.0000	0.280±0.0000	5.950±0.0000	29.800±0.0000
Tap water (100%)	51.130±2.0506	0.280±0.0000	6.600±0.7071	44.250±2.7577
Tap water with fertiliser (0.7 ml/l)	57.855±1.0960	0.280±0.0000	7.000±0.0000	50.575±1.0960
Wastewater (20%); tap water (80%)	45.630±0.0000	0.280±0.0000	5.100±0.0000	40.250±0.0000
Wastewater (100%)	41.930±2.4749	0.280±0.0000	5.350±0.3536	36.300±2.1213

[a] 11/10/13 to 25/09/14; [b] Second planting period: 11/10/13 to 07/11/13; [c] Final planting period before fruiting: 08/11/13 to 19/01/14; [d] Final planting period after fruiting: 20/01/14 to 25/09/14.

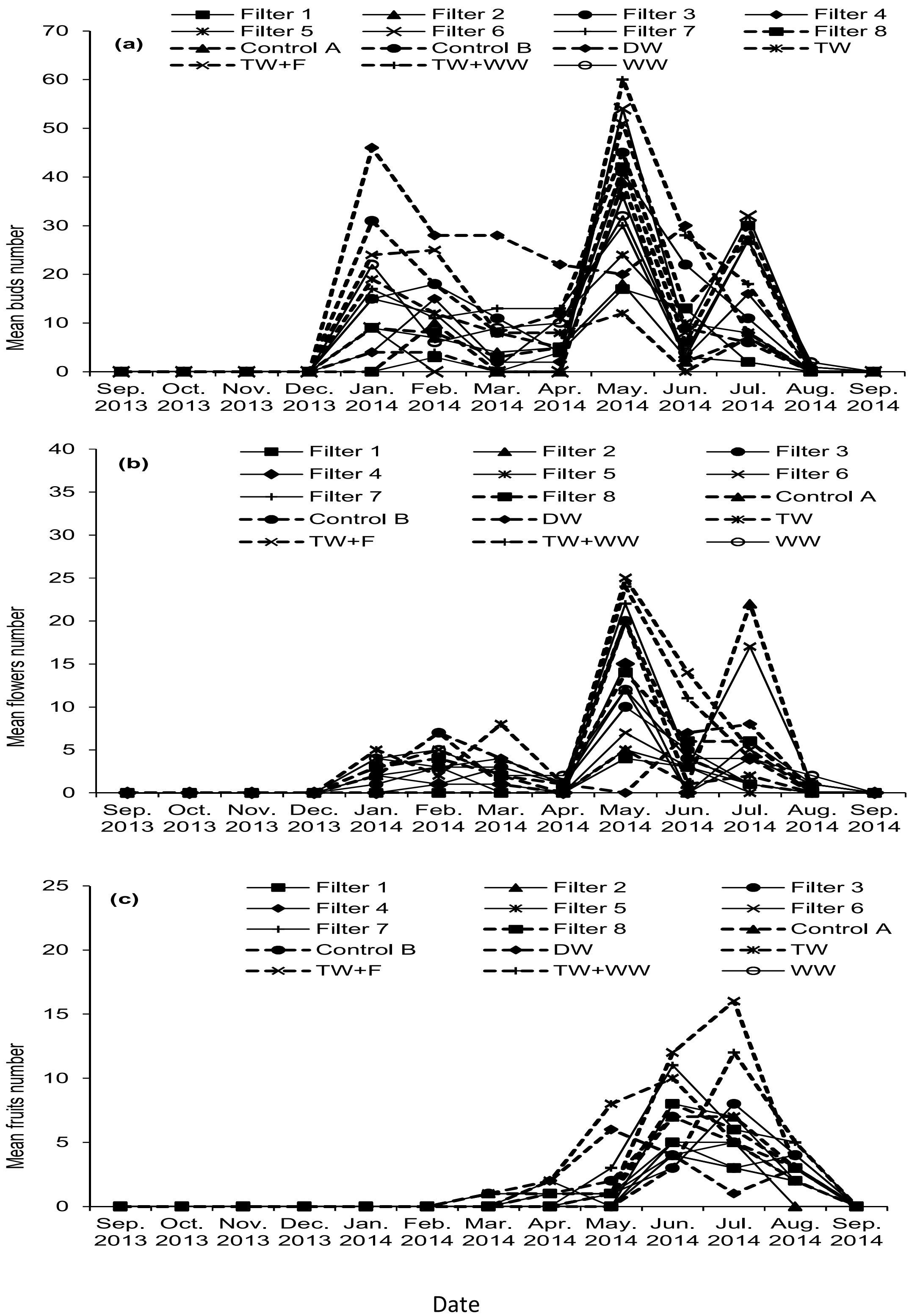

Figure 4.12: Overview of Chilli plant developments grown in organic media during whole experiment duration. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

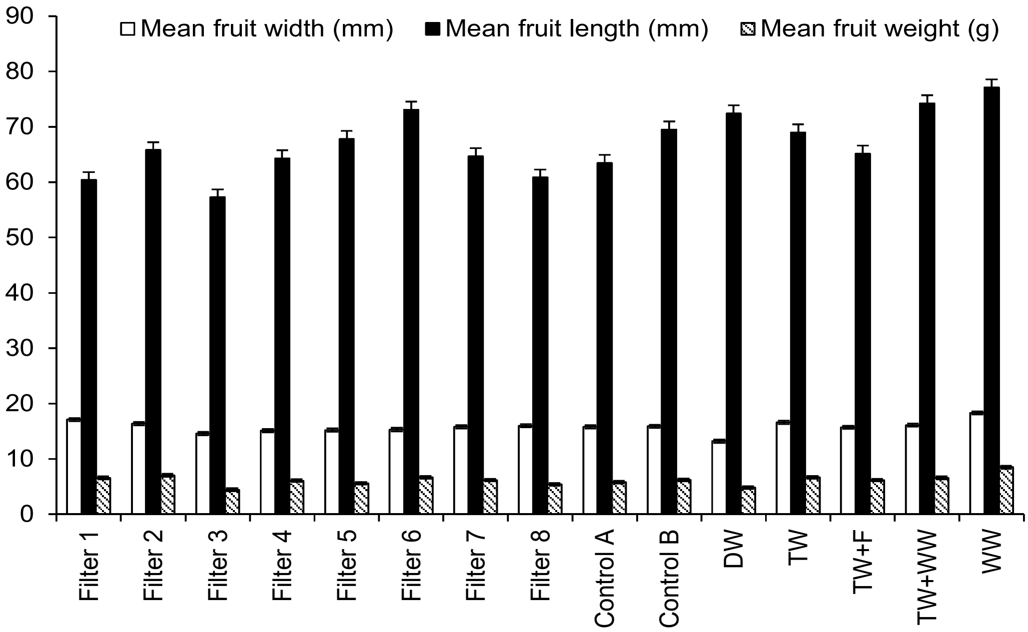

Figure 4.13: Differences in mean fruit width, mean fruit length and mean fruit weight linked to harvested Chilli plants irrigated with different water types and grown in organic media. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

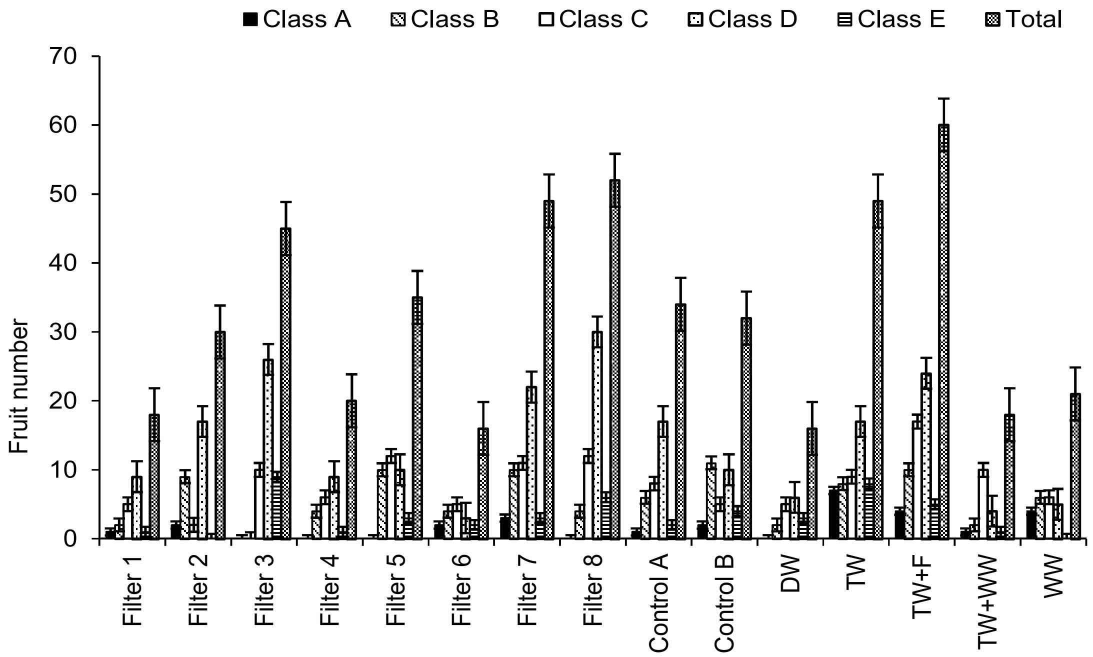

Figure 4.14: Overview of fruit numbers per class harvested from Chillies grown in organic media and subjected to different irrigation water types. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

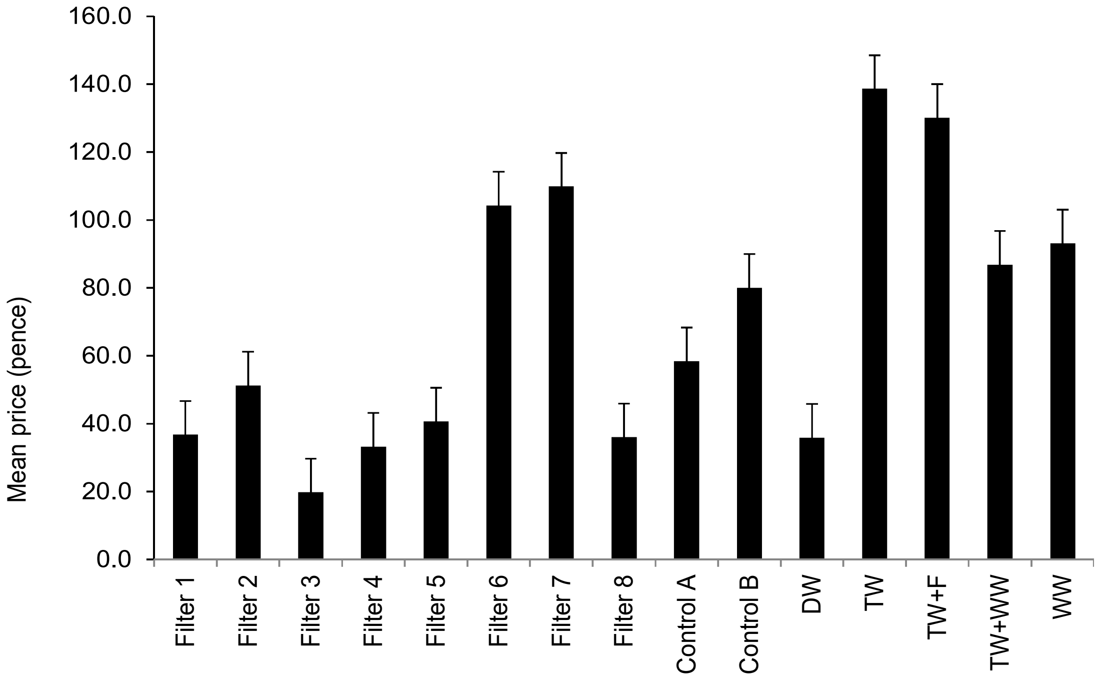

Figure 4.15: Chilli harvest outcome linked to plants grown in organic media (after classification scheme (Table 3.4) application). DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.33a: Overview of the statistically significant differences among Chilli fruit widths harvested from plants grown in organic media and subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

Treatment	Statistic (*p*-value)[a]														
	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5	Filter 6	Filter 7	Filter 8	Control A	Control B	DW[b]	TW[c]	TW+F[d]	TW+WW[e]	WW[f]
Filter 1	n.a	0.591	**0.029**	0.104	0.162	0.168	0.352	0.454	0.260	0.511	**0.002**	0.978	0.253	0.354	0.364
Filter 2	0.591	n.a	**0.023**	0.112	0.175	0.189	0.413	0.544	0.297	0.613	**0.002**	0.955	0.285	0.415	0.115
Filter 3	**0.029**	**0.023**	n.a	0.780	0.366	0.634	0.096	0.051	0.217	0.079	0.131	**0.011**	0.133	0.266	**0.001**
Filter 4	0.104	0.112	0.780	n.a	0.651	0.854	0.310	0.222	0.470	0.246	0.125	0.092	0.392	0.479	**0.007**
Filter 5	0.162	0.175	0.366	0.651	n.a	0.824	0.510	0.386	0.746	0.396	**0.033**	0.139	0.649	0.742	**0.009**
Filter 6	0.168	0.189	0.634	0.854	0.824	n.a	0.459	0.351	0.632	0.368	0.099	0.170	0.560	0.621	**0.017**
Filter 7	0.352	0.413	0.096	0.310	0.510	0.459	n.a	0.796	0.757	0.787	**0.007**	0.379	0.800	0.864	**0.028**
Filter 8	0.454	0.544	0.051	0.222	0.386	0.351	0.796	n.a	0.581	0.964	**0.008**	0.524	0.594	0.709	**0.043**
Control A	0.260	0.297	0.217	0.470	0.746	0.632	0.757	0.581	n.a	0.595	**0.017**	0.263	0.928	0.936	**0.020**
Control B	0.511	0.613	0.079	0.246	0.396	0.368	0.787	0.964	0.595	n.a	**0.006**	0.610	0.614	0.708	0.069
DW[b]	**0.002**	**0.002**	0.131	0.125	**0.033**	0.099	**0.007**	**0.008**	**0.017**	**0.006**	n.a	**0.001**	**0.009**	**0.028**	**0.000**
TW[c]	0.978	0.955	**0.011**	0.092	0.139	0.170	0.379	0.524	0.263	0.610	**0.001**	n.a	0.237	0.401	0.188
TW+F[d]	0.253	0.285	0.133	0.392	0.649	0.560	0.800	0.594	0.928	0.614	**0.009**	0.237	n.a	0.990	**0.014**
TW+WW[e]	0.354	0.415	0.266	0.479	0.742	0.713	0.864	0.709	0.936	0.708	**0.028**	0.401	0.990	n.a	**0.048**
WW[f]	0.364	0.115	**0.001**	**0.007**	**0.009**	**0.017**	**0.028**	**0.043**	**0.020**	0.069	**0.000**	0.188	**0.014**	**0.048**	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); [b], Deionised water; [c], Tap water; [d], Tap water with fertiliser (0.7 ml/l); [e], Wastewater (20%); tap water (80%); [f], Wastewater (100%) and n.a, not applicable as the treatment compared with itself. Bold values are significant ($p < 0.05$).

Table 4.33b: Overview of the statistically significant differences among Chilli fruit lengths harvested from plants grown in organic media and subjected to different irrigation water types using the parametric Games-Howell test.

Treatment	Statistic (*p*-value)[a]														
	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5	Filter 6	Filter 7	Filter 8	Control A	Control B	DW[b]	TW[c]	TW+F[d]	TW+WW[e]	WW[f]
Filter 1	n.a	0.980	0.998	1.000	0.605	0.158	0.984	1.000	1.000	0.429	0.205	0.479	0.948	0.054	**0.001**
Filter 2	0.980	n.a	0.297	1.000	1.000	0.905	1.000	0.950	1.000	1.000	0.948	1.000	1.000	0.722	0.105
Filter 3	0.998	0.297	n.a	0.769	**0.003**	**0.008**	0.073	0.847	0.560	**0.006**	**0.011**	**0.004**	**0.012**	**0.001**	**0.000**
Filter 4	1.000	1.000	0.769	n.a	1.000	0.806	1.000	0.999	1.000	0.992	0.870	0.997	1.000	0.595	0.101
Filter 5	0.605	1.000	**0.003**	1.000	n.a	0.978	0.998	0.237	0.982	1.000	0.992	1.000	0.999	0.870	0.113
Filter 6	0.158	0.905	**0.008**	0.806	0.978	n.a	0.585	0.078	0.479	1.000	1.000	0.999	0.607	1.000	0.999
Filter 7	0.984	1.000	0.073	1.000	0.998	0.585	n.a	0.925	1.000	0.962	0.683	0.981	1.000	0.291	**0.003**
Filter 8	1.000	0.950	0.847	0.999	0.237	0.078	0.925	n.a	1.000	0.177	0.106	0.187	0.736	**0.016**	**0.000**
Control A	1.000	1.000	0.560	1.000	0.982	0.479	1.000	1.000	n.a	0.898	0.574	0.934	1.000	0.228	**0.004**
Control B	0.429	1.000	**0.006**	0.992	1.000	1.000	0.962	0.177	0.898	n.a	1.000	1.000	0.972	0.994	0.564
DW[b]	0.205	0.948	**0.011**	0.870	0.992	1.000	0.683	0.106	0.574	1.000	n.a	1.000	0.706	1.000	0.992
TW[c]	0.479	1.000	**0.004**	0.997	1.000	0.999	0.981	0.187	0.934	1.000	1.000	n.a	0.987	0.983	0.405
TW+F[d]	0.948	1.000	**0.012**	1.000	0.999	0.607	1.000	0.736	1.000	0.972	0.706	0.987	n.a	0.301	**0.002**
TW+WW[e]	0.054	0.722	**0.001**	0.595	0.870	1.000	0.291	**0.016**	0.228	0.994	1.000	0.983	0.301	n.a	1.000
WW[f]	**0.001**	0.105	**0.000**	0.101	0.113	0.999	**0.003**	**0.000**	**0.004**	0.564	0.992	0.405	**0.002**	1.000	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); [b], Deionised water; [c], Tap water; [d], Tap water with fertiliser (0.7 ml/l); [e], Wastewater (20%); tap water (80%); [f], Wastewater (100%) and n.a, not applicable as the treatment compared with itself. Bold values are significant ($p < 0.05$).

Table 4.33c: Overview of the statistically significant differences among Chilli fruit weights harvested from plants grown in organic media and subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

Treatment	Statistic (*p*-value)[a]														
	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5	Filter 6	Filter 7	Filter 8	Control A	Control B	DW[b]	TW[c]	TW+F[d]	TW+WW[e]	WW[f]
Filter 1	n.a	0.622	**0.003**	0.757	0.329	0.926	0.708	0.155	0.358	0.909	0.057	0.815	0.699	0.955	0.068
Filter 2	0.622	n.a	**0.000**	0.399	0.087	0.697	0.289	**0.022**	0.101	0.485	**0.011**	0.626	0.270	0.662	0.123
Filter 3	**0.003**	**0.000**	n.a	**0.007**	**0.014**	**0.003**	**0.000**	**0.029**	**0.012**	**0.001**	0.545	**0.000**	**0.000**	**0.002**	**0.000**
Filter 4	0.757	0.399	**0.007**	n.a	0.525	0.693	0.994	0.285	0.562	0.817	0.103	0.540	0.992	0.710	**0.030**
Filter 5	0.329	0.087	**0.014**	0.525	n.a	0.295	0.423	0.626	0.949	0.315	0.261	0.118	0.404	0.289	**0.002**
Filter 6	0.926	0.697	**0.003**	0.693	0.295	n.a	0.639	0.140	0.322	0.831	0.052	0.909	0.629	0.970	0.112
Filter 7	0.708	0.289	**0.000**	0.994	0.423	0.639	n.a	0.160	0.467	0.765	0.058	0.414	0.998	0.625	**0.009**
Filter 8	0.155	**0.022**	**0.029**	0.285	0.626	0.140	0.160	n.a	0.581	0.121	0.351	**0.025**	0.138	0.129	**0.000**
Control A	0.358	0.101	**0.012**	0.562	0.949	0.322	0.467	0.581	n.a	0.349	0.200	0.138	0.449	0.317	**0.002**
Control B	0.909	0.485	**0.001**	0.817	0.315	0.831	0.765	0.121	0.349	n.a	**0.045**	0.671	0.754	0.856	**0.030**
DW[b]	0.057	**0.011**	0.545	0.103	0.261	0.052	0.058	0.351	0.200	0.045	n.a	**0.014**	0.053	**0.048**	**0.000**
TW[c]	0.815	0.626	**0.000**	0.540	0.118	0.909	0.414	0.025	0.138	0.671	**0.014**	n.a	0.388	0.866	**0.030**
TW+F[d]	0.699	0.270	**0.000**	0.992	0.404	0.629	0.998	0.138	0.449	0.754	0.053	0.388	n.a	0.641	**0.007**
TW+WW[e]	0.955	0.662	**0.002**	0.710	0.289	0.970	0.625	0.129	0.317	0.856	**0.048**	0.866	0.641	n.a	0.073
WW[f]	0.068	0.123	**0.000**	**0.030**	**0.002**	0.112	**0.009**	**0.000**	**0.002**	**0.030**	**0.000**	**0.030**	**0.007**	**0.073**	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); [b], Deionised water; [c], Tap water; [d], Tap water with fertiliser (0.7 ml/l); [e], Wastewater (20%); tap water (80%); [f], Wastewater (100%) and n.a, not applicable as the treatment compared with itself. Bold values are significant ($p < 0.05$).

Figure 4.16a–f shows Chilli growth details in terms of plant overall height, number of leaves, buds, flowers, fruits and total weight of fruits harvested from each treatment when using inorganic growth media and subjected to different irrigation water types. Regarding the overall height of plants growing in sand soil, Figure 4.16a shows that the maximum height was associated with plants irrigated with raw wastewater followed by those irrigated with tap water spiked with fertiliser, while the minimum plant height was observed for those plants irrigated with water harvested from Control B. This can be explained by the high nutrient load (Table 4.34) applied via irrigation water (Table 4.35) as discussed by FAO (2003) and Almuktar and Scholz (2016 b).

Regarding the total number of leaves (Figure 4.16b) linked to Chillies grown in inorganic media, findings indicated that Chillies irrigated with tap water spiked by fertiliser produced the highest leaf numbers followed by those plants irrigated with raw wastewater, wastewater diluted with tap water and water harvested from Filter 6 of high inflow loading rate (Table 3.1).

Figure 4.16c–f provides summaries of plant developments. The highest numbers of buds were recorded for Chillies irrigated with tap water spiked by fertiliser followed by those irrigated with water harvested from Filter 6 of high inflow loading rate and Filter 2 of large aggregate size. Moreover, Chillies irrigated with tap water spiked with fertiliser and Filter 3 outflow water showed the highest flower numbers, while the lowest numbers were recorded for plants irrigated with deionised water, tap waters and outflow waters from Controls A and B. However, the highest number of fruits was recorded for plants irrigated with tap water spiked with fertiliser followed by those plants irrigated with raw wastewater and outflow waters from Filter 7 of low contact time and Filter 6 of high inflow loading rate (Figure 4.16e), which subsequently produced the highest fruit total weight (Figure 4.16f).

Moreover, most buds and flowers fell down before reaching the fruiting stage. This can be explained by the adverse environmental conditions in the laboratory in terms of light intensity provided by the grow lights and the relative humidity which was elevated artificially by using humidifiers as explained in section 3.4. These may cause flower inhibition or cause flower abscission as reported by Wein and Zhang (1991).

Figure 4.17 shows that the plants started to produce buds and flowers in January 2014. However, fruit production took place between April 2014 and September 2014. This is possibly due to lack of nutrients available for plants from the poor inorganic soil (Table 4.27) and dependency mainly on fertiliser provided by irrigation water resulting in deprived plant development including fruit production (FAO, 1972. 1991, 1994; Almuktar & Scholz, 2016b).

Figure 4.18 summarises differences in fruit characteristics associated with sandy soil. Results show that the maximum fruit width was recorded for fruits harvested from plants irrigated with water drained from Filter 6 of high inflow loading rate (18 mm) followed by those for fruits harvested from plants irrigated with tap water spiked with fertiliser (16 mm) then wastewater diluted with tap water (15 mm). The latter produced fruits of mean widths similar to those of plants irrigated with water harvested from Filters 2 and 4, which were different in aggregate diameters. The minimum fruit width was recorded for fruits harvested from plants irrigated with deionised water and Control B outflow water (9 mm). Moreover, plants irrigated with water drained from Filters 3, 8 and Control A produced fruits of similar width (12 mm).

The maximum fruit length was observed for fruits harvested from plants irrigated with Filter 2 out flow water (80 mm) followed by those associated with pants irrigated with tap water spiked with fertiliser (75 mm) then wastewater diluted with tap water (60 mm), while the minimum fruit lengths were recorded for those plants irrigated with Control B outflow water (17 mm), and deionised and tap waters (18 mm).

Regarding mean fruit weight, results show that plants irrigated with outflow water of Filter 2 (large aggregate diameter) and tap water spiked with fertiliser produced fruits of maximum weight values, while the minimum weights were recorded for fruits harvested from plants irrigated with Control B outflow water, tap and deionised waters.

Figure 4.19 shows the number of harvested fruits categorised as Class A, B, C, D and E per treatment. Fruits categorised as Class A were only observed in the harvest associated with tap water spiked with fertiliser.

The highest Class B fruit number was recorded for plants irrigated with tap water mixed with fertiliser (6 fruits) followed by those linked to Filter 2 and 4 (1 fruit). Similarly, plants irrigated with tap water mixed with fertiliser produced the highest number of Class C fruits (4 fruits) followed by those irrigated with Filter 2 outflow water (2 fruits). At the same time 11 fruits from plants irrigated with tap water with fertiliser were categorised as Class D followed by those irrigated with raw wastewater and outflow water from Filter 8 (4 fruits). However, tap water with fertiliser irrigated plants produced the highest number of Class E fruits (3 fruits) followed by those irrigated with Filter 1 outflow water (2 fruits).

Figure 4.20 indicates the monetary value of the Chilli harvest linked to inorganic media. Results show that the highest mean harvest price was linked to plants irrigated with tap water with fertiliser followed by those irrigated with Filter 7 outflow water as they produced numerous fruits of high category classifications. Results show that Chilli yield quality for filters contaminated with hydrocarbons (Filters 1, 3, 5, and Control A) were noticeably lower than those associated with uncontaminated filters (Filter 2, 4, 6, and Control B) resulting in lower economic income. This confirms the results reported previously by Hester and Mendelssohn (2000) and Wyszkowska et al. (2001, 2002) indicating the negative impact of hydrocarbon contamination on marketable yield production as it could result in nutrient and trace element imbalance available for plant growth and development.

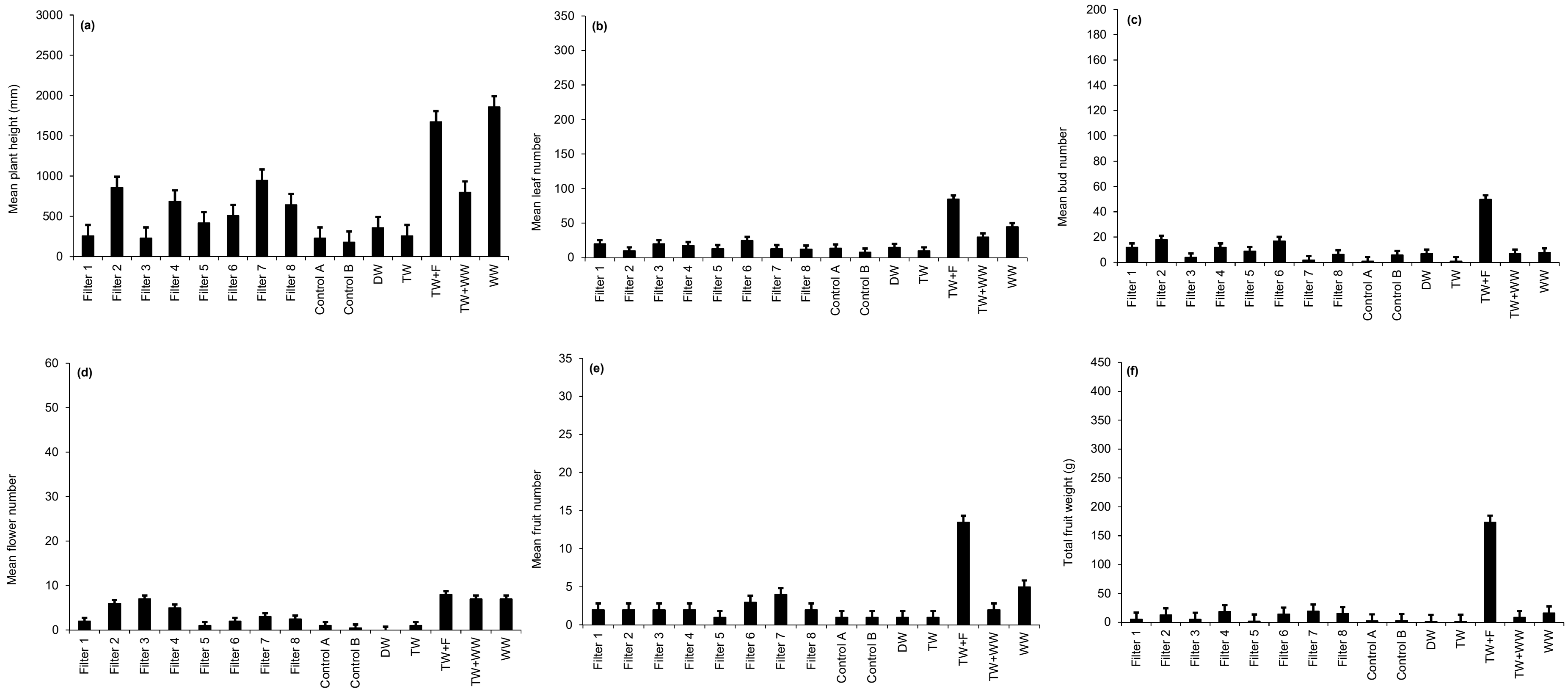

Figure 4.16: Growth of Chilli plants in inorganic media and subjected to different irrigation water types: (a) mean plant height; (b) mean leaf number; (c) mean bud number; (d) mean flower number; (e) mean fruit number; and (f) total fruit weight. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Table 4.34: Overview of total element mass applied to Chilli plants grown in inorganic media subjected to different irrigation water types.

Irrigation water type	Total applied mass (mg)									
	NH_4-N[a]	NO_3-N[b]	PO_4-P[c]	Ca[d]	Fe[e]	K[f]	Mg[g]	Mn[h]	Zn[i]	B[j]
Filter 1 outflow	98.3±0.00	8.2±0.00	81.9±0.00	647.1±0.00	21.6±0.00	158.5±0.00	107.5±0.00	2.7±0.00	0.9±0.00	1.2±0.00
Filter 2 outflow	104.8±0.00	39.8±0.00	59.6±0.00	550.5±0.00	21.4±0.00	127.8±0.00	90.6±0.00	0.8±0.00	1.0±0.00	0.8±0.00
Filter 3 outflow	64.3±0.00	6.9±0.00	57.3±0.00	1048.3±0.00	25.5±0.00	148.4±0.00	103.6±0.00	4.6±0.00	1.5±0.00	0.7±0.00
Filter 4 outflow	59.3±0.00	39.5±0.00	63.7±0.00	964.4±0.00	4.2±0.00	76.3±0.00	115.7±0.00	1.1±0.00	1.3±0.00	0.8±0.00
Filter 5 outflow	145.0±0.00	12.8±0.00	62.6±0.00	813.2±0.00	18.7±0.00	165.4±0.00	122.7±0.00	3.7±0.00	1.2±0.00	0.8±0.00
Filter 6 outflow	167.6±0.00	64.9±0.00	82.9±0.00	967.4±0.00	9.9±0.00	196.6±0.00	157.2±0.00	1.4±0.00	1.7±0.00	1.1±0.00
Filter 7 outflow	85.6±0.00	63.1±0.00	81.1±0.00	873.3±0.00	10.8±0.00	146.3±0.00	115.3±0.00	2.0±0.00	1.3±0.00	0.8±0.00
Filter 8 outflow	26.9±0.20	53.8±0.40	63.4±0.47	758.5±5.58	5.8±0.04	94.7±0.70	93.1±0.68	1.0±0.01	2.0±0.01	0.6±0.00
Control A outflow	24.6±0.00	8.9±0.00	40.2±0.00	548.2±0.00	2.7±0.00	34.6±0.00	35.4±0.00	1.4±0.00	1.0±0.00	0.2±0.00
Control B outflow	26.5±0.13	6.6±0.03	41.9±0.20	568.9±2.74	0.9±0.00	14.0±0.07	28.1±0.14	0.4±0.00	1.7±0.01	0.1±0.00
Deionised water	2.4±0.00	0.0±0.00	0.0±0.00	0.0±0.00	1.3±0.00	0.0±0.00	2.4±0.00	1.9±0.00	1.0±0.00	0.0±0.00
Tap water (100%)	2.3±0.00	4.7±0.00	18.7±0.00	242.4±0.00	17.4±0.00	9.7±0.00	22.6±0.00	1.9±0.00	2.8±0.00	0.0±0.00
Tap water with fertiliser (0.7 ml/l)	676.3±4.53	376.2±2.52	629.8±4.21	439.6±2.94	35.2±0.24	798.8±5.35	45.8±0.31	3.7±0.02	5.4±0.04	0.0±0.00
Wastewater (20%); tap water (80%)	183.4±0.00	13.7±0.00	82.1±0.00	617.9±0.00	14.5±0.00	98.1±0.00	83.0±0.00	1.7±0.00	4.1±0.00	0.8±0.00
Wastewater (100%)	884.4±0.00	63.2±0.00	392.2±0.00	1646.9±0.00	61.3±0.00	419.3±0.00	277.1±0.00	3.4±0.00	7.8±0.00	2.2±0.00

Note: [a] NH_4-N: ammonia-nitrogen; [b] NO_3-N: nitrate-nitrogen; [c] PO_4-P: ortho-phosphate-phosphorus; [d] Ca: calcium; [e] Fe: iron; [f] K: potassium; [g] Mg: magnesium; [h] Mn: manganese; [i] Zn: zinc; [j] B: boron. Total applied mass of each element calculated from irrigation water volume and average concentration.

Table 4.35: Overview of total water volume for Chilli plants grown in inorganic media during different planting periods.

Irrigation water type	Total irrigation water volume (l)			
	Overall[a]	FRP[b]	SRPBF[c]	SRPAF[d]
Filter 1 outflow	20.470±0.0000	0.270±0.0000	5.900±0.0000	14.300±0.0000
Filter 2 outflow	18.070±0.0000	0.270±0.0000	6.050±0.0000	11.750±0.0000
Filter 3 outflow	17.370±0.0000	0.270±0.0000	5.350±0.0000	11.750±0.0000
Filter 4 outflow	21.970±0.0000	0.270±0.0000	6.850±0.4243	14.850±0.4243
Filter 5 outflow	14.220±0.0000	0.270±0.0000	5.250±0.0000	8.700±0.0000
Filter 6 outflow	18.020±0.0000	0.270±0.0000	6.400±0.0000	11.350±0.0000
Filter 7 outflow	22.520±0.0000	0.270±0.0000	6.100±0.0000	16.150±0.0000
Filter 8 outflow	19.220±0.1414	0.270±0.0000	6.300±0.5657	12.650±0.4243
Control A outflow	22.320±0.0000	0.270±0.0000	5.250±0.0000	16.800±0.0000
Control B outflow	22.045±0.1061	0.270±0.0000	6.550±0.0000	15.225±0.1061
Deionised water	24.220±0.0000	0.270±0.0000	6.350±0.0000	17.600±0.0000
Tap water (100%)	23.370±0.0000	0.270±0.0000	6.200±0.0000	16.900±0.0000
Tap water with fertiliser (0.7 ml/l)	42.270±0.2828	0.270±0.0000	7.050±0.0707	34.950±0.0000
Wastewater (20%); tap water (80%)	27.370±0.0000	0.270±0.0000	6.100±0.0000	21.000±0.3536
Wastewater (100%)	26.320±0.0000	0.270±0.0000	5.900±0.0000	20.150±0.0000

[a] 11/10/13 to 25/09/14; [b] Second planting period: 11/10/13 to 07/11/13; [c] Final planting period before fruiting: 08/11/13 to 19/01/14; [d] Final planting period after fruiting: 20/01/14 to 25/09/14.

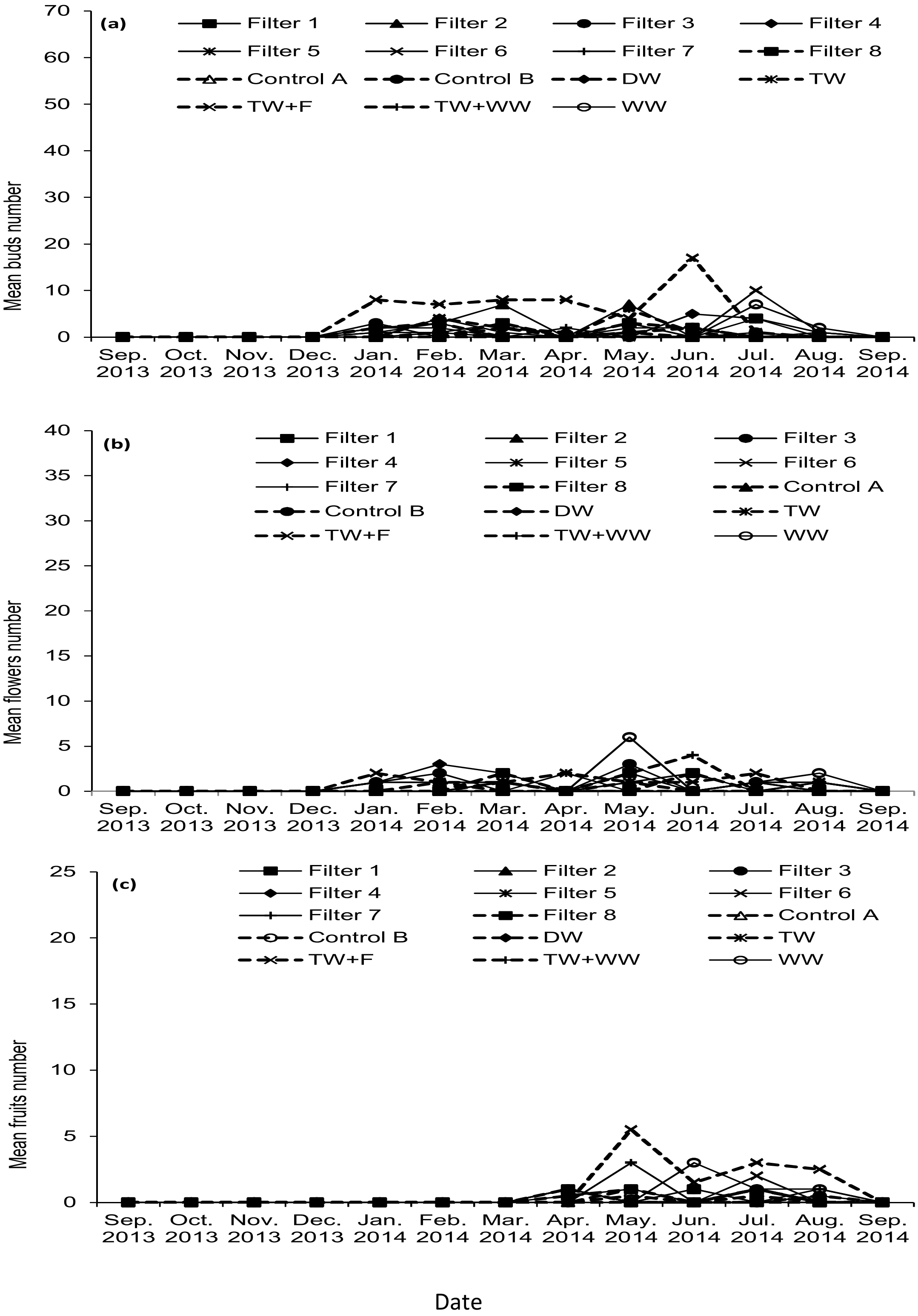

Figure 4.17: Overview of Chilli plant developments grown in inorganic media during whole experiment duration. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

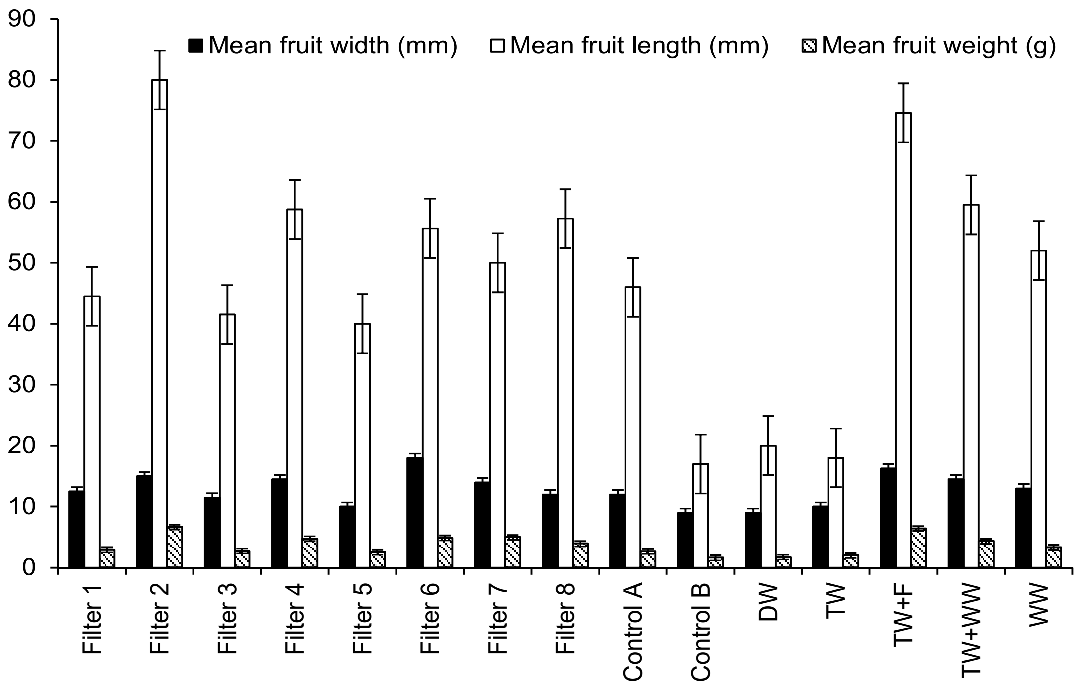

Figure 4.18: Differences in mean fruit width, mean fruit length and mean fruit weight linked to harvested Chilli plants irrigated with different water types and grown in inorganic media. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

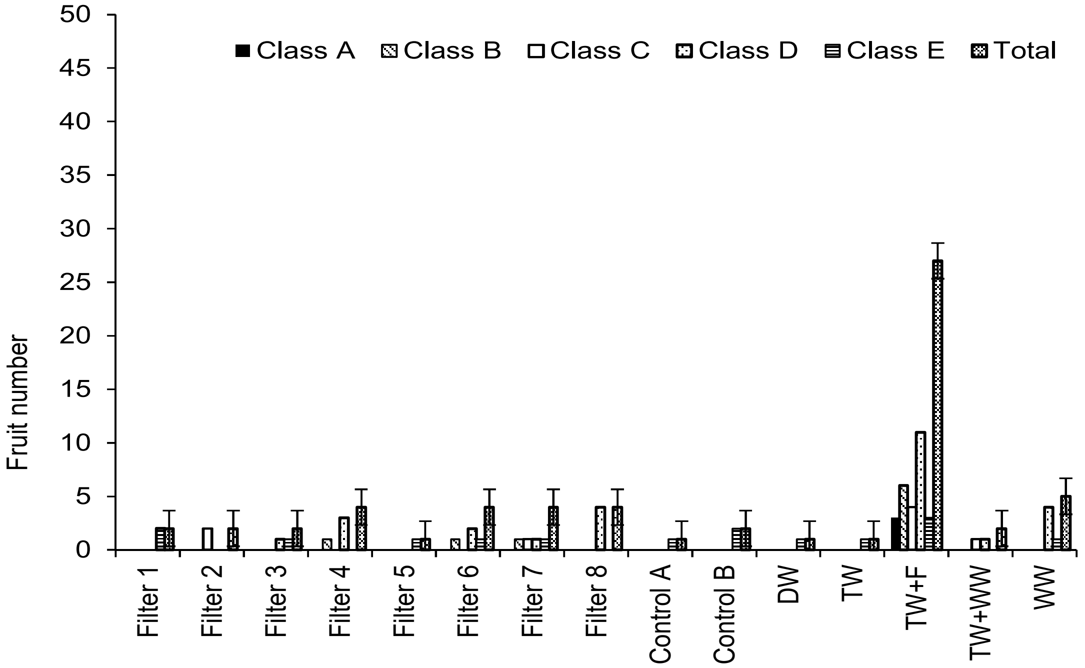

Figure 4.19: Overview of fruit numbers per class harvested from Chillies grown in inorganic media and subjected to different irrigation water types. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

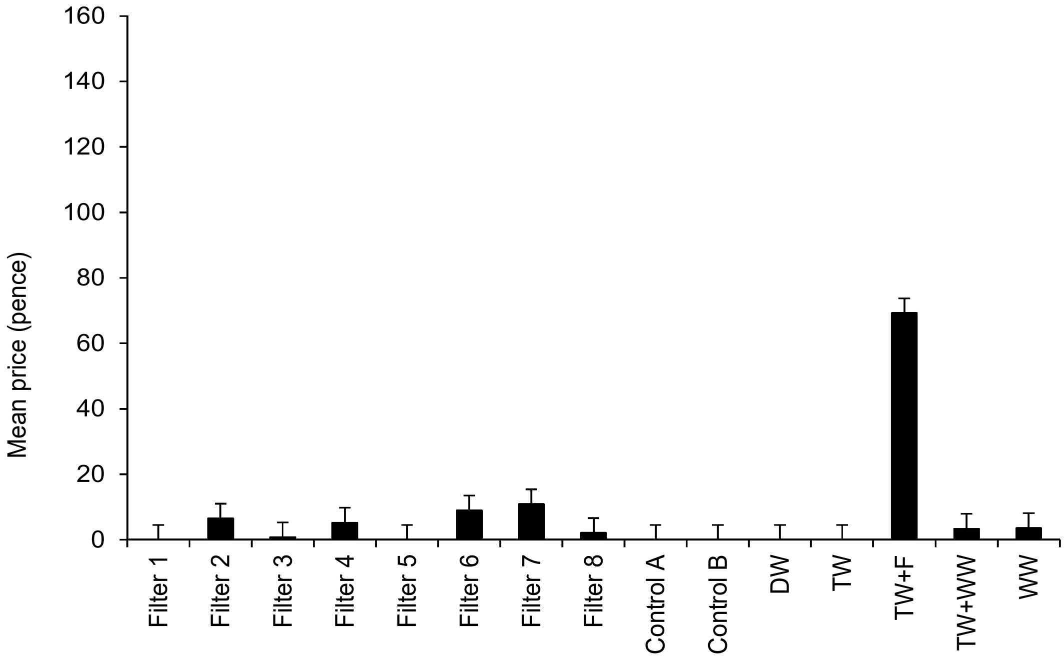

Figure 4.20: Chilli harvest outcome linked to plants grown in inorganic media (after classification scheme (Table 3.4) application). DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

Findings indicate that compost compared to sand is associated with considerably greater plant growth and productivity. This is due to the elevated nutrient availability in basic compost (Rahimi et al., 2013) compared to sand (Table 4.27). Moreover, organic substrates decompose over time, and subsequently release nutrients. The rate of decomposition and the physical conditions of the media vary with the parent material. That, in turn, will enhance crop growth and development. Moreover, better aeration of peat promotes vigorous root growth, which allows rapid development of foliage and therefore increases the whole plant yield. In contrast, for inorganic media such as sand, nutrient provision to the crops is limited to the nutrients that are part of the irrigation water resulting in a delay of plant foliage growth with a subsequent poor yield (Olle et al., 2012).

Generally, fruits harvested from plants grown in organic media had widths, lengths and weights greater than those from plants raised in inorganic media. These results, in addition to findings based on other research studies undertaken in greenhouse conditions to assess the effect of different growth media on *Capsicum annuum* growth rates and yields, indicate that seedlings benefited from peat moss media (Rahimi et al., 2013). Another study was undertaken, to determine the effects of peat and sand on variables such as fruit length, diameter and weight, as well as the total fruit number per plant and yield, by Gungor and Yildirim (2013) which showed that peat significantly increased length, diameter and weight of fruits in all cultivars grown in comparison to sand. Moreover, plants grown in compost consume more water than those grown in sand and subsequently increase the nutrient load applied to plants via irrigation water, leading to higher foliage and yield production.

4.4.4 Comparison of Sweet Pepper and Chilli productivity

Generally, Chillies produced more fruits than Sweet Peppers when using organic growth media (Figure 4.21a) indicating the positive impact of high nutrients and trace elements available by both compost and treated wastewater on growth and productivity of Chillies, and the negative impact on peppers, explaining the different tolerance of plants to the supplied nutrition (FAO, 1994, 2003). Nevertheless, a good balance in supplied nutrients is required for high marketable yield as the surplus will result in increasing the productivity at the expense of quality (Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 b). Based on that, Chillies harvested from organic media resulted in higher outcomes than Sweet Peppers harvested from the same media (Figure 4.22a).

Moreover, the growth and productivity of both Chillies and Sweet Peppers were rather disappointing when using inorganic media (Figure 4.21b) due to insufficiency of nutrition supplied only by the irrigation waters. Moreover, most fruits harvested from plants grown in the sandy soil were categorised with low classes leading to very low harvest outcomes as shown in Figure 4.22b.

However, considering that the monetary value of the Sweet Pepper harvest was low, it is unlikely to be chosen as a fruiting vegetable to be grown on recycled wastewater streams in the future. Moreover, sandy soil will not be preferable for growing Chillies later.

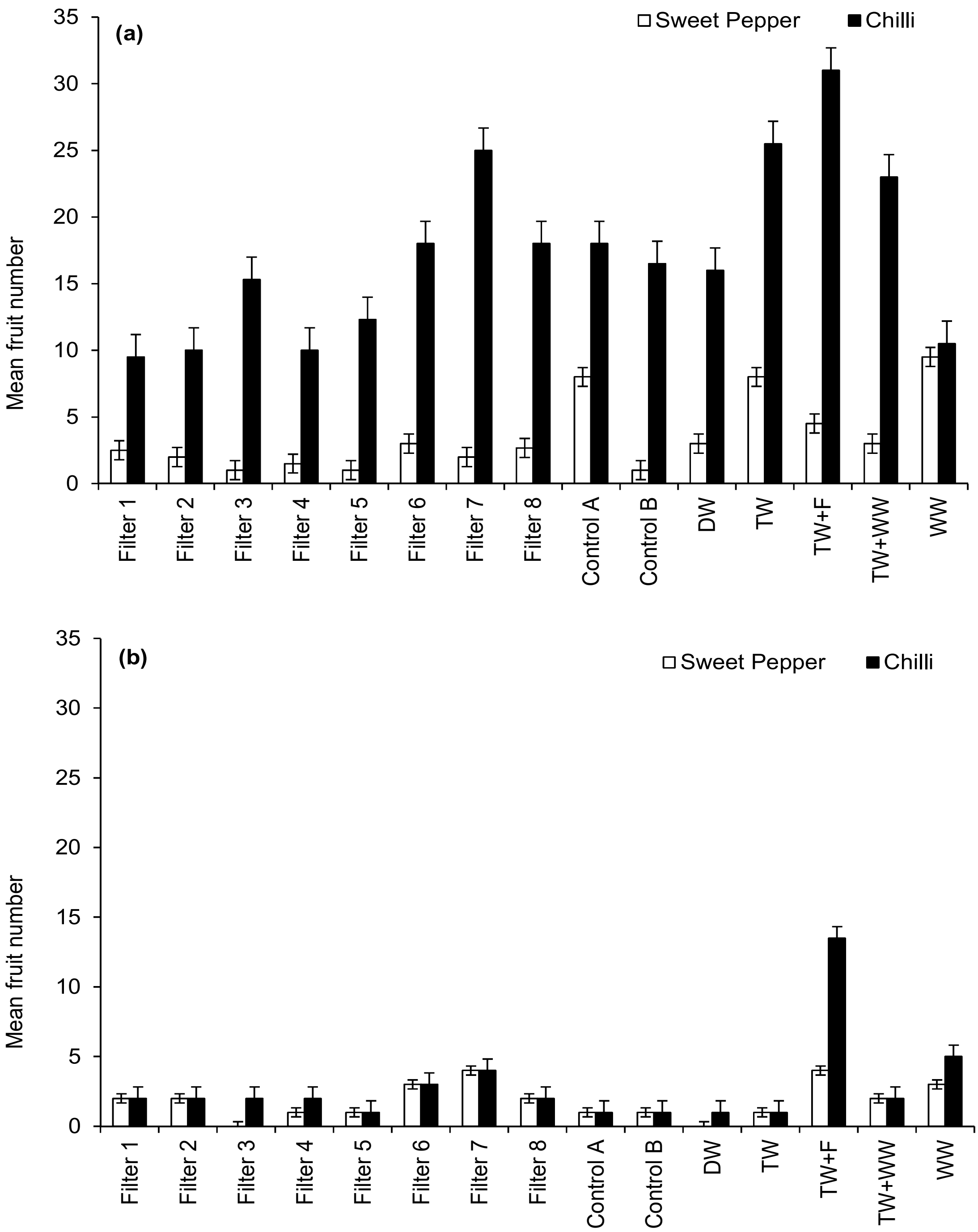

Figure 4.21: Overview of Sweet Pepper and Chilli plants mean fruit number when using: (a) organic growth media, and (b) inorganic growth media. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

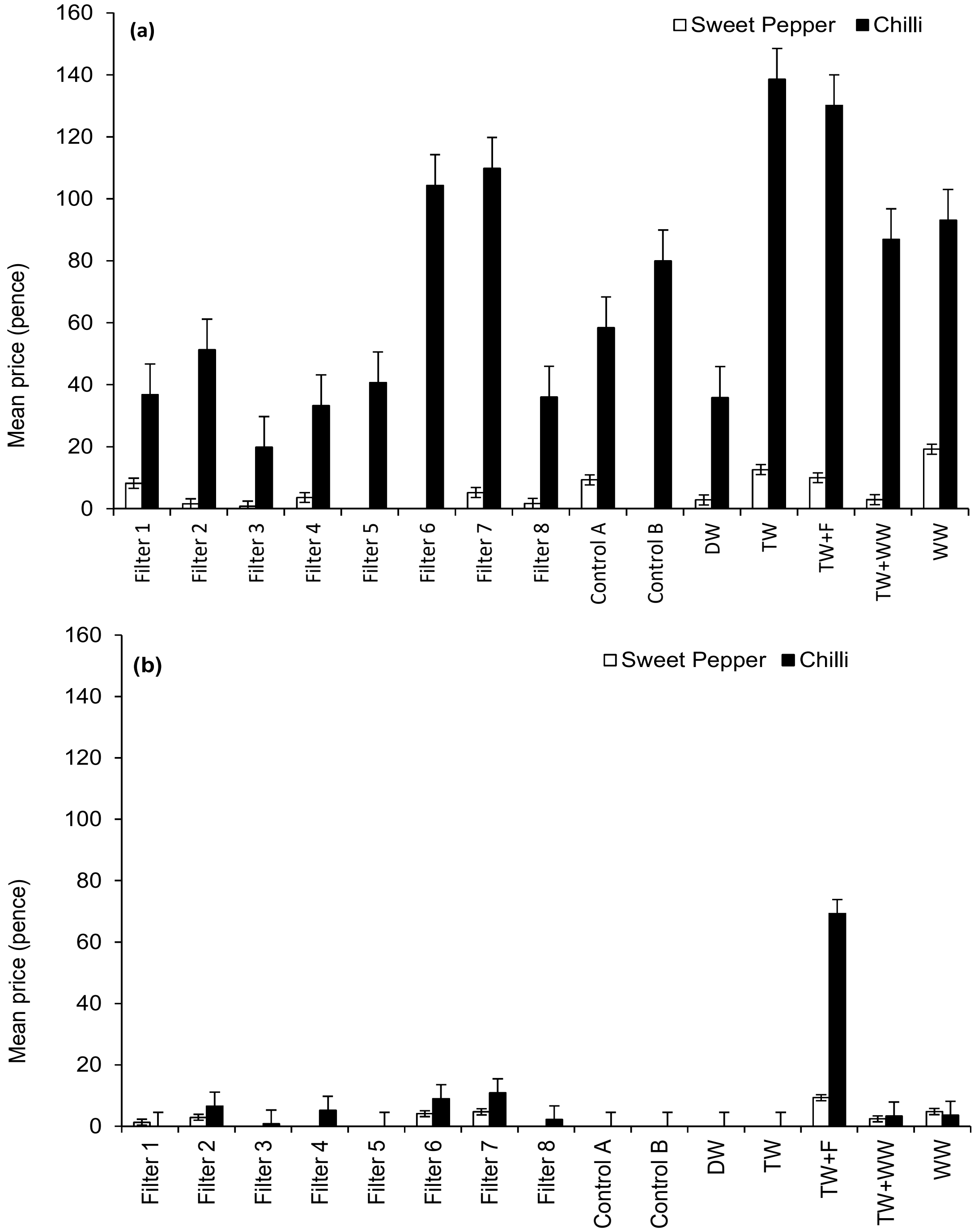

Figure 4.22: Overview of Sweet Pepper and Chilli plant harvest outcomes when using: (a) organic growth media, and (b) inorganic growth media. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

4.5 Summary

This chapter discusses the quality of different irrigation water types, mainly those associated with domestic wastewater treated by vertical-flow constructed wetlands for irrigation of various crops such as Sweet Peppers and Chillies grown in laboratory controlled conditions using different growth media. This includes the concentrations of nutrients, trace elements, organics, salinity and microbial contents of the treated wastewater compared with the irrigation water standards. The environmental boundary conditions available for plants are deliberated as well. Moreover, growth, productivity and marketable yields assessment of crops irrigated with various irrigation water types are discussed statistically in this chapter.

CHAPTER 5

MINERAL AND BIOLOGICAL CONTAMINATION OF SOIL AND *CAPSICUM ANNUUM* IRRIGATED WITH RECYCLED DOMESTIC WASTEWATER

Almuktar, S. A. A. A. N., & Scholz, M. (2015). Microbial contamination of *Capsicum annuum* irrigated with recycled domestic wastewater treated by vertical-flow wetlands. *Ecological Engineering, 82*, 404-414.

Almuktar, S. A. A. A. N., & Scholz, M. (2016). Mineral and biological contamination of soil and *Capsicum annuum* irrigated with recycled domestic wastewater. *Agricultural Water Management, 167*, 95-109

5.1 Overview

In this chapter, soil and Chillies irrigated with domestic wastewater treated by vertical-flow constructed wetlands were assessed for mineral and microbial contamination. Section 5.1 overviews this chapter, while sections 5.2 and 5.3 discuss the soil and Chilli fruit quality analysis for bacterial, trace element and heavy metal contents. Lastly, section 5.4 displays the summary of this chapter.

5.2 Soil quality analysis

5.2.1 Comparison of soil pH and redox potential

Table 5.1 shows pH, redox potential and electrical conductivity for organic and inorganic growth media irrigated with different water types. All pH values of organic media indicated acidic conditions (pH value < 7). In comparison, pH values for inorganic media were alkaline (pH value > 7). The soil pH can markedly affect the availability and consequently the plant uptake of trace elements (FAO, 1972, 2003).

The ability of plants to utilise trace elements decreases with decreasing acidity (increase in pH), while the utilisation at higher pH values remains constant (FAO, 1972). Table 5.1 lists the redox potential of organic and inorganic media. According to Husson (2013), soil could be classified as moderately reduced soil (redox potential values between +100 and +400 mV), reduced soil (redox potential values between −100 and +100 mV) and highly reduced soil (redox potential values between −100 and −300 mV). Based on this classification, Table 5.1 indicates that organic media irrigated with different water types could be considered as reduced soil. In comparison, inorganic media irrigated with outflow water from Filters 3, 4, 6, 7 and 8 as well as Control B could be classified as highly reduced soils, while others may be classified as reduced soils (Husson, 2013). The redox potential and pH are major drivers for change in soil, plant and microorganism systems. High levels of redox potentials can impact on system functioning as well as on plant health and production (Husson, 2013). However, climate conditions and soil moisture could directly affect pH and redox potential values, especially in organic soil.

5.2.2 Comparison of soil salinity

Generally, the electrical conductivity values (Table 5.1) of the organic media were higher than those for the inorganic ones. This can be explained by the acidic conditions of the organic media, which increase the dissolution of sodium, potassium, calcium and magnesium, and subsequently increase the salinity of the soil (FAO, 1972, 2003). However, irrigation with treated wastewater did not increase the salinity of organic media compared to the compost. In comparison, inorganic media showed higher salinity after irrigation with treated wastewater compared to sand. This can be explained by the pH values of different media and their relationship with the salinity as discussed above.

Furthermore, nutrient imbalances could result from excessive soil salinity leading to high accumulations of toxic elements, reducing water infiltration and subsequently limiting the growth of plants (FAO, 1972, 2003).

Table 5.1: Soil properties for pots irrigated with different water sources.

Inflow source and growth media	pH	Eh (mV)	EC (µS/cm)
Filter 1 and organic	6.36	66.3	2259.3
Filter 2 and organic	5.84	93.6	2374.5
Filter 3 and organic	6.18	76.0	1153.5
Filter 4 and organic	6.26	71.8	1764.0
Filter 5 and organic	6.49	59.8	800.0
Filter 6 and organic	6.82	45.5	2338.7
Filter 7 and organic	6.60	53.9	522.0
Filter 8 and organic	6.57	55.6	490.0
Control A and organic	6.44	62.2	976.5
Control B and organic	6.38	65.4	473.5
Deionised water and organic	6.16	77.1	1477.3
Tap water and organic	6.01	84.5	752.8
Tap water/fertiliser and organic	5.49	111.8	1378.0
Wastewater/tap water and organic	6.26	71.8	1032.0
Wastewater and organic	6.24	72.2	1611.0
Raw organic growth media	6.43	62.6	2438.5
Filter 1 and inorganic	8.13	-19.6	474.0
Filter 2 and inorganic	9.74	-95.1	374.0
Filter 3 and inorganic	11.01	-154.8	511.0
Filter 4 and inorganic	10.69	-139.4	581.0
Filter 5 and inorganic	8.91	-56.4	783.6
Filter 6 and inorganic	10.77	-143.4	874.2
Filter 7 and inorganic	10.99	-153.5	817.5
Filter 8 and inorganic	10.47	-129.2	528.8
Control A and inorganic	7.78	-3.3	835.3
Control B and inorganic	10.72	-141.2	370.0
Deionised water and inorganic	9.34	-76.3	996.4
Tap water and inorganic	9.47	-82.6	606.2
Tap water/fertiliser and inorganic	7.83	-5.8	404.5
Wastewater/tap water and inorganic	9.40	-79.1	598.7
Wastewater and inorganic	10.57	-134.1	2081.7
Raw inorganic growth media	9.40	-79.2	116.0

Note: Eh, redox potential; EC, Electrical conductivity; pH, Eh and EC entries are mean values of three samples.

5.2.3 Soil microbial content

Table 5.2 shows the results for soil microbial content. The findings showed that soils irrigated with water harvested from wetlands filters of large aggregate size (Filter 1 and 2) had lower contamination by total coliforms and Salmonellae than those of small aggregate diameter (Filters 3 and 4, respectively) in spite of their abundancy in the outflow waters of the former two (Figure 5.1). Similarly, soils irrigated with high inflow loading rate filters outflow waters (Filters 5 and 6) showed total coliform and Salmonella concentrations lower than those filters of diluted inflow loading rate (Filters 3 and 4, respectively) as shown in Table 5.2. Moreover, soil irrigated with Filter 7 outflow water had total coliform and Salmonella counts greater than those for soils irrigated with Filters 4 and 8 respectively, possibly due to the high irrigation water volume applied on soils irrigated with Filter 7 outflow water compared with others (Figure 5.2) as discussed by Almuktar and Scholz (2015; 2016a). Furthermore, Irrigation with water harvested from wetland filters contaminated with hydrocarbons (Filters 1, 3, 5, and Control A) showed total coliform and Salmonella contaminations which were considerably greater than those soils irrigated with outflow waters of uncontaminated filters (Filter 2, 4, 6, and Control B) confirming the results reported by Benedek et al. (2013) which indicated that hydrocarbons positively affected diversity of bacterial communities. Statistically, correlation analysis results showed that both total coliforms and Salmonella were significantly positively correlated with each other (R = 0.940 and p value of less than 0.001) confirming the results reported by Almuktar and Scholz (2015). Furthermore, the contamination by *E. coli* in soil irrigated with outflow water from wetland filters was not observed; with the exception of those soils associated with Filters 3 and 7, which were similarly contaminated. The highest contamination by E. coli was recorded for soil irrigated with raw wastewater. In contrast, the lowest contamination by E. coli was observed in soil irrigated with wastewater, which was diluted with 80% tap water. Contamination by Streptococci was not observed for soil irrigated with treated wastewater obtained from any of the wetland filters; with the exception of Filter 4. Soil irrigated with raw wastewater was reported to have higher contamination by Streptococci recordings than those soils irrigated by wastewater which was diluted with 80% tap water. However, the typical bacteria survival time in soil, fresh water and crops is less than 70, 60 and 30 days, respectively, according to EPA (1992).

Table 5.2: Microbiological results for soil irrigated by different water types (colony forming units per gram (CFU)/g).

Microbes	Mean	Standard deviation	Minimum	Maximum
Total coliforms				
Filter 1	1073	149.4	920	1270
Filter 2	648	101.4	570	790
Filter 3	1593	331.8	1210	1980
Filter 4	853	157.1	650	1030
Filter 5	910	706.3	210	1720
Filter 6	473	292.6	90	730
Filter 7	1503	1160.5	320	2810
Filter 8	1113	590.1	450	1620
Control A	1988	939.9	1180	2940
Control B	1293	645.9	670	2150
TW+WW	983	267.3	610	1200
WW	1118	293.9	890	1540
Escherichia coli				
Filter 1	0	0.0	0	0
Filter 2	0	0.0	0	0
Filter 3	5	10.0	0	20
Filter 4	0	0.0	0	0
Filter 5	0	0.0	0	0
Filter 6	0	0.0	0	0
Filter 7	5	5.8	0	10
Filter 8	0	0.0	0	0
Control A	0	0.0	0	0
Control B	0	0.0	0	0
TW+WW	3	5.0	0	10
WW	10	8.2	0	20

Table 5.2 (cont.)

Microbes	Mean	Standard deviation	Minimum	Maximum
***Streptococci* spp.**				
Filter 1	0	0.0	0	0
Filter 2	0	0.0	0	0
Filter 3	0	0.0	0	0
Filter 4	23	22.2	0	50
Filter 5	0	0.0	0	0
Filter 6	0	0.0	0	0
Filter 7	0	0.0	0	0
Filter 8	0	0.0	0	0
Control A	0	0.0	0	0
Control B	0	0.0	0	0
TW+WW	3	5.0	0	10
WW	20	33.7	0	70
***Salmonella* spp.**				
Filter 1	629	58.2	558	700
Filter 2	270	80.4	190	380
Filter 3	2262	115.3	2110	2380
Filter 4	405	165.0	290	640
Filter 5	1520	171.5	1280	1680
Filter 6	180	126.2	70	350
Filter 7	963	492.1	560	1610
Filter 8	713	495.7	240	1230
Control A	2190	1114.6	1130	3530
Control B	320	73.5	220	380
TW+WW	763	26.3	740	800
WW	1760	756.6	1130	2860

Note: 0 entries indicate absolutely no growth on the plate after incubation. TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). Ten soil samples were tested.

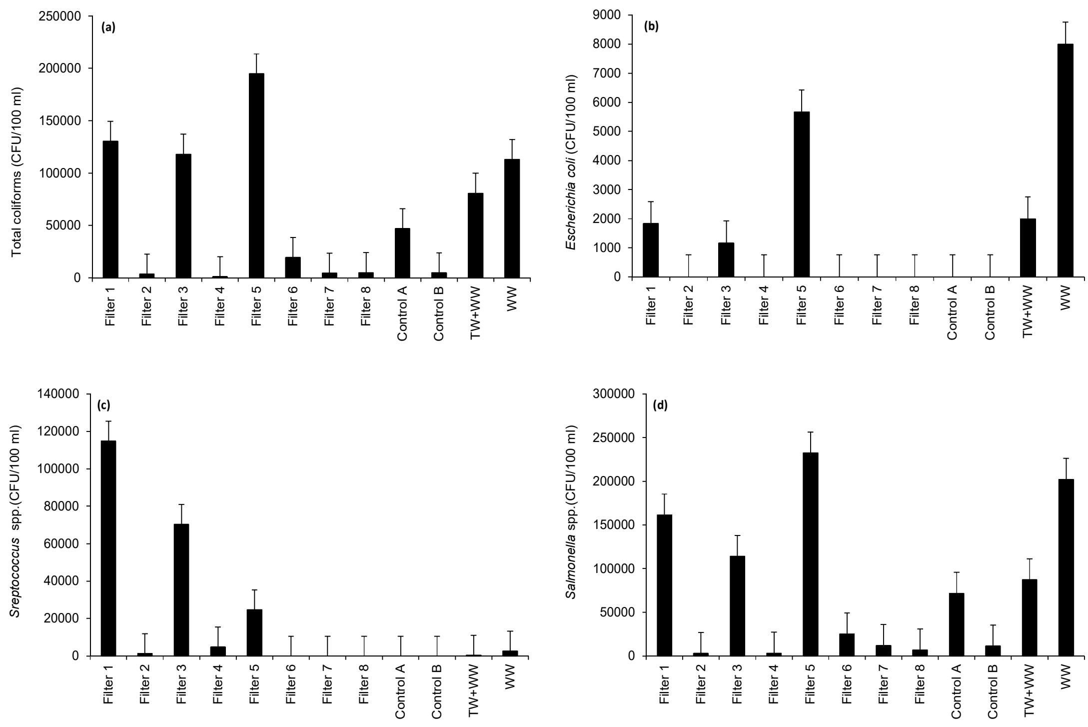

Figure 5.1: Microbiological characteristics of irrigation water: (a) Total coliforms; (b) *Escherichia coli*; (c) *Streptococci* spp.; and (d) *Salmonella* spp. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%). Twenty water samples were tested.

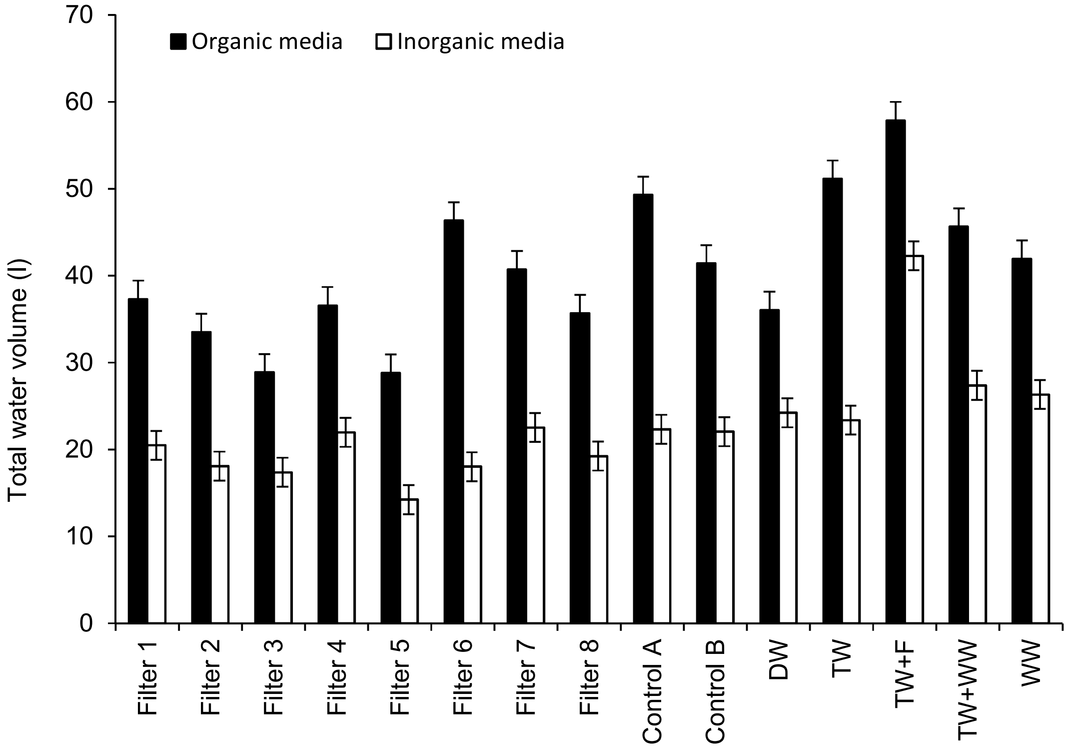

Figure 5.2: Overview of total irrigation water volume (l) for Chilli plants per water source. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); and WW, raw wastewater (100%).

5.2.4 Soil mineral content

Table 5.3 shows the concentrations of elements detected by ICP–OES analysis in the organic and inorganic media irrigated with different water types. The mineral content in the studied soils seems to be greater in the organic media than the inorganic ones as reported by FAO (1972).

This can be explained by the considerably higher total irrigation water volume (Figure 5.2) applied on the organic media compared to the inorganic ones which subsequently led to higher element mass applied on the former than the latter (Figure 5.3).

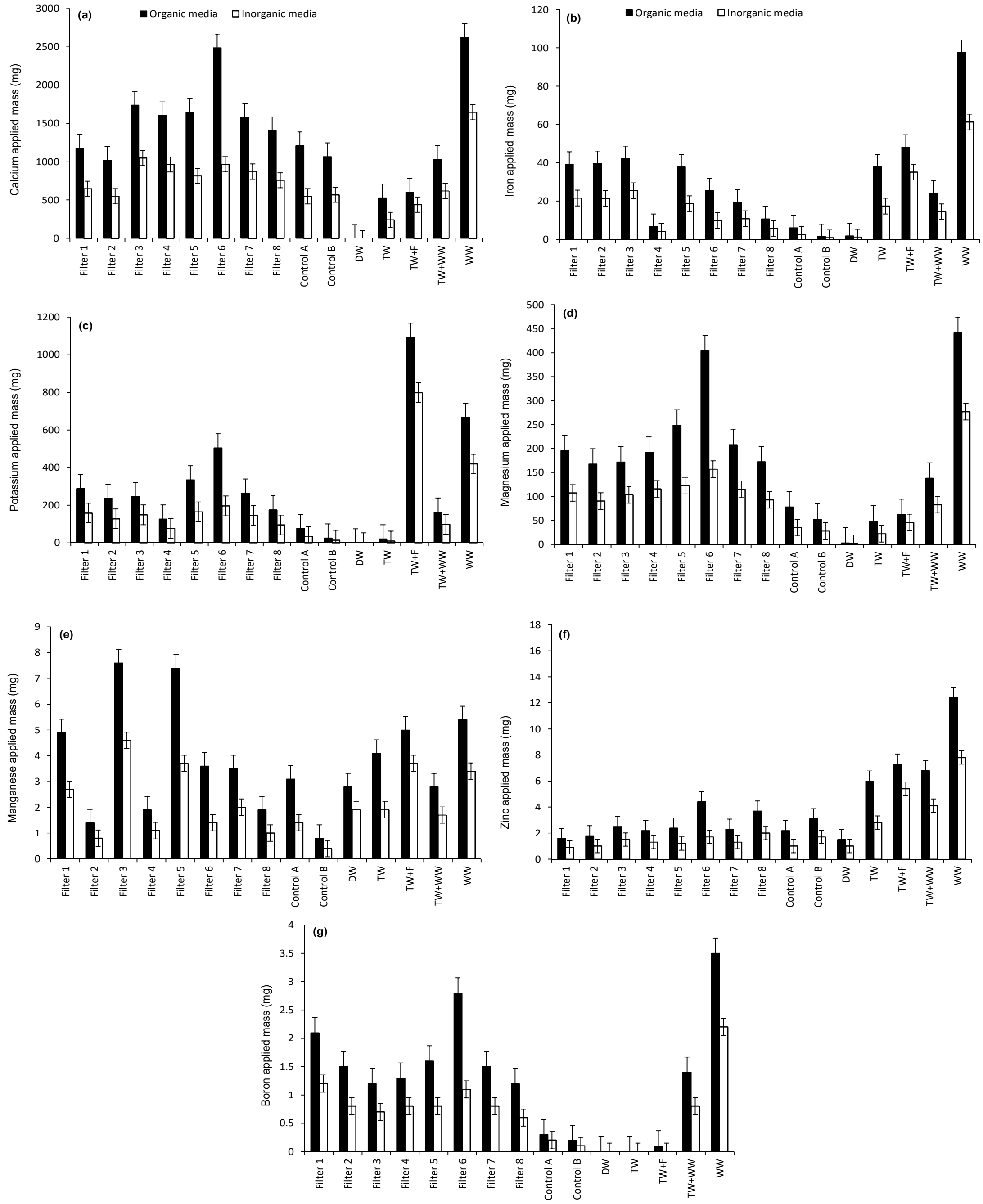

Figure 5.3: Overview of total element mass applied on growth media subjected to different irrigation water types: (a) calcium; (b) iron; (c) potassium; (d) magnesium; (e) manganese; (f) zinc; and (g) boron.

5.2.4.1 Soil aluminium

Aluminium solubility is mainly governed by soil pH, and by soil organic matter and clay contents. Exchangeable aluminium rapidly increases when pH decreases. However, irrigation of organic media with wetland filter outflow waters caused significant ($p < 0.05$) increases in aluminium concentrations compared to the raw organic media (Table 5.3 and Table 5.4a) with the exception of those plants irrigated with outflow waters from Filters 2, 3 and 5, possibly due to the irrigation water volumes applied on those soils (Figure 5.1).

Statistical analysis (Table 5.4a) showed that mean aluminium concentrations in soils irrigated with outflow water from Filter 4 were significantly ($p < 0.05$) greater than those irrigated with Filter 2 drain water, explaining the impact of aggregate size of wetland filters on aluminium concentrations of irrigated soils. Moreover, soil irrigated with water drained from Filters 1, 3, and 5 had aluminium concentrations which were significantly ($p < 0.05$) different from those soils irrigated with Filters 2, 4, and 6 outflow waters, explaining the impact of diesel contamination in the wetland system.

Furthermore, impacts of wetlands contact and resting time variables on irrigated soil aluminium concentrations were observed when comparing soil linked to Filter 7 with those of Filters 4 and 8, respectively. Wetland filters fed with high inflow loading rate (Filters 5 and 6) resulted in higher soil aluminium concentrations compared to those soils irrigated with outflow waters from diluted inflow loads filters (Filters 3 and 4, respectively).

Regarding sandy soil, no increase in aluminium concentration was observed for irrigated inorganic media compared to the raw sand (Table 5.3). Results show that soil irrigated with outflow from Filters 1 and 2 of large aggregate diameter had aluminium concentrations greater than those soils irrigated with outflow from small aggregate diameters (Filters 3 and 4, respectively). Statistical analysis results (Table 5.4b) showed that soil irrigated with water drained from Filter 4 of diluted inflow loading rate had aluminium concentrations which were significantly ($p < 0.05$) greater than that irrigated with water from Filter 6 of high inflow loading rate, possibly due to the difference in irrigation water volume (Figure 5.2). Moreover, significant differences in soil aluminium concentrations were observed when comparing soils linked to Filter 5 and Control A with those of Filter 6 and Control B, explaining the impact of hydrocarbons contamination of wetland filters on aluminium distribution in the irrigated soils (Almuktar & Scholz, 2016 a).

Generally, results showed that high soil aluminium concentrations were correlated well with higher irrigation water volume (Figure 5.2) as well as with higher microbial contents (Table 5.2), such as soils irrigated with water obtained from filters of high inflow loading rate and filters contaminated with hydrocarbons, due to the biodegradation activities (FAO, 1972, 2003).

Correlation analysis results (Table 5.5) showed that aluminium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with calcium, iron, potassium, magnesium, manganese and zinc values, while negatively correlated with boron levels in the soil, as reported by Essington (2015).

Table 5.3: Overview of the Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) analysis for selected elements (mean±standard deviation) for different growth media compared with common standard (e.g., FAO/WHO (2001)).

Inflow source	Detected element (mg/kg)							
	Aluminium	Calcium	Iron	Potassium	Magnesium	Manganese	Zinc	Boron
Organic growth media								
Filter 1	1141.34± 143.365	15554.13± 1208.662	1634.447± 253.567	640.35± 56.741	6107.43± 213.323	155.04± 32.744	25.11± 5.217	17.15± 2.681
Filter 2	913.46± 64.086	13806.61± 2237.932	1050.43± 44.932	276.48± 20.818	6208.70± 299.048	68.37± 10.931	21.40± 6.524	16.61± 1.667
Filter 3	960.74± 48.249	15512.25± 1175.662	2259.09± 1613.474	685.27± 54.573	6512.30± 231.303	75.89± 7.779	21.36± 4.760	14.40± 2.163
Filter 4	1676.36± 586.426	15740.05± 1292.311	1543.395± 109.391	1387.95± 175.304	6310.09± 499.148	145.81± 70.435	45.38± 21.034	12.38± 1.293
Filter 5	1023.91± 92.769	16260.76± 1246.058	3166.691± 1631.080	1284.76± 103.771	6603.18± 169.898	99.32± 10.370	30.92± 9.437	12.54± 1.517
Filter 6	1956.11± 240.293	22364.05± 2007.483	2482.217± 238.807	1277.07± 60.482	5974.29± 544.504	225.54± 18.675	68.60± 5.751	15.97± 1.731
Filter 7	2140.26± 134.789	20517.75± 2085.031	2944.728± 161.646	1322.30± 48.712	6136.23± 305.249	226.75± 12.272	67.69± 4.402	12.24± 1.261
Filter 8	2100.92± 90.244	20223.52± 1430.457	2622.924± 239.012	1211.13± 94.942	6871.78± 276.801	229.00± 17.052	65.91± 6.620	13.05± 1.660
Control A	2202.01± 380.332	19669.04± 2062.417	3413.248± 525.218	1228.29± 76.783	6595.67± 917.118	219.84± 62.985	69.22± 4.155	11.79± 1.863
Control B	2130.98± 357.459	19982.9± 1727.165	2640.14± 250.985	1433.50± 52.566	5691.65± 155.510	225.18± 11.480	65.17± 3.526	12.43± 1.739
DW	2195.95± 171.680	19766.78± 1992.668	2589.777± 229.591	1522.96± 69.650	6682.54± 179.246	199.04± 10.743	70.99± 4.234	11.89± 1.673
TW	2153.36± 103.995	17121.75± 1586.688	2933.564± 152.842	1207.39± 79.030	5908.86± 369.378	276.55± 32.959	73.07± 5.575	12.36± 2.018

Table 5.3 (cont.)

Inflow source	Detected element (mg/kg)							
	Aluminium	Calcium	Iron	Potassium	Magnesium	Manganese	Zinc	Boron
TW+F	1361.46± 89.065	14776.71± 849.099	1774.841± 65.299	279.96± 22.665	6035.08± 188.316	130.10± 11.260	36.01± 2.024	10.15± 1.635
TW+WW	980.86± 52.105	15044.78± 1087.789	1340.748± 123.923	223.13± 24.693	5826.97± 164.055	139.69± 24.636	31.56± 4.382	10.00± 1.511
WW	1035.42± 35.615	16063.89± 1144.098	1444.303± 59.790	475.38± 28.733	5635.63± 161.938	96.60± 7.622	26.0± 3.994	12.50± 1.680
RAW	1123.24± 51.999	20945.25± 7640.085	6042.014± 1664.159	2709.94± 100.734	5318.85± 89.221	203.91± 27.179	27.25± 3.397	12.44± 1.685
RM	-	-	50000	-	-	2000	300	-
Inorganic growth media								
Filter 1	1083.00±23.959	788.12±106.444	1148.88±144.650	176.47±18.335	350.91±21.605	15.15±4.866	11.62±2.502	n.d
Filter 2	1060.16±41.513	286.11±37.807	953.89±89.662	162.25±16.107	279.67±16.623	8.65±4.659	10.07±3.759	n.d
Filter 3	1002.69±180.967	452.48±68.986	1017.73±135.740	169.12±44.984	303.33±41.427	10.97±3.985	10.42±3.727	n.d
Filter 4	972.46±120.999	321.09±25.785	1050.70±116.493	131.19±20.291	303.71±26.603	7.89±2.560	10.12±3.057	n.d
Filter 5	960.38±38.649	850.59±71.851	1065.28±25.444	163.26±14.423	371.02±23.376	14.06±3.337	11.92±3.266	n.d
Filter 6	763.67±54.483	394.87±44.845	901.52±87.745	100.59±12.698	260.98±16.611	10.87±3.679	12.76±4.521	n.d
Filter 7	867.06±28.879	341.72±28.352	987.04±67.011	116.81±20.991	282.45±16.324	13.19±3.825	10.55±3.352	n.d
Filter 8	872.81±45.854	449.31±161.007	883.46±81.645	130.09±19.770	292.14±36.947	10.75±3.397	10.52±3.996	n.d
Control A	980.25±44.619	819.27±99.830	989.15±60.623	143.33±14.719	301.63±19.544	12.03±4.078	8.30±3.989	n.d
Control B	814.47±87.908	299.45±63.705	909.91±106.151	106.96±13.555	261.14±21.192	10.52±3.793	9.61±3.474	n.d
DW	862.04±177.883	203.74±28.652	934.09±220.618	107.71±26.363	262.71±46.661	10.03±3.812	9.97±2.690	n.d
TW	865.02±132.701	211.34±12.524	1067.13±194.906	118.03±22.759	272.82±32.165	12.90±3.572	10.77±4.089	n.d
TW+F	868.07±87.058	176.73±41.243	952.30±152.289	130.38±16.540	235.31±27.573	10.63±2.526	11.38±4.545	n.d
TW+WW	1292.01±49.349	338.91±85.406	1097.02±153.264	196.14±18.152	322.15±24.761	13.11±3.167	11.59±4.638	n.d
WW	1215.73±159.939	780.32±53.482	1136.82±127.457	246.84±43.936	401.72±42.336	16.94±3.578	15.05±5.904	n.d
RAW	1163.93±214.985	199.88±18.115	1150.75±195.617	157.95±42.735	303.96±38.134	12.28±3.386	11.12±2.567	n.d
RM	-	-	50000	-	-	2000	300	-

Note: Elements not listed in this table (i.e. arsenic, boron, barium, bismuth, cadmium, cobalt, chromium, copper, lithium, nickel, lead, strontium and titanium) were either below (or close to) the detection limits or could not be measured via the ICP–OES technology. Ten soil samples per treatment were analysed. DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); WW, raw wastewater (100%); RAW, raw media; RM, recommended maximum; and n.d, not detected.

Table 5.4a: Overview of the statistically significant differences in aluminium concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.024	0.059	0.019	0.293	0.006	<0.001	0.001	<0.001	0.001	<0.001	<0.001	0.220	0.113	0.388	0.851
2	0.024	n.a	0.711	<0.001	0.227	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.499	0.163	0.014
3	0.059	0.711	n.a	<0.001	0.402	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.760	0.305	0.038
4	0.019	<0.001	<0.001	n.a	0.001	0.689	0.195	0.297	0.246	0.283	0.103	0.165	0.264	<0.001	0.001	0.031
5	0.293	0.227	0.402	0.001	n.a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.023	0.549	0.851	0.216
6	0.006	<0.001	<0.001	0.689	<0.001	n.a	0.291	0.480	0.508	0.792	0.122	0.191	0.129	<0.001	<0.001	0.011
7	<0.001	<0.001	<0.001	0.195	<0.001	0.291	n.a	0.977	0.998	1.000	0.971	1.000	0.016	<0.001	<0.001	0.001
8	0.001	<0.001	<0.001	0.297	<0.001	0.480	0.977	n.a	0.966	1.000	0.627	0.836	0.031	<0.001	<0.001	0.001
9	<0.001	<0.001	<0.001	0.246	<0.001	0.508	0.998	0.966	n.a	0.999	1.000	0.999	0.023	<0.001	<0.001	0.001
10	0.001	<0.001	<0.001	0.283	<0.001	0.792	1.000	1.000	0.999	n.a	0.997	1.000	0.029	<0.001	<0.001	0.001
11	<0.001	<0.001	<0.001	0.103	<0.001	0.122	0.971	0.627	1.000	0.997	n.a	0.988	0.006	<0.001	<0.001	<0.001
12	<0.001	<0.001	<0.001	0.165	<0.001	0.191	1.000	0.836	0.999	1.000	0.988	n.a	0.012	<0.001	<0.001	<0.001
13	0.220	<0.001	0.002	0.264	0.851	0.129	0.016	0.031	0.023	0.029	0.006	0.012	n.a	0.005	0.037	0.299
14	0.113	0.499	0.760	<0.001	0.549	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	n.a	0.472	0.077
15	0.388	0.163	0.305	0.001	0.851	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.037	0.472	n.a	0.293
16	0.851	0.014	0.038	0.031	0.216	0.011	0.001	0.001	0.001	0.001	<0.001	<0.001	0.299	0.077	0.293	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.4b: Overview of the statistically significant differences in aluminium concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.561	0.212	0.061	0.035	<0.001	<0.001	<0.001	0.019	<0.001	<0.001	<0.001	<0.001	0.011	0.152	0.419
2	0.561	n.a	0.593	0.225	0.155	<0.001	0.005	0.006	0.127	<0.001	0.003	0.005	0.005	0.004	0.063	0.192
3	0.212	0.593	n.a	0.896	0.716	<0.001	0.054	0.064	0.268	0.006	0.041	0.058	0.059	<0.001	0.007	0.040
4	0.061	0.225	0.896	n.a	0.861	<0.001	0.072	0.086	0.913	0.008	0.056	0.077	0.079	0.002	0.017	0.098
5	0.035	0.155	0.716	0.861	n.a	0.001	0.118	0.137	0.733	0.016	0.093	0.125	0.127	0.001	0.009	0.059
6	<0.001	<0.001	<0.001	<0.001	0.001	n.a	0.089	0.075	<0.001	0.392	0.113	0.084	0.082	<0.001	<0.001	<0.001
7	<0.001	0.005	0.054	0.072	0.118	0.089	n.a	0.937	0.057	0.399	0.907	0.976	0.970	<0.001	<0.001	0.001
8	<0.001	0.006	0.064	0.086	0.137	0.075	0.937	n.a	0.068	0.356	0.845	0.961	0.967	<0.001	0.001	0.001
9	0.019	0.127	0.268	0.913	0.733	<0.001	0.057	0.068	n.a	0.006	0.043	0.061	0.062	<0.001	<0.001	0.002
10	<0.001	<0.001	0.006	0.008	0.016	0.392	0.399	0.356	0.006	n.a	0.467	0.382	0.378	<0.001	<0.001	<0.001
11	<0.001	0.003	0.041	0.056	0.093	0.113	0.907	0.845	0.043	0.467	n.a	0.884	0.878	<0.001	<0.001	<0.001
12	<0.001	0.005	0.058	0.077	0.125	0.084	0.976	0.961	0.061	0.382	0.884	n.a	0.994	<0.001	<0.001	0.001
13	<0.001	0.005	0.059	0.079	0.127	0.082	0.970	0.967	0.062	0.378	0.878	0.994	n.a	<0.001	<0.001	0.001
14	0.011	0.004	<0.001	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.a	0.216	0.080
15	0.152	0.063	0.007	0.017	0.009	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.216	n.a	0.533
16	0.419	0.192	0.040	0.098	0.059	<0.001	0.001	0.001	0.002	<0.001	<0.001	0.001	0.001	0.080	0.533	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.5: Overview of correlation coefficients and associated significances between soil elements using the non-parametric Spearman correlation test.

Element	Statistic	Element							
		Aluminium	Calcium	Iron	Potassium	Magnesium	Manganese	Zinc	Boron
Aluminium	R	1.000	0.686**	0.794**	0.811**	0.702**	0.768**	0.759**	-0.435
	p	n.a	0.000	0.000	0.000	0.000	0.000	0.000	0.092
Calcium	R	0.686**	1.000	0.839**	0.894**	0.817**	0.915**	0.853**	0.021
	p	0.000	n.a	0.000	0.000	0.000	0.000	0.000	0.940
Iron	R	0.794**	0.839**	1.000	0.909**	0.826**	0.890**	0.844**	-0.182
	p	0.000	0.000	n.a	0.000	0.000	0.000	0.000	0.499
Potassium	R	0.811**	0.894**	0.909**	1.000	0.867**	0.871**	0.847**	-0.082
	p	0.000	0.000	0.000	n.a	0.000	0.000	0.000	0.762
Magnesium	R	0.702**	0.817**	0.826**	0.867**	1.000	0.826**	0.821**	0.088
	p	0.000	0.000	0.000	0.000	n.a	0.000	0.000	0.745
Manganese	R	0.768**	0.915**	0.890**	0.871**	0.826**	1.000	0.933**	-0.191
	p	0.000	0.000	0.000	0.000	0.000	n.a	0.000	0.478
Zinc	R	0.759**	0.853**	0.844**	0.847**	0.821**	0.933**	1.000	-0.518*
	p	0.000	0.000	0.000	0.000	0.000	0.000	n.a	0.040
Boron	R	-0.435	0.021	-0.182	-0.082	0.088	-0.191	-0.518*	1.000
	p	0.092	0.940	0.499	0.762	0.745	0.478	0.040	n.a

Note: R, correlation coefficient; *p*, probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R=1). **, correlation is significant at the 0.01 level, and *, correlation is significant at the 0.05 level.

5.2.4.2 Soil calcium

Compared to the raw organic media, irrigation with outflow water of Filter 6 (high inflow loading rate) caused significant ($p < 0.05$) increase in the calcium concentration of the compost (Table 5.3 and Table 5.6a). However, statistical analysis (Table 5.6a) showed that the mean calcium concentration of soil irrigated with outflow water from Filter 6 was greater than the other concentrations due to the highest irrigation water volume (Figure 5.2) being applied on the soil irrigated with Filter 6 outflow, resulting in a high amount of calcium application (Figure 5.3).

Moreover, soil irrigated with Filter 4 drain water had calcium concentrations greater than those for soil irrigated with Filter 2 outflow water, indicating the impact of different aggregate diameters of the wetland system on treated water calcium levels (Figure 5.4) resulting in differences of applied calcium mass (Figure 5.3) via irrigation water (Figure 5.2). Impact of the wetland contact time variable on the irrigated soil calcium levels was observed when comparing soil irrigated with Filter 7 outflow water with that of Filter 4 which were significantly ($p < 0.05$) different from each other (Table 5.6a). Irrigation with outflow water of Filter 6 of high inflow loading rate resulted in calcium concentrations which were significantly greater than those of Filter 4 of diluted inflow water. Significant ($p < 0.05$) differences in soil calcium concentrations were also observed when comparing soil irrigated with Filter 6 outflow water with that of Filter 5, explaining the impact of diesel contamination.

Regarding inorganic media, irrigation with all irrigation water types caused a significant ($p < 0.05$) increase in the soil calcium compared to the raw sand with the exception of those soils irrigated with deionised water, tap water and tap water spiked with fertiliser (Table 5.3 and Table 5.6b). Kruskal-Wallis test results showed that significant ($p < 0.05$) differences in irrigated soil calcium concentrations were observed when comparing soils linked to Filters 1 and 3 to those of Filters 3 and 5, respectively, explaining the impact of aggregate size and inflow loading rate variables of the wetland system on outflow water calcium concentrations (Figure 5.4), leading to different calcium mass being applied to the irrigated soil (Figure 5.3) by irrigation water (Figure 5.2). Moreover, wetland filters which were contaminated with hydrocarbons (Filters 1, 3, 5 and Control A) significantly ($p < 0.05$) affect the calcium levels of the corresponding irrigated soil compared to those associated with standard (uncontaminated) filters (Filters 2, 4, 6 and Control B, respectively) as shown in Table 5.6b.

Moreover, correlation analysis results (Table 5.5) showed that calcium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, magnesium, manganese and zinc values, as reported by Essington (2015).

However, calcium is an important element required for the growth and development of plants, especially their roots and shoot tips (Haifa Chemical, 2014). Furthermore, the availability of high calcium levels will improve the effects of uptake of toxic cations like aluminium and sodium from the soil, while the presence of high levels of potassium and magnesium may reduce calcium uptake (FAO, 1972).

Table 5.6a: Overview of the statistically significant differences in calcium concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.235	0.954	0.752	0.349	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.082	0.318	0.549	0.506	0.021
2	0.235	n.a	0.258	0.133	0.034	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.851	0.557	0.064	<0.001
3	0.954	0.258	n.a	0.711	0.321	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.072	0.345	0.587	0.472	0.081
4	0.752	0.133	0.711	n.a	0.535	<0.001	<0.001	<0.001	0.002	<0.001	0.001	0.154	0.188	0.360	0.727	0.046
5	0.349	0.034	0.321	0.535	n.a	<0.001	0.001	0.003	0.014	0.004	0.007	0.421	0.053	0.125	0.786	0.171
6	<0.001	<0.001	<0.001	<0.001	<0.001	n.a	0.139	0.058	0.018	0.051	0.031	0.001	<0.001	<0.001	<0.001	0.004
7	<0.001	<0.001	<0.001	<0.001	0.001	0.139	n.a	0.677	0.372	0.634	0.502	0.016	<0.001	<0.001	<0.001	0.163
8	<0.001	<0.001	<0.001	<0.001	0.003	0.058	0.677	n.a	0.634	0.953	0.799	0.034	<0.001	<0.001	0.001	0.328
9	0.001	<0.001	0.001	0.002	0.014	0.018	0.372	0.634	n.a	0.677	0.825	0.097	<0.001	<0.001	0.006	0.616
10	<0.001	<0.001	<0.001	<0.001	0.004	0.051	0.634	0.953	0.677	n.a	0.845	0.036	<0.001	<0.001	0.002	0.358
11	<0.001	<0.001	<0.001	0.001	0.007	0.031	0.502	0.799	0.825	0.845	n.a	0.061	<0.001	<0.001	0.003	0.469
12	0.082	0.003	0.072	0.154	0.421	0.001	0.016	0.034	0.097	0.036	0.061	n.a	0.006	0.019	0.282	0.572
13	0.318	0.851	0.345	0.188	0.053	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	n.a	0.689	0.096	0.001
14	0.549	0.557	0.587	0.360	0.125	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.019	0.689	n.a	0.206	0.004
15	0.506	0.064	0.472	0.727	0.786	<0.001	<0.001	0.001	0.006	0.002	0.003	0.282	0.096	0.206	n.a	0.101
16	0.021	<0.001	0.081	0.046	0.171	0.004	0.163	0.328	0.616	0.358	0.469	0.572	0.001	0.004	0.101	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.6b: Overview of the statistically significant differences in calcium concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	<0.001	<0.001	<0.001	0.210	<0.001	<0.001	<0.001	0.549	<0.001	<0.001	<0.001	<0.001	<0.001	0.947	<0.001
2	<0.001	n.a	0.004	0.526	<0.001	0.023	0.196	0.037	<0.001	0.798	0.033	0.045	0.007	0.375	<0.001	0.018
3	<0.001	0.004	n.a	0.013	<0.001	0.420	0.079	0.454	<0.001	0.004	<0.001	<0.001	<0.001	0.027	<0.001	<0.001
4	<0.001	0.526	0.013	n.a	<0.001	0.066	0.460	0.105	<0.001	0.672	0.002	0.003	<0.001	0.778	<0.001	0.001
5	0.210	<0.001	<0.001	<0.001	n.a	<0.001	<0.001	<0.001	0.513	<0.001	<0.001	<0.001	<0.001	<0.001	0.187	<0.001
6	<0.001	0.023	0.420	0.066	<0.001	n.a	0.272	0.829	<0.001	0.024	<0.001	<0.001	<0.001	0.120	<0.001	<0.001
7	<0.001	0.196	0.079	0.460	<0.001	0.272	n.a	0.378	<0.001	0.245	<0.001	<0.001	<0.001	0.649	<0.001	<0.001
8	<0.001	0.037	0.454	0.105	<0.001	0.829	0.378	n.a	<0.001	0.041	<0.001	<0.001	<0.001	0.181	<0.001	<0.001
9	0.549	<0.001	<0.001	<0.001	0.513	<0.001	<0.001	<0.001	n.a	<0.001	<0.001	<0.001	<0.001	<0.001	0.505	<0.001
10	<0.001	0.798	0.004	0.672	<0.001	0.024	0.245	0.041	<0.001	n.a	0.008	0.011	0.001	0.480	<0.001	0.003
11	<0.001	0.033	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	0.008	n.a	0.892	0.542	0.001	<0.001	0.785
12	<0.001	0.045	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	0.892	n.a	0.456	0.001	<0.001	0.683
13	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.542	0.456	n.a	<0.001	<0.001	0.736
14	<0.001	0.375	0.027	0.778	<0.001	0.120	0.649	0.181	<0.001	0.480	0.001	0.001	<0.001	n.a	<0.001	<0.001
15	0.947	<0.001	<0.001	<0.001	0.187	<0.001	<0.001	<0.001	0.505	<0.001	<0.001	<0.001	<0.001	<0.001	n.a	<0.001
16	<0.001	0.018	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.785	0.683	0.736	<0.001	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

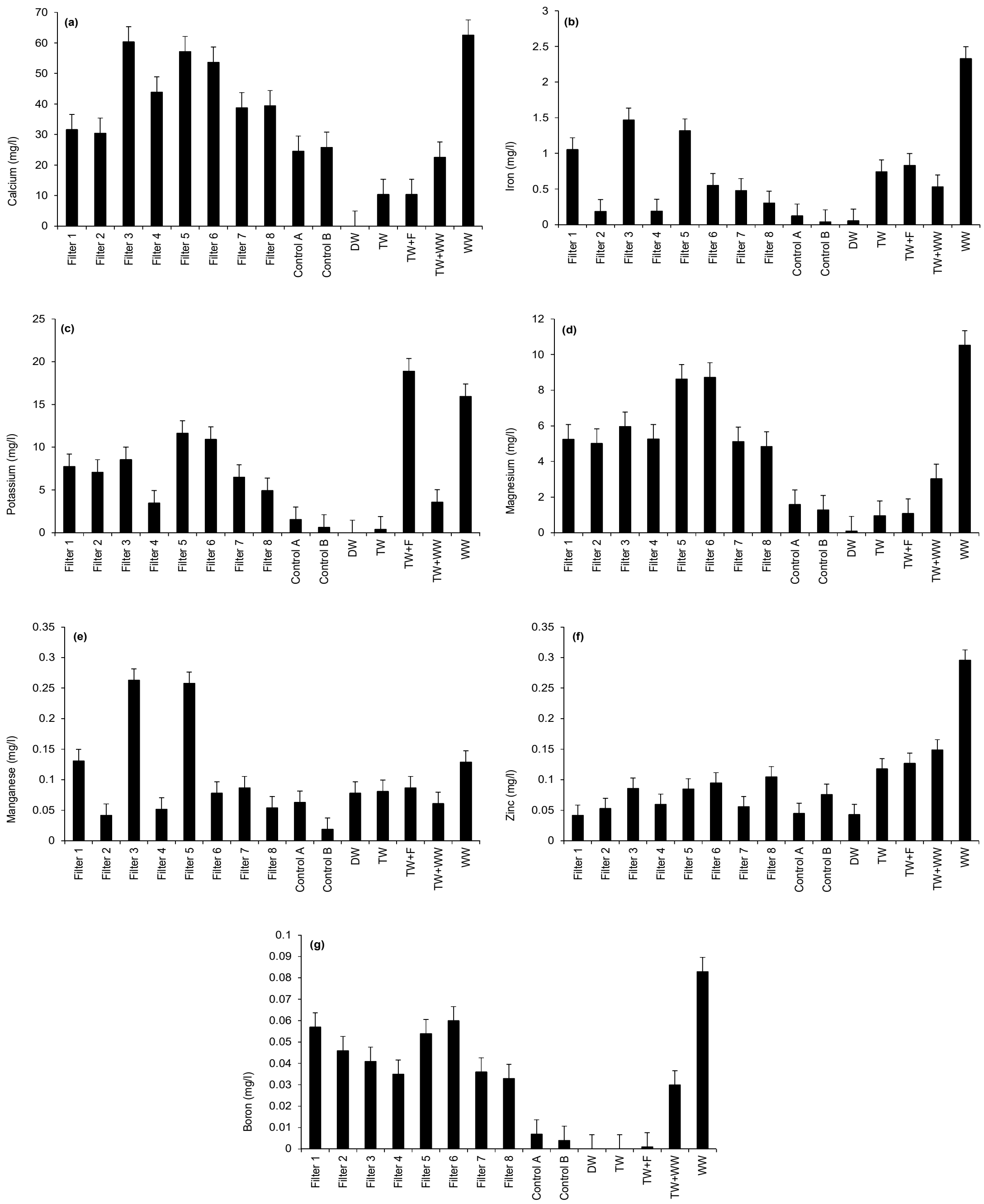

Figure 5.4: Overview of the Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) analysis for selected elements for irrigation water: (a) calcium; (b) iron; (c) potassium; (d), magnesium; (e) manganese; (f) zinc; and (g) boron. Note: elements not shown (i.e., arsenic, barium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, strontium and titanium) were not detected.

5.2.4.3 Soil iron

The solubility of iron is strongly influenced by both redox potential and pH. Iron toxicity is frequently observed at low redox potentials and pH values, (FAO, 1972). Table 5.3 shows that irrigation with treated wastewater did not increase the iron concentrations in the organic media compared to the raw compost. Findings indicate that irrigation with Filter 2 outflow water resulted in iron levels which were significantly ($p < 0.05$) lower than those for soil irrigated with Filter 4 outflow water (Table 5.7a). This can be explained by the impact of different aggregate diameter variables of the wetland system on treated water iron concentrations (Figure 5.4), which subsequently affect the iron mass applied on the soil (Figure 5.3) via irrigation water (Figure 5.2).

Impact of wetland system contact time variables was observed when comparing the iron levels of soil irrigated with Filter 7 drain water with those of soil linked to Filter 4, which were significantly ($p < 0.05$) different from each other (Table 5.7a). Moreover, soil irrigated with waters obtained from Filters 5 and 6 of high inflow loading rate had iron concentrations which were significantly ($p < 0.05$) greater than those of Filters 3 and 4, respectively, which were fed with diluted wastewater.

Furthermore, irrigation with hydrocarbon contaminated filter (Filters 1, 3, 5 and Control A) drain waters noticeably increased soil iron compared with uncontaminated filters (Filters 2, 4, 6 and Control B) as shown in Table 5.5. Iron concentrations in the irrigated inorganic media follow the same trend as the corresponding organic ones (Table 5.5 and Table 5.7b), in addition, the irrigation with treated wastewater did not cause any significant increase in the iron levels compared to the raw sand (Table 5.5).

Generally, results showed that high soil iron concentrations were correlated well with higher irrigation water volume (Figure 5.2) as well as with higher microbial contents (Table 5.2), such as soils irrigated with water obtained from filters of high inflow loading rate and filters contaminated with hydrocarbons, due to the biodegradation activities (FAO, 1972, 2003).

Correlation analysis results (Table 5.5) showed that iron concentration in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, calcium, potassium, magnesium, manganese and zinc values, while negatively correlated with boron levels in the soil, as reported by Essington (2015).

The high iron concentrations observed in irrigated soil can be explained by the already high iron concentration in the compost and the iron present in the irrigation water. Iron has a low bioavailability in terms of uptake by plants (FAO, 1972). This leads to the accumulation of iron in the irrigated soil. Moreover, iron concentrations in the irrigated soils did not exceed the corresponding metal threshold of 50000 mg/kg (FAO/WHO, 2001).

Table 5.7a: Overview of the statistically significant differences in iron concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.020	0.673	0.719	0.014	0.043	<0.001	0.008	<0.001	0.010	0.015	<0.001	0.589	0.224	0.388	<0.001
2	0.020	n.a	0.006	0.049	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.264	0.124	<0.001
3	0.673	0.006	n.a	0.434	0.043	0.110	0.001	0.026	<0.001	0.030	0.043	0.001	0.906	0.101	0.199	<0.001
4	0.719	0.049	0.434	n.a	0.005	0.017	<0.001	0.003	<0.001	0.003	0.005	<0.001	0.366	0.392	0.615	<0.001
5	0.014	<0.001	0.043	0.005	n.a	0.667	0.315	0.375	0.049	0.389	0.944	0.340	0.057	<0.001	0.001	<0.001
6	0.043	<0.001	0.110	0.017	0.667	n.a	0.088	0.532	0.031	0.567	0.673	0.095	0.139	0.001	0.004	0.001
7	<0.001	<0.001	0.001	<0.001	0.315	0.088	n.a	0.059	0.336	0.062	0.199	0.960	0.001	<0.001	<0.001	0.001
8	0.008	<0.001	0.026	0.003	0.375	0.532	0.059	n.a	0.004	0.980	0.840	0.066	0.035	<0.001	<0.001	<0.001
9	<0.001	<0.001	<0.001	<0.001	0.049	0.031	0.336	0.004	n.a	0.005	0.084	0.311	<0.001	<0.001	<0.001	0.016
10	0.010	<0.001	0.030	0.003	0.389	0.567	0.062	0.980	0.005	n.a	0.880	0.069	0.040	<0.001	0.001	<0.001
11	0.015	<0.001	0.043	0.005	0.944	0.673	0.199	0.840	0.084	0.880	n.a	0.213	0.057	<0.001	0.001	0.003
12	<0.001	<0.001	0.001	<0.001	0.340	0.095	0.960	0.066	0.311	0.069	0.213	n.a	0.002	<0.001	<0.001	0.001
13	0.589	0.004	0.906	0.366	0.057	0.139	0.001	0.035	<0.001	0.040	0.057	0.002	n.a	0.079	0.161	<0.001
14	0.224	0.264	0.101	0.392	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.079	n.a	0.724	<0.001
15	0.388	0.124	0.199	0.615	0.001	0.004	<0.001	<0.001	<0.001	0.001	0.001	<0.001	0.161	0.724	n.a	<0.001
16	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001	<0.001	0.016	<0.001	0.003	0.001	<0.001	<0.001	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.7b: Overview of the statistically significant differences in iron concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	<0.001	0.009	0.154	0.119	<0.001	0.001	<0.001	<0.001	<0.001	<0.001		<0.001	0.888	1.000	1.000
2	<0.001	n.a	0.132	0.028	0.003	0.305	0.432	0.142	0.504	0.380	0.705	0.031	0.657	0.001	<0.001	0.001
3	0.009	0.132	n.a	0.441	0.103	0.005	0.421	0.001	0.350	0.008	0.035	0.468	0.236	0.061	0.019	0.038
4	0.154	0.028	0.441	n.a	0.996	<0.001	0.115	<0.001	0.088	0.001	0.004	1.000	0.050	0.898	0.205	0.344
5	0.119	0.003	0.103	0.996	n.a	<0.001	0.015	<0.001	0.010	<0.001	<0.001	1.000	0.005	0.949	0.141	0.372
6	<0.001	0.305	0.005	<0.001	<0.001	n.a	0.043	0.619	0.058	0.868	0.469	<0.001	0.100	<0.001	<0.001	<0.001
7	0.001	0.432	0.421	0.115	0.015	0.043	n.a	0.012	0.896	0.063	0.193	0.126	0.703	0.007	0.002	0.004
8	<0.001	0.142	0.001	<0.001	<0.001	0.619	0.012	n.a	0.017	0.508	0.222	<0.001	0.032	<0.001	<0.001	<0.001
9	<0.001	0.504	0.350	0.088	0.010	0.058	0.896	0.017	n.a	0.084	0.242	0.097	0.802	0.005	0.001	0.003
10	<0.001	0.380	0.008	0.001	<0.001	0.868	0.063	0.508	0.084	n.a	0.577	0.001	0.140	<0.001	<0.001	<0.001
11	<0.001	0.705	0.035	0.004	<0.001	0.469	0.193	0.222	0.242	0.577	n.a	0.005	0.358	<0.001	<0.001	<0.001
12	0.652	0.031	0.468	1.000	1.000	<0.001	0.126	<0.001	0.097	0.001	0.005	n.a	0.056	0.997	0.763	0.753
13	<0.001	0.657	0.236	0.050	0.005	0.100	0.703	0.032	0.802	0.140	0.358	0.056	n.a	0.002	<0.001	0.001
14	0.888	0.001	0.061	0.898	0.949	<0.001	0.007	<0.001	0.005	<0.001	<0.001	0.997	0.002	n.a	0.956	0.936
15	1.000	<0.001	0.019	0.205	0.141	<0.001	0.002	<0.001	0.001	<0.001	<0.001	0.763	<0.001	0.956	n.a	1.000
16	1.000	0.001	0.038	0.344	0.372	<0.001	0.004	<0.001	0.003	<0.001	<0.001	0.753	0.001	0.936	1.000	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.2.4.4 Soil potassium

Potassium availability is mainly related to soil pH, and to clay content and type. An increase in pH increases potassium fixation to the soil (FAO, 1972). Irrigation with wetland filter outflow waters did not cause any increase of potassium concentration compared to the raw compost (Table 5.3). Statistical analysis results (Table 5.8a) showed that there were significant ($p < 0.05$) differences in mean potassium concentrations of soils irrigated with outflow water from Filter 4 compared to Filter 2, and Filter 5 compared to Filter 3, explaining the impacts of aggregate size and inflow loading rate, respectively, of wetland systems on potassium concentrations in the outflow waters (Figure 5.2), and subsequently their impacts on the distribution of potassium concentrations applied to the irrigated soils (Figure 5.3). Moreover, significant ($p < 0.05$) differences in mean potassium concentrations were observed between soils irrigated with Filter 3 and Control A compared to Filter 4 and Control B, respectively, due to the impact of irrigation water volume (Figure 5.2) and diesel contamination applied on those soils. Regarding the inorganic media, results (Table 5.3) showed that irrigation with the raw wastewater and wastewater diluted with tap water significantly ($p < 0.05$) increased potassium concentrations compared to the raw sand (Table 5.8b). Moreover, soil irrigated with outflow from filters with large aggregate diameters (Filter 1 and 2) had potassium concentrations greater than those for filters of small aggregates (Filters 3 and 4, respectively). Furthermore, diesel contamination caused considerable elevation of potassium concentrations in the irrigated soils compared to the standard filters as noticed when comparing soil linked to Filters 1, 3, 5 and Control A with that of Filters 2, 4, 6 and Control B (Table 5.5). Moreover, correlation analysis results (Table 5.5) showed that potassium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with other elements except boron, as reported by Essington (2015).

Table 5.8a: Overview of the statistically significant differences in potassium concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.129	0.831	<0.001	0.006	0.009	0.001	0.061	0.039	<0.001	<0.001	0.073	0.138	0.026	0.429	<0.001
2	0.129	n.a	0.084	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	0.974	0.474	0.406	<0.001
3	0.831	0.084	n.a	0.001	0.011	0.017	0.003	0.096	0.064	<0.001	<0.001	0.114	0.090	0.014	0.368	<0.001
4	<0.001	<0.001	0.001	n.a	0.178	0.082	0.412	0.104	0.152	0.152	0.013	0.088	<0.001	<0.001	<0.001	<0.001
5	0.006	<0.001	0.011	0.178	n.a	0.649	0.598	0.386	0.499	0.005	<0.001	0.341	<0.001	<0.001	0.001	<0.001
6	0.09	<0.001	0.017	0.082	0.694	n.a	0.357	0.469	0.594	0.002	<0.001	0.419	<0.001	<0.001	0.001	<0.001
7	0.001	<0.001	0.003	0.412	0.598	0.357	n.a	0.175	0.244	0.024	0.001	0.150	<0.001	<0.001	<0.001	<0.001
8	0.061	0.001	0.096	0.104	0.386	0.469	0.175	n.a	0.849	0.011	0.003	0.933	0.001	<0.001	0.010	<0.001
9	0.039	<0.001	0.064	0.152	0.499	0.594	0.244	0.849	n.a	0.018	0.005	0.783	<0.001	<0.001	0.006	<0.001
10	<0.001	<0.001	<0.001	0.152	0.005	0.002	0.024	0.011	0.018	n.a	0.295	0.008	<0.001	<0.001	<0.001	0.005
11	<0.001	<0.001	<0.001	0.013	<0.001	<0.001	0.001	0.003	0.005	0.295	n.a	0.002	<0.001	<0.001	<0.001	0.082
12	0.037	0.001	0.114	0.088	0.341	0.419	0.150	0.933	0.783	0.008	0.002	n.a	0.001	<0.001	0.013	<0.001
13	0.138	0.974	0.090	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	n.a	0.454	0.425	<0.001
14	0.026	0.474	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.454	n.a	0.122	<0.001
15	0.429	0.406	0.368	<0.001	0.001	0.001	<0.001	0.010	0.006	<0.001	<0.001	0.013	0.425	0.122	n.a	<0.001
16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.082	<0.001	<0.001	<0.001	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.8b: Overview of the statistically significant differences in potassium concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.837	1.000	0.039	0.797	<0.001	0.004	0.033	0.136	0.001	0.001	0.005	0.031	0.538	0.079	0.944
2	0.837	n.a	1.000	0.187	1.000	0.005	0.041	0.166	0.418	0.010	0.014	0.047	0.160	0.138	0.036	1.000
3	1.000	1.000	n.a	0.141	1.000	0.002	0.023	0.122	0.368	0.004	0.006	0.027	0.117	0.804	0.121	0.999
4	0.039	0.187	0.141	n.a	0.115	0.099	0.421	0.941	0.568	0.166	0.208	0.459	0.924	0.009	0.002	0.276
5	0.797	1.000	1.000	0.115	n.a	0.001	0.017	0.099	0.315	0.003	0.005	0.020	0.095	0.066	0.038	1.000
6	<0.001	0.005	0.002	0.099	0.001	n.a	0.397	0.115	0.026	0.791	0.695	0.363	0.120	<0.001	<0.001	0.006
7	0.004	0.041	0.023	0.421	0.017	0.397	n.a	0.465	0.169	0.561	0.649	0.949	0.478	0.001	<0.001	0.058
8	0.033	0.166	0.122	0.941	0.099	0.115	0.465	n.a	0.519	0.189	0.236	0.505	0.983	0.007	0.002	0.244
9	0.136	0.418	0.368	0.568	0.315	0.026	0.169	0.519	n.a	0.050	0.067	0.189	0.505	0.041	0.011	0.604
10	0.001	0.010	0.004	0.166	0.003	0.791	0.561	0.189	0.050	n.a	0.899	0.519	0.197	<0.001	<0.001	0.013
11	0.001	0.014	0.006	0.208	0.005	0.695	0.649	0.236	0.067	0.899	n.a	0.604	0.244	<0.001	<0.001	0.019
12	0.005	0.047	0.027	0.459	0.020	0.363	0.949	0.505	0.189	0.519	0.604	n.a	0.519	0.001	<0.001	0.067
13	0.031	0.160	0.117	0.924	0.095	0.120	0.478	0.983	0.505	0.197	0.244	0.519	n.a	0.007	0.001	0.236
14	0.538	0.138	0.804	0.009	0.066	<0.001	0.001	0.007	0.041	<0.001	<0.001	0.001	0.007	n.a	0.255	0.480
15	0.079	0.036	0.121	0.002	0.038	<0.001	<0.001	0.002	0.011	<0.001	<0.001	<0.001	0.001	0.255	n.a	0.056
16	0.944	1.000	0.999	0.276	1.000	<0.001	0.058	0.244	0.604	0.013	0.019	0.067	0.236	0.044	0.036	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.2.4.5 Soil magnesium

Irrigation of organic media with all water types caused significant ($p < 0.05$) increases in magnesium concentrations compared to the raw compost (Table 5.3 and Table 5.9a). Statistically, soils irrigated with Filter 5 and Control A outflow waters had mean magnesium concentrations, which were significantly ($p < 0.05$) greater than those irrigated with Filter 6 and Control B waters, respectively, showing the impact of the wetlands diesel contamination on the magnesium concentration of the outflow water (Figure 5.4), impacting on the distribution of magnesium applied on the irrigated soil (Figure 5.3).

In comparison, irrigation with Filters 1 and 5, which were contaminated with hydrocarbons, as well as with raw wastewater caused a significant ($p < 0.05$) increase in magnesium concentrations compared to the raw sand (Table 5.5 and Table 5.9b). Statistical analysis (Table 5.9b) showed that soil irrigated with water obtained from Filter 1 of large aggregate diameter had magnesium concentrations which were significantly ($p < 0.05$) greater than those of soil irrigated with Filter 3, of small aggregate diameter, outflow waters. Moreover, irrigation with water harvested from wetland filters of high inflow loading rate (Filters 5 and 6) caused significant ($p < 0.05$) increases in magnesium concentrations compared to those irrigated with Filters 3 and 4, respectively, which were fed with diluted wastewater.

Furthermore, irrigation with hydrocarbon contaminated wetland filters increased magnesium concentrations significantly ($p < 0.05$) in the soil compared to those irrigated with uncontaminated filters, as shown when comparing soils linked to Filters 1, 5, and Control A with those of Filters 2, 6 and Control B, respectively (Table 5.9b). However, sandy soils often have a low cation exchange capacity and may not contain adequate levels of magnesium (FAO, 1972).

Magnesium distribution in the soil is dependent on its supply and rate of uptake by plants. However, up-take of magnesium mainly depends on calcium and potassium as well as its levels in the soil. Plant magnesium uptake is usually a small portion of the total exchangeable magnesium available in the soil, which means that magnesium depletion from the soil by plant uptake is a minor factor, as discussed by Barber (1995). Moreover, correlation analysis results (Table 5.5) showed that magnesium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, calcium, manganese and zinc values, as reported by Essington (2015).

Table 5.9a: Overview of the statistically significant differences in magnesium concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.630	0.062	0.472	0.025	0.279	0.956	0.002	0.362	0.012	0.011	0.220	0.692	0.105	0.006	<0.001
2	0.630	n.a	0.080	0.288	0.019	0.118	0.670	<0.001	0.235	0.003	0.003	0.088	0.380	0.036	0.001	<0.001
3	0.062	0.080	n.a	0.493	0.547	0.003	0.070	0.020	0.575	<0.001	0.238	0.002	0.024	<0.001	<0.001	<0.001
4	0.472	0.288	0.493	n.a	0.197	0.071	0.506	0.003	0.900	0.001	0.062	0.052	0.264	0.019	0.001	<0.001
5	0.025	0.019	0.547	0.197	n.a	0.001	0.028	0.085	0.245	<0.001	0.564	0.001	0.008	<0.001	<0.001	<0.001
6	0.279	0.118	0.003	0.071	0.001	n.a	0.255	<0.001	0.046	0.155	<0.001	0.886	0.492	0.592	0.099	0.003
7	0.956	0.670	0.070	0.506	0.028	0.255	n.a	0.002	0.392	0.010	0.013	0.200	0.651	0.094	0.005	<0.001
8	0.002	<0.001	0.020	0.003	0.085	<0.001	0.002	n.a	0.004	<0.001	0.252	<0.001	<0.001	<0.001	<0.001	<0.001
9	0.362	0.235	0.575	0.900	0.245	0.046	0.392	0.004	n.a	0.001	0.082	0.033	0.191	0.011	<0.001	<0.001
10	0.012	0.003	<0.001	0.001	<0.001	0.155	0.010	<0.001	0.001	n.a	<0.001	0.201	0.035	0.376	0.820	0.125
11	0.011	0.003	0.238	0.062	0.564	<0.001	0.013	0.252	0.082	<0.001	n.a	<0.001	0.003	<0.001	<0.001	<0.001
12	0.220	0.088	0.002	0.052	0.001	0.886	0.200	<0.001	0.033	0.201	<0.001	n.a	0.406	0.694	0.123	0.005
13	0.692	0.380	0.024	0.264	0.008	0.492	0.651	<0.001	0.191	0.035	0.003	0.406	n.a	0.221	0.019	<0.001
14	0.105	0.036	<0.001	0.019	<0.001	0.592	0.094	<0.001	0.011	0.376	<0.001	0.694	0.221	n.a	0.266	0.015
15	0.006	0.001	<0.001	0.001	<0.001	0.099	0.005	<0.001	<0.001	0.820	<0.001	0.123	0.019	0.266	n.a	0.191
16	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.125	<0.001	0.005	<0.001	0.015	0.191	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.9b: Overview of the statistically significant differences in magnesium concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	<0.001	0.003	<0.001	0.132	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	0.022	0.001
2	<0.001	n.a	0.211	0.152	<0.001	0.150	0.843	0.528	0.161	0.176	0.264	0.551	0.010	0.013	<0.001	0.170
3	0.003	0.211	n.a	0.840	<0.001	0.003	0.238	0.487	0.867	0.004	0.008	0.039	<0.001	0.166	<0.001	0.894
4	<0.001	0.152	0.840	n.a	<0.001	0.001	0.167	0.370	0.736	0.002	0.004	0.023	<0.001	0.192	<0.001	0.612
5	0.132	<0.001	<0.001	<0.001	n.a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.192	0.438	<0.001
6	<0.001	0.150	0.003	0.001	<0.001	n.a	0.067	0.021	0.001	0.925	0.719	0.346	0.207	<0.001	<0.001	0.002
7	<0.001	0.843	0.238	0.167	<0.001	0.067	n.a	0.628	0.178	0.083	0.142	0.375	0.002	0.010	<0.001	0.189
8	<0.001	0.528	0.487	0.370	<0.001	0.021	0.628	n.a	0.388	0.026	0.051	0.170	<0.001	0.037	<0.001	0.408
9	<0.001	0.161	0.867	0.736	<0.001	0.001	0.178	0.388	n.a	0.002	0.005	0.025	<0.001	0.100	<0.001	0.398
10	<0.001	0.176	0.004	0.002	<0.001	0.925	0.083	0.026	0.002	n.a	0.790	0.396	0.175	<0.001	<0.001	0.002
11	<0.001	0.264	0.008	0.004	<0.001	0.719	0.142	0.051	0.005	0.790	n.a	0.560	0.105	<0.001	<0.001	0.005
12	<0.001	0.551	0.039	0.023	<0.001	0.346	0.375	0.170	0.025	0.396	0.560	n.a	0.027	0.001	<0.001	0.028
13	<0.001	0.010	<0.001	<0.001	<0.001	0.207	0.002	<0.001	<0.001	0.175	0.105	0.027	n.a	<0.001	<0.001	<0.001
14	0.016	0.013	0.166	0.192	<0.001	<0.001	0.010	0.037	0.100	<0.001	<0.001	0.001	<0.001	n.a	<0.001	0.424
15	0.022	<0.001	<0.001	<0.001	0.438	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.a	<0.001
16	0.001	0.170	0.894	0.612	<0.001	0.002	0.189	0.408	0.398	0.002	0.005	0.028	<0.001	0.424	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.2.4.6 Soil manganese

The availability of manganese is strongly influenced by the soil redox potential and pH (Husson, 2013). Manganese toxicity is quite common in association with a low soil pH (FAO, 1972). Statistical analysis results (Table 5.10a) showed no significant ($p < 0.05$) increase in the irrigated soil manganese concentrations compared to those of the raw compost. Moreover, results indicated that there were significant ($p < 0.05$) differences in mean manganese concentrations of soils irrigated with Filters 1, 3 and 5 compared to those irrigated with Filters 2, 4 and 6, explaining the impact of diesel contamination on manganese concentration values in the outflow waters (Figure 5.4) and resulting in differences in the manganese load (Figure 5.3) applied to the corresponding irrigated soils (Table 5.3 and Table 5.10a). Wetland aggregate size impacted on the manganese concentration variation of outflow waters (Figure 5.4), which led to significant ($p < 0.05$) differences in soil manganese concentration distribution, when comparing soils irrigated with Filters 1 and 2 to those soils irrigated by Filters 3 and 4 outflow waters, respectively. Moreover, soil irrigated with Filter 4 outflow water had manganese concentrations which were significantly ($p < 0.05$) different from those irrigated with Filter 6 outflow water, explaining the impact of the inflow loading rate of the wetland system on manganese concentrations in the outflow waters (Figure 5.4) and the subsequent impact on the manganese distribution in the irrigated soils (Almuktar & Scholz, 2016 a).

Regarding the inorganic media, statistical analysis results (Table 5.10b) showed that irrigation with raw wastewater and outflow water from Filter 1 (Table 5.3), which was contaminated with diesel, resulted in a significant ($p < 0.05$) increase in manganese concentrations compared to the raw sand. Moreover, soil irrigated with Filter 1 outflow water had manganese concentrations which were significantly ($p < 0.05$) greater than those for soil irrigated with Filter 3 outflow water (Table 5.10b), indicating the impact of the wetland aggregate diameter variable on the outflow manganese concentrations (Figure 5.4) and subsequent differences in the manganese mass applied on the soil (Figure 5.3) via irrigation water (Figure 5.2).

The impact of wetland contact and resting time variables on the irrigated soil manganese levels was observed when comparing soil linked to Filter 7 with those soils irrigated with Filters 4 and 8 outflow waters, which were significantly ($p < 0.05$) different from each other. Soils irrigated with waters drained from Filters 5 and 6 had manganese concentrations, which were significantly ($p < 0.05$) greater than those for Filters 3 and 4, respectively, due to differences in the inflow loading rate. Furthermore, hydrocarbon contamination in the wetland system resulted in a significant difference ($p < 0.05$) in manganese concentrations of soils irrigated with Filters 1, 3, and 5 compared to those of Filters 2, 4, and 6, respectively.

Correlation analysis results showed that manganese concentration in the soil correlated significantly positively with soil bacterial content (R = 0.758, $p = 0.011$ for total coliforms; R = 0.768, $p = 0.009$ for *Escherichia coli* and R = 0.842, $p = 0.002$ for *Salmonella* spp.). Furthermore, correlation analysis results (Table 5.5) showed that manganese concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, magnesium, calcium and zinc values, as reported by Essington (2015). However, for irrigated organic and inorganic media, manganese concentrations did not exceed the corresponding metal threshold of 2000 mg/kg (FAO/WHO, 2001).

Table 5.10a: Overview of the statistically significant differences in manganese concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.001	0.002	0.936	0.058	0.008	0.007	0.004	0.044	0.010	0.218	<0.001	0.501	0.597	0.042	0.120
2	0.001	n.a	0.808	0.001	0.163	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	0.005	0.209	<0.001
3	0.002	0.808	n.a	0.003	0.249	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.017	0.010	0.311	<0.001
4	0.936	0.001	0.003	n.a	0.070	0.006	0.005	0.003	0.036	0.008	0.190	<0.001	0.554	0.224	0.051	0.102
5	0.058	0.163	0.249	0.070	n.a	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.221	0.152	0.889	0.001
6	0.008	<0.001	<0.001	0.006	<0.001	n.a	0.949	0.733	0.359	0.907	0.155	0.063	0.001	<0.001	<0.001	0.111
7	0.007	<0.001	<0.001	0.005	<0.001	0.949	n.a	0.781	0.326	0.856	0.143	0.073	0.001	<0.001	<0.001	0.097
8	0.004	<0.001	<0.001	0.003	<0.001	0.733	0.781	n.a	0.208	0.646	0.098	0.130	<0.001	<0.001	<0.001	0.053
9	0.044	<0.001	<0.001	0.036	<0.001	0.359	0.326	0.208	n.a	0.423	0.432	0.006	0.007	0.001	<0.001	0.498
10	0.010	<0.001	<0.001	0.008	<0.001	0.907	0.856	0.646	0.423	n.a	0.180	0.048	0.001	<0.001	<0.001	0.139
11	0.218	<0.001	<0.001	0.190	0.002	0.155	0.143	0.098	0.432	0.180	n.a	0.007	0.057	0.035	0.001	0.747
12	<0.001	<0.001	<0.001	<0.001	<0.001	0.063	0.073	0.130	0.006	0.048	0.007	n.a	<0.001	<0.001	<0.001	0.001
13	0.501	0.009	0.017	0.554	0.221	0.001	0.001	<0.001	0.007	0.001	0.057	<0.001	n.a	0.834	0.173	0.026
14	0.597	0.005	0.010	0.224	0.152	<0.001	<0.001	<0.001	0.001	<0.001	0.035	<0.001	0.834	n.a	0.116	0.010
15	0.042	0.209	0.311	0.051	0.889	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.173	0.116	n.a	<0.001
16	0.120	<0.001	<0.001	0.102	0.001	0.111	0.097	0.053	0.498	0.139	0.747	0.001	0.026	0.010	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%), and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.10b: Overview of the statistically significant differences of manganese concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	<0.001	0.003	<0.001	0.976	0.002	0.614	0.001	0.034	0.001	<0.001	0.477	0.001	0.604	0.741	0.177
2	<0.001	n.a	0.137	0.389	<0.001	0.177	0.001	0.254	0.020	0.302	0.477	0.005	0.382	0.003	<0.001	0.018
3	0.003	0.137	n.a	0.028	0.012	0.896	0.071	0.747	0.369	0.638	0.409	0.139	0.603	0.097	<0.001	0.329
4	<0.001	0.389	0.028	n.a	<0.001	0.039	<0.001	0.061	0.003	0.073	0.129	0.001	0.106	<0.001	<0.001	0.003
5	0.976	<0.001	0.012	<0.001	n.a	0.009	0.994	0.006	0.102	0.003	0.001	0.972	0.005	0.991	0.183	0.776
6	0.002	0.177	0.896	0.039	0.009	n.a	0.055	0.848	0.308	0.737	0.492	0.112	0.692	0.077	<0.001	0.275
7	0.614	0.001	0.071	<0.001	0.994	0.055	n.a	0.039	0.365	0.024	0.009	1.000	0.030	1.000	0.019	0.992
8	0.001	0.254	0.747	0.061	0.006	0.848	0.039	n.a	0.234	0.892	0.634	0.081	0.833	0.055	<0.001	0.209
9	0.034	0.020	0.369	0.003	0.102	0.308	0.365	0.234	n.a	0.174	0.085	0.541	0.181	0.428	<0.001	0.914
10	0.001	0.302	0.638	0.073	0.003	0.737	0.024	0.892	0.174	n.a	0.728	0.055	0.931	0.036	<0.001	0.156
11	<0.001	0.477	0.409	0.129	0.001	0.492	0.009	0.634	0.085	0.728	n.a	0.023	0.817	0.014	<0.001	0.076
12	0.477	0.005	0.139	0.001	0.972	0.112	1.000	0.081	0.541	0.055	0.025	n.a	0.063	1.000	0.012	0.162
13	0.001	0.382	0.603	0.106	0.005	0.692	0.030	0.833	0.181	0.931	0.817	0.063	n.a	0.043	<0.001	0.162
14	0.604	0.003	0.097	<0.001	0.991	0.077	1.000	0.055	0.428	0.036	0.014	1.000	0.043	n.a	0.022	0.996
15	0.741	<0.001	<0.001	<0.001	0.183	<0.001	0.019	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	0.022	n.a	0.002
16	0.017	0.018	0.329	0.003	0.776	0.275	0.992	0.209	0.914	0.156	0.076	0.999	0.162	0.996	0.002	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.2.4.7 Soil zinc

Organic media irrigated with most water types had zinc concentrations which were significantly ($p < 0.05$) higher than those of the raw compost (Table 5.3 and Table 5.11a). Statistically, mean zinc concentrations in soil irrigated with Filter 2 drain water were significantly ($p < 0.05$) different from those irrigated with Filter 4, irrigation with Filter 7 water was significantly different from irrigation with Filter 4, and soil irrigated with Filter 4 water was significantly ($p < 0.05$) different from that irrigated with Filter 6 outflow waters (Table 5.11a), explaining the impact of aggregate size, contact time and inflow loading rate of the wetland systems on the zinc concentrations of the outflow waters (Figure 5.4), which subsequently impact on the zinc concentrations applied on the corresponding irrigated soils (Figure 5.3). Moreover, soil irrigated with waters from Filters 3 and 5 had mean zinc concentrations, which were significantly ($p < 0.05$) different from those of Filters 4 and 6, respectively, indicating the impact of diesel contamination on outflow water zinc concentrations (Figure 5.4) and the subsequent impact of the distribution of zinc values on the corresponding irrigated soils.

Regarding the inorganic media, results (Table 5.3 and Table 5.11b) showed that irrigation with raw wastewater significantly ($p < 0.05$) increased the zinc concentrations compared to the raw sand. No significant differences in zinc concentrations in the soil irrigated with outflow waters obtained from wetland system filters were observed. Correlation analysis results (Table 5.5) showed that zinc concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with calcium, iron, potassium, magnesium, manganese and aluminium values, while negatively correlated with boron levels in the soil, as reported by Essington (2015). However, zinc concentrations in irrigated soil did not exceed the corresponding metal threshold of 300 mg/kg (FAO/WHO,2001).

Table 5.11a: Overview of the statistically significant differences in zinc concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.463	0.402	0.005	0.376	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.030	0.206	0.860	0.641
2	0.463	n.a	0.918	<0.001	0.105	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.046	0.362	0.230
3	0.402	0.918	n.a	<0.001	0.085	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.036	0.311	0.192
4	0.005	<0.001	<0.001	n.a	0.055	0.029	0.036	0.200	0.041	0.266	0.015	0.008	0.520	0.123	0.009	0.019
5	0.376	0.105	0.085	0.055	n.a	<0.001	<0.001	0.001	<0.001	0.002	<0.001	<0.001	0.201	0.705	0.478	0.675
6	<0.001	<0.001	<0.001	0.069	<0.001	n.a	0.000	0.843	1.000	0.632	0.902	0.312	0.014	0.001	<0.001	<0.001
7	<0.001	<0.001	<0.001	0.096	<0.001	0.999	n.a	0.976	0.989	0.878	0.672	0.130	0.021	0.001	<0.001	<0.001
8	<0.001	<0.001	<0.001	0.200	0.001	0.843	0.976	n.a	0.668	1.000	0.178	0.013	0.054	0.005	<0.001	<0.001
9	<0.001	<0.001	<0.001	0.041	<0.001	1.000	0.989	0.668	n.a	0.432	0.976	0.496	0.007	<0.001	<0.001	<0.001
10	<0.001	<0.001	<0.001	0.266	0.002	0.632	0.878	1.000	0.432	n.a	0.078	0.004	0.074	0.008	<0.001	0.001
11	<0.001	<0.001	<0.001	0.015	<0.001	0.902	0.672	0.178	0.976	0.078	n.a	0.948	0.002	<0.001	<0.001	<0.001
12	<0.001	<0.001	<0.001	0.008	<0.001	0.312	0.130	0.013	0.496	0.004	0.948	n.a	0.001	<0.001	<0.001	<0.001
13	0.030	0.004	0.003	0.520	0.201	0.014	0.021	0.054	0.007	0.074	0.002	0.001	n.a	0.368	0.047	0.090
14	0.206	0.046	0.036	0.123	0.705	0.001	0.001	0.005	<0.001	0.008	<0.001	<0.001	0.368	n.a	0.277	0.425
15	0.860	0.362	0.311	0.009	0.478	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.047	0.277	n.a	0.772
16	0.641	0.230	0.192	0.019	0.675	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.090	0.425	0.772	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

Table 5.11b: Overview of the statistically significant differences in zinc concentrations in the inorganic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.166	0.249	0.149	1.000	0.980	0.189	0.267	0.012	0.067	0.093	0.329	1.000	1.000	0.339	0.997
2	0.166	n.a	0.785	0.984	0.216	0.169	0.856	0.723	0.327	0.773	0.838	0.629	0.286	0.253	0.008	0.284
3	0.249	0.785	n.a	0.789	0.312	0.250	0.915	0.938	0.190	0.549	0.617	0.834	0.414	0.374	0.014	0.411
4	0.149	0.984	0.789	n.a	0.202	0.154	0.864	0.723	0.290	0.743	0.812	0.625	0.268	0.234	0.005	0.267
5	1.000	0.216	0.312	0.202	n.a	0.998	0.250	0.334	0.023	0.104	0.134	0.400	1.000	1.000	0.543	0.991
6	0.980	0.169	0.250	0.154	0.998	n.a	0.194	0.268	0.015	0.074	0.099	0.327	0.979	0.990	0.888	0.894
7	0.189	0.856	0.915	0.864	0.250	0.194	n.a	0.848	0.206	0.606	0.678	0.741	0.334	0.294	0.007	0.332
8	0.267	0.723	0.938	0.723	0.334	0.268	0.848	n.a	0.151	0.482	0.552	0.891	0.445	0.402	0.014	0.442
9	0.012	0.327	0.190	0.290	0.023	0.015	0.206	0.151	n.a	0.440	0.414	0.116	0.028	0.020	<0.001	0.028
10	0.067	0.773	0.549	0.743	0.104	0.074	0.606	0.482	0.440	n.a	0.936	0.399	0.137	0.111	0.001	0.136
11	0.093	0.838	0.617	0.812	0.134	0.099	0.678	0.552	0.414	0.936	n.a	0.465	0.178	0.149	0.003	0.176
12	0.329	0.629	0.834	0.625	0.400	0.327	0.741	0.891	0.116	0.399	0.465	n.a	0.531	0.487	0.020	0.528
13	1.000	0.286	0.414	0.268	1.000	0.979	0.334	0.445	0.028	0.137	0.178	0.531	n.a	1.000	0.416	1.000
14	1.000	0.253	0.374	0.234	1.000	0.990	0.294	0.402	0.020	0.111	0.149	0.487	1.000	n.a	0.461	1.000
15	0.339	0.008	0.014	0.005	0.543	0.888	0.007	0.014	<0.001	0.001	0.003	0.020	0.416	0.461	n.a	0.201
16	0.997	0.284	0.411	0.267	0.991	0.894	0.332	0.442	0.028	0.136	0.176	0.528	1.000	1.000	0.021	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.2.4.8 Soil boron

Table 5.3 shows that boron was detected only in the organic media. However, the bioavailability of boron in the soil is affected by many factors such as soil parent material, texture, nature of minerals in the soil, content of organic matter, soil pH, irrigation water source, interrelationship with other elements and the environmental conditions (especially dry weather) and high light intensity (FAO, 1972).

Statistically, results (Table 5.12) showed that there were significant ($p < 0.05$) differences in mean boron concentrations in soils irrigated with outflow waters of Filters 1 and 2 (large aggregate size) compared to those irrigated with Filters 3 and 4 (small aggregate size), and in soils irrigated with outflow waters of Filter 4 (low inflow loads) compared to Filter 6 (high inflow load), explaining the impact of wetland design variables on boron concentrations of outflow waters (Figure 5.4), resulting in differences in boron application (Figure 5.3) and distributions in the irrigated soils (Table 5.3). Moreover, soils irrigated with water from Filters 3 and 5 had boron mean concentrations significantly ($p < 0.05$) different from those of Filters 4 and 6, explaining the impact of diesel contamination on boron values of the outflow water (Figure 5.4), resulting in differences in boron concentrations in the corresponding irrigated soils. Correlation analysis (Table 5.5) showed that boron concentrations in the soil correlated significantly negatively with other elements in the soil, as discussed by Essington (2015).

Table 5.12: Overview of the statistically significant differences in boron concentrations in the organic media subjected to different irrigation water types using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	n.a	0.942	0.022	<0.001	<0.001	0.475	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.942	n.a	0.026	<0.001	<0.001	0.521	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3	0.022	0.026	n.a	0.049	0.056	0.113	0.026	0.171	0.006	0.051	0.008	0.031	<0.001	<0.001	0.051	0.047
4	<0.001	<0.001	0.049	n.a	0.857	0.001	0.794	0.469	0.419	0.962	0.497	0.846	0.020	0.008	0.991	0.982
5	<0.001	<0.001	0.056	0.857	n.a	<0.001	0.659	0.588	0.323	0.971	0.390	0.708	0.012	0.005	0.866	0.840
6	0.475	0.521	0.113	0.001	<0.001	n.a	0.001	0.003	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.001	0.001
7	<0.001	<0.001	0.026	0.794	0.659	0.001	n.a	0.325	0.548	0.758	0.675	0.947	0.040	0.017	0.786	0.811
8	<0.001	<0.001	0.171	0.469	0.588	0.003	0.325	n.a	0.126	0.563	0.161	0.358	0.002	0.001	0.476	0.456
9	<0.001	<0.001	0.006	0.419	0.323	<0.001	0.548	0.126	n.a	0.392	0.898	0.540	0.131	0.066	0.413	0.432
10	<0.001	<0.001	0.051	0.962	0.971	<0.001	0.758	0.563	0.392	n.a	0.467	0.808	0.018	0.007	0.971	0.944
11	<0.001	<0.001	0.008	0.497	0.390	<0.001	0.675	0.161	0.898	0.467	n.a	0.628	0.101	0.049	0.490	0.511
12	<0.001	<0.001	0.031	0.846	0.708	0.001	0.947	0.358	0.540	0.808	0.628	n.a	0.034	0.014	0.837	0.863
13	<0.001	<0.001	<0.001	0.020	0.012	<0.001	0.040	0.002	0.131	0.018	0.101	0.034	n.a	0.744	0.020	0.022
14	<0.001	<0.001	<0.001	0.008	0.005	<0.001	0.017	0.001	0.066	0.007	0.049	0.014	0.744	n.a	0.008	0.009
15	<0.001	<0.001	0.051	0.991	0.866	0.001	0.786	0.476	0.413	0.971	0.490	0.837	0.020	0.008	n.a	0.974
16	<0.001	<0.001	0.047	0.982	0.840	0.001	0.811	0.456	0.432	0.944	0.511	0.863	0.022	0.009	0.974	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; 16, raw soil; n.a, not applicable as the treatment compared with itself.

5.3 Chilli quality and analysis

5.3.1 Chilli microbial content

Figure 5.1 shows the bacterial contamination of water, while Figure 5.5 indicates the bacterial contamination of fruits. No fruits harvested at a plant height equal to or above 50 cm were associated with microbial contamination. Figure 5.5 indicates that no microbial contamination of Chillies irrigated by wastewaters (treated by wetlands) was detected. Findings also show that no microbiological contamination was recorded for skin, flesh and washing solutions for the fruits harvested from plants irrigated with outflow water obtained from wetland filters. In contrast, the fruits harvested from plants irrigated with wastewater, which was diluted with 80% tap water, and with raw wastewater showed high contamination by total coliforms. Furthermore, high contamination levels with *Streptococcus* spp. and *Salmonella* spp. were recorded for Chilli fruits harvested from plants irrigated by raw wastewater. However, the fruits linked to wastewater and wastewater plus tap water treatments were contaminated due to the contact with contaminated soil, while other fruits, which were located far away from the soil, did not show any bacterial contamination. The approximate number of Chillies harvested below 50 cm was only about 5% of the total harvest for most plants. The results showed that there was no microbial contamination of Chillies located higher up on the plant branches. This can be explained by the relatively long distance between the fruits and the potentially contaminated soil (Cirelli et al., 2012). Moreover, vegetable pots receiving wastewater treated with wetlands acting as a biological filter bed can be considered safer than those receiving only preliminarily treated wastewater.

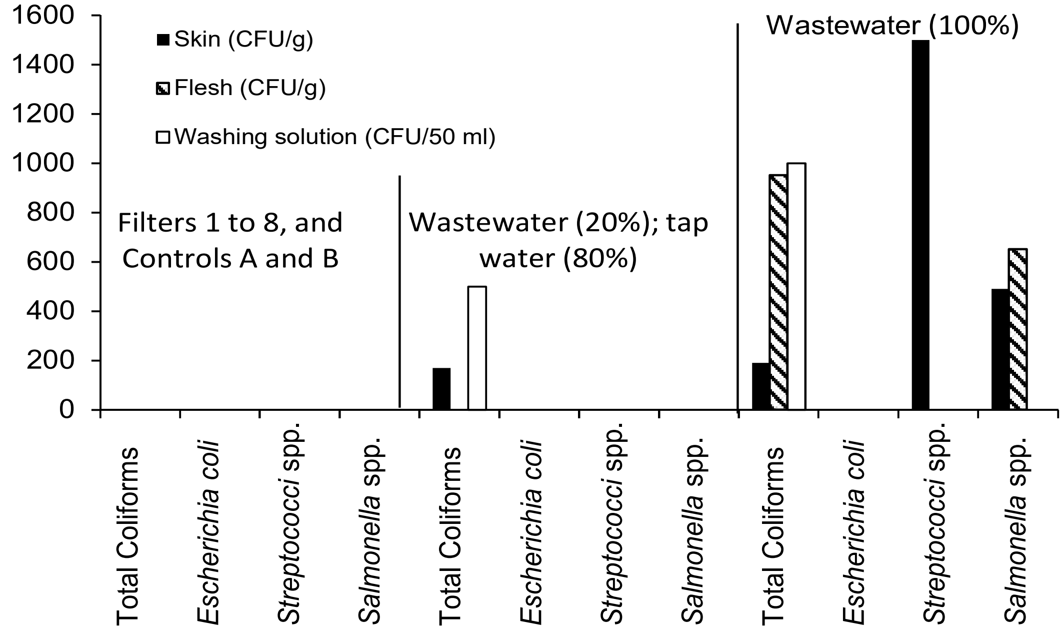

Figure 5.5: Contamination of fruits (only detected for location below a plant height of 50 cm) by bacteria as a function of water resource.

5.3.2 Chilli mineral content

Table 5.13 shows the concentrations of elements detected in Chillies harvested from plants grown in organic media. However, none of these elements were detected in fruits harvested from plants grown in inorganic media. This can be explained by the alkaline media condition, which limited most of the elements availability to be absorbed by the plant root systems (FAO, 1972, 2003). The high pH of the sand limited the uptake of nutrients by plants, explaining the poor fruit quality productions (see chapter 4 section 4.4.3). However, some element concentrations in fruits linked to sand media were too low to be detected by ICP–OES. Furthermore, compared to organic media, it is difficult to study the impact of inorganic media on the chemical composition of vegetables. This is due to the low cation exchange capacity of sandy soil, which may lead to the development of deficiencies regarding most elements in terms of their availability for plants. Moreover, the low cation exchange capacity of sandy soil causes high leaching of elements as reported by Olle et al. (2012).

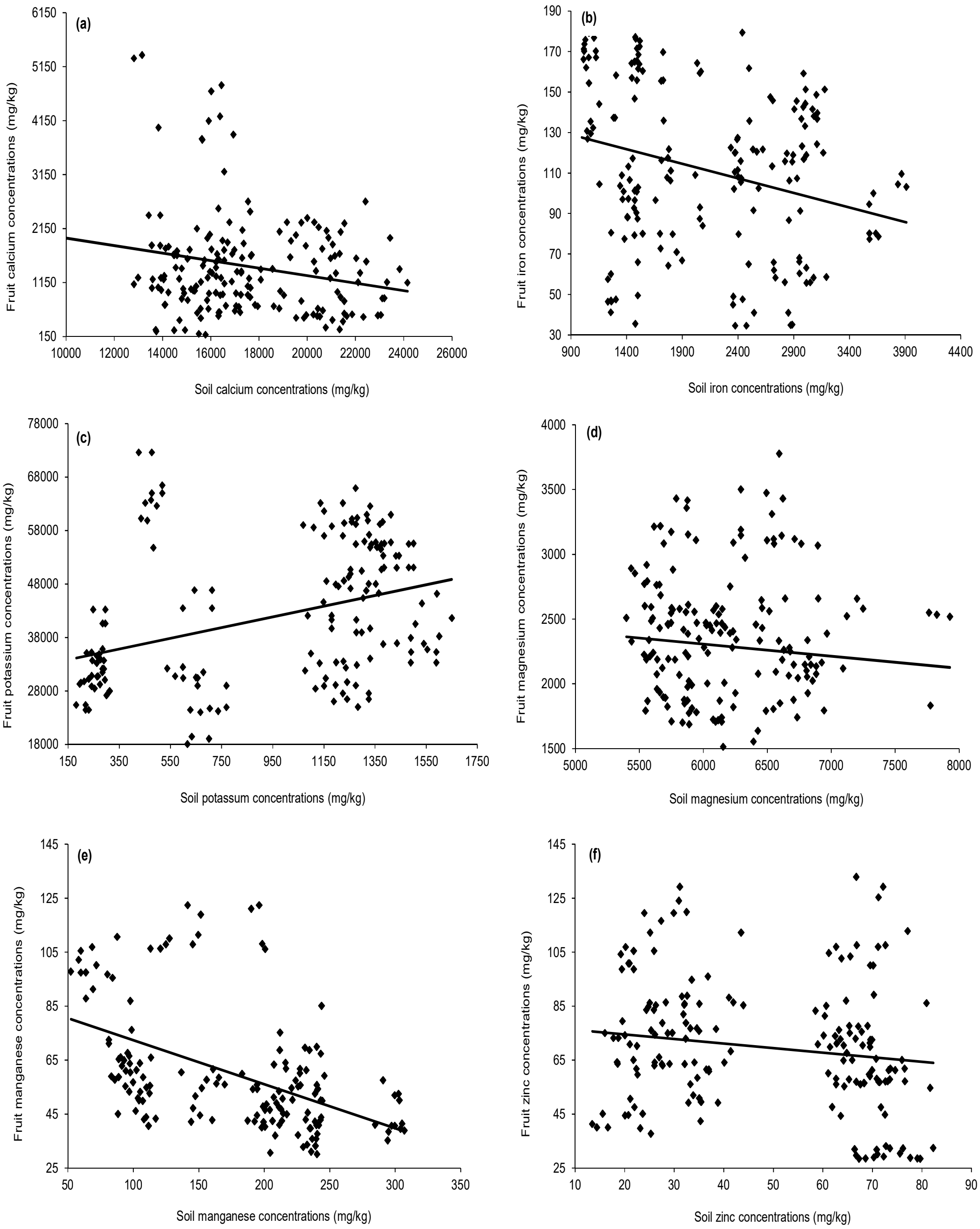

Figure 5.6: Overview of relationships between elements in the soil and harvested fruits: (a) calcium; (b) iron, (c) potassium, (d) magnesium, (e) manganese, and (f) zinc.

Table 5.13: Overview of the Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) analysis for selected elements (mean±standard deviation) compared with common standards for vegetables (e.g., EC (2001) and FAO/WHO (2001)) grown in organic media.

Inflow source	Detected element (mg/kg)						
	Aluminium	Calcium	Iron	Potassium	Magnesium	Manganese	Zinc
Filter 1	394.48±88.956	2792.80±1106.814	163.29±5.332	32588.44±10933.670	2195.05±343.970	112.44±6.719	85.32±38.139
Filter 2	139.92±25.267	2973.40±2122.403	171.84±5.889	35370.96±5749.271	2425.24±713.833	99.15±6.451	76.69±24.616
Filter 3	n.d	875.82±306.143	139.21±18.595	24863.58±4197.329	1767.40±574.813	n.d	55.62±22.723
Filter 4	n.d	1583.74±744.188	158.70±18.221	48319.10±4530.168	2513.00±682.086	67.33±9.603	57.00±21.943
Filter 5	n.d	1596.35±172.448	97.65±12.243	47196.91±9865.319	1986.40±229.150	59.63±10.842	97.76±11.002
Filter 6	253.39±24.710	1009.55±577.956	127.42±15.843	47657.11±6617.915	2202.71±533.415	34.79±8.725	57.19±9.076
Filter 7	n.d	1572.95±860.524	140.52±10.232	59353.41±3029.571	2978.20±561.676	58.84±7.996	110.58±20.816
Filter 8	n.d	1228.40±211.515	132.80±19.867	58657.90±2890.350	2351.36±247.065	40.50±3.880	89.84±16.034
Control A	n.d	1324.49±833.989	95.25±13.913	33766.63±6217.891	2410.43±227.174	51.40±6.271	56.23±2.915
Control B	n.d	1238.56±781.628	104.38±12.363	53065.69±2008.647	2262.87±128.407	55.33±6.308	75.29±5.170
DW	n.d	1255.93±615.983	40.47±10.227	36493.60±2100.030	1647.72±387.917	48.14±6.532	63.69±3.695
TW	n.d	900.47±119.918	60.79±4.296	28773.68±1620.890	2422.19±267.764	39.88±2.164	29.82±1.488
TW+F	n.d	600.68±386.359	91.33±21.075	30863.59±1908.317	1985.69±405.829	n.d	54.12±6.842
TW+WW	n.d	1458.03±319.501	54.87±14.453	29056.40±3598.387	2168.65±438.987	51.30±7.948	75.80±10.698
WW	n.d	1434.82±204.848	96.19±8.602	63367.78±5677.988	3001.33±238.871	57.51±7.050	71.04±9.999
RM	-	-	425.00	-	-	500.00	50.00

Note: DW, deionised water; TW, tap water; TW+F, tap water spiked with fertiliser (0.7 ml/l); TW+WW, tap water (80%) spiked with wastewater (20%); WW, raw wastewater (100%); and RM, recommended maximum. Elements not listed in this table (i.e. arsenic, boron, barium, bismuth, cadmium, cobalt, chromium, copper, lithium, nickel, lead, strontium and titanium) were either below (or close to) the detection limits or could not be measured via the ICP–OES technology. n.d, not detected. Fifteen fruit samples per treatment were analysed.

5.3.2.1 Comparison of aluminium

Table 5.13 shows that aluminium was detected only in fruits harvested from plants irrigated with outflow waters from Filters 1, 2 and 6. However, aluminium was found in abundancy in growth media (Table 5.3), since its solubility is mainly governed by the soil pH, and by soil organic matter and clay content. Exchangeable aluminium rapidly increases when pH decreases (Husson, 2013). In spite of that, aluminium was limited in terms of its transfer into fruit tissue. This can be explained by the high abundance of calcium in the growth media (compost) leading to the limited transport of aluminium to the plants (FAO, 1972). However, aluminium was not considered harmful to human health, because of its relatively low bioavailability (Stahl et al., 2011).

In acid mineral soils (pH < 7.0), aluminium buffers the soil pH at around 4, and is thus available to plants in the toxic form Al^3+. However, plant populations present in these soils normally evolve some degree of tolerance to aluminium in the soil solution and any aluminium present in these soils is likely to be as non-toxic organo-aluminium complexes (Kidd & Proctor, 2000).

5.3.2.2 Comparison of calcium

Table 5.13 shows that the highest calcium concentrations were observed in fruits harvested from plants irrigated with outflow water obtained from Filter 2, followed by those fruits irrigated with outflow waters from Filter 1. In comparison, the lowest iron concentrations were recorded for fruits of plants irrigated with outflow water received from Filter 3. However, calcium concentrations in tested fruits were higher than that reported by Ciju (2013) of 45 mg per 100 g of sun dried Chillies.

Statistical analysis (Table 5.14) showed that plants irrigated with water obtained from Filter 1 of large aggregate diameter produced Chillies of calcium concentrations which were significantly ($p < 0.05$) higher than those linked to Filter 3 of small aggregate diameter. Moreover, irrigation with Filters 5 and 6 of high inflow loading rate resulted in fruit calcium concentrations which were significantly ($p < 0.05$) different from those associated with Filters 3 and 4, respectively, which were fed with diluted wastewater (Table 5.14).

Diesel contamination in the wetland system caused significant differences ($p < 0.05$) in calcium concentrations of the harvested fruits as shown when comparing Chillies of Filters 3 and 5 with those of Filters 4 and 6, respectively. Correlation analysis indicated that calcium concentrations in soil and fruits were negatively correlated with each other ($R = -0.098$, $p = 0.190$). In addition, regression analysis results (Figure 5.6a) indicated that average concentrations of calcium in the fruits decreased linearly with corresponding concentrations in the soil.

However, calcium in the soil is in competition with other major cations such as sodium (Na^+), potassium (K^+), magnesium (Mg^{++}), ammonium (NH_4^+), iron (Fe^{++}), and aluminium (Al^{+++}) for uptake by crops (FAO, 1972). High potassium applications have been known to reduce the calcium uptake in plants (Barber, 1995). As the pH of a soil decreases, more of these elements (mainly Iron (Fe^{++}) and aluminium (Al^{+++})) become soluble and combine with calcium to form essentially insoluble compounds (Barber, 1995). High soil or plant calcium levels can inhibit B uptake and utilisation. Calcium is essential for many plant functions such as proper cell division and cell wall development as well as metabolism and enzyme activity (FAO, 1972).

Calcium is an essential mineral for human health as well, being especially important for metabolism processes, bone structure, muscle and nerve function control, and managing the balance of blood stream. This explains how food, which is rich in calcium, can play an important role in human health (Zhu & Prince, 2012).

Table 5.14: Overview of the statistically significant differences in calcium concentrations in the harvested fruits using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.334	<0.001	0.061	0.035	<0.001	0.017	0.002	0.002	0.001	0.002	<0.001	<0.001	0.033	0.027
2	0.334	n.a	0.002	0.364	0.254	0.010	0.387	0.113	0.114	0.071	0.103	0.002	<0.001	0.544	0.493
3	<0.001	0.002	n.a	0.011	0.002	0.578	0.023	0.120	0.118	0.182	0.130	0.928	0.335	0.011	0.014
4	0.061	0.364	0.011	n.a	0.815	0.047	0.790	0.324	0.327	0.228	0.303	0.014	<0.001	0.994	0.931
5	0.035	0.254	0.002	0.815	n.a	0.010	0.400	0.118	0.120	0.075	0.108	0.002	<0.001	0.559	0.508
6	<0.001	0.010	0.578	0.047	0.010	n.a	0.085	0.318	0.314	0.436	0.339	0.641	0.129	0.048	0.057
7	0.017	0.387	0.023	0.790	0.400	0.085	n.a	0.471	0.476	0.347	0.445	0.029	0.001	0.796	0.857
8	0.002	0.113	0.120	0.324	0.118	0.318	0.471	n.a	0.994	0.826	0.966	0.143	0.012	0.327	0.368
9	0.002	0.114	0.118	0.327	0.120	0.314	0.476	0.994	n.a	0.820	0.959	0.141	0.012	0.331	0.372
10	0.001	0.071	0.182	0.228	0.075	0.436	0.347	0.826	0.820	n.a	0.915	0.213	0.021	0.231	0.263
11	0.002	0.103	0.130	0.303	0.108	0.339	0.445	0.966	0.959	0.915	n.a	0.155	0.013	0.307	0.345
12	<0.001	0.002	0.928	0.014	0.002	0.641	0.029	0.143	0.141	0.213	0.155	n.a	0.292	0.015	0.018
13	<0.001	<0.001	0.335	<0.001	<0.001	0.129	0.001	0.012	0.012	0.021	0.013	0.292	n.a	<0.001	0.001
14	0.033	0.544	0.011	0.994	0.559	0.048	0.796	0.327	0.331	0.231	0.307	0.015	<0.001	n.a	0.938
15	0.027	0.493	0.014	0.931	0.508	0.057	0.857	0.368	0.372	0.263	0.345	0.018	0.001	0.938	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself.

5.3.2.3 Comparison of iron

Table 5.13 shows that the highest iron concentrations were observed in fruits harvested from plants irrigated with outflow water obtained from Filter 2, followed by those fruits irrigated with outflow waters from Filters 4 and 1. In comparison, the lowest iron concentrations were recorded for fruits of plants irrigated with outflow water received from Filter 5. However, the recorded iron concentrations in fruits harvested from all treatments (except fruits from deionised water plants) exceeded those reported by Ciju (2013) of 6.04 mg per 100 g dried Chillies. Statistical results (Table 5.15) showed that the impact of the wetland aggregate diameter was significant ($p < 0.05$) in comparison between the iron mean concentrations in fruits harvested from plants of Filters 2 compared to those of Filter 4. Fruits harvested from plants irrigated with Filters 3 and 4 had mean iron concentrations which were significantly ($p < 0.05$) different from those of Filters 5 and 7, respectively, due to the impact of wetlands inflow loading rate and contact time variables. Moreover, irrigation with hydrocarbon contaminated wetland filters produced fruits of mean iron concentrations which were significantly ($p < 0.05$) different from those of uncontaminated filters, as shown when comparing Chillies linked to Filters 1 and 5 with those of Filters 2 and 6, respectively. Correlation analysis results showed that mean iron concentrations in soil and fruits were significantly negatively correlated with each other (R = -0.256, p = 0.001). Moreover, regression analysis results (Figure 5.6b) indicated that iron concentrations in the fruits decreased linearly with the corresponding values in the soil due to its low bioavailability to the plants, as reported by FAO (1972). Moreover, iron concentrations in tested fruits did not exceed the threshold of 425 mg/kg (EC, 2001; FAO/WHO, 2001). Although iron is an essential element for human health, excessive iron amounts can lead to tissue damage (Abbaspour et al., 2014).

Table 5.15: Overview of the statistically significant differences in iron concentrations in the harvested fruits using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.038	0.178	0.919	<0.001	0.047	0.002	0.094	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.038	n.a	0.050	0.030	<0.001	0.009	<0.001	0.022	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3	0.178	0.050	n.a	0.256	0.006	0.523	0.922	0.745	0.004	0.023	<0.001	<0.001	0.003	<0.001	0.003
4	0.919	0.030	0.256	n.a	<0.001	0.076	0.003	0.144	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	<0.001	<0.001	0.006	<0.001	n.a	0.033	0.004	0.015	0.891	0.622	0.006	0.053	0.832	0.032	0.860
6	0.047	0.009	0.523	0.076	0.033	n.a	0.461	0.754	0.023	0.102	<0.001	<0.001	0.019	<0.001	0.021
7	0.002	<0.001	0.922	0.003	0.004	0.461	n.a	0.672	0.003	0.018	<0.001	<0.001	0.002	<0.001	0.002
8	0.094	0.022	0.745	0.144	0.015	0.754	0.672	n.a	0.010	0.051	<0.001	<0.001	0.008	<0.001	0.009
9	<0.001	<0.001	0.004	<0.001	0.891	0.023	0.003	0.010	n.a	0.528	0.009	0.072	0.931	0.044	0.969
10	<0.001	<0.001	0.023	0.001	0.622	0.102	0.018	0.051	0.528	n.a	0.001	0.015	0.473	0.008	0.503
11	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	0.009	0.001	n.a	0.422	0.012	0.554	0.010
12	<0.001	<0.001	<0.001	<0.001	0.053	<0.001	<0.001	<0.001	0.072	0.015	0.422	n.a	0.087	0.832	0.079
13	<0.001	<0.001	0.003	<0.001	0.832	0.019	0.002	0.008	0.931	0.473	0.012	0.087	n.a	0.054	0.963
14	<0.001	<0.001	<0.001	<0.001	0.032	<0.001	<0.001	<0.001	0.044	0.008	0.554	0.832	0.054	n.a	0.049
15	<0.001	<0.001	0.003	<0.001	0.860	0.021	0.002	0.009	0.969	0.503	0.010	0.079	0.963	0.049	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself.

5.3.2.4 Comparison of potassium

Potassium concentrations in all tested fruits (Table 5.13) were very high compared to those reported by Ciju (2013) who recommended a potassium value of 1870 mg per 100 g of sun dried Chillies. However, the highest potassium concentrations were observed in fruits harvested from plants irrigated with raw wastewater followed by those irrigated with Filters 8 and 7 outflow waters, while the lowest values were recorded for fruits harvested from plants irrigated with Filter 3 drain water (Table 5.13).

Moreover, statistical analysis results (Table 5.16) showed that fruits harvested from plants irrigated with water drained from Filter 2, of large aggregate diameter, had mean potassium concentrations which were significantly ($p < 0.05$) different from those linked to Filter 4, of small aggregate diameter. Moreover, results showed that significant ($p < 0.05$) differences were observed in the mean potassium concentration of fruits harvested from plants irrigated with water obtained from Filter 3 and Control A compared to those of Filter 4 and Control B, respectively, explaining the impact of diesel contamination, and Filter 3 compared to Filter 5, explaining the impact of inflow loading rate of the wetland system on average potassium concentrations of the yield. Correlation analysis results showed that potassium concentrations in soil and fruits were significantly positively correlated with each other ($R = 0.377$, $p < 0.001$).

Furthermore, regression analysis results (Figure 5.6c) indicated that potassium concentrations in the fruits increased linearly with the corresponding levels in the soil. This is due to high bioavailability of potassium for uptake by plants, as indicated by Barber (1995).

Potassium has an important role in the plants as it functions in many physiological processes such as photosynthesis, protein synthesis, and activation of some enzymes (FAO, 1972). However, from the human health point of view, potassium is an important mineral that can maintain the water balance and blood pressure within human bodies (FAO/WHO, 2001).

Table 5.16: Overview of the statistically significant differences in potassium concentrations in the harvested fruits using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.597	0.062	0.006	0.007	0.008	<0.001	<0.001	0.863	<0.001	0.308	0.229	0.624	0.310	<0.001
2	0.597	n.a	0.017	0.026	0.021	0.034	<0.001	<0.001	0.721	0.003	0.624	0.085	0.308	0.123	<0.001
3	0.062	0.017	n.a	<0.001	<0.001	<0.001	<0.001	<0.001	0.041	<0.001	0.004	0.505	0.168	0.393	<0.001
4	0.006	0.026	<0.001	n.a	0.934	0.913	0.058	0.072	0.010	0.447	0.081	<0.001	0.001	<0.001	0.019
5	0.007	0.021	<0.001	0.934	n.a	0.978	0.048	0.060	0.012	0.400	0.097	<0.001	0.002	<0.001	0.015
6	0.008	0.034	<0.001	0.913	0.978	n.a	0.045	0.056	0.013	0.384	0.102	<0.001	0.002	<0.001	0.014
7	<0.001	<0.001	<0.001	0.058	0.048	0.045	n.a	0.752	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	0.247
8	<0.001	<0.001	<0.001	0.072	0.060	0.056	0.752	n.a	<0.001	0.017	<0.001	<0.001	<0.001	<0.001	0.141
9	0.863	0.721	0.041	0.010	0.012	0.013	<0.001	<0.001	n.a	0.001	0.397	0.169	0.508	0.235	<0.001
10	<0.001	0.003	<0.001	0.447	0.400	0.384	0.007	0.017	0.001	n.a	0.012	<0.001	<0.001	<0.001	<0.001
11	0.308	0.624	0.004	0.081	0.097	0.102	<0.001	<0.001	0.397	0.012	n.a	0.026	0.131	0.042	<0.001
12	0.229	0.085	0.505	<0.001	<0.001	<0.001	<0.001	<0.001	0.169	<0.001	0.026	n.a	0.476	0.851	<0.001
13	0.624	0.308	0.168	0.001	0.002	0.002	<0.001	<0.001	0.508	<0.001	0.131	0.476	n.a	0.600	<0.001
14	0.310	0.123	0.393	<0.001	<0.001	<0.001	<0.001	<0.001	0.235	<0.001	0.042	0.851	0.600	n.a	<0.001
15	<0.001	<0.001	<0.001	0.019	0.015	0.014	0.247	0.141	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself.

5.3.2.5 Comparison of magnesium

Table 5.13 indicates that magnesium concentrations in tested fruits were higher than that reported by Ciju (2013) of 88 mg per 100 g of dried Chillies. The highest magnesium concentrations were observed in fruits harvested from plants irrigated with raw wastewater and Filter 7 outflow water followed by those of Filter 4 and 2, while the lowest concentration values were recorded for fruits of plants irrigated with Filter 3 outflow water.

Furthermore, statistical analysis (Table 5.17) showed that there are significant ($p < 0.05$) differences in mean fruit magnesium concentrations of plants irrigated with Filter 7 water compared to those of Filter 8, explaining the impact of the resting time variable in the wetland system. Moreover, a significant ($p < 0.05$) difference was observed between fruit magnesium mean concentrations of Filters 3 and 4, due to the impact of diesel contamination.

Correlation analysis results showed that magnesium concentrations in soil and fruits were correlated negatively with each other ($R = -0.124$, $p = 0.098$). Moreover, regression analysis results indicated that fruit magnesium concentrations linearly decreased with their corresponding levels in the soil (Figure 5.6d).

However, uptake of magnesium mainly depends on calcium and potassium as well as its levels in the soil. Plant magnesium uptake is usually a small portion of the total exchangeable magnesium available in the soil, which means that magnesium depletion from the soil by plant uptake is a minor factor, as discussed by Barber (1995).

Magnesium is an essential plant nutrient as it has a wide range of key roles in many plant functions. One of the well-known roles of magnesium is in the photosynthesis process, as it is a building block of chlorophyll, which makes leaves appear green.

Magnesium deficiency might be a significant limiting factor in crop production (FAO, 1972).

Considering human health, magnesium plays a role in the structural development of bones, and the active transport of calcium and potassium ions across cell membranes, which is important for nerve impulse conduction, muscle contraction, and a normal heart rhythm. Moreover, too much magnesium from food does not pose a health risk for healthy individuals (Musso, 2009).

Table 5.17: Overview of the statistically significant differences in magnesium concentrations in the harvested fruits using the parametric Games-Howell test.

	Statistic (*p*-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.999	0.655	0.969	0.892	1.000	0.032	0.990	0.868	1.000	0.067	0.871	0.983	1.000	<0.001
2	0.999	n.a	0.487	1.000	0.756	1.000	0.717	1.000	1.000	1.000	0.147	1.000	0.846	0.998	0.415
3	0.655	0.487	n.a	0.027	0.991	0.819	0.002	0.179	0.101	0.305	1.000	0.100	0.998	0.818	<0.001
4	0.969	1.000	0.272	n.a	0.475	0.992	0.864	1.000	1.000	0.988	0.059	1.000	0.601	0.966	0.582
5	0.892	0.756	0.991	0.475	n.a	0.987	0.003	0.055	0.010	0.082	0.425	0.018	1.000	0.989	<0.001
6	1.000	1.000	0.819	0.992	0.987	n.a	0.097	1.000	0.990	1.000	0.268	0.989	0.997	1.000	0.013
7	0.032	0.717	0.002	0.864	0.003	0.097	n.a	0.019	0.178	0.039	0.000	0.217	0.005	0.040	1.000
8	0.990	1.000	0.179	1.000	0.055	1.000	0.109	n.a	1.000	0.997	0.003	1.000	0.391	0.990	<0.001
9	0.868	1.000	0.101	1.000	0.010	0.990	0.178	1.000	n.a	0.796	0.001	1.000	0.188	0.908	<0.001
10	1.000	1.000	0.305	0.988	0.082	1.000	0.039	0.997	0.796	n.a	0.008	0.842	0.631	1.000	<0.001
11	0.067	0.147	1.000	0.059	0.425	0.268	0.000	0.003	0.001	0.008	n.a	0.001	0.731	0.199	<0.001
12	0.871	1.000	0.100	1.000	0.018	0.989	0.217	1.000	1.000	0.842	0.001	n.a	0.198	0.905	0.001
13	0.983	0.846	0.998	0.601	1.000	0.997	0.005	0.391	0.188	0.631	0.731	0.198	n.a	0.998	<0.001
14	1.000	0.998	0.818	0.966	0.989	1.000	0.040	0.990	0.908	1.000	0.199	0.905	0.998	n.a	0.001
15	<0.001	0.415	<0.001	0.582	<0.001	0.013	1.000	0.000	0.000	<0.001	0.000	0.001	<0.001	0.001	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the *p*-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself.

5.3.2.6 Comparison of manganese

Manganese concentrations in tested fruits are shown in Table 5.13. The highest manganese concentrations were observed in fruit of plants irrigated with Filter 1 followed by Filter 2 outflow waters, while the lowest values were observed in those fruits of plants irrigated with Filter 6 drain water. Statistical analysis (Table 5.18) showed that fruit mean manganese concentrations of plants irrigated with Filters 4 and 7 outflow waters were significantly ($p < 0.05$) different from those of plants irrigated with Filters 6 and 8 outflow waters indicating the impact of inflow loading rate and resting time of wetlands on corresponding growth media. Furthermore, fruits of plants irrigated with Filter 5 had average magnesium concentrations significantly ($p < 0.05$) different from those of Filter 6, explaining the impact of diesel contamination. However, the differences in manganese concentrations in the harvested fruits can be explained by the differences in manganese values regarding the corresponding growth media (section 5.2.4.6). Correlation analysis results showed that manganese concentrations in the soil and fruits were significantly ($p < 0.05$) negatively correlated with each other ($R = -0.545$, $p < 0.001$). Moreover, regression analysis results (Figure 5.6e) indicted that fruit manganese concentrations linearly decreased with their corresponding values in the soil due to its low bioavailability, as reported by Barber (1995). Manganese is an essential plant mineral nutrient, playing a key role in several physiological processes, particularly photosynthesis. The impact of manganese deficiencies on crops includes reduced dry matter production and yield, weaker structural resistance against pathogens and a reduced tolerance to drought and heat stress (Hakala et al., 2006). Manganese concentrations in tested fruits did not exceed the corresponding metal threshold of 500 mg/kg (EC, 2001; FAO/WHO, 2001).

Table 5.18: Overview of the statistically significant differences in manganese concentrations in the harvested fruits using the non-parametric Kruskal-Wallis test.

	Statistic (*p*-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.575	n.d.	0.061	0.002	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	n.d.	<0.001	0.001
2	0.575	n.a	n.d.	0.189	0.010	<0.001	0.013	<0.001	<0.001	0.002	<0.001	<0.001	n.d.	<0.001	0.009
3	n.d.	n.d.	n.a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	0.061	0.189	n.d.	n.a	0.201	<0.001	0.242	<0.001	0.010	0.076	0.001	<0.001	n.d.	0.012	0.189
5	0.002	0.010	n.d.	0.201	n.a	<0.001	0.914	<0.001	0.199	0.691	0.052	<0.001	n.d.	0.217	0.971
6	<0.001	<0.001	n.d.	<0.001	<0.001	n.a	<0.001	0.721	0.008	0.001	0.045	0.964	n.d.	0.007	<0.001
7	0.002	0.013	n.d.	0.242	0.914	<0.001	n.a	<0.001	0.164	0.545	0.040	<0.001	n.d.	0.180	0.885
8	<0.001	<0.001	n.d.	<0.001	<0.001	0.721	<0.001	n.a	0.021	0.002	0.100	0.853	n.d.	0.019	<0.001
9	<0.001	<0.001	n.d.	0.010	0.199	0.008	0.164	0.021	n.a	0.432	0.509	0.013	n.d.	0.960	0.212
10	<0.001	0.002	n.d.	0.076	0.691	0.001	0.545	0.002	0.432	n.a	0.148	0.001	n.d.	0.461	0.645
11	<0.001	<0.001	n.d.	0.001	0.052	0.045	0.040	0.100	0.509	0.148	n.a	0.067	n.d.	0.478	0.057
12	<0.001	<0.001	n.d.	<0.001	<0.001	0.964	<0.001	0.853	0.013	0.001	0.067	n.a	n.d.	0.011	<0.001
13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	n.d.	n.d.
14	<0.001	<0.001	n.d.	0.012	0.217	0.007	0.180	0.019	0.960	0.461	0.478	0.011	n.d.	n.a	0.231
15	0.001	0.009	n.d.	0.189	0.971	<0.001	0.885	<0.001	0.212	0.645	0.057	<0.001	n.d.	0.231	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself; and n.d., not detected.

5.3.2.7 Comparison of zinc

Table 5.13 shows that the highest zinc concentrations were observed in fruits harvested from plants watered with Filter 7 outflow water followed by those of Filters 5 and 1, while the lowest concentrations were observed in fruits of plants irrigated with Filter 3 outflow water. However, detected zinc concentrations exceeded those reported by Ciju (2013) of 1.02 mg for 100 g of dried Chillies.

Statistical analysis (Table 5.19) showed that average zinc concentrations in fruits of Filter 4 irrigated plants were significantly ($p < 0.05$) different from those of Filter 7, explaining the impact of the contact time variable on the corresponding zinc concentrations of the growth media (section 5.2.4.7). Inflow loading rate impact was observed due to the significant ($p < 0.05$) differences in mean zinc concentrations of Filter 3 plants compared to those of Filter 5. Moreover, the impact of diesel spill filter contamination on mean zinc concentration of harvested fruits was observed due to the significant ($p < 0.05$) differences when comparing fruits from Filter 5 and Control A plants with those of Filter 6 and Control B, respectively. Differences in zinc concentrations in harvested fruits are likely due to the differences in zinc values in the corresponding growth media.

Correlation analysis results showed that zinc concentrations in the soil and fruits were significantly negatively correlated with each other (R = -0.183, p = 0.014). Moreover, regression analysis results (Figure 5.6f) indicated that Chilli zinc concentrations decreased linearly with their corresponding values in the soil due to low bioavailability of zinc, as discussed by Barber (1995). Zinc is an essential micronutrient for plants as it acts as a functional, structural, or regulatory cofactor of a large number of enzymes, also, zinc may play a role in controlling gene expression, as discussed by Brown et al. (1993).

However, zinc concentrations in fruits from all irrigated plants (except for those irrigated with Filter 6 outflow water and tap water) exceeded the corresponding metal threshold of 50 mg/kg (EC, 2001; FAO/WHO, 2001). Lactase (1998) assessed the levels of heavy metals in vegetables by evaluating the contamination/pollution (C/P) index. This index is based on the metal concentration in vegetables (or water or soil) divided by the corresponding maximum permissible concentration levels (thresholds). Lacatusu (1998) listed the significance intervals of the C/P index. Based on this, all fruits tested for zinc were slightly polluted (C/P value between 1.1 and 2.0) with the exception of those harvested from plants irrigated with Filter 7 outflow water, which were moderately polluted (C/P values between 2.1 and 4.0). Considering human health, zinc is an essential micronutrient in the body and can be used in numerous pharmaceutics. Nevertheless, zinc is toxic, when taken long-term in high doses (FAO/WHO, 2001).

Table 5.19: Overview of the statistically significant differences in zinc concentrations in the harvested fruits using the non-parametric Kruskal-Wallis test.

	Statistic (p-value)[a]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	n.a	0.947	0.059	0.097	0.027	0.017	0.035	0.149	0.007	0.832	0.162	<0.001	0.007	0.802	0.684
2	0.947	n.a	0.051	0.085	0.012	0.014	0.042	0.169	0.006	0.885	0.143	<0.001	0.006	0.854	0.635
3	0.059	0.051	n.a	0.817	<0.001	0.616	<0.001	0.001	0.417	0.036	0.624	0.008	0.417	0.032	0.139
4	0.097	0.085	0.817	n.a	<0.001	0.464	<0.001	0.002	0.297	0.062	0.790	0.004	0.297	0.056	0.211
5	0.027	0.012	<0.001	<0.001	n.a	<0.001	0.927	0.583	<0.001	0.046	<0.001	<0.001	<0.001	0.005	0.009
6	0.017	0.014	0.616	0.464	<0.001	n.a	<0.001	<0.001	0.757	0.009	0.322	0.031	0.757	0.008	0.047
7	0.035	0.042	<0.001	<0.001	0.927	<0.001	n.a	0.247	<0.001	0.058	<0.001	<0.001	<0.001	0.001	0.012
8	0.149	0.169	0.001	0.002	0.583	<0.001	0.247	n.a	<0.001	0.219	0.005	<0.001	<0.001	0.123	0.064
9	0.007	0.006	0.417	0.297	<0.001	0.757	<0.001	<0.001	n.a	0.004	0.193	0.065	1.000	0.003	0.022
10	0.832	0.885	0.036	0.062	0.046	0.009	0.058	0.219	0.004	n.a	0.107	<0.001	0.004	0.969	0.536
11	0.162	0.143	0.624	0.796	<0.001	0.322	<0.001	0.005	0.193	0.107	n.a	0.002	0.193	0.099	0.322
12	<0.001	<0.001	0.008	0.004	<0.001	0.031	<0.001	<0.001	0.065	<0.001	0.002	n.a	0.065	<0.001	<0.001
13	0.007	0.006	0.417	0.297	<0.001	0.757	<0.001	<0.001	1.000	0.004	0.193	0.065	n.a	0.003	0.022
14	0.802	0.854	0.032	0.056	0.005	0.008	0.001	0.123	0.003	0.969	0.099	<0.001	0.003	n.a	0.510
15	0.684	0.635	0.139	0.211	0.009	0.047	0.012	0.064	0.022	0.536	0.322	<0.001	0.022	0.510	n.a

Note: [a] *P*-value, probability of statistical test (values are statistically significantly different only if the p-value < 0.05); 1 to 8, soils irrigated with wetland filters (1-8), respectively; 9 & 10, soil irrigated with wetland controls (A & B); 11 to 15, soils irrigated with deionised water; tap water; tap water with fertiliser (0.7 ml/l); wastewater (20%) with tap water (80%); and wastewater (100%), respectively; n.a, not applicable as the treatment compared with itself.

5.3.2.8 Comparison of boron

In spite of boron availability in growth media (Table 5.3), results showed that boron was not detected in the tested fruits harvested from all treatments. Boron can be available in the soil at different concentrations and compositions, but only a relatively small proportion is obtainable by plants (Diana, 2006). However, boron is an essential micronutrient for plant growth and development as it has an important role in metabolism processes, mainly related to stabilisation of cell membranes (Cakmak & Römheld, 1997; Blevins & Lukaszewski, 1998). Regarding human health, boron is considered as an essential mineral that can positively affect bone growth and reduce the risk of some cancer types (Nielsen, 2014).

5.4 Summary

This chapter discusses the quality of soil used for growing Chilli plants and irrigated with different irrigation water types. The mineral content of these soils as well as the microbial contamination are studied. Harvested Chilli fruit quality, in terms of trace element and heavy metal content, is investigated and assessed compared to the standards of human and public health.

CHAPTER 6
RECYCLING OF DOMESTIC WASTEWATER TREATED BY VERTICAL-FLOW WETLANDS FOR IRRIGATION OF CHILLI GENERATIONS

Almuktar, S. A. A. A. N, **Abed, S.N., & Scholz, M.** Recycling of domestic wastewater treated by vertical-flow wetlands for irrigation of two consecutive *Capsicum annuum* generations. *Ecological Engineering*

Almuktar, S. A. A. A. N., Abed, S. N., & Scholz, M. Contamination of Chillies generation irrigated with recycled domestic wastewater treated by vertical-flow constructed wetlands. *Journal of Environmental Management*

6.1 Overview

This chapter discusses the impact of recycling urban wastewater treated by vertical-flow wetlands on growth of Chilli generations (experiment No. 3). Section 6.1 introduces this chapter, while section 6.2 discusses the comparisons of irrigation water qualities in terms of different variables. Environmental conditions available for growing plants are explained in section 6.3, while the growth and yield production comparisons are provided in section 6.4. Soil and fruit contamination in terms of microbes, heavy metals and trace elements are investigated in sections 6.5 and 6.6, respectively. Lastly, the summary of this chapter is provided in section 6.7.

6.2 Comparison of irrigation water qualities

Tables 6.1 to 6.5 show the inflow water quality received by the plants and corresponding analysis. Note that the wetland effluent was used as the influent for the vegetable pots. Figure 6.1 explains the comparison strategy of irrigation water qualities according to wetland design variables.

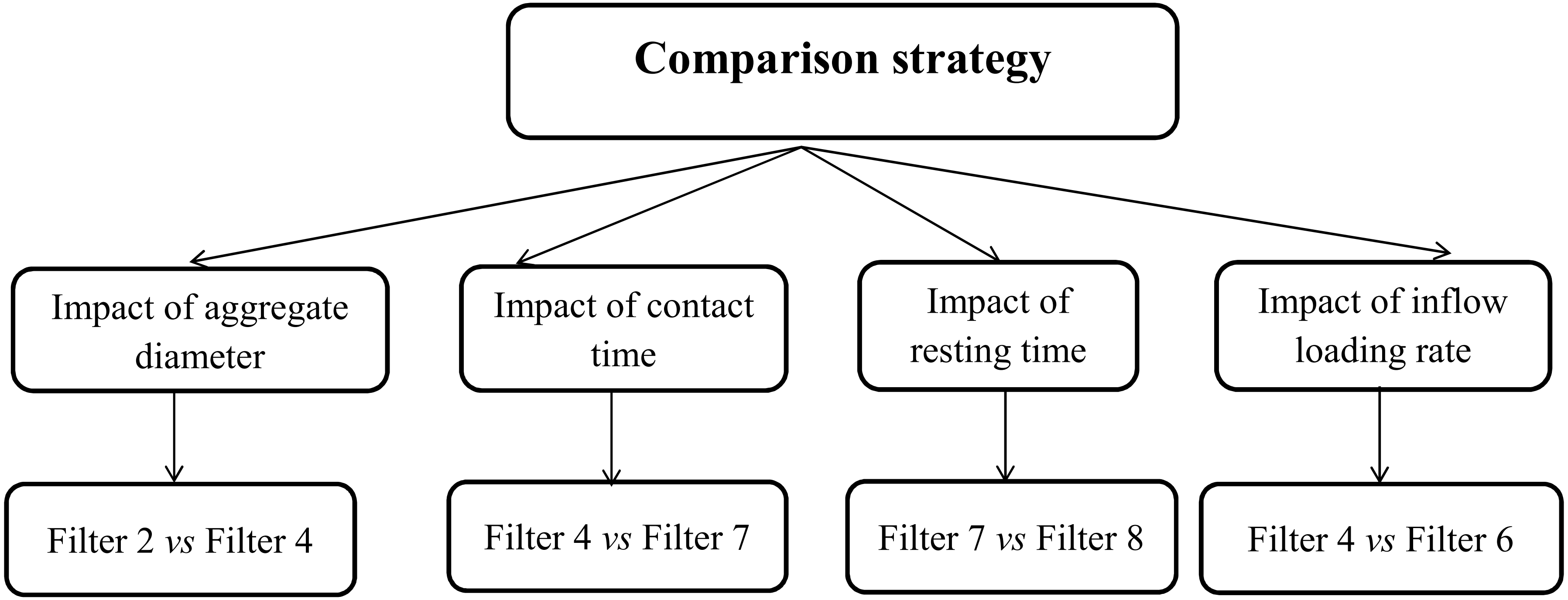

Figure 6.1: Comparison strategy of irrigation water qualities.

6.2.1 Comparison of oxygen demand variables

The chemical oxygen demand (COD) can be used as an indicator for organic pollutants that may induce lipid peroxidation and toxicity to plants. Table 6.1 shows that COD values were the highest for Filter 6 outflow water followed by that for Filter 8. In contrast, the lowest mean values were noted for Filter 7 drain water. Statistical analysis results (Table 6.2) showed that there were no significant differences ($p > 0.05$) in mean COD values of Filters 2 and 4 compared to those of Filters 4 and 7, respectively, indicating that aggregate size and contact time wetland design variables may not matter with regard to COD treatment. Moreover, Filters 7 and 4 outflow waters had COD values, which were significantly ($p < 0.05$) different from those of Filters 8 and 6, respectively, explaining the impact of resting time and inflow loading rate variables on outflow water COD values. Furthermore, COD values for different filter outflows were considerably different during various vegetable growth periods (Table 6.1). This can be explained by the seasonal variation in wetland systems, confirming the results observed by Song et al. (2006) and Sani et al. (2013) which indicated a clear seasonal trend with high COD values in autumn and low COD values in summer.

Table 6.1 shows that the five-day biochemical oxygen demand (BOD) was the highest for Filter 6 outflow water followed by that of Filter 2, while Filter 7 outflow water had the lowest five-day BOD values. Filters 2 and 4 had BOD values which were higher than those for Filters 4 and 7, respectively, indicating the impact of aggregate size and contact time variables of the wetland system on outflow five-day BOD values (Figure 6.1). Moreover, Filter 7 showed biochemical oxygen demand levels which were lower than those for Filter 8, explaining the impact of the resting time variable. Furthermore, the five-day BOD for Filter 6 outflow water of high inflow load was significantly ($p < 0.05$) greater than that for Filter 4 of low inflow loading rate (Table 6.2) confirming the results reported by Sani et al. (2013) indicating that high load filters tend to be overloaded. Furthermore, the five-day BOD values for different filter outflows were noticeably different during various vegetable growth periods (Table 6.1) confirming the results from previous studies (Scholz, 2011; Sani et al., 2013) which reported a seasonal trend with high BOD values in summer and low BOD values in winter.

Table 6.1 shows that dissolved oxygen mean values were the highest in outflow water of Filter 4 followed by those for Filters 8 and 7, while the lowest values were observed in Filters 2 and 6 outflow waters. Statistical analysis results (Table 6.2) did not show any significant differences in dissolved oxygen values for the various filter outflows.

Correlation analysis results show that COD and BOD values were significantly positively ($R = 0.815$, $p = 0.004$) correlated with each other in the system, confirming the results reported previously by Scholz (2010).

Moreover, correlation analysis results indicated that dissolved oxygen was negatively correlated with chemical oxygen demand ($R = -0.574$, $p = 0.083$) and biochemical oxygen demand ($R = -0.351$, $p = 0.320$) in the system. Furthermore, dissolved oxygen values were significantly ($p < 0.05$) negatively correlated with microorganisms (e.g., total coliforms; $R = -0.726$; $p = 0.017$ and Salmonella; $R = -0.751$, $p = 0.012$). This negative correlation can be explained by an improvement of the chemical oxygen demand and biochemical oxygen demand removal efficiencies as microorganisms responsible for biodegradation acclimatised, resulting in a reduction in the amount of available dissolved oxygen in the system (Song et al. 2006; Scholz, 2010).

Table 6.1: Comparison of the water quality of the inflow waters received by the vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Chemical oxygen demand (mg/l)				
Filter 2	45.0±18.14 (19)	37.1±7.28 (4)	50.2±6.60 (4)	46.1±22.94 (11)
Filter 4	43.3±13.10 (18)	45.7±12.07 (4)	51.8±4.60 (4)	39.0±14.58 (10)
Filter 6	63.9±36.81 (18)	49.4±13.15 (4)	61.0±2.65 (4)	70.8±48.46 (10)
Filter 7	42.2±23.82 (16)	37.2±20.53 (4)	27.6±2.83 (4)	52.0±27.99 (8)
Filter 8	60.4±34.41 (17)	85.9±61.87 (4)	46.5±21.85 (4)	55.3±17.41 (9)
Biochemical oxygen demand (mg/l)				
Filter 2	18.9±14.98 (41)	15.7±10.42 (7)	16.3±8.51 (8)	20.5±17.46 (26)
Filter 4	15.6±15.10 (42)	14.9±9.30 (7)	10.3±6.27 (8)	17.4±17.80 (27)
Filter 6	29.4±26.99 (41)	19.4±12.53 (7)	42.1±19.57 (8)	28.2±30.66 (26)
Filter 7	14.2±12.76 (48)	7.5±3.70 (11)	10.2±5.25 (11)	18.7±15.53 (26)
Filter 8	17.2±13.46 (53)	12.4±5.43 (11)	14.4±4.85 (14)	20.4±17.40 (28)
Dissolved oxygen (mg/l)				
Filter 2	2.8±1.56 (45)	1.1±0.68 (7)	2.2±0.99 (6)	3.3±1.48 (32)
Filter 4	3.1±1.96 (44)	1.2±0.87 (7)	2.7±1.11 (6)	3.7±2.00 (31)
Filter 6	2.9±1.53 (45)	1.3±0.85 (7)	2.4±0.89 (6)	3.3±1.51 (32)
Filter 7	3.1±2.13 (46)	1.0±1.06 (11)	3.7±0.77 (7)	3.8±2.17 (28)
Filter 8	3.1±2.39 (50)	1.1±0.70 (12)	1.9±1.13 (10)	4.5±2.32 (28)
Ammonia-nitrogen (mg/l)				
Filter 2	4.6±4.57 (23)	5.5±6.57 (5)	7.6±4.78 (6)	2.8±2.57 (12)
Filter 4	2.9± 2.86 (22)	2.1±2.59 (5)	5.2±2.43 (6)	2.0±2.69 (11)
Filter 6	8.5±7.30 (24)	6.9±6.12 (6)	12.4±5.06 (6)	7.4±8.30 (12)
Filter 7	3.5±4.23 (17)	4.6±5.26 (7)	2.6±4.26 (3)	2.9±3.44 (7)
Filter 8	1.6±2.33 (18)	1.4±1.56 (7)	1.5±2.49 (3)	1.7±3.04 (8)
Nitrate-nitrogen (mg/l)				
Filter 2	1.6±2.06 (22)	0.1±0.02 (5)	2.0±3.13 (5)	2.1±1.86 (12)
Filter 4	0.4±0.48 (21)	0.1±0.03 (5)	0.4±0.37 (5)	0.6±0.57 (11)
Filter 6	3.9±3.57 (24)	1.0±1.72 (5)	6.8±3.01 (6)	3.7±3.45 (13)
Filter 7	4.0±3.36 (15)	4.7±4.52 (5)	5.2±2.57 (3)	2.9±2.82 (7)
Filter 8	3.0±2.29 (16)	2.2±1.87 (5)	5.4±0.88 (3)	2.6±2.44 (8)
Ortho-phosphate-phosphorus (mg/l)				
Filter 2	4.1±1.47 (24)	4.3±0.91 (6)	5.3±1.99 (4)	3.7±1.41 (14)
Filter 4	3.7±1.24 (23)	4.3±0.58 (6)	5.3±0.28 (4)	2.9±0.96 (13)
Filter 6	5.0±2.01 (23)	6.3±1.67 (6)	4.8±2.91 (4)	4.4±1.73 (13)
Filter 7	4.3±2.53 (19)	5.8±1.88 (5)	4.4±0.43 (3)	3.6±2.92 (11)
Filter 8	4.0±2.09 (20)	4.6±1.86 (5)	3.8±0.41 (3)	3.8±2.45 (12)
Suspended solids (mg/l)				
Filter 2	12.1±10.07 (53)	5.6±6.42 (11)	20.6±7.89 (8)	12.2±10.10 (34)
Filter 4	4.7±6.25 (52)	4.2±5.55 (11)	5.6±2.83 (8)	4.7±7.11 (33)
Filter 6	11.0±13.00 (53)	5.1±3.92 (11)	4.9±2.90 (8)	14.4±15.07 (34)
Filter 7	4.7±8.16 (56)	0.9±1.03 (14)	0.5±0.52 (11)	7.9±9.88 (31)
Filter 8	3.1±3.71 (56)	1.7±3.01 (15)	1.0±1.12 (9)	4.3±4.04 (32)

Table 6.1 (cont.)

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Turbidity (NTU)				
Filter 2	9.2±8.07 (53)	4.8±2.60 (11)	17.4±7.87 (8)	8.6±8.02 (34)
Filter 4	3.5±4.19 (51)	2.6±0.81 (11)	4.0±1.04 (8)	3.7±5.24 (32)
Filter 6	8.2±9.05 (51)	4.1±1.91 (11)	4.4±1.59 (8)	10.6±10.71 (32)
Filter 7	3.8±3.71 (55)	2.4±1.62 (14)	2.6±0.63 (10)	4.8±4.60 (31)
Filter 8	3.1±2.76 (55)	2.6±1.62 (13)	2.3±0.94 (10)	3.5±3.40 (32)
pH (-)				
Filter 2	6.6±0.26 (52)	6.4±0.10 (11)	6.6±0.18 (8)	6.7±0.28 (33)
Filter 4	6.6±0.28 (51)	6.4±0.08 (11)	6.6±0.20 (8)	6.7±0.30 (32)
Filter 6	6.8±0.28 (52)	6.6±0.12 (11)	6.9±0.19 (8)	6.9±0.32 (33)
Filter 7	6.7±0.32 (58)	6.5±0.21 (15)	6.7±0.19 (13)	6.9±0.35 (30)
Filter 8	6.6±0.38 (61)	6.4±0.13 (16)	6.5±0.23 (10)	6.8±0.41 (35)
Electrical conductivity (µS/cm)				
Filter 2	462.6±146.89 (44)	539.8±178.63 (6)	626.8±55.54 (5)	423.7±129.35 (33)
Filter 4	483.4±155 (43)	527.2±288.88 (6)	568.8±80.73 (5)	461.8±122.57 (32)
Filter 6	832.4±298.17 (44)	1067.7±366.91 (6)	1119.6±129.55 (5)	746.1±255.69 (33)
Filter 7	582.9±442.86 (44)	521.5±222.25 (10)	636.2±73.24 (8)	590.2±561.77 (26)
Filter 8	584.1±459.11 (47)	692.5±242.73 (11)	641.9±62.91 (7)	529.0±561.95 (29)

Note: [a] 11/10/14 to 25/09/15; [b] SPP, second planting period: 11/10/14 to 07/11/14; [c] FPPBF, final planting period before fruiting: 08/11/14 to 19/01/15; and [d] FPPAF, final planting period after fruiting: 20/01/15 to 25/09/15.

Table 6.2: Assessment of the statistically significant differences between the overall water quality variables of different irrigation water types.

Parameter	Aggregate diameter Filters 2 & 4			Contact time Filters 4 & 7			Resting time Filters 7 & 8			Inflow loading rate Filters 4 & 6		
	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b]	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b]	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b]	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values[b]
COD	0.010	M-W-U	0.832	0.017	M-W-U	0.408	<0.001	M-W-U	0.017	<0.001	M-W-U	0.009
BOD	<0.001	M-W-U	0.144	<0.001	M-W-U	0.884	<0.001	M-W-U	0.122	<0.001	M-W-U	0.008
DO	0.010	M-W-U	0.706	0.004	M-W-U	0.965	<0.001	M-W-U	0.883	0.006	M-W-U	0.749
NH_4-N	0.010	M-W-U	0.023	0.001	M-W-U	0.824	<0.001	M-W-U	0.031	<0.001	M-W-U	0.015
NO_3-N	<0.001	M-W-U	0.008	<0.001	M-W-U	0.001	0.031	M-W-U	0.566	<0.001	M-W-U	<0.001
PO_4-P	0.035	M-W-U	0.530	<0.001	M-W-U	0.723	<0.001	M-W-U	0.500	0.029	M-W-U	0.022
Fe	<0.001	M-W-U	0.450	<0.001	M-W-U	0.040	<0.001	M-W-U	0.032	<0.001	M-W-U	0.032
Mn	0.024	M-W-U	0.501	<0.001	M-W-U	0.037	<0.001	M-W-U	0.045	0.006	M-W-U	0.027
Zn	0.219	I-T	0.080	0.253	I-T	0.566	0.558	I-T	0.786	0.104	I-T	0.054
K	0.029	M-W-U	0.082	0.063	I-T	0.181	0.234	I-T	0.223	0.039	M-W-U	0.013
B	0.057	I-T	0.528	0.095	I-T	0.832	0.063	I-T	0.910	0.132	I-T	0.274
Na	0.197	I-T	0.935	0.095	I-T	0.890	0.278	I-T	0.728	0.683	I-T	<0.001
Ca	0.453	I-T	0.073	0.629	I-T	0.477	0.838	I-T	0.939	0.601	I-T	0.199
Mg	0.224	I-T	0.970	0.220	I-T	0.673	0.373	I-T	0.347	0.100	I-T	<0.001
SS	<0.001	M-W-U	<0.001	<0.001	M-W-U	0.227	<0.001	M-W-U	0.755	<0.001	M-W-U	0.002
NTU	<0.001	M-W-U	<0.001	<0.001	M-W-U	0.683	<0.001	M-W-U	0.363	<0.001	M-W-U	<0.001
PH	0.076	I-T	0.822	0.142	I-T	0.018	0.008	M-W-U	0.040	0.023	M-W-U	<0.001
EC	0.223	I-T	0.523	<0.001	M-W-U	0.425	<0.001	M-W-U	0.751	0.005	M-W-U	<0.001
TC	<0.001	M-W-U	0.597	<0.001	M-W-U	0.007	<0.001	M-W-U	0.417	<0.001	M-W-U	0.043
Salm.	<0.001	M-W-U	0.577	<0.001	M-W-U	0.002	<0.001	M-W-U	0.349	<0.001	M-W-U	0.013

Note: COD, chemical oxygen demand (mg/l); BOD, biochemical oxygen demand (mg/l); DO, dissolved oxygen (mg/l); NH4-N, ammonia nitrogen (mg/l); NO3-N, nitrate-nitrogen; PO4-P, ortho-phosphate-phosphorus (mg/l); Fe, iron (mg/l); Mn, manganese (mg/l); Zn, zinc (mg/l); K, potassium (mg/l); B, boron (mg/l); Na, sodium (mg/l); Ca, calcium (mg/l); Mg, magnesium (mg/l); SS, suspended solids (mg/l), NTU, turbidity unit (Nephelometric Turbidity Unit); EC, electrical conductivity (µS/cm); TC, total coliforms (colony forming unit (CFU)/100 ml); and *salm.*, Salmonella spp. [a] Test of normality (if *p*-value > 0.05, data are normally distributed; if *p*-value < 0.05, data are not normally distributed. [b] *p*-value, probability of the statistical test. Filters are statistically significantly different only if the *p*-value < 0.05 for the corresponding water quality parameter. M-W-U, the non-parametric Mann–Whitney U-test, and I-T, the parametric Independent samples T-test.

6.2.2 Comparison of nutrients variables

Table 6.1 shows that the highest ammonia-nitrogen values were observed for Filter 6 outflow water which was fed with high inflow loading rate, followed by those for Filter 2 and Filter 7, while the lowest values were recorded for Filter 8 followed by Filter 4 outflow waters. Filter 2 outflow water had ammonia-nitrogen significantly ($p < 0.05$) greater than that for Filter 4 (Tables 6.1 and 6.2) indicating the impact of aggregate size on the ammonia-nitrogen removal process.

Filter 4 outflow water had ammonia-nitrogen values lower than those for Filter 7; while the latter outflow water had ammonia-nitrogen values significantly ($p < 0.05$) higher than those for Filter 8, indicating the impact of contact and resting time wetland design variables on ammonia nitrogen values of the outflow waters. The inflow water was high for chemical oxygen demand, resulting in statistically significant ($p < 0.05$) differences between the overall mean daily ammonia-nitrogen values of Filter 4 compared to those of Filter 6 (Table 6.2).

This could be explained by the fact that, it is likely that high rate filters are overloaded (Sani et al., 2013). Table 6.1 indicates that ammonia-nitrogen values for irrigation water types were considerably varied during different periods of crop growth due to wetlands seasonal behaviour variation (Sani et al. 2013). Compared to the standards of 5 mg/l (Pescod, 1992; FAO, 2003), statistical analysis results (Table 6.3) showed that higher ammonia-nitrogen values which significantly ($p < 0.05$) exceeded the standards were observed in outflow from Filter 6, which was fed with high inflow loading rate. However, ammonia-nitrogen has a negative effect on plant fruit, leaf and stem development as discussed by Bar-Tal, Aloni, Karni, and Rosenberg (2001).

Moreover, correlation analysis results show that ammonia-nitrogen values were negatively correlated with dissolved oxygen and positively correlated with chemical oxygen demand, biochemical oxygen demand and nitrate-nitrogen in the system. This can be explained by the increasing of nitrification processes by oxidation of ammonia-nitrogen to nitrate-nitrogen via microorganisms with the abundancy of organic sources (Scholz, 2010), which will reduce the oxygen availability in the system (Cooper et al., 1996; Scholz, 2010; Fan et al., 2013; Vymazal, 2014).

Table 6.1 shows that the highest nitrate-nitrogen values were observed in outflow water from Filter 6, while the lowest values were recorded for Filter 4 outflow water. Statistical analysis results (Table 6.2) showed that Filter 2 outflow water had nitrate-nitrogen values which were significantly ($p < 0.05$) greater than those for Filter 4, indicating the impact of aggregate diameter. Moreover, significant differences ($p < 0.05$) in nitrate-nitrogen values in the outflow of Filters 4 and 7 were observed, explaining the impact of contact time on the nitrate-nitrogen removal process.

Moreover, filters of high inflow loading rate showed nitrate-nitrogen concentrations which were significantly ($p < 0.05$) greater than those of diluted inflow (Filter 4 compared to Filter 6), confirming the results reported previously by Sani et al. (2013).

Table 6.1 shows that nitrate-nitrogen values for irrigation waters were considerably varied during different crop growth periods due to seasonal change in wetlands system behaviour as discussed by Sani et al. (2013), indicating high nitrate-nitrogen values in winter and low values in summer confirming previous results (Werker et al., 2002; Kuschk et al., 2003; Gikas et al., 2007).

Compared to the standards of 30 mg/l (Pescod, 1992; FAO, 2003), nitrate-nitrogen for all filter outflow waters was significantly ($p < 0.05$) less than the maximum threshold value as shown in Table 6.3. However, the total yield increases as the nitrate-nitrogen to ammonia-nitrogen ratio increases. This can be explained by a reduction in fruit physiological disorders, which usually reduce fruit mean weight (Bar-Tal et al., 2001). Correlation analysis results show that nitrate-nitrogen values were negatively correlated with organic material in the system. This can be explained by the consuming of nitrogen by microorganisms during organic matter degradation, simulating nitrogen removal via additional carbon supplied via organics, as reported by Scholz (2010). Furthermore, nitrate-nitrogen correlated positively with ammonia-nitrogen and dissolved oxygen in the system. This is because with high availability of ammonia-nitrogen and oxygen in the treatment plant, more oxidation of ammonia to nitrate will occur (Cooper et al., 1996, Scholz, 2010; Fan et al., 2013; Vymazal, 2014).

Table 6.1 shows that the highest ortho-phosphate-phosphorus values were observed for outflow water from Filter 6 which was fed with high inflow loads followed by those for Filter 7, while the lowest ortho-phosphate-phosphorus values were recorded for Filter 4 outflow water. Moreover, outflow water from Filter 2 had ortho-phosphate-phosphorus values greater than those for Filter 4 (Table 6.1), indicating the impact of aggregate diameter.

Filter 4 outflow water showed lower ortho-phosphate-phosphorus values compared to that of Filter 7, while the latter had values higher than those for Filter 8 explaining the impact of wetlands contact and resting time, respectively, on outflow water ortho-phosphate-phosphorus values. Moreover, irrigation water linked to Filter 6 showed ortho-phosphate-phosphorus values which were significantly ($p < 0.05$) greater than those of Filter 4 (Table 6.2), explaining the impact of the wetland inflow loading rate variable on the outflow water ortho-phosphate-phosphorus values.

In general, phosphorus is one of the most difficult pollutants to remove by mature constructed wetlands (Pant, Reddy, & Lemon, 2001). This can be explained by the fact that phosphorus is usually present in particulate form, and does not dissolve well in filters that are not yet saturated by phosphorus or other compounds competing for adsorption sites (Scholz, 2006, 2010).

Compared to standards of 2 mg/l (Table 6.3), all irrigation waters were reported with ortho-phosphate-phosphorus values which significantly ($p < 0.05$) exceeded the threshold (Pescod, 1992; FAO, 2003).

However, phosphorus deficiency has been identified to limit crop yields. Little research has been undertaken regarding the effects of high phosphorus on plants. High phosphorus levels are known to interfere with plant normal metabolisms. Also, it is known to promote manganese uptake by plants (McCauly et al., 2011; FAO, 1972).

Table 6.3: Statistical assessment of irrigation water qualities compared to standards (e.g. FAO (1994, 2003); USEPA (2004)).

Irrigation water type	Shapiro-Wilk test (*p*-value)[a]	Statistical test	Mean value	Standard	Statistic (*p*-value)[b]
Ammonia-nitrogen (mg/l)					
Filter 2 outflow	0.050	O-S-W	4.6	5.0	0.794
Filter 4 outflow	0.020	O-S-W	2.9	5.0	0.011
Filter 6 outflow	0.014	O-S-W	8.5	5.0	0.013
Filter 7 outflow	0.007	O-S-W	3.5	5.0	0.234
Filter 8 outflow	0.001	O-S-W	1.6	5.0	0.002
Nitrate-nitrogen (mg/l)					
Filter 2 outflow	<0.001	O-S-W	1.6	30.0	<0.001
Filter 4 outflow	0.000	O-S-W	0.4	30.0	<0.001
Filter 6 outflow	0.110	O-S-T	3.9	30.0	<0.001
Filter 7 outflow	0.139	O-S-T	4.0	30.0	<0.001
Filter 8 outflow	0.157	O-S-T	3.0	30.0	<0.001
Ortho-phosphate-phosphorus (mg/l)					
Filter 2 outflow	0.011	O-S-W	4.1	2.0	<0.001
Filter 4 outflow	0.110	O-S-T	3.7	2.0	<0.001
Filter 6 outflow	0.540	O-S-T	5.0	2.0	<0.001
Filter 7 outflow	0.001	O-S-W	4.3	2.0	<0.001
Filter 8 outflow	0.000	O-S-W	4.0	2.0	<0.001
Iron (mg/l)					
Filter 2 outflow	0.003	O-S-W	0.232	5.0	0.005
Filter 4 outflow	0.032	O-S-W	0.173	5.0	0.005
Filter 6 outflow	<0.001	O-S-W	0.475	5.0	0.005
Filter 7 outflow	<0.001	O-S-W	0.533	5.0	0.005
Filter 8 outflow	<0.001	O-S-W	0.266	5.0	0.005

Table 6.3 (cont.)

Irrigation water type	Shapiro-Wilk test (p-value)[a]	Statistical test	Mean value	Standard	Statistic (p-value)[b]
		Manganese (mg/l)			
Filter 2 outflow	0.119	O-S-T	0.062	0.2	0.001
Filter 4 outflow	0.337	O-S-T	0.067	0.2	<0.001
Filter 6 outflow	0.081	O-S-T	0.100	0.2	0.050
Filter 7 outflow	0.002	O-S-W	0.138	0.2	0.237
Filter 8 outflow	0.065	O-S-T	0.092	0.2	0.074
		Zinc (mg/l)			
Filter 2 outflow	0.225	O-S-T	0.051	2.0	<0.001
Filter 4 outflow	0.342	O-S-T	0.043	2.0	<0.001
Filter 6 outflow	0.063	O-S-T	0.058	2.0	<0.001
Filter 7 outflow	0.107	O-S-T	0.046	2.0	<0.001
Filter 8 outflow	0.951	O-S-T	0.045	2.0	<0.001
		Potassium (mg/l)			
Filter 2 outflow	0.177	O-S-T	6.667	2.0	0.002
Filter 4 outflow	0.003	O-S-W	4.472	2.0	0.021
Filter 6 outflow	0.256	O-S-T	12.097	2.0	0.001
Filter 7 outflow	0.557	O-S-T	7.017	2.0	0.001
Filter 8 outflow	0.053	O-S-T	4.961	2.0	0.046
		Boron (mg/l)			
Filter 2 outflow	0.432	O-S-T	0.084	0.75	<0.001
Filter 4 outflow	0.106	O-S-T	0.066	0.75	<0.001
Filter 6 outflow	0.557	O-S-T	0.096	0.75	<0.001
Filter 7 outflow	0.520	O-S-T	0.061	0.75	<0.001
Filter 8 outflow	0.016	O-S-W	0.063	0.75	0.005
		Sodium (mg/l)			
Filter 2 outflow	0.587	O-S-T	41.737	920	<0.001
Filter 4 outflow	0.376	O-S-T	41.276	920	<0.001
Filter 6 outflow	0.833	O-S-T	65.321	920	<0.001
Filter 7 outflow	0.518	O-S-T	42.117	920	<0.001
Filter 8 outflow	0.532	O-S-T	39.891	920	<0.001
		Calcium (mg/l)			
Filter 2 outflow	0.862	O-S-T	31.972	400	<0.001
Filter 4 outflow	0.586	O-S-T	43.054	400	<0.001
Filter 6 outflow	0.428	O-S-T	54.106	400	<0.001
Filter 7 outflow	0.295	O-S-T	39.065	400	<0.001
Filter 8 outflow	0.830	O-S-T	39.355	400	<0.001
		Magnesium (mg/l)			
Filter 2 outflow	0.521	O-S-T	5.045	60	<0.001
Filter 4 outflow	0.490	O-S-T	5.066	60	<0.001
Filter 6 outflow	0.091	O-S-T	9.019	60	<0.001
Filter 7 outflow	0.119	O-S-T	5.280	60	<0.001
Filter 8 outflow	0.969	O-S-T	4.776	60	<0.001

Table 6.3 (cont.)

Irrigation water type	Shapiro-Wilk test (*p*-value)[a]	Statistical test	Mean value	Standard	Statistic (*p*-value)[b]
Electrical conductivity (μS/cm)					
Filter 2 outflow	0.513	O-S-T	462.6	3000	<0.001
Filter 4 outflow	0.125	O-S-T	483.4	3000	<0.001
Filter 6 outflow	0.017	O-S-W	832.4	3000	<0.001
Filter 7 outflow	<0.001	O-S-W	582.9	3000	<0.001
Filter 8 outflow	<0.001	O-S-W	584.1	3000	<0.001
Total coliforms (CFU/100 ml)					
Filter 2 outflow	<0.001	O-S-W	100071	1000	0.001
Filter 4 outflow	0.001	O-S-W	79500	1000	0.003
Filter 6 outflow	0.051	O-S-W	157071	1000	0.001
Filter 7 outflow	0.003	O-S-W	7154	1000	0.032
Filter 8 outflow	0.003	O-S-W	4438	1000	0.056

Note: [a] Test of normality (if *p*-value > 0.05, data are normally distributed; if *p*-value < 0.05, data are not normally distributed. [b] *p*-value, probability of the statistical test (values are statistically significantly different only if the *p*-value < 0.05 for the corresponding water quality parameter). O-S-T, the parametric one sample T-test, O-S-W, the non-parametric Wilcoxon signed rank test. CFU, colony forming units.

6.2.3 Comparison of trace elements

Table 6.4 provides an overview of the ICP–OES analysis for selected elements determined in the irrigation waters. Results showed that all detected element concentrations were considerably varied during different plant growth periods due to wetland seasonal behaviour variation, as reported by Sani et al. (2013) and Scholz (2010, 2011). Table 6.4 shows that iron concentrations were observed with highest values in Filter 7 outflow water followed by Filter 6, while the lowest values were recorded for outflow water from Filter 4. Filter 2 outflow waters had iron concentrations greater than those for Filter 4, indicating the impact of wetland aggregate diameter on iron removal processes. Filters 4 and 7 outflow waters showed iron levels which were significantly ($p < 0.05$) different from those of Filters 7 and 8, respectively, explaining the impact of both contact and resting time on iron removal processes.

Moreover, irrigation water obtained from Filter 6 of high inflow loading rate had iron concentrations which were significantly ($p < 0.05$) greater than those of Filter 4 of low inflow loading rate.

Compared to the standards of 5.0 mg/l (Table 6.3), all irrigation water types were reported with iron concentrations which were significantly lower than the threshold (Pescod, 1992; FAO, 2003). Moreover, correlation analysis results show that iron concentration values were significantly positively ($p < 0.05$) correlated with chemical oxygen demand (R = 0.705, p = 0.023), biochemical oxygen demand (R = 0.830, p = 0.003), ortho-phosphate-phosphorus (R =0.794, p = 0.006), manganese (R = 0.915, p < 0.001), potassium (R = 0.867, p = 0.001, boron (R = 0.709, p = 0.022), calcium (R = 0.818, p = 0.004) and magnesium (R = 0.806, p = 0.005), while negatively correlated with the dissolved oxygen (R = -0.283, p = 0.428) values in the treatment system (Essington, 2015; Almuktar & Scholz, 2016 a,b).

Furthermore, iron values were significantly ($p < 0.05$) positively correlated with the monitored microorganisms in the system such as total coliforms (R = 0.636, p = 0.048), *Escherichia coli* (R = 0.768, p = 0.009) and *Salmonella* spp. (R = 0.673, p = 0.033), as reported by Almuktar and Scholz (2015, 2016a). According to the previous studies (Wiseman & Edwards, 2004; Lesley et al., 2008), iron can be removed from the wastewater treated by wetland systems mainly through oxidative processes and iron hydroxides formation. Moreover, biotic processes can be significantly considered in iron removal processes in the treatment system, as reported by Lesley et al. (2008).

Oxygen availability and water pH can be also considered as crucial factors in iron removal processes as discussed by Goulet and Pick (2001) contributing to the impact of plant photosynthesis which may considerably control the oxygen levels in the wetland systems and subsequent impact on iron removal rate mainly for concentrations of less than 2 mg/l as confirmed by Batty and Younger (2002) Furthermore, bacterial communities also can mediate the iron oxidation in the system as reported by Lesley et al. (2008). Table 6.4 shows that manganese concentrations were the highest for outflow waters from Filter 7 followed by those for Filter 6, while the lowest values were observed for Filter 2 outflow water. Filter 2, of large aggregate size, outflow waters showed manganese concentrations which were lower than those for Filter 4 of small aggregate diameter.

Filter 4 outflow water had manganese levels which were significantly ($p < 0.05$) lower than those for Filter 7 outflow, while the latter showed values which were significantly ($p < 0.05$) greater than those for Filter 8 drain water, explaining the impact of contact and resting times of the wetlands system on manganese removal processes. Furthermore, high inflow loading rate significantly ($p < 0.05$) impacts outflow water iron concentrations, as shown when comparing Filters 4 and 6 with each other (Table 6.2). Compared to the standards of 0.2 mg/l for manganese (Table 6.3), most irrigation water types had manganese concentrations which were significantly ($p < 0.05$) lower than the threshold (Pescod, 1992; FAO, 2003).

Correlation analysis results show that manganese values were significantly ($p < 0.05$) positively correlated with chemical oxygen demand (R = 0.766, $p = 0.010$), biochemical oxygen demand (R = 0.697, $p = 0.025$), ortho-phosphate-phosphorus (R = 0.648, $p = 0.043$), iron (R = 0.915, $p < 0.001$), potassium (R = 0.733, $p = 0.016$) and magnesium (R = 0.648, $p = 0.043$) values, as discussed by Essington (2015). Moreover, manganese values were significantly ($p < 0.05$) positively correlated with the monitored microorganisms in the system such as total coliforms (R = 0.758, $p = 0.011$), *Escherichia coli* (R = 0.768, $p = 0.009$) and *Salmonella* spp. (R = 0.842, $p = 0.002$), confirming the results reported by another study (Burdige, Dhakar, & Nealson, 1992) indicating that there is a significantly positively correlation (R = 0.61; $p < 0.01$) between manganese and heterotrophic bacteria recovered on different strengths of nutrient agar. Manganese removal in the wetland system was reported to be related to the oxygenic photosynthesis resulting in high removal rates during the summer season (Hallberg & Johnson, 2005).

Lesley et al. (2008) indicated that manganese can be removed successfully using wetland systems referring that iron needs to be removed before manganese removal is able to take place. High manganese concentrations resulted in low growth rates of plants (Rahimi et al., 2013). However, manganese is an essential trace element for most plants, intervening in several metabolic processes, mainly in photosynthesis. Nevertheless, an excess of this micronutrient is often toxic for plants. Manganese phyto-toxicity is exhibited in a reduction of biomass and photosynthesis, and biochemical disorders including oxidative stress (Millaleo, Reyes-Díaz, Ivanov, Mora, & Alberdi, 2010).

Table 6.4 shows that zinc concentrations in irrigation waters were the highest for Filter 6 outflow water followed by that for Filter 2, while the lowest value was observed for Filter 4 outflow water. Outflow waters from Filter 2 of large aggregate diameter showed zinc concentrations which were higher than those for Filter 4, which had small aggregate size. Filter 4 outflow water had zinc concentrations lower than those for Filter 7, while the latter had zinc concentrations greater than those for Filter 8, due to differences in both contact and resting time, respectively, in the design of the wetland system.

Moreover, irrigation water harvested from Filter 6 had zinc concentrations which were higher than those for Filter 4 due to differences in inflow loading rate. However, statistical analysis results (Table 6.2) did not show any significant differences in irrigation water zinc concentrations associated with wetland filters of different design, indicating that aggregate diameter, contact time, resting time and inflow loading rate may not matter in zinc removal processes (Almuktar & Scholz, 2016 a, b). This agreed with the results reported by Ying et al. (2001) who investigated the efficiency of eight laboratory-scale constructed wetlands to treat heavy metals in synthetic mine water, which indicated that hydraulic loading, and substrate composition usually did not affect the treatment efficiency.

According to Sheoran and Sheoran (2006), heavy metals can be removed in wetland systems via physical, chemical and biological processes including sedimentation, settling, filtration, adsorption, precipitation, and co-precipitation into insoluble compounds. Compared to the standard of 2.0 mg/l for zinc (Table 6.3), statistical analysis showed that all irrigation water types had zinc levels which were significantly ($p < 0.05$) lower than the threshold (Pescod, 1992; FAO, 2003).

Table 6.4 shows that the highest potassium concentration values were recorded for Filter 6 outflow water followed by those for Filter 7, while the lowest potassium values were observed for Filter 4 outflow water. Filter 2 of large aggregate diameter had outflow water with potassium concentrations greater than those for Filter 4 of small aggregate size. Filter 7 outflow water had potassium concentrations greater than those for Filters 4 and 8 due to differences in contact and resting time, respectively. This explains the impact of aggregate diameter, contact and resting time variables of wetland design on potassium treatment, as indicated by Almuktar and Scholz (2016 a, b).

Wetland filters which were fed with undiluted wastewater had outflow water with potassium concentrations which were significantly ($p < 0.05$) greater than those of diluted inflow wastewater, as shown when comparing Filter 6 potassium concentrations with those of Filter 4. This agreed with the results reported by Sani et al. (2013)

indicating that filters of high inflow loading rate tent to be overloaded. Compared to the standards of 2.0 mg/l of potassium (FAO,1994, 2003), all irrigation water types were observed with high potassium concentrations which significantly ($p < 0.05$) exceeded the threshold (Table 6.3).

Correlation analysis results showed that potassium concentration values were significantly ($p < 0.05$) positively correlated with most other variables in the system such as biochemical oxygen demand (R = 0.794, $p = 0.006$), ammonia-nitrogen (R = 0.891, $p = 0.001$), ortho-phosphate-phosphorus (R = 0.915, $p < 0.001$), iron (R = 0.867, $p = 0.001$), manganese (R = 0.733, $p = 0.016$), boron (R = 0.903, $p < 0.001$), sodium (R = 0.697, $p = 0.025$, calcium (R = 0.721, $p = 0.019$) and magnesium (R = 0.855, $p = 0.002$), as discussed by Almuktar and Scholz (2016 a, b).

This result confirmed findings from other studies (Choi, Yu, Lee, & Yu, 2011), which explained that there are linear correlation coefficients between the pairs potassium and ortho-phosphate-phosphorus, and magnesium and ortho-phosphate-phosphorus while assessing the role of potassium, magnesium and calcium ions in enhanced biological phosphorus removal from wastewaters using membrane bioreactors. Cakmak (2005) reported that increasing potassium concentration in irrigation water provided important protection against stem damage from low night temperatures in plants. Furthermore, decreases in yield and increases in leaf damage induced by frost under field conditions could be alleviated by high application of potassium fertiliser.

Hakerlerler, Oktay, Eryüce, and Yagmur (1997) indicated that improving low-temperature-stress tolerance of plants by increasing potassium supply was also shown in tomato, pepper, and eggplant seedlings growing outside, with temperatures ranging from 4 to 16 °C. Depending on the source of potassium fertilisers, potassium supply enhanced total plant yield by 2.4-fold, 1.9-fold, and 1.7-fold in tomato, pepper, and eggplant, respectively. Moreover, potassium supply also reduced the rate of seedling death due to low temperature (Cakmak, 2005).

Table 6.4 shows that the highest boron concentration values were observed in outflow waters of Filters 6 followed by those of Filter 2, while the lowest boron values were observed in Filter 7 outflow water. Filter 2 outflow waters had boron concentrations higher than those for Filter 4, due to differences in aggregate diameter. Filters 4 and 7 harvested waters had boron concentrations which were relatively similar to those for Filters 7 and 8, respectively.

Moreover, irrigation waters harvested from Filter 6 of high inflow loading rate had boron concentrations greater than those for Filter 4 of diluted inflow waters. However, statistical results (Table 6.2) did not show any significant ($p > 0.05$) differences among boron concentrations associated with wetland filters of different variable design, indicating that aggregate diameter, contact time, resting time and inflow loading rate may not matter in boron treatment (Almuktar & Scholz, 2016 a, b).

Compared to the standard of 0.75 mg/l for boron (FAO, 1994, 2003), statistical analysis (Table 6.3) showed that all irrigation water types had boron concentration values which were significantly ($p < 0.05$) lower than the threshold. Correlation analysis results showed than boron concentrations were significantly ($p < 0.05$) positively correlated with most other elements in the system such as biochemical oxygen demand (R = 0.745, $p = 0.013$), ammonia-nitrogen (R = 0.915, $p < 0.001$), ortho-phosphate-phosphorus (R = 0.915, $p < 0.001$), iron (R = 0.709, $p = 0.022$), potassium (R = 0.903, $p < 0.001$), sodium (R = 0.709, $p = 0.022$) and magnesium (R = 0.806, $p = 0.005$), as reported by Almuktar and Scholz (2016 a).

Table 6.4 shows that sodium concentrations were observed with the highest values in Filter 6 outflow water followed by those for Filter 7, while the lowest sodium concentrations were recorded for Filter 8 outflow water. Drain waters from Filters 2, 4, 7 and 8 showed relatively similar sodium concentrations. Statistical analysis results (Table 6.2) showed that waters harvested from Filter 4 had sodium concentrations which were significantly ($p < 0.05$) lower than those of Filter 6, explaining the impact of high inflow loading rate of wetlands on outflow water sodium concentrations resulting in filter overload (Sani et al., 2013).

Compared to the standard of 920 mg/l for sodium (Table 6.3), statistical analysis results showed that all irrigation water types had sodium concentrations which were significantly ($p < 0.05$) lower than the threshold (Pescod, 1992; FAO, 2003). Moreover, correlation analysis results showed that sodium concentrations were significantly ($p < 0.05$) positively correlated with other elements in the system such as ammonia-nitrogen ($R = 0.794$, $p = 0.006$), ortho-phosphate-phosphorus ($R = 0.721$, $p = 0.019$), potassium ($R = 0.697$, $p = 0.025$), boron ($R = 0.709$, $p = 0.022$), calcium ($R = 0.673$, $p = 0.033$) and magnesium ($R = 0.867$, $p = 0.001$), as indicated previously by Almuktar and Scholz (2016 a, b).

Calcium concentrations were observed with highest values in Filter 6 outflow water followed by those for Filter 4, while the lowest values were recorded for Filter 2 outflow water (Table 6.4). Irrigation water obtained from Filter 2 of large aggregate diameter had calcium concentrations lower than those for Filter 4 of small aggregate size, while the latter had calcium concentrations greater than those for Filter 7 outflow water due to differences in contact time. Moreover, water harvested from Filter 4 which was fed with diluted wastewater had calcium concentrations lower than that for Filter 6 of high inflow loading rate. Compared to the standards of 400 mg/l for calcium (FAO, 2003, Pescod, 1992), statistical analysis results (Table 6.3) showed that all irrigation water types had calcium concentrations which were significantly ($p < 0.05$) lower than the threshold. Moreover, correlation analysis results showed that calcium concentrations were significantly ($p < 0.05$) positively correlated with iron (R = 0.818, $p = 0.004$), zinc (R = 0.661, $p = 0.038$), potassium (R = 0.721, $p = 0.019$, sodium (R = 0.673, $p = 0.033$), and magnesium (R = 0.855, $p = 0.002$) values in the system (Essington, 2015). The highest magnesium concentrations were observed in irrigation waters harvested from Filter 6 of high inflow loading rate, while the lowest concentrations were recorded for Filter 8 outflow water (Table 6.4).

Filters 2 and 4 drain waters had similar magnesium concentrations, while magnesium values in irrigation water obtained from Filter 7 were higher than those harvested from Filters 4 and 8, explaining the impact of contact and resting times on magnesium treatment by wetland system. Statistical analysis results (Table 6.2) showed that outflow waters from Filter 6 of high inflow loading rate had magnesium levels which were significantly ($p < 0.05$) greater than those from Filter 4 which was fed with diluted wastewater. Compared to the standard of 60 mg/l for magnesium (Pescod, 1992; FAO, 2003), statistical analysis results (Table 6.3) showed that all irrigation water types had magnesium levels which were significantly ($p < 0.05$) lower than the threshold. Moreover, correlation analysis results showed that magnesium concentrations were significantly ($p < 0.05$) positively correlated with most other variables in the treatment system (Essington, 2015).

However, in constructed wetlands, heavy metals and trace elements can be removed by various mechanisms. For example, Denga, Yea, and Wonga (2004); Galletti, Verlicchi, and Ranieri (2010) and Guittonny-Philippe et al. (2014) reported that these elements can be removed via different physical, chemical, and biological processes performed in the wetland systems such as settling, sedimentation sorption, adsorption, complexation, cation and anion exchange, oxidation and reduction, chemical precipitation and co-precipitation as insoluble salts, photo-degradation, phyto-accumulation, biodegradation, microbial activity, and plant uptake. In vertical-flow wetlands, these elements are most likely to accumulate in the litter layer at the top of the system, while in horizontal-flow wetlands, heavy metals and trace elements tend to accumulate near the system inlet regardless of elimination pathways (Cheng et al., 2002). However, most of those elements available in the wastewater are removed in wetlands through the interaction with system media after treatment by wetland plants which is considered as a polishing system, as stated by Matagi, Swai, and Muganbe (1998) and Guittonny-Philippe et al. (2014).

Moreover, in wetland systems, heavy metals can be removed effectively by settling and sedimentation processes after a series of dynamic transformations performed in the system (Prestes et al., 2006; Terzakis et al., 2008; Matagi et al., 1998). However, the sedimentation of those heavy metals will occur after their agglomeration to bigger particles that can be trapped by wetland sediment as reported by Walker and Hurl (2002). According to Scholz (2006, 2010), Wetlands macrophytes can also be considered as a trapper to the metal solids available in the wastewater while it passes through the surface of system plants.

Moreover, the accumulation of heavy metals in wetland biomass can be considered as a predominant way to eliminate those metals in the wetland system, as reported by Madera-Parra et al. (2015) who agreed with the observation reported by Guittonny-Philippe et al. (2014) showing that the heavy metals in the wetland system can be removed by accumulation in the system sediment as well as in different parts of macrophyte tissue such as roots, stems, leaves and shoots.

Furthermore, the sorption process in wetland systems, which includes adsorption, absorption and precipitation reactions, can be considered as the main chemical means of heavy metal removal (Marchand et al., 2010). However, wetland macrophytes can uptake heavy metals with different capacities depending on several factors, such as plants species, heavy metal levels, sediment chemistry and pH, in addition to the temperature and organic matter content, as reported by Sheoran (2004), Sheoran and Sheoran (2006), Liu et al. (2007) and Marchand et al. (2010).

Table 6.4: Overview of the Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) analysis for the detected trace elements in the irrigation waters received by vegetable pots (mean±standard deviation (number of samples)).

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Iron (mg/l)				
Filter 2	0.232±0.2442 (15)	0.485±0.3373 (3)	0.488±0.5323 (3)	0.123±0.0746 (9)
Filter 4	0.173±0.1720 (15)	0.392±0.1518 (3)	0.206±0.1288 (3)	0.079±0.0498 (9)
Filter 6	0.475± 0.7163 (15)	1.337±0.8403 (3)	1.172±1.5704 (3)	0.106±0.0632 (9)
Filter 7	0.533±1.1726 (15)	1.617±1.9142 (3)	1.937±2.6485 (3)	0.069±0.0473 (9)
Filter 8	0.2660±0.5850 (15)	0.762±1.0062 (3)	0.994±1.3059 (3)	0.054±0.0237 (9)
Manganese (mg/l)				
Filter 2	0.062±0.0692 (15)	0.130±0.0625 (3)	0.106±0.1359 (3)	0.021±0.0297 (9)
Filter 4	0.067±0.0659 (15)	0.135±0.0648 (3)	0.142±0.0969 (3)	0.033±0.0336 (9)
Filter 6	0.100±0.1301 (15)	0.227±0.1583 (3)	0.216±0.2655 (3)	0.037±0.0506 (9)
Filter 7	0.138±0.2597 (15)	0.313±0.3490 (3)	0.358±0.5055 (3)	0.006±0.0068 (9)
Filter 8	0.092±0.1172 (15)	0.171±0.1242 (3)	0.160±0.2169 (3)	0.013±0.0147 (9)
Zinc (mg/l)				
Filter 2	0.051±0.0055 (15)	0.053±0.0021 (3)	0.052±n.a (1)	0.049±0.0099 (11)
Filter 4	0.043±0.0073 (15)	0.044±0.0064 (3)	0.051±n.a (1)	0.042±0.0113 (11)
Filter 6	0.058±0.0132 (15)	0.052±0.0046 (3)	0.057±n.a (1)	0.068±0.0191 (11)
Filter 7	0.046±0.0104 (15)	0.051±0.0052 (3)	0.054±n.a (1)	0.040±0.0148 (11)
Filter 8	0.045±0.0098 (15)	0.047±0.0128 (3)	0.058±n.a (1)	0.041±0.0042 (11)

Table 6.4 (cont.)

Parameter	Overall[a]	SPP[b]	FPPBF[c]	FPPAF[d]
Potassium (mg/l)				
Filter 2	6.667±3.2904 (15)	5.757±1.1359 (3)	9.835±3.9599 (3)	7.057±3.9012 (9)
Filter 4	4.472±4.8224 (15)	1.502±0.4315 (3)	6.118±7.0371 (3)	5.744±5.3408 (9)
Filter 6	12.097±6.5985 (15)	10.902±2.9090 (3)	18.868±6.5520 (3)	12.609±7.8402 (9)
Filter 7	7.017±3.1901 (15)	6.565±1.0562 (3)	9.168±1.9706 (3)	7.211±3.8403 (9)
Filter 8	4.961±4.0407 (15)	2.676±1.6215 (3)	8.142±5.8077 (3)	5.941±4.4592 (9)
Boron (mg/l)				
Filter 2	0.084±0.0723 (15)	0.000±0.0000 (3)	0.064±0.0908 (3)	0.120±0.0531 (9)
Filter 4	0.066±0.0506 (15)	0.000±0.0000 (3)	0.066±0.0934 (3)	0.094±0.0272 (9)
Filter 6	0.096±0.0665 (15)	0.012±0.02606 (3)	0.114±0.1110 (3)	0.132±0.0388 (9)
Filter 7	0.061±0.0525 (15)	0.003±0.0046 (3)	0.062±0.0766 (3)	0.086±0.0413 (9)
Filter 8	0.063±0.0469 (15)	0.000±0.0000 (3)	0.054±0.0771 (3)	0.091±0.0205 (9)
Sodium (mg/l)				
Filter 2	41.737±11.3778 (15)	37.031±3.1002 (3)	47.986±11.8149 (3)	43.754±13.2349 (9)
Filter 4	41.276±13.4063 (15)	34.924±5.5490 (3)	44.956±20.2390 (3)	43.998±15.1826 (9)
Filter 6	65.321±10.9490 (15)	76.591±8.3651 (3)	76.238±12.4346 (3)	60.492±8.1107 (9)
Filter 7	42.117±13.4779 (15)	35.682±6.9387 (3)	47.165±5.2208 (3)	44.874±15.0618 (9)
Filter 8	39.891±14.7070 (15)	30.763±16.6967 (3)	49.660±4.9424 (3)	43.804±13.1153 (9)
Calcium (mg/l)				
Filter 2	31.972±7.6851 (15)	32.983±n.a (1)	30.934±2.8977 (3)	31.972±7.6851 (11)
Filter 4	43.054±12.7731 (15)	41.875±n.a (1)	40.328±2.1888 (3)	43.054±12.7731 (11)
Filter 6	54.106±17.2773 (15)	53.366±n.a (1)	51.717±2.3315 (3)	54.106±17.2773 (11)
Filter 7	39.065±6.5656 (15)	35.152±n.a (1)	37.168±2.8523 (3)	39.065±6.5656 (11)
Filter 8	39.355±7.2702 (15)	40.909±n.a (1)	40.050±1.2149 (3)	39.355±7.2702 (11)

Table 6.4 (cont.)

FPPBF[c]	FPPAF[d]			
		Magnesium (mg/l)		
Filter 2	5.045±1.0367 (15)	5.433±0.4193 (3)	6.268±0.5282 (3)	4.879±1.2026 (9)
Filter 4	5.066±1.3099 (15)	5.028±0.1917 (3)	5.786±1.2973 (3)	5.082±1.6001 (9)
Filter 6	9.019±2.3105 (15)	10.203±1.0255 (3)	11.675±0.4340 (3)	8.512±2.5802 (9)
Filter 7	5.280±0.8894 (15)	5.487±0.9686 (3)	6.221±0.5406 (3)	5.192±0.9184 (9)
Filter 8	4.776±1.3933 (15)	4.767±2.4392 (3)	6.728±0.5648 (3)	4.780±0.9636 (9)

Note: [a] 11/10/14 to 25/09/15; [b] SPP, second planting period: 11/10/14 to 07/11/14; [c] FPPBF, final planting period before fruiting: 08/11/14 to 19/01/15; and [d] FPPAF, final planting period after fruiting: 20/01/15 to 25/09/15. Detection limits (mg/l) are: 0.10×10^{-3}, 0.03×10^{-3}, 0.20×10^{-3}, 0.30×10^{-3}, 0.10×10^{-3}, 0.15×10^{-3}, 0.01×10^{-3} and 0.01×10^{-3} for iron, manganese, zinc, potassium, boron, sodium, calcium and magnesium, respectively. Elements not listed in this table (i.e., arsenic, barium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, strontium and titanium) were either below (or close to) the detection limits or could not be measured via the ICP–OES technology. n.a, not applicable.

6.2.4 Comparison of particles

Table 6.1 shows that the maximum suspended solids and turbidity values were observed in Filter 2 outflow water followed by those for Filter 6, while the minimum values were recorded for Filter 8 outflow water. Irrigation water harvested from Filter 2 had suspended solids and turbidity values, which were significantly ($p < 0.05$) greater than those for Filter 4 (Table 6.2) explaining the impact of wetland aggregate size on the particle removal process (Sani et al., 2013). Moreover, Filter 7 outflow water had suspended solids and turbidity values higher than those of Filter 8, due to differences in resting time. Inflow loading rate of the wetland system impact significantly ($p < 0.05$) on filter outflow particle levels, as shown when comparing Filter 6 with Filter 4 (Table 6.2).

Correlation analysis results showed that suspended solids, turbidity, chemical oxygen demand, biochemical oxygen demand and monitored microorganisms in the system (Total coliforms, *Escherichia coli, Streptococci* spp. and *Salmonella* spp.) were significantly ($p < 0.05$) positively correlated with each other in the treatment system. This can be explained by the fact that increasing the microorganisms in the treatment system will increase the biodegradation process for the organic matter resulting in high suspended solids and turbidity concentrations in the outflow waters.

This indicates a good relationship between suspended solids, turbidity and indicator microorganisms activity due to degradation of organic matter and a subsequent increase in particles (Sani et al., 2013; Almuktar & Scholz, 2015). Suspended solids and turbidity values for irrigation waters obtained from the wetland system greatly fluctuated during different crop growth periods due to seasonal behaviour change in the wetland system. For example, as above-ground *p.australis* plant parts decay in winter and early spring, more particles are created as by-products of the biodegradation process (Scholz, 2010, 2011; Sani et al., 2013; Almuktar & Scholz, 2016 b).

However, high values of suspended solids and turbidity associated with irrigation water will considerably increase the development of hydrophobicity in the soil, and subsequently affect plant growth (Travis et al., 2010; Almuktar et al., 2015 a, b; Almuktar & Scholz, 2016 a, b). Previous studies (Kadlec & Knight, 1996; Green et al., 1997; Garcia et al., 2010; Hua et al., 2013) reported that most solids can be removed using constructed wetlands (CWs) technology through sedimentation, settling, adsorption and biological degradation processes performed in CWs systems. Moreover, in surface flow constructed wetlands, solids removal will occur through flocculation, sedimentation and filtration processes undertaken in the system, as reported by Kadlec and Wallace (2009).

In addition, interaction and adhering of suspended solids with other constituents available in wetlands systems, such as heavy metals and nutrients, pathogens and organic matter, will improve their removal from wastewater (Sundaravadivel & Vigneswaran, 2001). In subsurface vertical-flow constructed wetlands, the removal of solids will depend on characteristics of the substrate, hydraulic load and microorganisms available in the system (Manios, Stentiford, & Millner, 2003).

6.2.5 Comparison of pH and salinity

Table 6.1 shows Filter 6 outflow water had the highest pH values followed by those for Filter 7, while the lowest values were recorded for Filter 2 outflow water. Table 6.2 showed that there is a statistically significant ($p < 0.05$) difference in pH values of outflow waters from filters compared in terms of contact and resting times as well as the inflow loading rate. However, the pH values for all irrigation water types were within the normal range of between 6.0 and 8.5 (Pescod, 1992; FAO, 2003) .

Moreover, pH values varied throughout the day due to respiration (after sunset) and photosynthesis (after sunrise) of plants in the wetland systems. This directly affected the dissolved oxygen and carbon dioxide concentrations in the systems leading to fluctuations in pH values. For example, after sunset, dissolved oxygen concentrations decline as photosynthesis stops and all plants and animals in the system consume oxygen (respiration) resulting in increasing of carbon dioxide concentrations. The latter will react with the water producing carbonic acid leading to reduce pH values.

On the other hand, during day time the dissolved oxygen will increase and carbon dioxide will decrease due to photosynthesis, leading to increased pH values of water in the system (Zang et al., 2011). Wastewater pH is an important factor that may affect the performance of wetlands, mainly in terms of nitrogen and organic matter removal.

For example, consumption of most of the alkalinity during the nitrification process will lead to a significant drop in pH values in the system, subsequently affecting the denitrification rate as discussed by Kadlec and Knight (1996). However, the optimum pH value for the denitrification process can range between 6.0 and 8.0, while the highest rate can occur at a pH value of 7.0 to 7.5, as reported by Saeed and Sun (2012).

Moreover, Vymazal (2007) noted that the slower rate of denitrification process can occur at a pH value of 5.0, while insignificant denitrification rate can be observed at pH values below 4. Wastewater pH value is also important for organic matter, mainly for anaerobic degradation processes (Saeed & Sun, 2012). This is because of the high sensitivity of the bacteria responsible for formation of methane gas in the system to the narrow ranges of pH values; they can survive only in pH values of between 6.5 and 7.5. As a result, the anaerobic degradation process will not complete if the pH value is not in this range, leading to volatile fatty acid accumulation in the system and a subsequent drop in the pH value which will kill all methanogens available in the wetland system, as reported by Copper et al. (1996) and Vymazal (1999).

Electrical conductivity is the most important indirect measure of salinity, posing a great hazard to crops and determining the suitability of water for irrigation use (FAO, 1994, 2003). Table 6.1 shows that the highest electrical conductivity values were recorded for Filter 6 outflow water followed by those of Filter 8, while the lowest values were recorded for Filter 2 drain water. Outflow water from Filter 2 had electrical conductivity values lower than those for Filter 4 explaining the impact of the aggregate diameter of wetlands on outflow water salinity concentrations (Almuktar & Scholz, 2016 a, b). Filter 4 drain water had electrical conductivity values lower than those for Filter 7 due to differences in contact time.

Filter 6 which was fed with undiluted wastewater had outflow waters of salinity values which were significantly ($p < 0.05$) greater than those for Filter 4 of low inflow loading rate, confirming the results obtained by other researchers which indicated that filters of high inflow loading rate tend to be overloaded (Sani et al., 2013). Compared to the standards of 3000 µS/cm for electrical conductivity (Pescod, 1992; FAO, 2003), all irrigation water types had salinity values which were significantly ($p < 0.05$) lower than the threshold (Table 6.3). High levels of electrical conductivity in irrigation water create saline soil.

Salts negatively impact on the growth of plants, soil structure and soil permeability which indirectly affects plant growth as well (Maas & Grattan, 1999). Correlation analysis results showed that salinity values in the treatment system were significantly ($p < 0.05$) positively correlated with the values of other elements such as ammonia-nitrogen (R = 0.685, $p = 0.029$), ortho-phosphate-phosphorus (R = 0.782, $p = 0.008$), iron (R = 0.806, $p = 0.005$), manganese (R = 0.648, $p = 0.043$), potassium (R = 0.794, $p = 0.006$), sodium (R = 0.818, $p = 0.004$), calcium (R = 0.927, $p < 0.001$) and magnesium (R = 0.891, $p = 0.001$), as discussed by Almuktar and Scholz (2016 a, b).

6.2.6 Comparison of microbial content

Microbial characteristics of irrigation waters are summarised in Table 6.5. The results show that the highest total coliforms values were recorded for the outflow from Filter 6 followed by those of Filter 2, while the lowest values were observed for Filter 8 outflow water. Irrigation waters harvested from Filter 2 had total coliforms greater than those for Filter 4, indicating the impact of aggregate diameter on total coliforms removal processes.

Harvested water from Filter 4 had total coliforms which were significantly ($p < 0.05$) different from those for Filters 6 and 7, due to differences in filter loading rate and contact time. Moreover, Filter 7 outflow water had total coliforms higher than those for Filter 8, indicating the impact of the resting time variable of the wetland system. Compared to the standards of 1000 CFU/100 ml (USEPA, 2004) for total coliforms to irrigate crops, which are often eaten uncooked, all irrigation waters were too highly contaminated by total coliforms, significantly ($p < 0.05$) exceeding the threshold (Table 6.3).

Correlation analysis results showed that total coliforms in the treatment system were significantly ($p < 0.05$) positively correlated with other variables such as chemical oxygen demand, biochemical oxygen demand, iron, suspended solids, turbidity, *Escherichia coli and Salmonella* spp., while total coliforms were significantly ($p < 0.05$) negatively correlated with dissolved oxygen in the system, as discussed previously (Almuktar & Scholz, 2015, 2016 a).

Contamination with *Escherichia coli was* detected only in outflow water from Filters 2, 6, and 8 following this order: Filter 6 > Filter 2 > Filter 8 (Table 6.5). Irrigation water harvested from Filter 2 of large aggregate diameter had *Escherichia coli* values (71 CFU/100ml) greater than those for Filter 4 of small aggregate diameter (0 CFU/100ml). Filter 6 of high inflow loading rate had *Escherichia coli* values (5572 CFU/100 ml), which were considerably greater than those for Filter 4 of low inflow loading rate (0 CFU/100ml). Moreover, water harvested from Filter 8 of long resting time had *Escherichia coli* values (59 CFU/100 ml) greater than those for Filter 7 (0 CFU/100 ml) of short resting time. No contamination by *Escherichia coli* was detected for outflow waters from Filters 4 and 7.

However, this result contradicted findings reported by Cirelli et al. (2012), who presented results of a reuse scenario where municipal wastewater was treated by constructed wetlands (tertiary treatment step), and reused for the supply of irrigation water for vegetables in Eastern Sicily, Italy. They found increased numbers of *Escherichia coli* in the irrigation water, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 ml for secondary treated urban wastewater effluents.

Moreover, correlation analysis results showed that *Escherichia coli* values in the treatment system were significantly ($p < 0.05$) positively correlated with other variables, such as chemical and biochemical oxygen demands, iron, manganese, suspended solids and turbidity, as well as with other microbes in the system, such as total coliforms, *Streptococcus* spp. and *Salmonella* spp., while being significantly ($p < 0.05$) negatively correlated with dissolved oxygen in the system, as reported by Almuktar and Scholz (2015, 2016 a).

The highest *Salmonella* spp. count was observed in the outflow water from Filter 6 followed by that for Filter 2 (Table 6.5), while the lowest values were observed for Filter 7. Filter 2 outflow water was associated with higher *Salmonella* spp. contamination than the water from Filter 4 due to differences in aggregate diameter. Outflow waters from Filter 4 had *Salmonella* spp. colonies which were significantly ($p < 0.05$) greater than those for Filter 7 (Table 6.2), while the latter had colonies lower than those associated with Filter 8, indicating the impact of contact and resting times of wetland design on outflow microbe levels. Moreover, Filter 6 of high inflow loading rate had *Salmonella* spp. contamination which was significantly ($p < 0.05$) greater than that for Filter 4 of low inflow loading rate, as shown in Tables 6.1 and 6.2, confirming the results reported previously (Sani et al., 2013; Almuktar & Scholz, 2015).

Correlation analysis results showed that *Salmonella* spp. values in the treatment system were significantly ($p < 0.05$) positively correlated with chemical and biochemical oxygen demands, iron, manganese, suspended solids, and turbidity as well as with other microbes such as total coliforms and *Escherichia coli*, confirming the results reported previously (Almuktar & Scholz, 2015, 2016 a, b).

Generally, wetland filters fed with undiluted inflow water showed higher microbial contamination levels than those fed with diluted inflow. This confirms results by Sani et al. (2013) that high rate filters tend to be overloaded. Filters of large aggregate diameter showed microbial contamination levels higher than those of small aggregate diameter. This is because a large aggregate size allows for more microorganisms to colonise the empty spaces between the filter media (Almuktar & Scholz 2015, 2016 a, b). Constructed wetland systems have been reported to remove various types of pathogens effectively (Scholz, 2006, 2010). Arias et al. (2003), Hansen et al. (2004) and Molleda et al. (2008) demonstrated that in subsurface flow constructed wetlands, pathogens can be removed through different mechanisms such as antibiotics excretion (Garcia et al., 2013).

However, this mechanism cannot be evidenced, as reported by Stottmeister et al., (2003). Moreover, in constructed wetlands, pathogens can be removed directly or indirectly via different processes such as filtration, sedimentation, adsorption, and predation by protozoa and bacteriophages (Kadlec & Wallace, 2008). An investigation of the role of sedimentation in pathogen removal in wetland systems was performed by Karim et al. (2004). The authors' results showed that, statistically, there are no significant differences in faecal coliform and coliphage numbers in effluent water compared to those in the sediment, indicating that macrophyte roots of wetland systems play an important role in pathogen removal. These results agreed with those obtained by Garcia et al., (2013) who reported that *E-Coli* were removed well by wetland plants.

Table 6.5: Microbiological examination for the irrigation waters (colony forming units (CFU)/100 ml).

Microbes	Mean	Standard deviation	Minimum	Maximum
Total coliforms				
Filter 2	100 071	127 775.3	2000	386 000
Filter 4	79 500	117 307.5	0	449 000
Filter 6	157 071	145 767.7	10 000	550 000
Filter 7	7154	9590.3	0	35 000
Filter 8	4438	6663.0	0	20 000
Escherichia coli				
Filter 2	71	267.3	0	1000
Filter 4	0	0.0	0	0
Filter 6	5571	19 991.2	0	75 000
Filter 7	0	0.0	0	0
Filter 8	59	242.5	0	1000
***Streptococci* spp.**				
Filter 2	0	0.0	0	0
Filter 4	0	0.0	0	0
Filter 6	0	0.0	0	0
Filter 7	0	0.0	0	0
Filter 8	0	0.0	0	0
***Salmonella* spp.**				
Filter 2	85 462	100 888.2	0	323 000
Filter 4	73 143	90 000.7	0	295 000
Filter 6	176 000	178 138.7	0	450 000
Filter 7	5333	9209.2	0	31 000
Filter 8	7529	15 391.2	0	60 000

Note: 0 entries indicate absolutely no growth on the plate after incubation. Twenty water samples were tested.

6.3 Environmental boundary conditions

Table 6.6 shows an overview of the environmental boundary conditions associated with the vegetable pots. The light intensity records for this experiment during the flowering and fruiting stage were below the proposed range from about 8600 and 17200 lux (Deli & Tiessen, 1969). Low light intensity may lead to flower inhibition or cause flower abscission (Wein & Zhang, 1991). Moreover, low light intensity applied to plants will produce leggy plants growing toward light, which is necessary for photosynthesis (Ciju, 2013).

Concerning the vegetative growth stage, the temperature records for this experiment were higher than the recommended optimum values of between 21 and 23 °C (Bakker & Ufflen, 1988). However, temperature records for this stage complied with the values associated with the highest photosynthesis rate, which can be achieved at temperatures between 24 and 29 °C (Bhatt & Srinivasa Rao, 1989; Nilwik, 1980). Table 6.6 shows that the relative humidity before and after fruiting was low (45% and 55%, respectively). Humidity values below 50% could have a negative impact on the fruit development, as a humid atmosphere is necessary for flowers to successfully pollinate, otherwise, the unfertilised flowers will drop off, as reported elsewhere (Nilwik, 1980).

Table 6.6: Overview of environmental boundary conditions associated with the vegetable pots.

Parameter	Overall (11/10/2014 to25/09/2015)					SPP (11/10/2014 to 7/11/2014)					FPPBF (8/11/2014 to 19/01/2015)					FPPAF (20/01/15 to 25/09/2015)				
	Mean	STD	Min	Max	No.	Mean	STD	Min	Max	No.	Mean	STD	Min	Max	No.	Mean	STD	Min	Max	No.
A	2785	1004	1328	9930	1376	3990	1136	2532	6680	104	2838	1415	1330	6680	216	2656	799	1328	9930	1056
B	23.9	3.21	14.9	29.6	176	23.5	1.68	20.6	26.1	14	22.0	2.81	14.9	26.1	27	24.4	3.27	17.7	29.6	135
C	19.5	2.93	13.3	25.5	175	19.4	2.88	16.4	25.5	13	18.3	3.17	13.3	25.5	27	19.8	2.84	13.9	25.1	135
D	25.6	3.10	17.3	32.1	175	24.7	1.65	21.9	27.7	13	23.2	2.54	17.3	27.7	27	26.2	3.06	18.1	32.1	135
E	53.0	12.61	31.0	89.0	175	46.3	8.45	31	61	13	45.2	7.56	31	61	27	55.2	12.94	33	89	135
F	38.0	4.90	30.0	60.0	175	39.2	4.99	31	45	13	39.1	4.30	31	49	27	37.7	4.98	30	60	135
G	59.0	12.48	35.0	99.0	175	53.3	7.34	41	71	13	51.1	7.59	35	71	27	61.1	12.88	35	99	135

Note: SPP, second planting period; FPPBF, final planting period before fruiting; FPPAF, final planting period after fruiting; A, illuminance (one-off record during lab visit); B, temperature (one-off record during lab visit); C, temperature (minimum within a 24-hour period); D, temperature (maximum within a 24-hour period); E, relative humidity (one-off record during lab visit); F, relative humidity (minimum within a 24-hour period); G, relative humidity (maximum within a 24-hour period), STD, standard deviation; Min, minimum value; Max, maximum value; and No., number of readings.

6.4 Chilli generation growth comparisons and marketable yield assessment

Figure 6.2a–f show Chilli growth details in terms of plant overall height, number of leaves, buds, flowers, fruits and total weight of fruits harvested from each treatment compared to their mother plants. Regarding the overall height of generation plants, Figure 6.2a shows that the maximum height was associated with plants irrigated with outflow from Filter 8 (502 mm) followed by those plants irrigated with Filter 2 drain water (442 mm), while the minimum height was recoded for plants irrigated with water harvested from Filter 4 (266 mm).

Table 6.7 shows that the growth rate for plant height compared to the base line follows this order: Filter 8 > Filter 2 > Filter 7 > Filter 6 > Filter 4. Statistical analysis results (Table 6.8) showed that plants irrigated with Filters 2 and 7 outflow waters had mean heights which were significantly ($p < 0.05$) greater than those for Filter 4, explaining the impact of wetland aggregate diameter and contact time variables on outflow water qualities and subsequently on the total elements mass applied on plants via irrigation water (Figures 6.3 and 6.4).

Moreover, plants irrigated with Filters 8 and 6 outflow waters had mean height greater than those for Filters 7 and 4, respectively, explaining the impact of wetland system resting time and inflow loading rate variables on nutrients and elements supplied to the irrigated plants. However, all Chilli generation plants (except those linked to Filter 4) showed mean height values which complied with those reported by Nickels (2012) which indicated that Chilli plants can grow to about 45 cm in height. Compared to their mothers (Figure 6.2a), generation plants showed considerably shorter heights explaining the impact of reducing irrigation water volume applied on generation plants (Figure 6.3), resulting in noticeable reduction in total elements applied mass (Figure 6.4) and subsequent shorter plants as reported by FAO (1994, 2003).

Regarding the total number of leaves (Figure 6.2b), results showed that the maximum leaf number was associated with plants irrigated with outflow from Filters 7 and 8, while the minimum values were recoded for plants irrigated with water harvested from Filter 4.

Table 6.7 shows that the growth rate of plants in terms of leaf number compared to the base line follows this order: Filter 7 > Filter 8 > Filter 6 > Filter 2 > Filter 4. Plants irrigated with Filters 2 and 4 outflow waters had relatively similar mean leaf numbers, while Chillies irrigated with Filters 7 and 6 drain water showed mean leaf numbers which were greater than those of Filter 4.

This can be explained by the differences in contact time and inflow loading rate variables of the wetland system which subsequently impact on total element mass applied via irrigation water (Figures 6.3 and 6.4). However, statistical analysis results (Table 6.8) showed that there were no significant differences ($p > 0.05$) among Chilli generation leaf numbers. Furthermore, all generation plants (except those linked to Filter 2) produced a higher leaf number than their mother plants (Figure 6.2b).

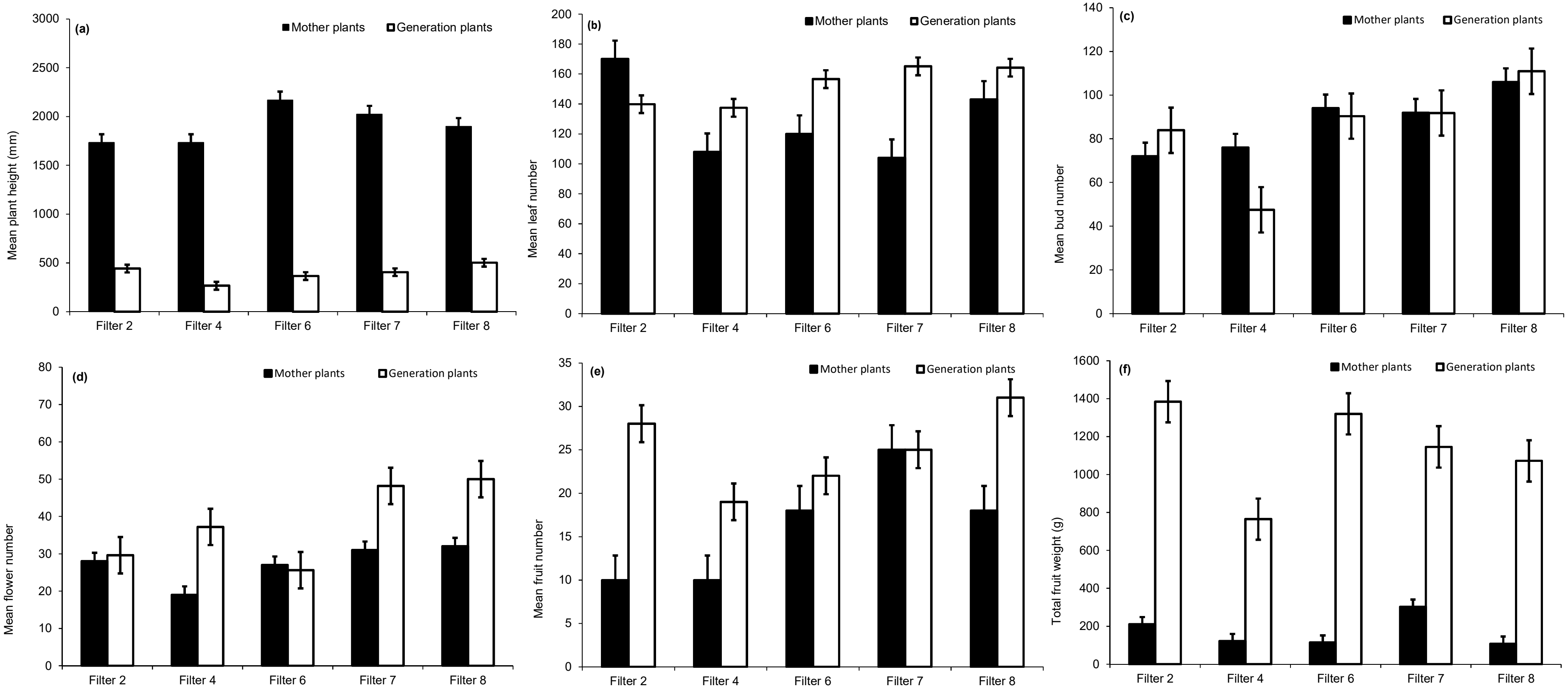

Figure 6.2: Overview of growth of Chilli mother and generation plants subjected to different irrigation water types: (a) mean plant height; (b) mean leaf number; (c) mean bud number; (d) mean flower number; (e) mean fruit number; and (f) total fruit weight.

Table 6.7: Overview of Chilli generation plant growth rate in terms of height and leaf number.

Water source	Measurement	Replicate number										Mean	Standard deviation
		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
Plant height (mm)													
Filter 2 outflow	Baseline	30	35	35	25	30	25	45	30	30	25	31	6.1
	Final	450	420	400	410	350	410	510	310	450	710	442	108.9
	Growth rate	420	385	365	385	320	385	465	280	420	685	411	109.4
Filter 4 outflow	Baseline	30	40	30	30	20	30	30	30	30	30	30	4.7
	Final	300	210	270	140	230	390	190	370	310	250	266	78.6
	Growth rate	270	170	240	110	210	360	160	340	280	220	236	79.0
Filter 6 outflow	Baseline	50	50	40	60	40	40	50	30	40	40	44	8.4
	Final	580	220	490	390	370	320	240	260	380	400	365	112.4
	Growth rate	530	170	450	330	330	280	190	230	340	360	321	111.7
Filter 7 outflow	Baseline	30	50	30	40	35	40	40	40	40	40	39	5.8
	Final	320	520	420	450	360	340	540	470	330	300	405	87.0
	Growth rate	290	470	390	410	325	300	500	430	290	260	367	84.5
Filter 8 outflow	Baseline	30	30	40	40	20	20	25	20	20	20	27	8.2
	Final	450	560	540	460	430	490	610	540	550	390	502	68.8
	Growth rate	420	530	500	420	410	470	585	520	530	370	476	68.4
Plant leaf number													
Filter 2 outflow	Baseline	7	8	7	6	6	6	7	5	4	5	6	1.2
	Final	185	102	91	70	182	155	209	134	163	107	140	46.2
	Growth rate	178	94	84	64	176	149	202	129	159	102	134	46.2
Filter 4 outflow	Baseline	6	6	5	5	5	6	5	6	6	6	6	0.5
	Final	136	109	118	49	89	124	121	212	174	242	137	57.4
	Growth rate	130	103	113	44	84	118	116	206	168	236	132	57.1
Filter 6 outflow	Baseline	6	6	6	7	7	7	6	6	9	7	7	0.9
	Final	141	88	134	285	204	132	137	86	182	177	157	58.9
	Growth rate	135	82	128	278	197	125	131	80	173	170	150	58.4

Table 6.7 (cont.)

Water source	Measurement	Replicate number										Mean	Standard deviation
		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
Filter 7 outflow	Baseline	7	7	7	7	7	6	6	7	6	6	7	0.5
	Final	217	127	146	212	197	142	236	122	145	107	165	45.9
	Growth rate	210	120	139	205	190	136	230	115	139	101	159	45.8
Filter 8 outflow	Baseline	6	5	8	5	6	7	5	5	6	7	6	1.1
	Final	130	143	195	181	184	193	225	122	157	112	164	37.0
	Growth rate	124	138	187	176	178	186	220	117	151	105	158	36.9

Note: Growth rate was calculated based on difference between baseline and final measurements.

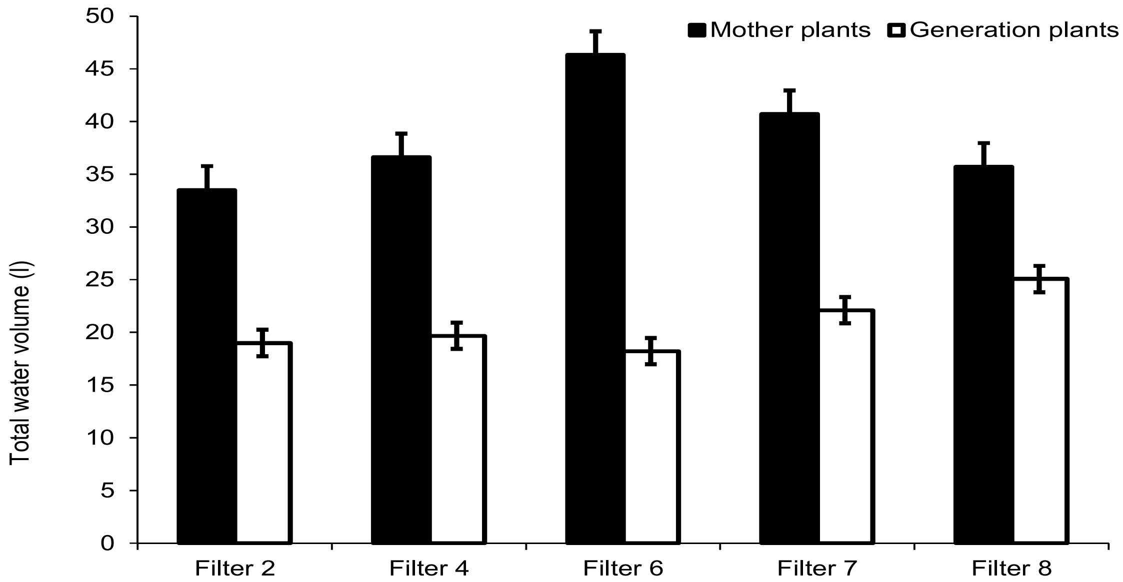

Figure 6.3: Overview of total water volumes for Chilli mother and generation plants.

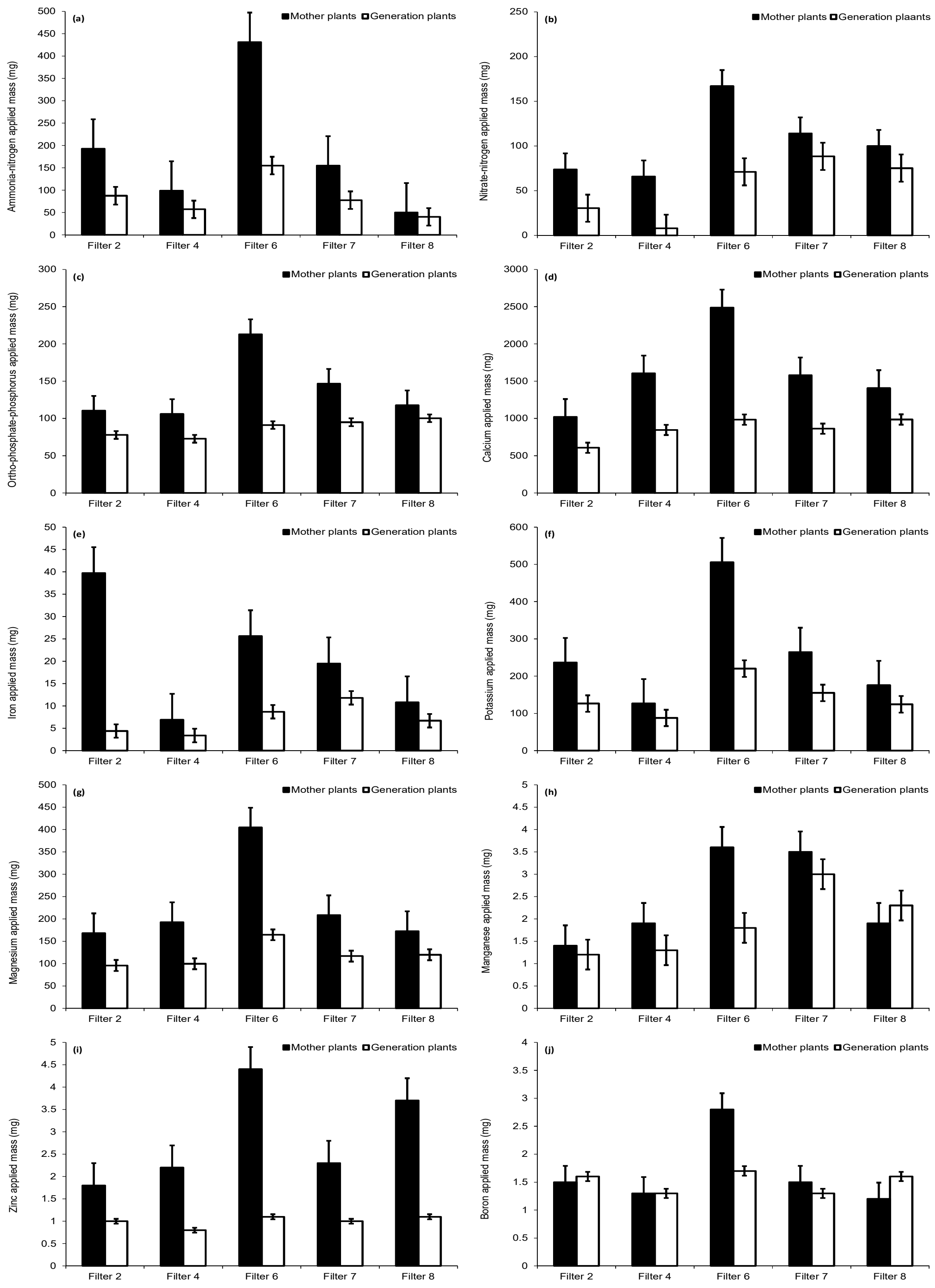

Figure 6.4: Overview of total element mass applied on Chillies: (a) ammonia-nitrogen; (b) nitrate-nitrogen; (c) ortho-phosphate-phosphorus; (d) calcium; (e) iron; (f) potassium; (g) magnesium; (h) manganese; (i) zinc; and (j) boron.

Figure 6.2c–f provides summaries of plant developments. Very high numbers of buds were recorded for Chilli generations. However, most of the buds fell down before reaching the flowering stage. Also, many flowers died before producing any fruits. This can be explained by the elevated nutrient concentrations, mainly ammonia-nitrogen, supplied to those plants grown in rich organic media (Table 6.9) and irrigated by wastewater (Table 6.1) as indicated by FAO (1994, 2003) and Almuktar et al. (2016 a, b).

Moreover, falling of most buds and flowers before reaching the fruiting stage can also be explained by the adverse environmental conditions in the laboratory in terms of light intensity provided by the grow lights and the relative humidity which was elevated artificially by using humidifiers, as explained in section 3.4. These may cause flower inhibition or cause flower abscission, as reported by Wein and Zhang (1991).

Figure 6.2c shows that the highest mean bud numbers were observed for plants irrigated with outflow water from Filters 8 and 7, while the minimum values were recorded for Filter 4 plants. Statistical analysis results (Table 6.8) showed that plants irrigated with water harvested from Filter 2 of large aggregate diameter produced bud numbers which were significantly ($p < 0.05$) greater than those for plants irrigated with drain water from Filter 4 of small aggregate diameter.

Furthermore, Chillies irrigated with Filters 4 and 7 outflow waters had mean bud numbers which were significantly ($p < 0.05$) different from those of Filters 7 and 8, respectively, which were different in terms of contact and resting time variables. Furthermore, plants irrigated with outflow water from Filter 6 of high inflow loading rate produced significantly ($p < 0.05$) more buds than those for Filter 4 of diluted inflow wastewater (Table 6.8).

In addition, Figure 6.2d indicates that the highest mean flower numbers were observed in plants irrigated with Filters 8 and 7, while the lowest values were recorded for Chillies irrigated with Filter 6 outflow water.

Irrigation with Filter 4 outflow water resulted in higher mean flower numbers than those obtained for plants irrigated with Filters 2 and 6, while plants irrigated with Filters 7 and 8 outflow waters produced more flowers than those plants irrigated with Filters 4 and 7, respectively. Moreover, Figure 6.2e shows that the highest mean fruit numbers were harvested from plants irrigated with Filter 8 outflow water followed by Filter 2, while the minimum values were recorded for Filter 4 plants. Statistical analysis results (Table 6.8) indicated a significant difference ($p < 0.05$) in mean harvested fruit numbers when comparing plants linked to Filter 4 with those associated with Filters 2 and 7. Also, Chillies irrigated with Filters 7 and 4 outflow waters produced fruit numbers lower than those irrigated with Filters 8 and 6 outflows, respectively.

Figure 6.4 indicates that the high elements load applied to mother Chillies resulted in high bud numbers compared to their generations. However, the elevated nitrogen levels (mainly in terms of ammonia) caused most of these buds to fall before the flowering stage. In comparison, reducing the irrigation water volume applied on Chilli generations, which subsequently reduced the ammonia mass applied on plants, resulted in higher flower numbers which fruited successfully compared to their mothers (Figure 6.2c–e).

Figure 6.5 shows that Chilli generation pants began producing buds, flowers and fruits earlier than their mothers. Moreover, the peak period for bud production was between January and April for generation plants, while it was between April and June for mother plants. The peak flowering time for generation plants was between February and April, while it was between April and June for their mothers.

Furthermore, the highest numbers of harvested fruits were recorded between March and August for Chilli generations, while between May and August for Chilli mothers. This can be explained by the lower element mass load applied on Chilli generations via irrigation water compared to their mothers (Figures 6.3 and 6.4) resulting in a better balance in supplied nutrients and trace elements in combination with the organic growth media supporting yield development, confirming the results obtained by Almuktar and Scholz (2016b) reporting that the combination of fresh compost and treated wastewater is usually too high in a particular nutrient to produce a good pepper harvest.

Figure 6.2f shows that the maximum total harvested fruit weight was recorded for plants linked to Filter 2 of large aggregate diameter followed by those of Filter 6 of high inflow loading rate, as they provided plants with higher nutrients and trace elements mass (Figure 6.4), while the minimum total harvested weight was observed in plants irrigated with Filter 4 outflow water. Statistically, results (Table 6.8) showed that the total fruit weight harvested from Filter 4 was significantly ($p < 0.05$) lower than those for Filters 2, 7 and 6 of large aggregate diameter, short contact time and high inflow loading rate, respectively. Furthermore, plants linked to Filter 7, of long resting time, produced fruits of total weight greater than those of Filter 8 of short resting time. However, compared to their mothers, Chilli generations produced the greatest total harvested fruit weights.

Figure 6.6 summarises the differences in Chilli generation fruit characteristics. Results show that mean fruit widths harvested from plants irrigated with Filter 4 outflow water were significantly ($p < 0.05$) lower than those linked to Filters 2 and 6 of large aggregate diameter and high inflow loading rate, respectively. Moreover, fruits harvested from plants linked to Filters 4 and 7 had mean widths which were similar to those associated with Filters 7 and 8, respectively.

However, compared to the Chilli mothers, generation plants had mean fruit widths which were significantly ($p < 0.05$) the lowest with the exception of those fruits linked to Filter 6 of high inflow loading rate which had fruit mean widths similar to their mothers (Figure 6.7 and Table 6.10).

Figure 6.6 indicates that the longest fruits were harvested from plants irrigated with Filter 6 outflow water followed by those of Filter 2, while the shortest fruits were observed in plants associated with Filters 4 and 8. Statistical analysis results (Table 6.8) indicated that fruits harvested from plants irrigated with Filter 4 of small aggregate diameter, low inflow loading rate and long contact time were significantly ($p < 0.05$) shorter than those of Filters 2, 6 and 7 of large aggregate diameter, high inflow loading rate and short contact time, respectively.

Moreover, Filter 7 irrigated plants had mean fruit lengths which were significantly ($p < 0.05$) greater than those for Filter 8 due to differences in the filter resting time of the wetland system. However, Figure 6.7 and Table 6.10 show that Chilli mothers produced fruits of mean lengths which were significantly ($p < 0.05$) greater than those for their generations.

Regarding mean fruit weight (Figure 6.6), results show that irrigation with Filter 6 of high inflow loading rate produced the highest mean fruit weights, while the minimum values were recorded for those fruits harvested from plants irrigated with Filter 8 outflow water. Statistically, Table 6.8 shows that fruit weight harvested from plants irrigated with Filters 4 and 7 were significantly ($p < 0.05$) different from those of Filters 6 and 8, respectively, due to differences in inflow loading rate and resting time variables of the corresponding filters in the wetland system.

It can be concluded that the best harvest quality in terms of width, length and weight is correlated directly with the filters of nutrient rich outflow water, such as Filters 2 and 6, confirming the results reported previously by Gungor and Yildirim (2013) which indicated the impact of nutrients and trace element load supplied to the plants on the fruit dimensions. In comparison to the Chilli mothers, generation plants had mean fruit weights which were significantly ($p < 0.05$) lower, with the exception of those fruits linked to Filter 6 of high inflow loading rate which had fruit mean weights similar to their mothers (Figure 6.7 and Table 6.10).

Table 6.8: Overview of the statistically significant differences in growth parameters of Chilli generations subjected to different irrigation water types.

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Mean plant height (mm)								
0.990	ANOVA	<0.001	Filter 2	n.a	0.001	0.355	0.898	0.601
			Filter 4	0.001	n.a	0.137	0.013	<0.001
			Filter 6	0.355	0.137	n.a	0.869	0.015
			Filter 7	0.898	0.013	0.869	n.a	0.151
			Filter 8	0.601	< 0.001	0.015	0.151	n.a
Plant height growth rate (mm)*								
0.966	ANOVA	<0.001	Filter 2	n.a	0.001	0.205	0.816	0.528
			Filter 4	0.001	n.a	0.255	0.022	< 0.001
			Filter 6	0.205	0.255	n.a	0.804	0.004
			Filter 7	0.816	0.022	0.804	n.a	0.079
			Filter 8	0.528	< 0.001	0.004	0.079	n.a
Mean leaf number								
0.680	ANOVA	0.590	Filter 2	n.a	1.000	0.942	0.786	0.807
			Filter 4	1.000	n.a	0.909	0.725	0.748
			Filter 6	0.942	0.909	n.a	0.995	0.997
			Filter 7	0.786	0.725	0.995	n.a	1.000
			Filter 8	0.807	0.748	0.997	1.000	n.a
Plant leaf growth rate (number)*								
0.701	ANOVA	0.606	Filter 2	n.a	1.000	0.948	0.795	0.802
			Filter 4	1.000	n.a	0.924	0.748	0.756
			Filter 6	0.948	0.924	n.a	0.995	0.996
			Filter 7	0.795	0.748	0.995	n.a	1.000
			Filter 8	0.802	0.756	0.996	1.000	n.a

Table 6.8 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Mean bud number								
0.848	ANOVA	<0.001	Filter 2	n.a	0.001	0.922	0.874	0.187
			Filter 4	0.001	n.a	< 0.001	< 0.001	0.001
			Filter 6	0.922	< 0.001	n.a	1.000	0.400
			Filter 7	0.874	< 0.001	1.000	n.a	0.486
			Filter 8	0.187	0.001	0.400	0.486	n.a
Mean flower number								
0.233	ANOVA	<0.001	Filter 2	n.a	0.635	0.948	0.011	0.004
			Filter 4	0.635	n.a	0.227	0.275	0.149
			Filter 6	0.948	0.227	n.a	0.001	< 0.001
			Filter 7	0.011	0.275	0.001	n.a	0.997
			Filter 8	0.004	0.149	< 0.001	0.997	n.a
Mean fruit number								
0.959	ANOVA	<0.001	Filter 2	n.a	< 0.001	0.043	0.614	0.832
			Filter 4	< 0.001	n.a	0.437	0.021	< 0.001
			Filter 6	0.043	0.437	n.a	0.584	0.002
			Filter 7	0.614	0.021	0.584	n.a	0.115
			Filter 8	0.832	< 0.001	0.002	0.115	n.a
Total fruit weight (g)								
0.708	ANOVA	<0.001	Filter 2	n.a	< 0.001	0.987	0.358	0.127
			Filter 4	< 0.001	n.a	0.001	0.037	0.137
			Filter 6	0.987	0.001	n.a	0.660	0.317
			Filter 7	0.358	0.037	0.660	n.a	0.978
			Filter 8	0.127	0.137	0.317	0.978	n.a

Table 6.8 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Mean fruit width (mm)								
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.001	< 0.001	0.058	0.534
			Filter 4	0.001	n.a	< 0.001	0.084	0.004
			Filter 6	< 0.001	< 0.001	n.a	< 0.001	< 0.001
			Filter 7	0.058	0.084	< 0.001	n.a	0.205
			Filter 8	0.534	0.004	< 0.001	0.205	n.a
Mean fruit length (mm)								
0.016	Kruskal-Wallis	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.001	< 0.001
			Filter 4	< 0.001	n.a	< 0.001	0.028	0.814
			Filter 6	< 0.001	< 0.001	n.a	< 0.001	< 0.001
			Filter 7	0.001	0.028	< 0.001	n.a	0.028
			Filter 8	< 0.001	0.814	< 0.001	0.028	n.a
Mean fruit weight (g)								
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.235	< 0.001	0.001	< 0.001
			Filter 4	0.235	n.a	< 0.001	0.562	0.153
			Filter 6	< 0.001	< 0.001	n.a	< 0.001	< 0.001
			Filter 7	0.001	0.562	< 0.001	n.a	0.026
			Filter 8	< 0.001	0.153	< 0.001	0.026	n.a
Class A fruit number								
< 0.001	Kruskal-Wallis	0.168	Filter 2	n.a	> 0.05	> 0.05	> 0.05	> 0.05
			Filter 4	> 0.05	n.a	> 0.05	> 0.05	> 0.05
			Filter 6	> 0.05	> 0.05	n.a	> 0.05	> 0.05
			Filter 7	> 0.05	> 0.05	> 0.05	n.a	> 0.05
			Filter 8	> 0.05	> 0.05	> 0.05	> 0.05	n.a

Table 6.8 (cont.)

Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values for treatment combinations	Treatment	Statistic (*p*-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Class B fruit number								
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.004	0.085	0.209	0.005
			Filter 4	0.004	n.a	< 0.001	0.029	0.917
			Filter 6	0.085	< 0.001	n.a	0.003	< 0.001
			Filter 7	0.209	0.029	0.003	n.a	0.012
			Filter 8	0.005	0.917	< 0.001	0.012	n.a
Class C fruit number								
0.100	ANOVA	0.002	Filter 2	n.a	0.012	1.000	0.701	0.061
			Filter 4	0.012	n.a	0.012	0.232	0.967
			Filter 6	1.000	0.012	n.a	0.701	0.061
			Filter 7	0.701	0.232	0.701	n.a	0.587
			Filter 8	0.061	0.967	0.061	0.587	n.a
Class D fruit number								
0.389	ANOVA	< 0.001	Filter 2	n.a	0.320	0.002	0.948	0.093
			Filter 4	0.320	n.a	0.001	0.587	< 0.001
			Filter 6	0.002	0.001	n.a	0.001	< 0.001
			Filter 7	0.948	0.587	0.001	n.a	0.006
			Filter 8	0.093	< 0.001	< 0.001	0.006	n.a
Class E fruit number								
0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.425	0.027	0.349	0.046
			Filter 4	0.425	n.a	0.157	0.083	0.008
			Filter 6	0.027	0.157	n.a	0.002	< 0.001
			Filter 7	0.349	0.083	0.002	n.a	0.289
			Filter 8	0.046	0.008	< 0.001	0.289	n.a

Table 6.8 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Mean price (pence)								
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	< 0.001	0.276	0.125	0.008
			Filter 4	< 0.001	n.a	< 0.001	0.029	0.297
			Filter 6	0.276	< 0.001	n.a	0.009	< 0.001
			Filter 7	0.125	0.029	0.009	n.a	0.256
			Filter 8	0.008	0.297	< 0.001	0.256	n.a

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). n.a, not applicable as the treatment compared with itself. ANOVA, the parametric one-way analysis of variance test; Kruskal-Wallis, the non-parametric Kruskal-Wallis test. *, growth rates were calculated based on differences between the base line and final measurements.

Table 6.9: Basic soil properties based on three replicates each (14 /09/2014).

Parameter	Raw Soil	Total Per Pot (mg)
pH	6.43	-
Redox potential (mV)	62.60	-
Electrical conductivity (μS/cm)	2438.50	-
Total nitrogen (mg/kg)	998.75	3495.63
Total phosphor (mg/kg)	367.50	1286.25
Aluminium (mg/kg)	1118.38	3914.33
Calcium (mg/kg)	18 421.96	64 476.86
Iron (mg/kg)	6233.15	21,816.03
Potassium (mg/kg)	2776.02	9716.07
Magnesium (mg/kg)	5287.67	18 506.85
Manganese (mg/kg)	201.59	705.57
Zinc (mg/kg)	26.59	93.07
Boron (mg/kg)	12.29	43.02
Organic matter (%)	89.00	-
Bulk density(g/L)	350	-

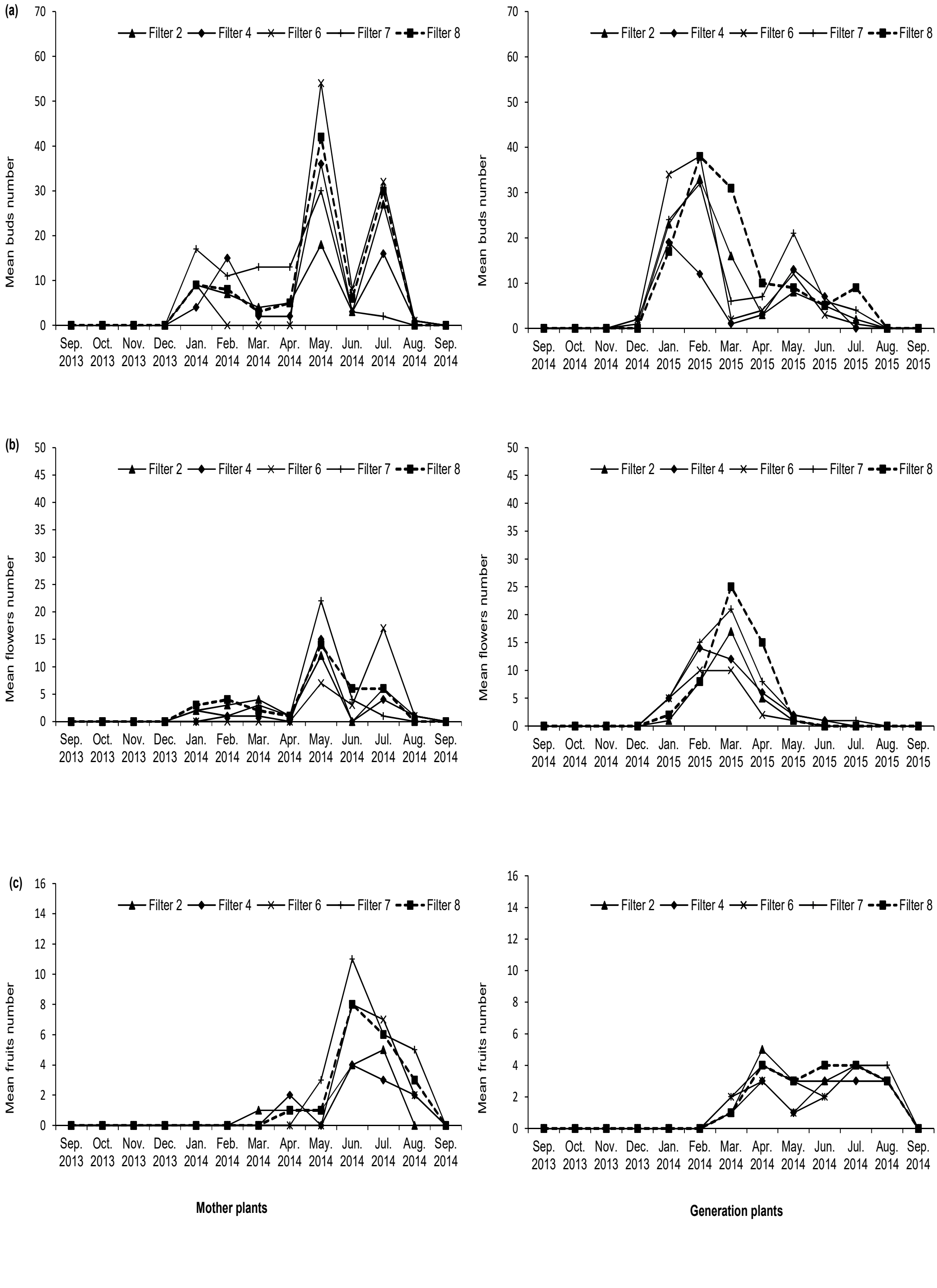

Figure 6.5: Overview of Chilli plant developments during the whole experiment duration for both mother and generation plants.

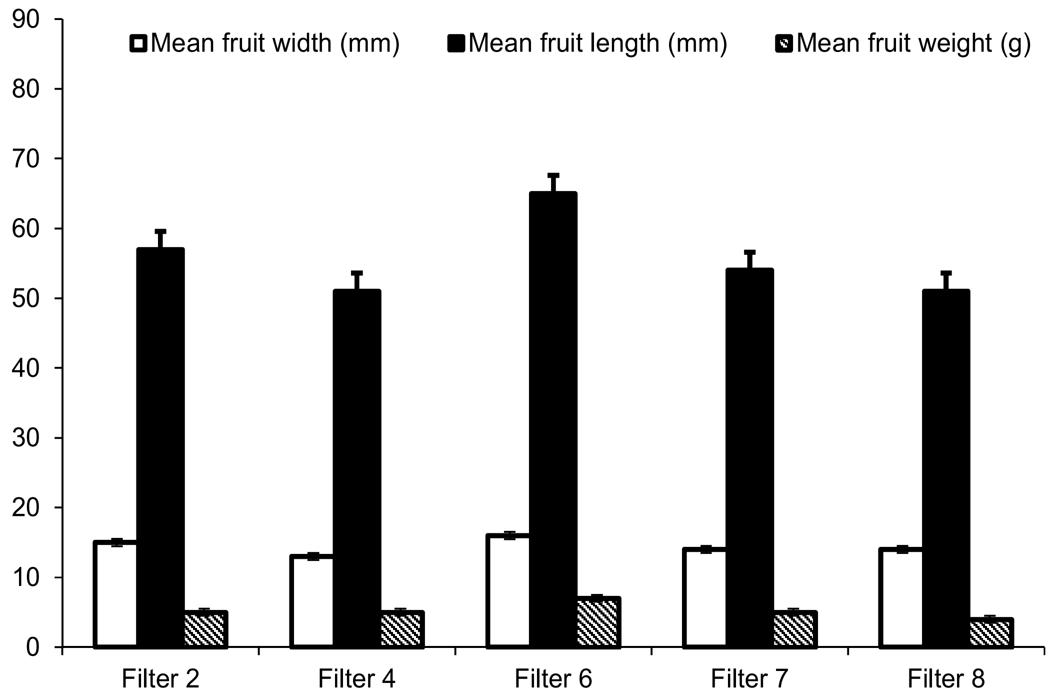

Figure 6.6: Differences in mean fruit width, mean fruit length and mean fruit weight linked to harvested Chilli generation plants irrigated with different water types.

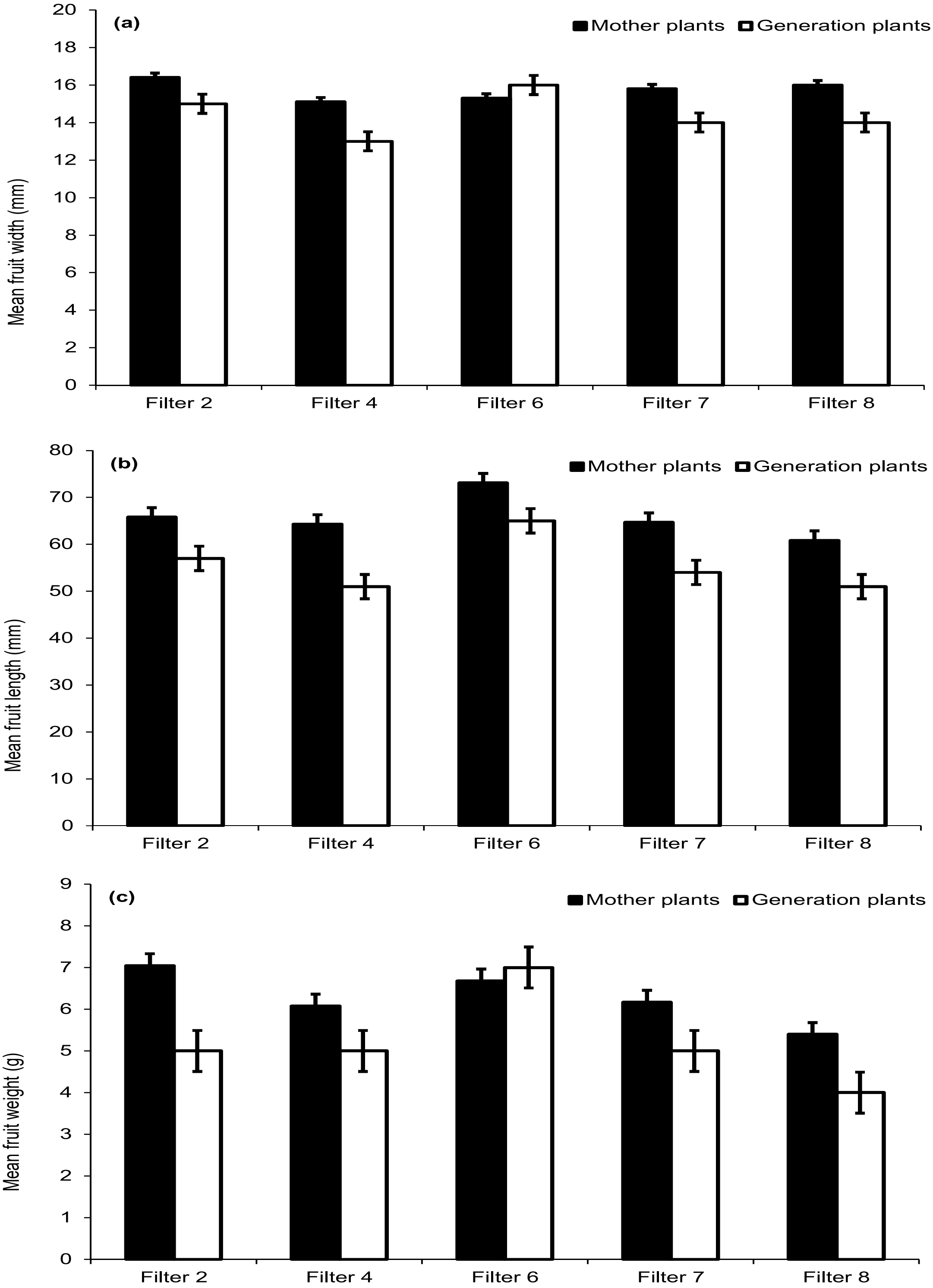

Figure 6.7: Overview of yield quality of mother and generation plants.

Table 6.10: Overview of the statistically significant differences in Chilli fruit mean widths, lengths, and weights harvested from mother and generation plants subjected to different irrigation water types.

Parameter	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values between mother and generation plants
Filter 2 outflow			
Fruit widths (mm)	< 0.001	M-W-U	0.002
Fruit lengths (mm)	0.014	M-W-U	0.003
Fruit weights (g)	< 0.001	M-W-U	0.002
Filter 4 outflow			
Fruit widths (mm)	< 0.001	M-W-U	0.021
Fruit lengths (mm)	0.070	I-T	0.001
Fruit weights (g)	< 0.001	M-W-U	0.002
Filter 6 outflow			
Fruit widths (mm)	0.004	M-W-U	0.240
Fruit lengths (mm)	0.001	M-W-U	0.001
Fruit weights (g)	0.167	I-T	0.730
Filter 7 outflow			
Fruit widths (mm)	< 0.001	M-W-U	< 0.001
Fruit lengths (mm)	0.152	I-T	< 0.001
Fruit weights (g)	< 0.001	M-W-U	< 0.001
Filter 8 outflow			
Fruit widths (mm)	< 0.001	M-W-U	< 0.001
Fruit lengths (mm)	0.445	I-T	< 0.001
Fruit weights (g)	< 0.001	M-W-U	< 0.001

Note: [a] Test of normality (if *p*-value > 0.05, data are normally distributed; if *p*-value < 0.05, data are not normally distributed. [b] *p*-value, probability of the statistical test (values are statistically significantly different only if the *p*-value < 0.05 for the corresponding parameter). M-W-U, the non-parametric Mann-Whitney U-test and I-T, the parametric Independent samples T-test.

All harvested fruits from each treatment were categorised according to Table 3.4, which shows a novel harvest classification scheme for Chillies. Only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, bending and weight. The lowest variable class entry for any individual Chilli fruit assessment determined the final class. If a fruit is categorised, for example, as class A with respect to length, class B in terms of diameter and E regarding weight, then the final class for this fruit is class E. It follows that the corresponding price for this Chilli sample will be zero pence (Table 3.4).

However, the estimated prices are dependent on global commodity market developments. Figure 6.8 shows the number of Chilli fruits categorised as class A, B, C, D or E per treatment as well as the total harvested fruits number. Results show that irrigation with Filter 8 outflow water produced the highest total harvested fruits number (305) which was categorised as follows, according to Table 3.4: Class A (0 fruit), Class B (3 fruits), Class C (37 fruits), Class D (193 fruits) and Class E (72 fruits). In comparison, the lowest total harvested fruit number was recorded for plants irrigated with Filter 4 outflow water, which was categorised as follows: Class A (0 fruit), Class B (2 fruits), Class C (30 fruits), Class D (117 fruits) and Class E (37 fruits). However, Figure 6.8 indicates that the highest fruit number categorised as Class A was observed in plants irrigated with Filter 6 outflow water resulting in the highest mean price (Figure 6.9).

The highest Class B fruits number was recorded for plants linked to Filter 6 (58 fruits) followed by Filter 2 (29 fruits), while the lowest value was observed for Filter 4 plants (2 fruits). Statistical analysis results (Table 6.8) showed that the number of Class B fruits linked to Filter 4 (small aggregate diameter, low inflow loading rate and long contact time) plants was significantly ($p < 0.05$) lower than those associated with Filters 2, 6 and 7 of large aggregate size, high inflow loading rate and short contact time, respectively. Moreover, plants irrigated with Filters 7 and 8 outflow waters produced numbers of Class B fruits which were significantly ($p < 0.05$) different from each other, due to differences in corresponding filter resting times in the wetland system.

Figure 6.8 shows that the highest number of fruits categorised as Class D was recorded for Filters 6 and 2 irrigated plants (67 fruits), while the lowest value was noted for Filter 4 plants (30 fruits). Table 6.8 indicates that there is a significant ($p < 0.05$) difference in the number of Class B fruits harvested from Filter 4 plants compared to those of Filters 2 and 6.

Regarding Class D fruit numbers, results showed that plants irrigated with Filters 8 and 2 outflow waters had the highest fruit numbers from this category (193 and 147, respectively), while plants linked to Filter 6 showed the lowest Class D fruit number (Figure 6.8). However, plants irrigated with Filter 4 outflow water produced fewer fruits categorised as Class D than those irrigated from Filters 2 and 7. Statistical analysis results (Table 6.8) indicated significant differences ($p < 0.05$) in mean Class D fruit numbers when comparing Filters 4 and 7 plants with those associated with Filters 6 and 8, respectively.

Lastly, the greatest Class E fruit number (Figure 6.8) was recorded for plants irrigated with Filter 8 outflow water (72 fruits) followed by those of Filter 7 (52 fruits), while the lowest number was linked to Filter 6 plants (20 fruits). Moreover, plants irrigated with Filters 2 and 4 outflow waters produced similar fruit numbers of this category. Filters 4 and 7 plants had Class E mean fruit numbers lower than those for Filters 7 and 8 plants, respectively, while Filter 6 plants had a lower number than those for Filter 4.

Based on these analyses, the highest mean price was recorded for plants irrigated with Filter 6 outflow water (Figure 6.9) followed by those irrigated with Filter 2 outflow water as they produced more fruits of high category classification than the others, while Filter 4 plants were significantly ($p < 0.05$) the lowest (Table 6.8) as they produced an abundancy of low category classification fruits.

Although Chilli generations produced total fruit numbers which were considerably higher than their mothers (Figure 6.10), most of these fruits received low category classification (Class C, D and E) resulting in a mean price for Chilli generations lower than their mothers (except those linked to Filter 2) as shown in Figure 6.9. This can be explained by the impact of high mass of nutrients and trace elements applied on Chilli mothers (Figure 6.4) via irrigation water (Figure 6.3) resulting in better fruit quality in terms of width, length and weight (Gungor & Yildirim, 2013; Almuktar & Scholz, 2016a) and subsequent greater price compared to their generations. However, correlation analysis findings indicated that fruit weights were significantly positively correlated with total water volumes used for irrigation (R = 0.821, $p < 0.001$). Potential water stress might have reduced cell division and caused cell enlargement to cease.

This could have led to a slowdown of the growth rate and might have been the reason for the relatively low weight, width and length of fruits (Tedesse, 1997). However, the abundancy of element load applied on Chilli mothers in combination with rich organic soil caused imbalanced nutrition leading to high foliage growth (mainly in terms of plant heights) in expense of fruit production, as reported by FAO (1994, 2003). Based on this, a smart irrigation system is highly recommended to guarantee good plant growth and subsequent better yield quality in terms of economic outcomes.

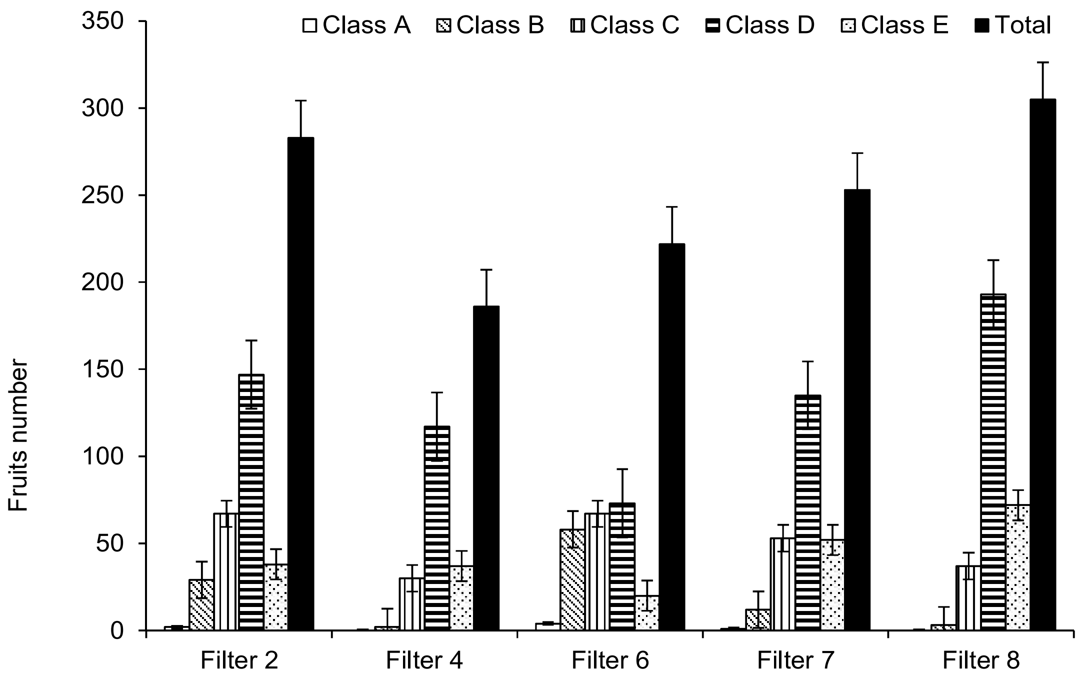

Figure 6.8: Overview of fruit numbers per class harvested from Chilli generation plants subjected to different irrigation water types.

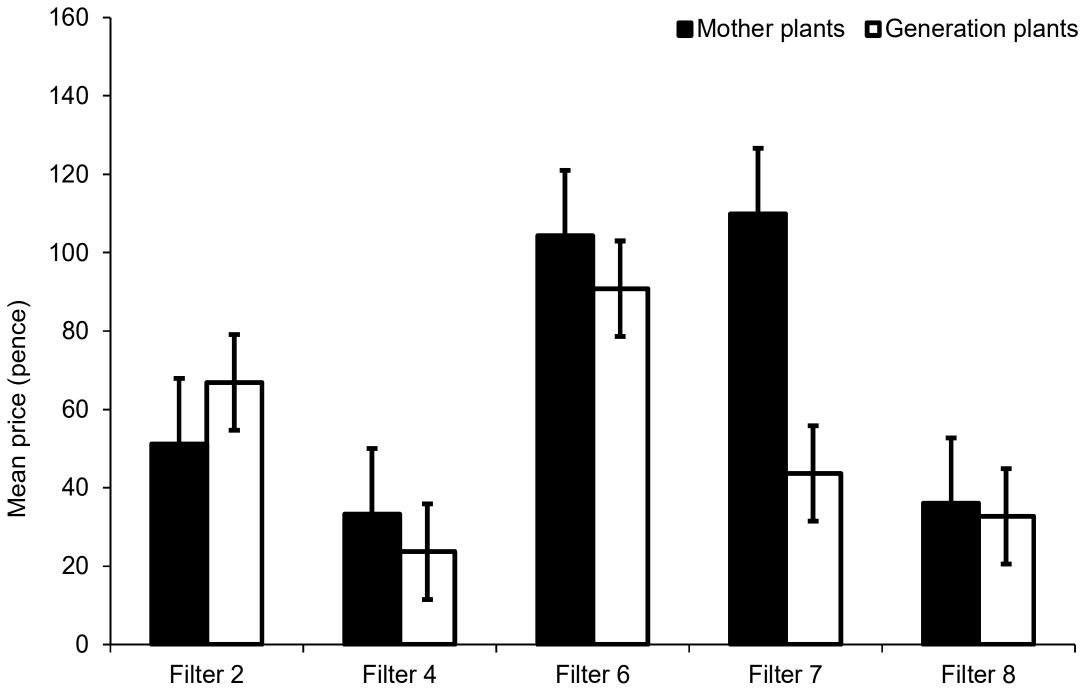

Figure 6.9: Chilli harvest outcome linked to mother and generation plants (after classification scheme (Table 3.4) application).

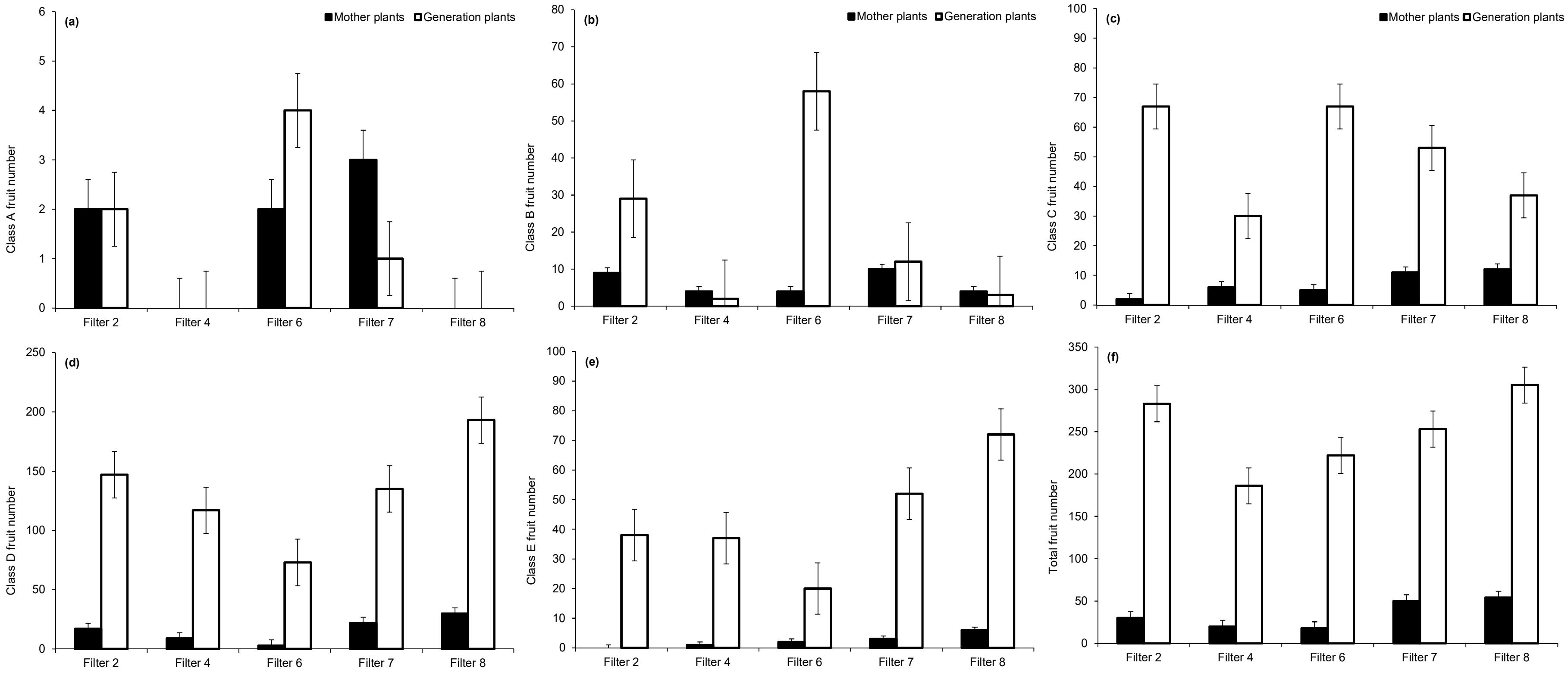

Figure 6.10: Overview of fruit number per class harvested from Chilli mother and generation plants subjected to different irrigation water types.

6.5 Soil quality analysis

6.5.1 Comparison of soil pH and redox potential

Table 6.11 shows qualities for soils irrigated with different water types. All soil pH values indicated acidic conditions (pH value < 7). The soil pH can markedly affect the availability and consequently the plant uptake of trace elements (FAO, 1972, 2003). The ability of plants to utilise trace elements decreases with decreasing acidity (increase in pH), while the utilisation at higher pH values remains constant (FAO, 1972).

Table 6.12 indicates that compared with raw soil (pH = 6.2), irrigation with treated wastewater did not cause significant differences ($p < 0.05$) with the exception of soil irrigated with Filter 4 outflow water which showed the highest pH values of 6.7 followed by those of Filter 2 (pH = 6.4), while the minimum values were reported for soils irrigated with Filter 6 outflow water showing a pH value of 6.0. Furthermore, irrigation with Filter 4 drain water showed pH values which were significantly ($p < 0.05$) greater than those for Filters 2, 6 and 7, while no significant differences ($p > 0.05$) were observed when comparing pH values of soil irrigated with Filters 7 and 8 outflow waters with each other.

However, all soil pH values were within the optimal range of between 6.0 and 7.5 for growth of many plants and microbial health of soil (FAO, 1972, 2003).

Regarding soil redox potential values, according to Husson (2013), soil could be classified as moderately reduced soil (redox potential values between +100 and +400 mV), reduced soil (redox potential values between −100 and +100 mV) and highly reduced soil (redox potential values between −100 and −300 mV). Based on this classification, Table 6.11 indicates that all soil irrigated with different water types could be considered as reduced soil.

The highest redox potential values were recorded for soils irrigated with Filter 6 outflow water followed by those linked to Filter 7 showing values of 90.8 mV and 79.1 mV, respectively, while the lowest values were associated with soils irrigated with water harvested from Filter 4 (redox potential value of 56.8 mV). Statistically, results showed that irrigation with treated wastewaters did not change soil redox potential values significantly ($p > 0.05$) compared with the raw soil (Table 6.12). Moreover, soils irrigated with Filter 4 outflow water showed redox potential values which were significantly different ($p < 0.05$) from those of Filters 2, 6 and 7, while no significant differences ($p > 0.05$) were observed when comparing redox potential values of soil irrigated with Filter 7 with those of Filter 8.

The redox potential and pH are major drivers for change in soil, plant and microorganism systems. High levels of redox potentials can impact on system functioning as well as on plant health and production (Husson, 2013). However, climate conditions and soil moisture could directly affect pH and redox potential values, especially in organic soil. Furthermore, correlation analysis results showed that both pH and redox potential values were significantly negatively (R = -0.986, $p < 0.001$) correlated with each other, as reported by FAO (1972) and Essington (2015).

6.5.2 Comparison of soil salinity

Table 6.11 shows that irrigation with treated wastewater did not increase the soil salinity compared to the raw soil. However, among the irrigated soils, application of outflow water from Filter 6 of high inflow loading rate resulted in the highest electrical conductivity value of 1108 µS/cm, while soil linked to Filter 4 showed the lowest value of 493.8 µS/cm. Moreover, salinity values for soil irrigated with Filters 2 and 7 outflow waters were greater than those of Filters 4 and 8, respectively.

Irrigation with Filters 6 and 7 outflow waters resulted in soil salinity values which were significantly ($p < 0.05$) greater than that for Filter 4 (Table 6.12), explaining the impact of wetland system design in terms of contact time and inflow loading rate on outflow water salinity (Table 6.1) applied on the irrigated soils. Furthermore, correlation analysis results showed that soil electrical conductivity values were significantly negatively correlated with soil pH values (R = -0.899, p = 0.015). For example, in acidic conditions, of low pH values, the dissolution of elements such as sodium, potassium, calcium and magnesium will increase and subsequently increase the salinity of the soil. However, excessive soil salinity could result in nutrient imbalances leading to high accumulations of toxic elements, reducing water infiltration and subsequently limiting the growth of plants (FAO, 1972, 2003).

Table 6.11: Comparison of qualities of soil subjected to different irrigation water types.

Parameter	Mean	Minimum	Maximum	Standard deviation	Number
pH (-)					
Filter 2	6.4	6.3	6.5	0.08	10
Filter 4	6.7	6.6	6.7	0.06	10
Filter 6	6.0	5.8	6.1	0.13	10
Filter 7	6.2	6.1	6.3	0.08	10
Filter 8	6.3	6.2	6.4	0.07	10
Raw soil	6.2	5.5	6.4	0.31	10
Redox potential (mV)					
Filter 2	70.5	65.5	75.7	3.73	10
Filter 4	56.8	52.5	62.3	3.37	10
Filter 6	90.8	83.6	98.6	6.26	10
Filter 7	79.1	74.9	84.6	4.19	10
Filter 8	73.0	69.2	77.9	3.56	10
Raw soil	75.9	62.2	111.8	16.39	10
Electrical conductivity (µS/cm)					
Filter 2	833.6	789.0	862.0	31.28	10
Filter 4	493.8	449.0	530.0	32.67	10
Filter 6	1108.4	1050.0	1204.0	59.43	10
Filter 7	1107.0	1072.0	1143.0	25.01	10
Filter 8	1042.0	1013.0	1062.0	17.32	10
Raw soil	2297.0	2081.7	2438.5	119.92	10

Table 6.11 (cont.)

Parameter	Mean	Minimum	Maximum	Standard deviation	Number
Total coliforms (CFU/g)					
Filter 2	2090.0	1290.0	2720.0	558.79	10
Filter 4	3846.0	3370.0	4340.0	354.73	10
Filter 6	4818.0	3730.0	5410.0	700.12	10
Filter 7	2656.0	2390.0	3080.0	276.37	10
Filter 8	2000.0	1650.0	2290.0	300.58	10
Raw soil	nm	nm	nm	nm	nm
Salmonella spp. (CFU/g)					
Filter 2	658.0	270.0	1380.0	448.91	10
Filter 4	2062.0	1810.0	2550.0	303.60	10
Filter 6	2214.0	1890.0	3040.0	477.94	10
Filter 7	1278.0	1110.0	1400.0	120.08	10
Filter 8	524.0	200.0	930.0	262.35	10
Raw soil	nm	nm	nm	nm	nm
Aluminium (mg/kg)					
Filter 2	1616.68	784.82	2190.89	352.770	24
Filter 4	1462.15	467.61	2411.32	552.153	24
Filter 6	1625.17	807.60	2314.08	350.074	24
Filter 7	1462.92	797.06	1895.49	275.540	24
Filter 8	1787.51	892.98	2582.59	395.871	24
Raw soil	1123.24	1046.41	1194.90	51.999	24
Calcium (mg/kg)					
Filter 2	23046.24	16870.10	26088.60	2606.972	24
Filter 4	26292.01	17951.45	32556.95	4198.267	24
Filter 6	20722.51	15734.55	22953.15	1997.241	24
Filter 7	22508.54	17240.15	24789.15	2198.997	24
Filter 8	25874.47	19760.90	28660.15	2742.057	24
Raw soil	20945.25	14616.40	34222.65	7640.085	24
Iron (mg/kg)					
Filter 2	2245.91	1909.80	2591.80	299.687	24
Filter 4	1826.04	802.02	2683.21	754.206	24
Filter 6	2116.55	2008.71	2259.81	74.092	24
Filter 7	1964.20	1807.22	2202.09	142.272	24
Filter 8	2479.04	2169.97	2927.85	282.124	24
Raw soil	6042.01	4463.66	8566.25	1664.159	24
Potassium (mg/kg)					
Filter 2	1197.39	991.65	1453.11	184.054	24
Filter 4	2182.72	1589.70	2795.76	425.563	24
Filter 6	1527.28	1232.91	1785.84	220.323	24
Filter 7	1677.80	1442.59	1926.87	214.510	24
Filter 8	1604.47	1374.95	1822.90	208.238	24
Raw soil	2709.94	2520.64	2852.39	100.734	24
Magnesium (mg/kg)					
Filter 2	9298.52	6444.75	10830.15	1160.616	24
Filter 4	10195.92	6413.05	13004.90	1819.783	24
Filter 6	8090.41	5822.65	9076.20	896.252	24
Filter 7	8950.89	6506.50	9997.35	973.506	24
Filter 8	10205.45	7295.95	11523.20	1154.870	24
Raw soil	5318.85	5216.70	5451.15	89.221	24

Table 6.11 (cont.)

Parameter	Mean	Minimum	Maximum	Standard deviation	Number
Manganese (mg/kg)					
Filter 2	161.85	149.44	198.51	13.035	24
Filter 4	152.14	54.81	264.43	66.393	24
Filter 6	137.12	103.62	195.22	21.585	24
Filter 7	169.43	125.53	208.25	15.606	24
Filter 8	193.56	163.87	245.34	19.964	24
Raw soil	203.91	177.25	243.29	27.179	24
Zinc (mg/kg)					
Filter 2	37.01	17.78	58.89	12.464	24
Filter 4	35.31	13.30	55.44	14.227	24
Filter 6	31.04	13.11	46.54	7.972	24
Filter 7	36.71	13.74	56.47	11.537	24
Filter 8	31.27	11.40	42.02	9.989	24
Raw soil	27.25	22.81	35.16	3.397	24
Boron (mg/kg)					
Filter 2	5.53	2.86	10.75	2.913	24
Filter 4	6.24	3.28	10.04	2.142	24
Filter 6	4.34	2.64	7.68	1.763	24
Filter 7	4.19	2.17	7.10	1.529	24
Filter 8	4.95	3.24	7.53	1.706	24
Raw soil	12.44	9.83	15.50	1.685	24

Note: *Escherichia coli and Streptococci* spp. were not detected in the tested soils; nm, not measured.

Table 6.12: Overview of the statistically significant differences in properties of soil subjected to different irrigation water types.

Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values for treatment combinations	Treatment	Statistic (*p*-value)[b]					
				Filter 2	**Filter 4**	**Filter 6**	**Filter 7**	**Filter 8**	**Raw soil**
Soil pH (-)									
0.180	ANOVA	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.008	0.731	0.618
			Filter 4	< 0.001	n.a	< 0.001	< 0.001	< 0.001	0.025
			Filter 6	< 0.001	< 0.001	n.a	0.009	< 0.001	0.465
			Filter 7	0.008	< 0.001	0.009	n.a	0.070	1.000
			Filter 8	0.731	< 0.001	< 0.001	0.070	n.a	0.845
			Raw soil	0.618	0.025	0.465	1.000	0.845	n.a
Soil redox potential (mV)									
0.220	ANOVA	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.007	0.735	0.931
			Filter 4	< 0.001	n.a	< 0.001	< 0.001	< 0.001	0.092
			Filter 6	< 0.001	< 0.001	n.a	0.008	< 0.001	0.250
			Filter 7	0.007	< 0.001	0.008	n.a	0.067	0.993
			Filter 8	0.735	< 0.001	< 0.001	0.067	n.a	0.995
			Raw soil	0.931	0.092	0.250	0.993	0.995	n.a
Soil electrical conductivity (µS/cm)									
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.253	0.009	0.003	0.189	< 0.001
			Filter 4	0.253	n.a	< 0.001	< 0.001	0.014	< 0.001
			Filter 6	0.009	< 0.001	n.a	0.755	0.192	0.051
			Filter 7	0.003	< 0.001	0.755	n.a	0.106	0.100
			Filter 8	0.189	0.014	0.192	0.106	n.a	< 0.001
			Raw soil	< 0.001	< 0.001	0.051	0.100	< 0.001	n.a

Table 6.12 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]					
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8	Raw soil
Soil Total coliforms (CFU/g)									
0.107	ANOVA	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.343	0.998	nm
			Filter 4	< 0.001	n.a	0.027	0.005	< 0.001	nm
			Filter 6	< 0.001	0.027	n.a	< 0.001	< 0.001	nm
			Filter 7	0.343	0.005	< 0.001	n.a	0.214	nm
			Filter 8	0.998	< 0.001	< 0.001	0.214	n.a	nm
			Raw soil	nm	nm	nm	nm	nm	n.a
Soil *Salmonella* spp. (CFU/g)									
0.331	ANOVA	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.071	0.972	nm
			Filter 4	< 0.001	n.a	0.956	0.015	< 0.001	nm
			Filter 6	< 0.001	0.956	n.a	0.003	< 0.001	nm
			Filter 7	0.071	0.015	0.003	n.a	0.020	nm
			Filter 8	0.972	< 0.001	< 0.001	0.020	n.a	nm
			Raw soil	nm	nm	nm	nm	nm	n.a
Soil aluminium (mg/kg)									
0.015	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.539	0.846	0.130	0.106	0.003
			Filter 4	0.539	n.a	0.374	0.323	0.014	0.008
			Filter 6	0.846	0.374	n.a	0.061	0.118	0.001
			Filter 7	0.130	0.323	0.061	n.a	0.001	0.004
			Filter 8	0.106	0.014	0.118	0.001	n.a	< 0.001
			Raw soil	0.003	0.008	0.001	0.065	< 0.001	n.a

Table 6.12 (cont.)

Shapiro-Wilk test (p-value)[a]	**Statistical test**	***P*-values for treatment combinations**	**Treatment**	**Statistic (p-value)**[b]					
				Filter 2	**Filter 4**	**Filter 6**	**Filter 7**	**Filter 8**	**Raw soil**
Soil calcium (mg/kg)									
0.028	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.023	0.013	0.579	0.009	0.163
			Filter 4	0.023	n.a	< 0.001	0.005	0.716	0.001
			Filter 6	0.013	< 0.001	n.a	0.053	< 0.001	0.465
			Filter 7	0.579	0.005	0.053	n.a	0.001	0.357
			Filter 8	0.009	0.716	< 0.001	0.001	n.a	< 0.001
			Raw soil	0.163	0.001		0.357	< 0.001	n.a
Soil iron (mg/kg)									
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.269	0.579	0.011	0.016	< 0.001
			Filter 4	0.269	n.a	0.539	0.110	< 0.001	< 0.001
			Filter 6	0.579	0.539	n.a	0.027	0.001	< 0.001
			Filter 7	0.011	0.110	0.027	n.a	< 0.001	< 0.001
			Filter 8	0.016	< 0.001	0.001	< 0.001	n.a	0.020
			Raw soil	< 0.001	< 0.001	< 0.001	< 0.001	0.020	n.a
Soil potassium (mg/kg)									
0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.001	0.279	0.054	0.158	< 0.001
			Filter 4	0.001	n.a	0.032	0.023	0.070	0.020
			Filter 6	0.279	0.032	n.a	0.397	0.742	< 0.001
			Filter 7	0.054	0.023	0.397	n.a	0.605	0.006
			Filter 8	0.158	0.070	0.742	0.605	n.a	0.001
			Raw soil	< 0.001	0.020	< 0.001	0.006	0.001	n.a

Table 6.12 (cont.)

Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values for treatment combinations	Treatment	Statistic (*p*-value)[b]					
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8	Raw soil
Soil magnesium (mg/kg)									
0.040	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.203	< 0.001	0.430	0.037	< 0.001
			Filter 4	0.203	n.a	< 0.001	0.039	0.413	< 0.001
			Filter 6	< 0.001	< 0.001	n.a	0.005	< 0.001	0.021
			Filter 7	0.430	0.039	0.005	n.a	0.004	< 0.001
			Filter 8	0.037	0.413	< 0.001	0.004	n.a	< 0.001
			Raw soil	< 0.001	< 0.001	0.021	< 0.001	< 0.001	n.a
Soil manganese (mg/kg)									
0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.222	0.058	0.178	< 0.001	< 0.001
			Filter 4	0.222	n.a	< 0.001	0.889	0.001	0.002
			Filter 6	0.058	< 0.001	n.a	< 0.001	< 0.001	< 0.001
			Filter 7	0.178	0.889	< 0.001	n.a	0.001	0.003
			Filter 8	< 0.001	0.001	< 0.001	0.001	n.a	0.681
			Raw soil	< 0.001	0.002	< 0.001	0.003	0.681	n.a
Soil zinc (mg/kg)									
0.090	ANOVA	0.083	Filter 2	n.a	0.999	0.542	1.000	0.668	0.059
			Filter 4	0.999	n.a	0.847	0.999	0.908	0.192
			Filter 6	0.542	0.847	n.a	0.422	1.000	0.446
			Filter 7	1.000	0.999	0.422	n.a	0.595	0.120
			Filter 8	0.668	0.908	1.000	0.595	n.a	0.621
			Raw soil	0.059	0.192	0.446	0.120	0.621	n.a

Table 6.12 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	*P*-values for treatment combinations	Treatment	Statistic (p-value)[b]					
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8	Raw soil
Soil boron (mg/kg)									
0.001	Kruskal-Wallis	<0.001	Filter 2	n.a	0.379	0.418	0.469	0.921	< 0.001
			Filter 4	0.379	n.a	0.091	0.109	0.327	0.006
			Filter 6	0.418	0.091	n.a	0.932	0.478	< 0.001
			Filter 7	0.469	0.109	0.932	n.a	0.532	< 0.001
			Filter 8	0.921	0.327	0.478	0.532	n.a	< 0.001
			Raw soil	< 0.001	0.006	< 0.001	< 0.001	< 0.001	n.a

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). n.a, not applicable as the treatment compared with itself. ANOVA, the parametric one-way analysis of variance test; Kruskal-Wallis, the non-parametric Kruskal-Wallis test. mV, millivolts; µS/cm, micro-Siemens per centimetre; CFU/g, colony forming units per gram; and nm, not measured.

Table 6.13: Overview of correlation coefficients and associated significances between soil elements using the non-parametric Spearman correlation test.

Element	Statistic	Element							
		Aluminium	Calcium	Iron	Potassium	Magnesium	Manganese	Zinc	Boron
Aluminium	R	1.000	0.686**	0.794**	0.811**	0.702**	0.768**	0.759**	-0.435
	p	n.a	0.000	0.000	0.000	0.000	0.000	0.000	0.092
Calcium	R	0.686**	1.000	0.839**	0.894**	0.817**	0.915**	0.853**	0.021
	p	0.000	n.a	0.000	0.000	0.000	0.000	0.000	0.940
Iron	R	0.794**	0.839**	1.000	0.909**	0.826**	0.890**	0.844**	-0.182
	p	0.000	0.000	n.a	0.000	0.000	0.000	0.000	0.499
Potassium	R	0.811**	0.894**	0.909**	1.000	0.867**	0.871**	0.847**	-0.082
	p	0.000	0.000	0.000	n.a	0.000	0.000	0.000	0.762
Magnesium	R	0.702**	0.817**	0.826**	0.867**	1.000	0.826**	0.821**	0.088
	p	0.000	0.000	0.000	0.000	n.a	0.000	0.000	0.745
Manganese	R	0.768**	0.915**	0.890**	0.871**	0.826**	1.000	0.933**	-0.191
	p	0.000	0.000	0.000	0.000	0.000	n.a	0.000	0.478
Zinc	R	0.759**	0.853**	0.844**	0.847**	0.821**	0.933**	1.000	-0.518*
	p	0.000	0.000	0.000	0.000	0.000	0.000	n.a	0.040
Boron	R	-0.435	0.021	-0.182	-0.082	0.088	-0.191	-0.518*	1.000
	p	0.092	0.940	0.499	0.762	0.745	0.478	0.040	n.a

Note: R, correlation coefficient; *p*, probability of the statistical test (if *p*-value > 0.05, the variables are not statistically significantly correlated, if *p*-value < 0.05, the variables are statistically significantly correlated); n.a, not applicable since the variable is tested to be correlated with itself (R = 1). **, correlation is significant at the 0.01 level, and *, correlation is significant at the 0.05 level.

6.5.3 Soil microbial content

Table 6.11 shows that total coliforms were detected with the highest values in the soil irrigated with Filter 6 outflow water (4818 CFU/g) followed by those linked to Filter 4 showing a value of 3846 CFU/g, while the lowest values were reported for soils irrigated with Filter 8 outflow water (2000 CFU/g). Statistical analysis results (Table 6.12) showed that soil irrigated with Filter 4 outflow water had total coliforms colonies significantly ($p < 0.05$) greater than those soils irrigated with Filters 2 and 7 drain waters, indicating the impact of aggregate diameter and contact time of the wetland system on outflow water total coliforms (Table 6.5) and subsequently on the irrigated soils. Irrigation with outflow water from an undiluted inflow wetland filter significantly ($p < 0.05$) increased soil total coliform levels compared with those of diluted inflow wastewater as shown when comparing soils irrigated with Filter 6 with those irrigated with Filter 4 drain waters (Table 6.12).

Similarly to the total coliforms, Table 6.11 shows that the highest Salmonella colonies were detected in the soil irrigated with Filter 6 followed by those irrigated with Filter 4 outflow waters, showing values of 2214 CFU/g and 2062 CFU/g, respectively, while the lowest values were recorded for soils irrigated with Filer 8 outflow water (524 CFU/g). Statistically, soil irrigated with water harvested from Filter 2 of large aggregate size had Salmonellae significantly ($p < 0.05$) lower than those linked to Filter 4 of small aggregate diameter, in spite of their abundancy in the outflow waters of the former (Table 6.5). Significant differences ($p < 0.05$) in irrigated soil Salmonella counts were also observed when comparing soils associated with Filters 4 and 7 with those of Filters 7 and 8, respectively, due to differences in contact and resting times design variables for the corresponding filters in the wetland system (Figure 6.1).

Correlation analysis results showed that both total coliforms and Salmonella were significantly positively correlated with each other (R = 0.954 and p value of less than 0.001), confirming the results reported by Almuktar and Scholz (2015). However, the typical bacteria survival time in soil, fresh water and crops is less than 70, 60 and 30 days, respectively, according to EPA (1992).

6.5.4 Soil mineral content

6.5.4.1 Soil aluminium

Aluminium solubility is mainly governed by soil pH, and by soil organic matter and clay contents. Exchangeable aluminium rapidly increases when pH decreases. However, irrigation of soil with wetland filter outflow waters caused significant ($p < 0.05$) increases in aluminium concentrations compared to raw soil (Table 6.11 and 6.12) because of its relatively low bioavailability (Stahl et al., 2011).

Results showed that the maximum aluminium concentrations were detected in soil irrigated with Filter 8 followed by Filter 6 outflow waters, while the minimum values were recorded for Filters 4 and 7 irrigated soils. Irrigation with Filter 4 outflow water resulted in mean aluminium concentrations lower than those for soils linked to Filters 2 and 6, explaining the impact of wetland filter aggregate diameter and inflow loading rate on outflow water aluminium concentrations and subsequently on its levels in the irrigated soils.

Moreover, soils irrigated with Filter 7 outflow water had aluminium concentrations which were significantly ($p < 0.05$) lower than those for Filter 8 (Table 6.11 and 6.12), possibly due to the difference in applied irrigation water volumes (Figure 6.3). Correlation analysis results (Table 6.13) show that aluminium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with calcium, iron, potassium, magnesium, manganese and zinc values, while negatively correlated with boron levels in the soil, as reported by Essington (2015).

6.5.4.2 Soil calcium

Table 6.11 shows that the highest calcium concentrations were observed in the soil irrigated with Filter 4 outflow water which caused a significant ($p < 0.05$) increment in levels compared to the raw soil, while the lowest values were recorded for Filter 6 outflow water irrigated soils. However, these observations could be explained by the best growth rate and production obtained when irrigation with Filter 6 outflow water (section 6.4) caused uptake of most of the calcium in the soil rather than accumulation of it in the soil, as shown with Filter 4 for which plants showed the worst growth case.

Statistical analysis results (Table 6.12) showed that calcium concentrations in soils irrigated with Filter 4 outflow water were significantly ($p < 0.05$) greater than those in soils irrigated with Filters 2, 6 and 7 outflow waters which were different from the former in terms of aggregate diameter, inflow loading rate and contact time variables in the wetland system.

Furthermore, soil irrigated with water harvested from Filter 7 and 8 had calcium concentrations which were significantly ($p < 0.05$) different from each other (Table 6.12). Moreover, correlation analysis results (Table 6.13) show that calcium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, magnesium, manganese and zinc values, as reported by Essington (2015). However, calcium is an important element required for the growth and development of plants, especially their roots and shoot tips (Haifa Chemical, 2014). Furthermore, the availability of high calcium levels will improve the effects of uptake of toxic cations like aluminium and sodium from the soil, while the presence of high levels of potassium and magnesium may reduce calcium uptake (FAO, 1972).

6.5.4.3 Soil iron

The solubility of iron is strongly influenced by both redox potential and pH. Iron toxicity is frequently observed at low redox potentials and pH values, (FAO, 1972). Table 6.11 shows that the highest iron concentrations were observed in the soils irrigated with Filter 8 followed by those of Filter 2 outflow waters, while the lowest values were recorded for those soils irrigated with Filter 4 outflow water.

However, irrigation with treated wastewater did not increase the iron concentrations in the soil compared to the raw soil. Findings indicate that irrigation with Filter 4 outflow water resulted in iron levels which were lower than those for soil irrigated with Filters 2, 6 and 7 outflow waters (Table 6.11). This can be explained by the impact of different aggregate diameter, inflow loading rate and contact time variables of the wetland system on treated water iron concentrations (Table 6.4), which subsequently affect the iron mass applied on the soil (Figure 6.4) via irrigation water (Figure 6.3).

Statistical analysis results (Table 6.12) showed that irrigation with Filters 7 and 8 outflow waters caused significant ($p < 0.05$) differences in soil iron concentrations, possibly due to differences in irrigation water volumes (Figure 6.3) and subsequent impact on iron mass applied on the corresponding soils (Figure 6.4). Correlation analysis results (Table 6.13) show that iron concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, calcium, potassium, magnesium, manganese and zinc values, while negatively correlated with boron levels in the soil, as reported by Essington (2015).

However, the high iron concentrations observed in irrigated soil can be explained by the already high iron concentration in the raw soil and the iron present in the irrigation water. Iron has a low bioavailability in terms of uptake by plants (FAO, 1972). This leads to the accumulation of iron in the irrigated soil. Moreover, iron concentrations in the irrigated soils did not exceed the corresponding metal threshold of 50000 mg/kg (FAO/WHO, 2001).

6.5.4.4 Soil potassium

Potassium availability is mainly related to soil pH, and to clay content and type. An increase in pH increases potassium fixation to the soil (FAO, 1972). Irrigation with wetland filter outflow waters did not cause any increase of potassium concentration compared to the raw soil (Table 6.11). Potassium concentrations were observed with the highest values in the soils irrigated with Filter 4 outflow water, which were significantly ($p < 0.05$) different from the others.

In comparison, the lowest values were recorded for those soils irrigated with Filter 2 harvested water. This observation could be linked to the corresponding plant growth rate and productivity (section 6.4). For example, the high growth rate and productivity of plants linked to Filters 2, 6 and 7 resulted in higher uptake of potassium from the soil leading to low levels compared to those plants which did not grow well, such as those linked to Filter 4 resulting in higher potassium levels in the corresponding soils. Moreover, potassium concentrations in soil irrigated with Filter 7 outflow water showed higher values than those soils irrigated with Filter 8 harvested water, possibly due to elevated irrigation water volumes (Figure 6.4) applied by the former resulting in higher potassium applied mass in the irrigated soil (Figure 6.3).

Moreover, correlation analysis results (Table 6.13) show that potassium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with other elements except boron, as reported by Essington (2015).

6.5.4.5 Soil magnesium

Table 6.11 shows that the highest magnesium concentrations were observed in the soil irrigated with water harvested from Filter 8 followed by Filter 4, while the lowest values were recorded for those soils irrigated with water obtained from Filter 6, indicating the impact on corresponding plant growth rate and production of the magnesium concentrations remaining in the soil as discussed previously (section 6.4). Table 6.11 and 6.12 indicate that irrigation with treated wastewater caused a significant ($p < 0.05$) increasing of soil magnesium levels compared with the raw soil. Moreover, soils irrigated with Filter 4 outflow water had magnesium concentrations which were significantly ($p < 0.05$) greater than those for soil irrigated with Filters 6 and 7 drain waters. Furthermore, soil irrigated with Filters 7 and 8 outflow waters had magnesium levels which were significantly ($p < 0.05$) different from each other, due to differences in irrigation water volumes applied on these soils (Figure 6.3).

Magnesium distribution in the soil is dependent on its supply and rate of uptake by plants. However, uptake of magnesium mainly depends on calcium and potassium as well as its levels in the soil. Plant magnesium uptake is usually a small portion of the total exchangeable magnesium available in the soil, which means that magnesium depletion from the soil by plant uptake is a minor factor, as discussed by Barber (1995). Moreover, correlation analysis results (Table 6.13) show that magnesium concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, calcium, manganese and zinc values, as reported by Essington (2015).

6.5.4.6 Soil manganese

The availability of manganese is strongly influenced by the soil redox potential and pH (Husson, 2013). Manganese toxicity is quite common in association with a low soil pH (FAO, 1972). Table 6.11 shows that irrigation with treated wastewater did not increase soil manganese levels compared with the raw soil. However, manganese concentrations were recorded with the maximum values in soils irrigated with Filters 8 and 7 outflow waters, while the minimum values were linked to those soils irrigated with Filter 6 outflow water.

Soils irrigated with water harvested from Filter 2 of large aggregate diameter had manganese concentrations greater than those soils irrigated with water drained from Filter 4 of small aggregate size. Moreover, the higher manganese mass applied on soils irrigated with Filter 7 outflow water (Figure 6.4) compared to those irrigated with Filter 4 outflow water caused differences in manganese levels in their corresponding irrigated soils as shown in Table 6.11.

Statistical analysis (Table 6.12) showed that there were significant ($p < 0.05$) differences in manganese levels for soils irrigated with Filters 4 and 7 outflow waters compared to those irrigated with Filters 6 and 8 harvested water, respectively, indicating the impact of high inflow loading rate and resting time of the wetland system on outflow water manganese concentrations resulting in differences in its distribution in irrigated soils (Table 6.4).

Furthermore, correlation analysis results (Table 6.13) showed that manganese concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with aluminium, iron, potassium, magnesium, calcium and zinc values, as reported by Essington (2015). However, for all irrigated soils, manganese concentrations did not exceed the corresponding metal threshold of 2000 mg/kg (FAO/WHO, 2001).

6.5.4.7 Soil zinc

Table 6.11 indicates that zinc concentrations were observed with the highest values in the soil irrigated with water harvested from Filters 2 and 7, while the lowest values were recorded for those soils irrigated with Filter 6 outflow water. Compared to the raw soil, there was no significant ($p > 0.05$) increase in the irrigated soil zinc concentrations (Table 6.12). Irrigation with outflow water from Filter 2 of large aggregate diameter caused higher soil zinc concentrations compared to those soils irrigated with water obtained from Filter 4 of small aggregate diameter (Table 6.11).

Moreover, soils irrigated with Filters 4 and 7 outflow waters had zinc concentrations which were different from those of Filters 7 and 8, respectively, due to differences in their contact and resting time variables of the wetland system (Figure 6.1). However, there were no significant differences ($p < 0.05$) in mean soil zinc concentrations among soils irrigated with different treated wastewaters (Table 6.12).

These results indicate the impact of wetland system design variables on the outflow water zinc concentrations (Table 6.4) leading to differences in distribution of this element in the irrigated soils. Correlation analysis results (Table 6.13) showed that zinc concentrations in the irrigated soil were significantly ($p < 0.001$) positively correlated with calcium, iron, potassium, magnesium, manganese and aluminium values, while negatively correlated with boron levels in the soil, as reported by Essington (2015).

However, zinc concentrations in irrigated soil did not exceed the corresponding metal threshold of 300 mg/kg (FAO/WHO, 2001)

6.5.4.8 Soil boron

The bioavailability of boron in the soil is affected by many factors such as soil parent material, texture, nature of minerals in the soil, content of organic matter, soil pH, irrigation water source, interrelationship with other elements and the environmental conditions (especially dry weather) and high light intensity (FAO, 1972). Table 6.11 shows that the highest boron concentrations were observed in soil irrigated with Filter 4 followed by Filter 2 outflow waters, while the lowest values were reported for the soil irrigated with water obtained from Filter 7.

Compared with the raw soil, irrigation with treated wastewaters did not increase the soil boron levels (Table 6.11). Statistically, results showed that there were no significant differences in irrigated soil boron concentrations (Table 6.12). However, soil irrigated with water harvested from Filter 4 had boron concentrations greater than those soils irrigated with Filters 2, 6 and 7 outflow waters.

Moreover, soils irrigated with Filter 7 outflow water had boron concentrations lower than those irrigated with Filter 8 drain water. This indicates the impact of wetland system design variables on treated water boron concentrations (Table 6.4) which caused differences in the element distribution when applied on the soils. Correlation analysis (Table 6.13) showed that boron concentrations in the soil correlated negatively with other elements in the soil, as discussed by Essington (2015).

6.6 Chilli generation quality and analysis

6.6.1 Chilli generation microbial and mineral contents

None of the Chilli generation harvested fruits were found to have microbial contamination. Findings also show that no microbiological contamination was recorded for skin, flesh and washing solutions for the fruits harvested from plants irrigated with different waters obtained from wetland filters.

This can be explained by the relatively long distance between the fruits and the potentially contaminated soil (Cirelli et al., 2012). This confirmed the results reported by Almuktar and Scholz (2015) which indicated that Chilli contamination by various microbes such as total coliforms, *Streptococcus* spp. and *Salmonella* spp. was detected only in Chilli fruits which were in contact with contaminated soils, while fruits located far away from the soil surface did not show any bacterial contamination. Regarding Chilli mineral contamination, Tables 6.14 and 6.15 show the concentrations of elements detected in Chillies harvested from generation plants and the associated statistical analysis results, respectively. The bioavailability of each element was indicated by calculating the Concentration Factor (CF) value. This factor is defined as the relationship, as a percentage, between element concentration in the plant organ and its concentration in the soil (Q.Li et al., 2012) as follows:

$$\mathbf{CF=100^{*}\ (C_{fruit}/C_{soil})}$$

Where,

CF: concentration factor (%)

C_{fruit}: element concentration in the fruit (mg/kg)

C_{soil}: element concentration in the soil (mg/kg)

However, results showed that the CF values in the Chillies followed the order of:

potassium > boron > zinc > magnesium > manganese > calcium > iron, with values (%)

of 3819, 314, 169, 30, 14, 3 and 1, respectively. More details on fruit element contents are shown in the subsections below:

6.6.1.1 Comparison of aluminium

Table 6.14 shows that aluminium was not detected in any of the fruits harvested from generation plants irrigated with different water types, confirming the results reported by Almuktar and Scholz (2016a). However, aluminium was found in abundancy in irrigated soils (Table 6.11), since its solubility is mainly governed by the soil pH, and by soil organic matter and clay content. Exchangeable aluminium rapidly increases when pH decreases (Husson, 2013). In spite of this, aluminium was limited in terms of its transfer into fruit tissue. This can be explained by the high abundance of calcium in the soil leading to the limited transport of aluminium to the plants (FAO, 1972). However, aluminium is not considered harmful to human health, because of its relatively low bioavailability (Stahl et al., 2011).

In acid mineral soils (pH < 7.0), aluminium buffers the soil pH at around 4, and is thus available to plants in the toxic form Al^{3}+. However, plant populations present in these soils normally evolve some degree of tolerance to aluminium in the soil solution and any aluminium present in these soils is likely to be as non-toxic organo-aluminium complexes (Kidd & Proctor, 2000).

Table 6.14: Overview of the Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) analysis for selected elements compared with common standards for vegetables (e.g., EC (2001) and FAO/WHO (2001)).

Element	Mean	Minimum	Maximum	Standard deviation
Calcium (mg/kg)				
Filter 2	415.08	334.58	570.78	55.060
Filter 4	422.24	329.77	632.54	70.283
Filter 6	681.02	562.90	905.28	84.787
Filter 7	578.64	447.47	805.03	82.518
Filter 8	958.67	787.58	1297.70	118.701
Standards	-	-	-	-
Iron (mg/kg)				
Filter 2	14.29	3.62	37.50	10.242
Filter 4	33.44	23.86	55.95	8.772
Filter 6	31.46	22.45	45.05	7.532
Filter 7	16.03	10.89	28.08	4.789
Filter 8	8.09	3.02	12.60	3.180
Standards	425.00	425.00	425.00	-
Potassium (mg/kg)				
Filter 2	33143.63	31819.73	35566.00	1508.906
Filter 4	41398.00	37162.67	45830.00	3297.548
Filter 6	77930.56	46516.67	146472.33	46526.582
Filter 7	41993.72	40354.33	43473.67	1138.769
Filter 8	109481.67	46674.67	147138.00	48252.728
Standards	-	-	-	-
Magnesium (mg/kg)				
Filter 2	2446.07	1817.23	2874.30	290.248
Filter 4	2540.75	1867.74	2953.76	296.377
Filter 6	2915.42	2233.62	3238.17	310.426
Filter 7	2709.31	2053.97	3023.17	294.989
Filter 8	3262.31	2424.44	3599.10	363.250
Standards	-	-	-	-
Manganese (mg/kg)				
Filter 2	21.05	16.07	29.96	3.762
Filter 4	19.12	13.91	25.72	3.200
Filter 6	23.73	19.84	35.35	4.025
Filter 7	21.43	16.52	32.04	4.030
Filter 8	24.19	19.77	35.99	4.711
Standards	500.00	500.00	500.00	-
Zinc (mg/kg)				
Filter 2	35.87	18.70	54.93	8.229
Filter 4	66.60	39.62	86.93	12.552
Filter 6	65.04	38.63	77.52	11.998
Filter 7	65.48	43.96	88.32	12.326
Filter 8	53.16	30.37	80.21	12.029
Standards	50.00	50.00	50.00	-
Boron (mg/kg)				
Filter 2	14.77	11.25	18.74	2.567
Filter 4	16.04	12.72	18.82	1.867
Filter 6	17.73	15.67	20.27	1.453
Filter 7	14.89	13.01	17.13	1.277
Filter 8	13.90	11.19	16.82	1.589
Standards	-	-	-	-

Note: Aluminium was not detected in the tested fruits. Thirty fruit samples per treatment were analysed.

Table 6.15: Overview of the statistically significant differences in elements in the harvested fruits.

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Fruit calcium (mg/kg)								
0.008	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.936	< 0.001	< 0.001	< 0.001
			Filter 4	0.936	n.a	< 0.001	< 0.001	< 0.001
			Filter 6	< 0.001	< 0.001	n.a	0.034	0.006
			Filter 7	< 0.001	< 0.001	0.034	n.a	< 0.001
			Filter 8	< 0.001	< 0.001	0.006	< 0.001	n.a
Fruit iron (mg/kg)								
0.007	Kruskal-Wallis	< 0.001	Filter 2	n.a	< 0.001	< 0.001	0.275	0.147
			Filter 4	< 0.001	n.a	0.852	< 0.001	< 0.001
			Filter 6	< 0.001	0.852	n.a	< 0.001	< 0.001
			Filter 7	0.275	< 0.001	< 0.001	n.a	0.012
			Filter 8	0.147	< 0.001	< 0.001	0.012	n.a
Fruit potassium (mg/kg)								
< 0.001	Kruskal-Wallis	< 0.001	Filter 2	n.a	0.082	< 0.001	0.071	< 0.001
			Filter 4	0.082	n.a	0.030	0.948	0.009
			Filter 6	< 0.001	0.030	n.a	0.036	0.646
			Filter 7	0.071	0.948	0.036	n.a	0.011
			Filter 8	< 0.001	0.009	0.646	0.011	n.a
Fruit magnesium (mg/kg)								
0.124	ANOVA	< 0.001	Filter 2	n.a	0.799	< 0.001	0.020	< 0.001
			Filter 4	0.799	n.a	< 0.001	0.280	< 0.001
			Filter 6	< 0.001	< 0.001	n.a	0.115	0.001
			Filter 7	0.020	0.280	0.115	n.a	< 0.001
			Filter 8	< 0.001	< 0.001	0.001	< 0.001	n.a

Table 6.15 (cont.)

Shapiro-Wilk test (p-value)[a]	Statistical test	P-values for treatment combinations	Treatment	Statistic (p-value)[b]				
				Filter 2	Filter 4	Filter 6	Filter 7	Filter 8
Fruit manganese (mg/kg)								
0.042	Kruskal-Wallis	<0.001	Filter 2	n.a	0.009	0.019	0.881	0.010
			Filter 4	0.009	n.a	< 0.001	0.072	< 0.001
			Filter 6	0.019	< 0.001	n.a	0.028	0.826
			Filter 7	0.881	0.072	0.028	n.a	0.015
			Filter 8	0.010	< 0.001	0.826	0.015	n.a
Fruit zinc (mg/kg)								
0.012	Kruskal-Wallis		Filter 2	n.a	< 0.001	< 0.001	< 0.001	0.001
			Filter 4	< 0.001	n.a	0.852	0.761	0.003
			Filter 6	< 0.001	0.852	n.a	0.914	0.007
			Filter 7	< 0.001	0.761	0.914	n.a	0.008
			Filter 8	0.001	0.003	0.007	0.008	n.a
Fruit boron (mg/kg)								
0.463	ANOVA	<0.001	Filter 2	n.a	0.637	0.020	1.000	0.856
			Filter 4	0.637	n.a	0.136	0.417	0.045
			Filter 6	0.020	0.136	n.a	< 0.001	< 0.001
			Filter 7	1.000	0.417	< 0.001	n.a	0.469
			Filter 8	0.856	0.045	< 0.001	0.469	n.a

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding water quality parameter). n.a, not applicable as the treatment compared with itself. ANOVA, the parametric one-way analysis of variance test; Kruskal-Wallis, the non-parametric Kruskal-Wallis test.

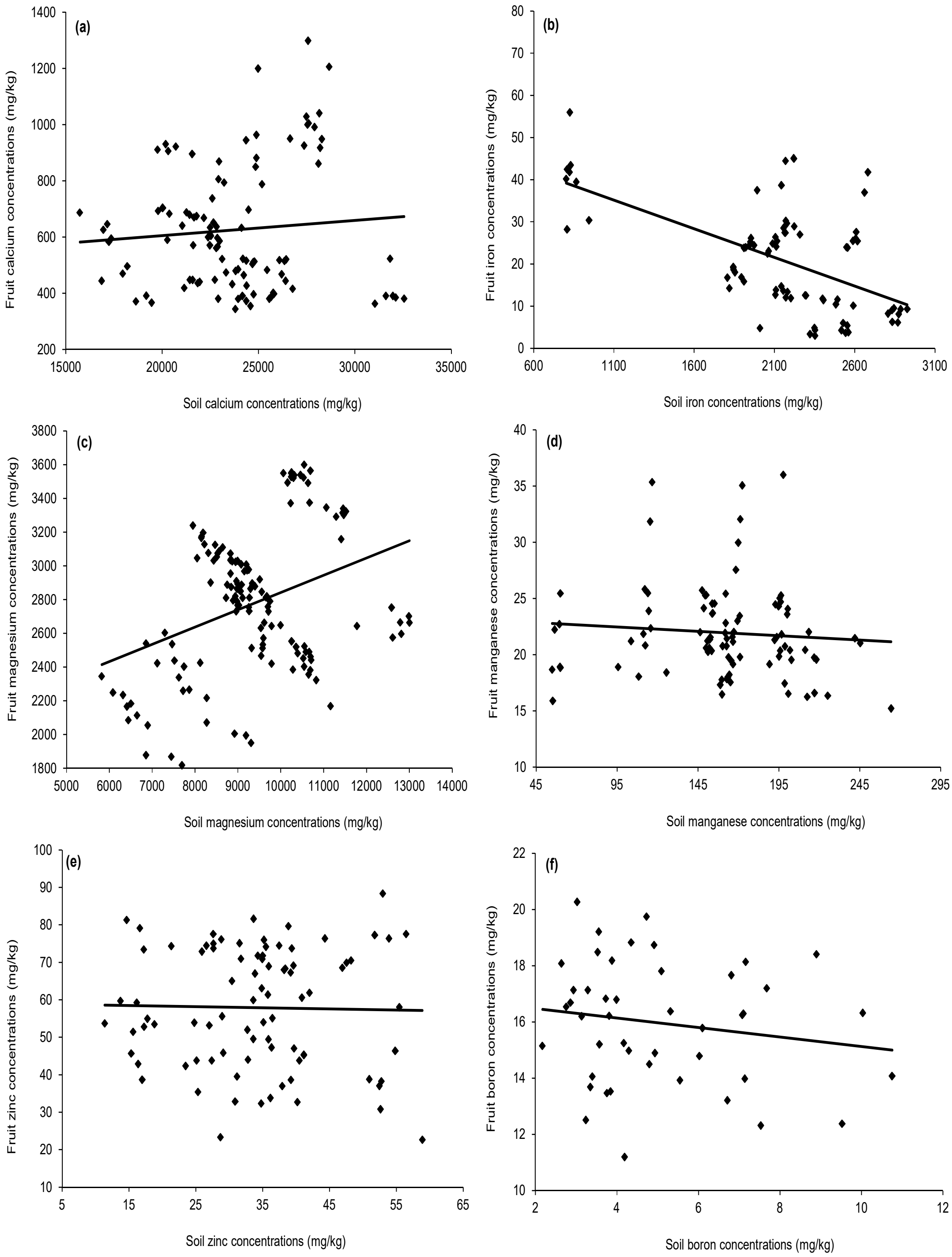

Figure 6.11: Overview of relationships between elements in the soil and harvested fruits: (a) calcium; (b) iron, (c) magnesium, (d) manganese, (e) zinc, and (f) boron.

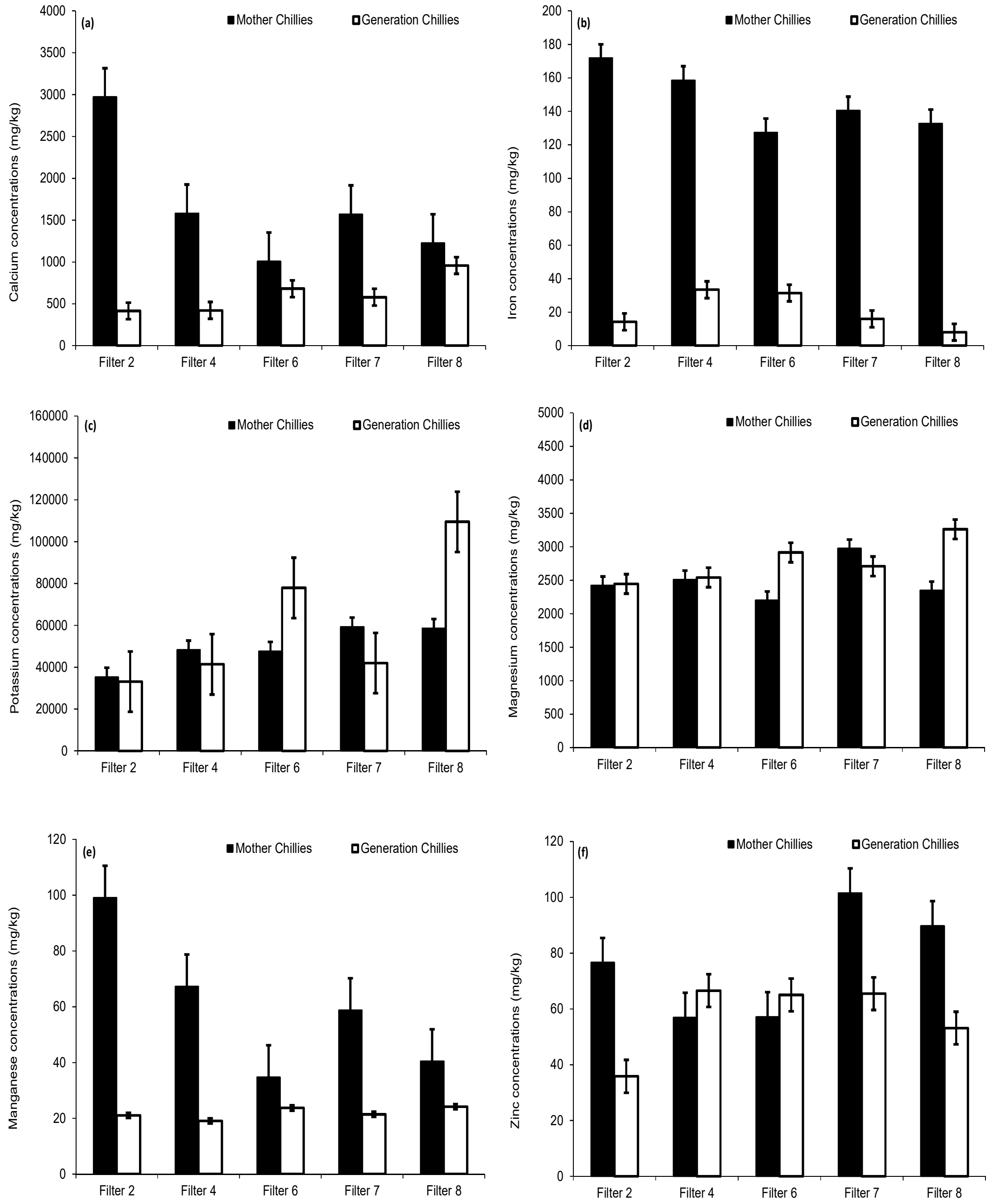

Figure 6.12: Comparison between Chilli mother and generation fruit element concentrations: (a) calcium, (b) iron, (c) potassium, (d) magnesium, (e) manganese, and (f) zinc.

Table 6.16: Overview of the statistically significant differences in Chilli fruit mean element concentrations harvested from mother and generation plants subjected to different irrigation water types.

Element	Shapiro-Wilk test (*p*-value)[a]	Statistical test	*P*-values between mother and generation plants
Fruit calcium (mg/kg)			
Filter 2	< 0.001	M-W-U	< 0.001
Filter 4	< 0.001	M-W-U	< 0.001
Filter 6	< 0.001	M-W-U	< 0.001
Filter 7	< 0.001	M-W-U	< 0.001
Filter 8	0.001	M-W-U	0.001
Fruit iron (mg/kg)			
Filter 2	< 0.001	M-W-U	< 0.001
Filter 4	< 0.001	M-W-U	< 0.001
Filter 6	< 0.001	M-W-U	< 0.001
Filter 7	< 0.001	M-W-U	< 0.001
Filter 8	< 0.001	M-W-U	< 0.001
Fruit potassium (mg/kg)			
Filter 2	0.207	I-T	0.229
Filter 4	0.814	I-T	0.004
Filter 6	< 0.001	M-W-U	0.261
Filter 7	0.004	M-W-U	0.001
Filter 8	< 0.001	M-W-U	0.261
Fruit magnesium (mg/kg)			
Filter 2	0.031	M-W-U	1.000
Filter 4	0.283	I-T	0.894
Filter 6	< 0.001	M-W-U	< 0.001
Filter 7	0.179	I-T	0.140
Filter 8	< 0.001	M-W-U	< 0.001
Fruit manganese (mg/kg)			
Filter 2	< 0.001	M-W-U	< 0.001
Filter 4	< 0.001	M-W-U	< 0.001
Filter 6	< 0.001	M-W-U	< 0.001
Filter 7	< 0.001	M-W-U	< 0.001
Filter 8	< 0.001	M-W-U	< 0.001
Fruit zinc (mg/kg)			
Filter 2	< 0.001	M-W-U	< 0.001
Filter 4	0.001	M-W-U	0.383
Filter 6	0.020	M-W-U	0.018
Filter 7	0.012	M-W-U	< 0.001
Filter 8	0.014	M-W-U	< 0.001

Note: [a] Test of normality (if p-value > 0.05, data are normally distributed; if p-value < 0.05, data are not normally distributed. [b] p-value, probability of the statistical test (values are statistically significantly different only if the p-value < 0.05 for the corresponding parameter). M-W-U, the non-parametric Mann-Whitney U-test and I-T, the parametric Independent samples T-test.

6.6.1.2 Comparison of calcium

Table 6.14 shows that the highest calcium concentrations were observed in fruits harvested from plants irrigated with outflow water obtained from Filter 8, followed by those fruits irrigated with outflow waters from Filter 6. In comparison, the lowest iron concentrations were recorded for fruits of plants irrigated with outflow water received from Filter 2. However, calcium concentrations in all tested fruits (except those for Filter 2 and 4) were higher than that reported by Ciju (2013) of 45 mg per 100 g of sun dried Chillies.

Statistical analysis (Table 6.15) showed that plants irrigated with water obtained from Filter 2 of large aggregate diameter produced Chillies of calcium concentrations which were similar to those linked to Filter 4 of small aggregate diameter. Moreover, irrigation with Filter 6 of high inflow loading rate resulted in fruit calcium concentrations which were significantly ($p < 0.05$) greater than those associated with Filter 4, which was fed with diluted wastewater (Table 6.11). Furthermore, Chillies harvested from plants irrigated with Filter 7 outflow water showed calcium concentrations which were significantly ($p < 0.05$) different from those linked to Filters 4 and 8. No significant ($p > 0.05$) positive correlation relationship was observed in calcium concentrations between soil and fruits (Figure 6.11a).

Moreover, the calcium concentration factor (CF) in the tested fruits order was: Filter 8 > Filter 6 > Filter 7 > Filter 2 > Filter 4 with values of 3.7%, 3.3%, 2.6%, 1.8% and 1.6%, respectively.

Compared with their mothers, Chilli generation fruits had calcium concentrations which were significantly ($p < 0.05$) lower (Table 6.16 and Figure 6.12a) explaining the impact of higher irrigation water volumes applied on mother plants than those for generations (Figure 6.3).

However, calcium in the soil is in competition with other major cations such as sodium (Na+), potassium (K+), magnesium (Mg++), ammonium (NH4+), iron (Fe++), and aluminium (Al+++) for uptake by the crop (FAO, 1972). High potassium applications have been known to reduce the calcium uptake in plants (Barber, 1995). As the pH of a soil decreases, more of these elements (mainly Iron (Fe++) and aluminium (Al+++)) become soluble and combine with calcium to form essentially insoluble compounds (Barber, 1995).

High soil or plant calcium levels can inhibit boron uptake and utilisation. Calcium is essential for many plant functions such as proper cell division and cell wall development as well as metabolism and enzyme activity (FAO, 1972). Calcium is an essential mineral for human health as well, being especially important for metabolism processes, bone structure, muscle and nerve function control, and managing the balance of blood stream. This explains how food which is rich in calcium can play an important role in human health (Zhu & Prince, 2012).

6.6.1.3 Comparison of iron

Table 6.14 shows that the highest iron concentrations were observed in fruits harvested from plants irrigated with outflow water obtained from Filter 4, followed by those fruits irrigated with outflow waters from Filter 6. In comparison, the lowest iron concentrations were recorded for fruits of plants irrigated with outflow water received from Filter 8. However, the recorded iron concentrations in fruits harvested from all treatments were considerably lower than that reported by Ciju (2013) of 6.04 mg per 100 g dried Chillies. Statistical results (Table 6.15) showed that the impact of the wetland aggregate diameter was significant ($p < 0.05$) in comparison between the iron mean concentrations in fruits harvested from plants of Filter 2 compared to those of Filter 4.

Fruit harvested from plants irrigated with Filters 4 and 7 had mean iron concentrations which were significantly ($p < 0.05$) different from those of Filters 7 and 8, respectively, due to the impact of wetland contact and resting time variables. Correlation analysis results showed that mean iron concentrations in soil and fruits were significantly negatively correlated with each other ($R = -0.475$, $p < 0.001$). Moreover, regression analysis results (Figure 6.11b) indicated that iron concentrations in the fruits decreased linearly with the corresponding values in the soil due to its low bioavailability to the plants, as reported by FAO (1972).

Moreover, the iron concentration factor (CF) in the tested fruits order was: Filter 4 > Filter 6 > Filter 7 > Filter 2 > Filter 8 with values of 1.8%, 1.5%, 0.8%, 0.6% and 0.3%, respectively. Compared with their mothers, Chilli generation fruits had iron concentrations which were significantly ($p < 0.05$) lower (Table 6.16 and Figure 6.12b), explaining the impact of higher irrigation water volumes applied on mother plants than those for generations (Figure 6.3). Moreover, iron concentrations in tested fruits did not exceed the threshold of 425 mg/kg (EC, 2001; FAO/WHO, 2001). Although iron is an essential element for human health, excessive iron amounts can lead to tissue damage (Abbaspour et al., 2014).

6.6.1.4 Comparison of potassium

Table 6.14 shows that the highest mean potassium concentrations were observed in fruit harvested from plants irrigated with Filter 8 followed by Filter 6 outflow waters, while the lowest values were recorded for those fruits harvested from Filter 2 plants.

However, potassium concentrations in all tested fruits were very high compared to those reported by Ciju (2013) who recommended a potassium value of 1870 mg per 100g of sun dried Chillies. Fruits harvested from plants irrigated with Filter 2 (large aggregate diameter) outflow water had potassium concentrations lower than those of Filter 4 (small aggregate size), while the latter fruits had potassium concentrations which were similar to those of Filter 7 (Table 6.14). Moreover, statistical analysis results (Table 6.15) showed that fruit harvested from plants irrigated with water drained from Filters 4 and 7 had potassium concentrations which were significantly ($p < 0.05$) lower than those of Filters 6 and 8, respectively.

Moreover, the potassium concentration factor (CF) in the tested fruits order was: Filter 8 > Filter 6 > Filter 2 > Filter 7 > Filter 4 with values of 6826%, 5103%, 2768%, 2503% and 1897%, respectively. Compared with their mothers (Figure 6.12), generation fruits showed some significant ($p < 0.05$) differences in mean potassium concentrations (Table 6.16), possibly due to differences in element mass applied on both plants (Figure 6.4) via irrigation water leading to differences in corresponding soil potassium concentrations available for uptake by pants.

Potassium has an important role in the plants as it functions in many physiological processes such as photosynthesis, protein synthesis, and activation of some enzymes (FAO, 1972). However, from the human health point of view, potassium is an important mineral that can maintain the water balance and blood pressure within human bodies (FAO/WHO, 2001).

6.6.1.5 Comparison of magnesium

Table 6.14 indicates that magnesium concentrations in tested fruits were higher than that reported by Ciju (2013) of 88 mg per 100 g of dried Chillies. The highest magnesium concentrations were observed in fruits harvested from plants irrigated with Filter 8 followed by Filter 6 outflow waters, while the lowest concentration values were recorded for fruits of plants irrigated with Filter 2 outflow water.

Fruits harvested from plants irrigated with Filter 2 drain water had mean magnesium concentrations similar to those of Filter 4 plants, while the latter plants produced fruits of mean concentrations which were lower than those associated with Filter 7 plants. Furthermore, statistical analysis (Table 6.15) showed that there were significant ($p < 0.05$) differences in mean fruit magnesium concentrations of plants irrigated with Filter 7 water compared to those of Filter 8, explaining the impact of the resting time variable in the wetland system. Moreover, a significant ($p < 0.05$) difference was observed between fruit magnesium mean concentrations of Filter 4 fruits compared to those linked to Filter 6, due to inflow loading rate of the corresponding wetland filters.

Correlation analysis results showed that magnesium concentrations in soil and fruits were significantly positively correlated with each other ($R = 0.248$, $p = 0.004$). Moreover, regression analysis results indicated that fruit magnesium concentrations linearly increased with their corresponding levels in the soil (Figure 6.11c).

Furthermore, the magnesium concentration factor (CF) in the tested fruits order was: Filter 6 > Filter 8 > Filter 7 > Filter 2 > Filter 4 with values of 36%, 32%, 30%, 26% and 25%, respectively. Moreover, Chilli generation magnesium mean concentrations were observed to be different from their mothers, as shown in Figure 6.12 and Table 6.16.

However, uptake of magnesium mainly depends on calcium and potassium as well as its levels in the soil. Plant magnesium uptake is usually a small portion of the total exchangeable magnesium available in the soil, which means that magnesium depletion from the soil by plant uptake is a minor factor, as discussed by Barber (1995). Magnesium is an essential plant nutrient as it has a wide range of key roles in many plant functions. One of the well-known roles of magnesium is in the photosynthesis process, as it is a building block of chlorophyll, which makes leaves appear green. Magnesium deficiency might be a significant limiting factor in crop production (FAO, 1972).

Considering human health, magnesium plays a role in the structural development of bones, and the active transport of calcium and potassium ions across cell membranes, which is important for nerve impulse conduction, muscle contraction, and a normal heart rhythm. Moreover, too much magnesium from food does not pose a health risk for healthy individuals (Musso, 2009).

6.6.1.6 Comparison of manganese

Manganese concentrations in tested fruits are shown in Table 6.14. The highest manganese concentrations were observed in fruit of plants irrigated with Filter 8 followed by Filter 6 outflow waters, while the lowest values were observed in those fruits of plants irrigated with Filter 4 drain water. Statistical analysis (Table 6.15) showed that fruit mean manganese concentrations of plants irrigated with Filters 4 and 7 outflow waters were significantly ($p < 0.05$) different from those of plants irrigated with Filters 2 and 8 outflow waters, indicating the impact of aggregate diameter and resting time of wetlands on corresponding soil element distribution available for uptake by plants.

Furthermore, fruits of plants irrigated with Filter 4 had average magnesium concentrations which were significantly ($p < 0.05$) different from those of Filter 6, explaining the impact of wetland inflow loading rate. However, the differences in manganese concentrations in the harvested fruits can be explained by the differences in manganese values regarding the corresponding growth media (section 6.6.4.6).

Correlation analysis results showed that manganese concentrations in the soil and fruits were negatively correlated with each other (R = -0.135, p = 0.206). Moreover, regression analysis results (Figure 6.11d) indicated that fruit manganese concentrations linearly decreased with their corresponding values in the soil due to its low bioavailability, as reported by Barber (1995).

The manganese concentration factor (CF) in the tested fruits order was: Filter 6 > Filter 4 = Filter 2 = Filter 7 > Filter 8 with values of 17%, 13%, 13%, 13% and 12%, respectively. Compared to their mother plants, Chilli generation fruits showed mean manganese concentrations which were significantly ($p < 0.05$) lower (Figure 6.12 and Table 6.16).

Manganese is an essential plant mineral nutrient, playing a key role in several physiological processes, particularly photosynthesis. The impact of manganese deficiencies on crops includes reduced dry matter production and yield, weaker structural resistance against pathogens and a reduced tolerance to drought and heat stress (Hakala et al., 2006). Manganese concentrations in tested fruits did not exceed the corresponding metal threshold of 500 mg/kg (EC, 2001; FAO/WHO, 2001).

6.6.1.7 Comparison of zinc

Table 6.14 shows that the highest zinc concentrations were observed in fruit harvested from plants watered with Filter 4 outflow water, while the lowest concentrations were observed in fruits of plants irrigated with Filter 2 outflow water. However, detected zinc concentrations exceeded that reported by Ciju (2013) of 1.02 mg for 100 g of dried Chillies.

Statistical analysis (Table 6.15) showed that average zinc concentrations in fruits of Filters 4 and 8 irrigated plants were significantly ($p < 0.05$) different from those of Filters 2 and 7, respectively, explaining the impact of the aggregate diameter and resting time variables on the corresponding zinc concentrations of the growth media (section 6.6.4.7). Differences in zinc concentrations in harvested fruits are likely due to the differences in zinc values in the corresponding growth media. No significant ($p > 0.05$) negative correlation relationship was observed in zinc concentrations between soil and fruits (Figure 6.11e) ($R = -0.005$, $p = 0.961$).

The zinc concentration factor (CF) in the tested fruits order was: Filter 6 > Filter 4 > Filter 7 > Filter 8 > Filter 2 with values of 210%, 189%, 178%, 170% and 97%, respectively. However, Chilli generations had mean zinc concentrations which were significantly ($p < 0.05$) different from their mothers (Figure 6.12 and Table 6.16).

Zinc is an essential micronutrient for plants as it acts as a functional, structural, or regulatory cofactor of a large number of enzymes, also, zinc may play a role in controlling gene expression, as discussed by Brown et al. (1993).

However, zinc concentration in fruits harvested from all irrigated plants (except for those irrigated with Filter 2 outflow) exceeded the corresponding metal threshold of 50 mg/kg (EC, 2001; FAO/WHO, 2001).

Lactase (1998) assessed the levels of heavy metals in vegetables by evaluating the contamination/pollution (C/P) index. This index is based on the metal concentration in vegetables (or water or soil) divided by the corresponding maximum permissible concentration levels (thresholds). Lacatusu (1998) listed the significance intervals of the C/P index. Based on this, all fruits tested for zinc were slightly polluted (C/P value between 1.1 and 2.0). Considering human health, zinc is an essential micronutrient in the body and can be used in numerous pharmaceutics. Nevertheless, zinc is toxic, when taken long-term in high doses (FAO/WHO, 2001).

6.6.1.8 Comparison of boron

Table 6.14 indicates that boron was detected with the highest levels in the fruits harvested from plants irrigated with outflow water from Filter 6 followed by Filter 4, while the lowest values were recorded for those fruits harvested from Filter 8 plants. Moreover, Filters 2 and 7 plants produced fruits with mean boron concentrations which were lower than those of Filter 4, while the latter fruits had boron concentrations lower than those for Filter 6.

Moreover, fruits of plants irrigated with Filters 7 and 8 outflow waters were different from each other (Table 6.14). Results showed that boron concentrations in fruits and soils were negatively correlated with each other ($R = -0.147$, $p = 0.337$) as shown in Figure 6.11f which indicates that boron in the fruits decreased linearly with their corresponding soil values.

The boron concentration factor (CF) in the tested fruits order was: Filter 6 > Filter 7 > Filter 8 > Filter 2 > Filter 4 with values of 408%, 355%, 281%, 267% and 257%, respectively. This result contradicts that reported by Diana (2006) which indicated that boron can be available in the soil at different concentrations and compositions, but only a relatively small proportion is obtainable by plants. However, boron was not detected in the mother Chillies. This is possibly due to the higher calcium concentrations in soils associated with the mother plants than the generations, because of the higher irrigation water volume applied on the former (Figure 6.3), which will inhibit boron uptake and utilisation (Barber, 1995).

However, boron is an essential micronutrient for plant growth and development as it plays an important role in metabolism processes, mainly related to stabilisation of cell membranes (Cakmak & Römheld, 1997; Blevins & Lukaszewski, 1998). Regarding human health, boron is considered an essential mineral that can positively affect bone growth and reduce the risk of some cancer types (Nielsen, 2014).

6.7 Summary

This chapter discusses the quality of different irrigation water types associated with domestic wastewater treated by vertical-flow constructed wetlands for irrigation of Chilli generations grown in laboratory controlled conditions using organic growth media. This includes the concentrations of nutrients, trace elements, organics, salinity and microbial contents of the treated wastewater compared with the irrigation water standards. The environmental boundary conditions available for plants are discussed as well. Moreover, growth, productivity and marketable yield assessment of Chilli generations irrigated with various irrigation water types are discussed statistically compared to their mothers. Furthermore, the quality of soil used for growing Chilli generation plants and irrigated with different water types is assessed. The mineral content of these soils as well as the microbial contamination are studied. Harvested Chilli fruit quality in terms of trace element and heavy metal content is investigated and assessed compared to their mothers as well as with the standards of human and public health.

CHAPTER 7
CONCLUSION AND RECOMMENDATIONS FOR FURTHER RESEARCH

7.1 Conclusion

There are many studies which focus on irrigation of plants with different treated and untreated wastewater. However, most research on irrigation with treated wastewater has dealt with traditional treatment processes which are known to be highly cost effective. According to the literature, due to the high efficiency of wetlands in treating wastewater, there is an interest in recycling the effluent for different purposes, particularly in the agricultural field. Moreover, traces of hydrocarbons from diesel spills associated with urban runoff or industrial effluent are a more recent challenge.

Despite the numerous studies on recycling of urban wastewater treated with different technologies for irrigation purposes, there are few long-term and controlled studies involving domestic wastewater due to health and safety concerns. Moreover, there are few studies in the literature on recycling of domestic wastewater treated by wetlands in general and vertical-flow ones in particular. According to the literature, few studies, if any, give attention to long-term evaluation of wetlands effluent suitability, especially that of vertical-flow (VF) ones, for irrigation purposes. Moreover, no research has been undertaken to monitor the impact of different wetlands system designs on treated domestic wastewater (contaminated and uncontaminated with diesel) and the subsequent effect on plant growth, productivity and safety in terms of human consumption.

In this study, effluent from different types of wetland systems treating domestic wastewater was selected to irrigate vegetables grown in the laboratory (controlled environmental conditions). Some of the wetlands received standard wastewater while others received wastewater that was subject to a one-off diesel fuel spill. The treated wastewater from all wetland types was recycled for the irrigation of Bell Peppers and Chillies, which are commonly seen as two popular, relatively expensive and easy-to-grow vegetables with high nutritional value; also, they can be grown in greenhouses in the UK.

This study provides the scientific justification for integrating treatment wetlands into agricultural food production. Moreover, it fills gaps in knowledge and understanding by assessing the impact of different wetland (some contaminated with diesel) system designs in terms of their suitability in providing irrigation water for an example crop, which should be safe for human consumption and lead to a good economic return, and its corresponding water management should not result in soil contamination. Therefore, the overall aim of this study is to assess if vegetables can be grown successfully using recycled domestic wastewater treated by constructed wetlands.

The overall results showed that the example crops can be grown successfully using wastewater treated by constructed wetland systems despite irrigation water contamination by metals and pathogens, indicating that recycling of domestic wastewater treated by wetlands for irrigation purposes seems to be a viable alternative to the use of drinking water and fertiliser application. The key research findings have been compared to the objectives set out in chapter 1 (section 1.6). The main conclusions emanating from this research are summarised as follows:

- Vertical flow constructed wetlands treated the domestic wastewater well, meeting the irrigation water quality standards for most water quality parameters with exception of phosphorus, ammonia-nitrogen, potassium and total coliforms, which showed high values significantly ($p < 0.05$) exceeding the thresholds set for irrigation purposes.
- Sweet Peppers can be grown using wastewater treated by constructed wetlands. However, the marketable yield was too low to make a decent profit. The highest number of fruits was linked to raw wastewater and an organic growth medium (10 fruits), while the highest fruit quality, indicated by diameter (45 mm), length (65 mm) and weight (54 g), was observed for peppers grown in organic media and irrigated with outflow water from wetlands with large aggregates, long contact and resting times, and a low inflow loading rate. As the nutrients within the degraded compost got depleted after the start of the experiment, the harvest increased for pots that received pre-treated wastewater in comparison to those depending only on the remaining nutrients obtained from the almost exhausted compost. Results show that nutrient concentrations supplied to the peppers by biodegrading compost and nutrient-rich wastewater were too high to produce a reasonable harvest, because Sweet Peppers are sensitive to too high nutrient concentrations leading to plant development challenges. A high marketable yield (harvest of Sweet Peppers, which are of good quality) related to the most suitable provision of nutrients to the peppers rather than the volume of applied irrigation water volume. A good pepper harvest was linked to a wetland system with a large aggregate diameter and diesel spill. This can be explained by the fact that the degradation of hydrocarbon requires the presence of considerable nitrogen resources.

In the absence of diesel, too much nitrogen increases leaf development and decreases fruit development.

- Chillies can be grown successfully using wastewater treated by constructed wetlands. Chilies did reasonably well but the growth of foliage was excessive due to high nitrogen concentrations in the inflow water and the harvest was delayed. The highest number of fruits was associated with tap water and an organic growth medium (51 fruits). However, the best fruit quality, in terms of length (73 mm), width (15 mm) and weight (7 g), was observed for plants grown in organic media and irrigated with outflow water from wetlands containing small aggregates with long contact and resting times and fed with a high inflow loading rate (undiluted wastewater), releasing more nutrients into their effluent resulting in a greater marketable profit. High Chilli yields in terms of economic return were associated with tap water and an organic growth medium (138.6 pence), and a wetland with a small aggregate size, short contact time and long resting times with a low inflow loading rate (109.9 pence). Low fruit numbers correlate well with inorganic growth media. Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated wastewater were usually too high to produce a good harvest. However, as the compost was depleted of nutrients such as nitrogen, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost. Filters contaminated with hydrocarbon were usually associated with a substantially lower marketable yield than those filters lacking hydrocarbon pollution. The productivity of Chillies in terms of harvest was independent of the wastewater consumption volume, but may have depended on the water quality.

Nevertheless, higher foliage production due to excess nutrients and trace minerals required more water. A high yield was related with the most suitable provision of nutrients and trace elements. Chillies harvested at a plant height below 50 cm were often contaminated by potentially pathogenic microbes. However, the overall proportion of contaminated harvest was less than 5%. No bacteriological contamination was detected for any Chilli fruits harvested from a plant height equal to or above 50 cm. The mineral content of organic soil was significantly ($p < 0.05$) higher than that for inorganic soil before and after irrigation with treated wastewater. Compared to the raw growth media, irrigation with treated wastewater led to concentration increases of some elements such as magnesium, aluminium, zinc and boron. However, no substantial mineral contamination was observed in the soils due to irrigation with treated wastewater. Slight to moderate zinc contamination was detected in harvested Chillies based on common standards for vegetables.

- Generally, Chillies produced more fruits than Sweet Peppers when using organic growth media, indicating the positive impact of high nutrients and trace elements available by both compost and treated wastewater on growth and productivity of Chillies, but the negative impact on Peppers explaining the different tolerance of plants to the supplied nutrition. Nevertheless, a good balance in supplying nutrients is required for high marketable yield as the surplus will result in increasing the productivity at the expense of quality. Based on this, outcomes for Chillies harvested from organic media were higher than those associated with Sweet Pepper.

Moreover, the growth and productivity of both Chillies and Sweet Peppers were rather disappointing when using inorganic media, due to insufficiency of nutrition supplied only by the irrigation waters. Moreover, most fruits harvested from plants grown in the sandy soil were categorised with low classes leading to very low harvest outcomes. However, considering that the monetary value of the Sweet Pepper harvest was low, it is unlikely to be the fruiting vegetable choice of to be grown using recycled wastewater streams in the future.

- Chilli generations can be grown successfully using wastewater treated by constructed wetlands and organic soil. The highest number of fruits is associated with wetlands of small aggregate diameter, short resting and contact times, and diluted inflow wastewater (305 fruits). However, the best fruit quality, in terms of length (65 mm), width (16 mm) and weight (7 g), was observed for plants irrigated with outflow water from wetlands containing small aggregates with long contact and resting times and fed with a high inflow loading rate (undiluted wastewater), releasing more nutrients into their effluent resulting in a greater marketable profit. High Chilli yields in terms of economic return were associated with wetlands containing small aggregates and where the contact and resting time were high with high inflow loading rate (90.78 pence). The productivity of Chillies in terms of harvest was independent of the wastewater consumption volume, but may have depended on the water quality. A high yield was related with the most suitable provision of nutrients and trace elements. No microbial content was observed in the harvested fruits. Compared to the raw growth media, irrigation with treated wastewater led to concentration increases of some elements such as magnesium, aluminium and zinc.

However, no substantial mineral contamination was observed in the soils due to irrigation with treated wastewater. Slight zinc contamination was detected in harvested Chillies based on common standards for vegetables.

- Reducing irrigation water volumes applied on Chilli generations compared to their mothers resulted in a noticeable reduction in total elements applied mass and subsequent shorter plants with abundant fruit numbers which were harvested earlier than their mothers. However, excessive nutrients applied on mother plants resulted in better fruit quality in terms of dimensions and weights compared with their generations leading to a greater marketable profit. This reduction in irrigation water volume also resulted in a significant reduction in Chilli generation mineral content compared to their mothers increasing safety for human consumption.

7.2 Recommendations for future work

Although this research verified the potential use of vertical-flow constructed wetlands for treating wastewater and subsequent recycling for irrigation purposes, a long-term controlled experiment is recommended for further investigation. Moreover, the experimental constructed wetlands system used in this study is not similar to that of large-scale ones used in industries, since it was operated under semi-real (controlled) conditions in greenhouse. Therefore, it cannot be compared to those in the real field, which utilise a large land area with a high natural energy resulting in a suitable environment for biodiversity, mainly in terms of various microorganism types as well as different types of animals which have a significant impact on the wetland processes.

Based on this, further research on the possibility for recycling of the treated wastewater by real constructed wetlands for irrigation purposes is recommended. In addition, the abundancy of phosphorus in the treated wastewater, which significantly exceeded the standards for irrigation, needs to be moderated. This can be either by modifying the wetland system design or undertaking post-treatment procedures such as using sustainable ochre sludge filters, which has recently been found to be a highly phosphorus-absorbent material.

Considering that the findings indicate the unsuitability for the example crops to be grown in the tested manner, further research to optimise nutrient and trace mineral provision using precision agriculture, which is, however, too inexpensive for most developing countries, is recommended; for example, using a mixture of different growth media and different dilutions of outflow water from wetland filters to optimise the concentrations of nutrients applied to plants, supporting the production of the highest yield and best quality. Smart irrigation systems are also recommended, in order to provide each plant with the required necessary irrigation water amount based on the plants and/or crops needs.

An assessment of the long-term impact of soil and fruit enrichment with minerals is highly recommended in the future. However, the author recommend undertaking long-term field trials in the agricultural industry to assess changes in the soil properties due to different irrigation management schemes. Considering the fact that field trial results are very variable, because each field is an uncontrolled open system, the findings of this research do not necessarily extrapolate well to field conditions.

Although the new cultivar generated from Chilli plants was grown successfully without showing any risk of metal or microbial contamination compared to their mothers, long-term investigation into potential contamination due to irrigation with recycled wastewater treated by constructed wetlands, using more advance techniques, is highly recommended; for example investigation into the potential of new generation biological contamination via genes using the DNA extraction method techniques to obtain a new cultivar adapted to urban wastewater.

Considering that the soil became depleted of nitrogen, additional research on regeneration soils used for growing crops should be investigated by using different plants in combination with the treated wastewater, such as Alfalfa and Clover, to reintroduce nitrogen to the exhausted soils. In addition, further research with other vegetables receiving recycled treated wastewater from other wastewater treatment units should also be undertaken to select the best and most cost-effective technology, in order to obtain the greatest crop yield.

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APPENDIX A

A-1) Davyhulme Sewage Works operated by the water company United Utilities in Greater Manchester (www.unitedutilities.com).

A-2) Irrigation water quality analysis equipments.

Spectrophotometer DR 2800 Hach Lange

BOD_5 manometric measurement device

Turbidity (NTU) Meter

ICP-OES device (Varian 720-ES)

pH and redox potential meter.

Electrical conductivity meter

Dissolved oxygen meter

Microwave digester

A-3) Plants and harvest measurements

Measuring plant heights

Measuring plant leaf, bud, flower and fruit numbers

Fruits monitoring, harvesting and measuring.

COD cuvette test 15-150 mg/L O_2

Product #: LCK314
GBP Price: £55.00
Available

Hazardous

LCK cuvette tests: Minimum amounts of reagents, maximum safety.

Pre-dosed reagents for maximum safety. Easy to use and photometric evaluation, packaged in a fully equipped box. Officially approved for consent limits. Environmentally friendly due to less chemicals.

Precise and reliable measurement values

Maximum safety for users, thanks to the closed system and low amounts of reagents

Convenient and error-free dosing of the reagents

Barcode label for automatic recognition in the photometer

Differentiation between tests and measuring ranges by means of colour-coding

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Specifications

According to standard:	ISO 6060-1989, DIN 38409-H41-H44
Description:	Chemical Oxygen Demand
Digestion required:	Yes
Instrument:	DR3900, DR6000, DR1900, DR2800, DR3800, DR5000
Method:	Dichromate
Number of tests:	25
Parameter:	COD
Platform:	LCK
Shelf life:	24 months from production date
Storage conditions:	15 - 25 °C (protect from light)

Ammonium cuvette test 2.0-47.0 mg/L NH4-N

Product #: LCK303
GBP Price: £69.40
Available

Hazardous

LCK cuvette tests: Minimum amounts of reagents, maximum safety.

Pre-dosed reagents for maximum safety. Easy to use and photometric evaluation, packaged in a fully equipped box. Officially approved for consent limits. Environmentally friendly due to less chemicals.

Precise and reliable measurement values

Maximum safety for users, thanks to the closed system and low amounts of reagents

Convenient and error-free dosing of the reagents

Barcode label for automatic recognition in the photometer

Differentiation between tests and measuring ranges by means of colour-coding

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Specifications

According to standard:	ISO 7150-1, DIN 38406 E5-1
Instrument:	DR3900, DR6000, DR1900, DR2800, DR3800, DR5000
Measuring range (2):	2.5 - 60 mg/L NH_4
Method:	Indophenol Blue
Number of tests:	25
Parameter:	Ammonium
Platform:	LCK
Shelf life:	18 months from production date
Storage conditions:	2 - 8 °C (keep refrigerated)

Nitrate cuvette test 0.23-13.5 mg/L NO3-N

Product #: LCK339
GBP Price: £79.90
Available

Hazardous



LCK cuvette tests: Minimum amounts of reagents, maximum safety.

Pre-dosed reagents for maximum safety. Easy to use and photometric evaluation, packaged in a fully equipped box. Officially approved for consent limits. Environmentally friendly due to less chemicals.

Precise and reliable measurement values

Maximum safety for users, thanks to the closed system and low amounts of reagents

Convenient and error-free dosing of the reagents

Barcode label for automatic recognition in the photometer

Differentiation between tests and measuring ranges by means of colour-coding

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Specifications

According to standard:	ISO 7890-1-2-1986, DIN 38405 D9-2
Instrument:	DR3900, DR6000, DR1900, DR2800, DR3800, DR5000
Measuring range (2):	1.0 - 60.0 mg/L NO_3
Method:	2.6-Dimethylphenol
Number of tests:	25
Parameter:	Nitrate
Platform:	LCK
Shelf life:	24 months from production date
Standard method:	EN 38405 D-2
Storage conditions:	15 - 25 °C

Orthophosphate cuvette test 1.6-30 mg/L PO4-P

Product #: LCK049
GBP Price: £52.00
Available

Hazardous

LCK cuvette tests: Minimum amounts of reagents, maximum safety.

Pre-dosed reagents for maximum safety. Easy to use and photometric evaluation, packaged in a fully equipped box. Environmentally friendly due to less chemicals.

Precise and reliable measurement values

Maximum safety for users, thanks to the closed system and low amounts of reagents

Convenient and error-free dosing of the reagents

Barcode label for automatic recognition in the photometer

Differentiation between tests and measuring ranges by means of colour-coding

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Specifications

Description:	Orthophosphate
Instrument:	DR3900, DR6000, DR1900, DR2800, DR3800, DR5000
Measuring range (2):	5 - 90 mg/L PO_4
Method:	Vanadate-Molybdate
Number of tests:	25
Parameter:	Phosphate, ortho
Platform:	LCK
Shelf life:	36 months from production date
Storage conditions:	15 - 25 °C

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Recycling of domestic wastewater treated by vertical-flow wetlands for irrigating Chillies and Sweet Peppers

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ABSTRACT

Due to water scarcity in many arid countries, there is considerable interest in recycling various wastewater streams such as treated urban wastewater for irrigation in the agricultural sector. The aim was therefore to assess if domestic wastewater treated by different wetlands can be successfully recycled to water commercially grown crops. The objectives were to assess variables and boundary conditions impacting on the growth of two different types of peppers fed by domestic wastewater pre-treated by diverse mature constructed treatment wetlands. The growth of both Sweet Pepper (California Wonder; cultivar of *Capsicum annuum* Linnaeus Grossum Group) and Chilli (De Cayenne; *C. annuum* (Linnaeus) Longum Group 'De Cayenne') fed with different treated and untreated wastewater types were assessed. A few plants suffered from either a shortage and/or excess of some nutrients and trace minerals. The overall growth development of Sweet Peppers was poor due to the high concentrations of nutrients and trace minerals. In contrast, Chilies did reasonably well, but the growth of foliage was excessive and the harvest was delayed. High yields were associated with tap water and an organic growth medium, and a wetland with a high aggregate size, leaving sufficient space for biomass. Low fruit numbers correlated well with inorganic growth media and irrigation water contaminated by hydrocarbons. Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated waste water are usually too high to produce a good harvest. However, as the compost is depleted of nutrients after about eight months, the harvest increased for pots that received pre-treated wastewater. The project contributes to ecological sanitation understanding by closing the loop in the food and water chain. Findings will lead to a better understanding of the effects of different wetland treatment processes on the recycling potential of their outflow waters.

1. Introduction

1.1. Background

Since water resources are limited, particularly in dry climatic regions, wastewater treatment and subsequent recycling is a viable alternative (Asano, 1994). Treated wastewater can be used for agricultural land irrigation, aquaculture, landscape irrigation, urban and industrial applications, recreational and ecosystem purposes, and artificial recharging of ground water (Asano et al., 2007). Around 20 M ha of agricultural land is irrigated by both treated and untreated wastewater (Jiménez and Asano, 2008). Advantageous associated with wastewater recycling include the supply of nutrients and trace minerals to plants, potentially leading to higher yields and a decrease in the demand for inorganic fertilisers (Bichai et al., 2012). For more detailed information on treated wastewater reuse and planning in warm countries, the reader may wish to consult Kalavrouziotis and Drakatos (2001), Kalavrouziotis et al. (2008, 2011, in press), Pedrero et al. (2009) and Kalavrouziotis (2011).

1.2. Constructed wetlands

Constructed treatment wetlands are implemented for environmental pollution control to treat a variety of wastewaters including industrial effluents, urban and agricultural runoff, animal wastewaters, sludge and mine drainage (Sani et al., 2013; Scholz, 2010). Recently, some large-scale wetland systems have also been successfully applied to treat domestic wastewater (Dong et al., 2011).

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1.3. Wastewater recycling for crop irrigation

The earliest documented sewage farms, where wastewater has been applied to land for disposal and for agricultural use, were operated in the 16th and 17th centuries in Bunzlau, Germany, and Edinburgh, Scotland (Shuval et al., 1986). Treated urban wastewater can be recycled and reused in arid regions that are confronting increasing water shortages. The evaluation of the effects of treated wastewater reuse on crops intended for human consumption is of particular interest (Aiello et al., 2007; Asano and Levine, 1996; Cirelli et al., 2012). Unfavourable concentrations of certain nutrients and trace elements are a challenge to the growth of plants fed by recycled pre-treated wastewater. Moreover, traces of hydrocarbons from diesel spills associated with urban runoff or industrial effluent are a more recent challenge (García-Delgado et al., 2012; Scholz, 2010).

García-Delgado et al. (2012) undertook a greenhouse study in Spain to assess the effect of treated urban waste water on soil and pepper quality. The wastewater application saved fertiliser (37% nitrogen, 66% phosphorus and 12% potassium). Total polyaromatic hydrocarbons and the heavy metals cadmium, lead and arsenic within the pepper fruits were low. The highest concentration (lower than proposed threshold concentrations for carcinogenicity) was recorded for phenathrene.

Boyden and Rababah (1996) assessed the recycling of nutrients from settled primary domestic wastewater (not disinfected) to produce value-added crops including capsicum and tomato. The crops grown in these systems considerably removed nitrogen and phosphorous from settled primary sewage, and appeared healthy compared to the control using commercial nutrients.

Cheng et al. (2004) assessed an integrated system that recycles waste organics and treats wastewater from a swine farm to grow vegetables. An anaerobic digester with ambient temperature has been used to treat the swine wastewater and to produce biogas. A trickling nitrification biofilter has been developed to convert ammonium in the effluent into nitrate. The nitrified anaerobic effluent is used as both fertiliser and irrigation water.

Morari and Giardini (2009) assessed the treatment efficiency of pilot-scale vertical-flow constructed wetlands on municipal wastewaters and their suitability for irrigation reuse in Italy. Only water quality parameters with high removal efficiencies fulfilled the Italian guidelines for irrigation reuse, whereas parameters with lower efficiencies such as suspended solids and total phosphorus limited the potential water reuse.

Vertical-flow constructed wetlands treating septic tank effluent in Guangzhou (China) achieved removal rates for chemical oxygen demand, five-day biochemical oxygen demand, suspended solids, total nitrogen and total phosphorus of 60, 80, 74, 49 and 79, respectively (Cui et al., 2003). After that the treated effluent was used for hydroponic cultivation of water spinach and romaine lettuce. The removal efficiencies of the whole system for chemical oxygen demand, five-day biochemical oxygen demand, suspended solids, total nitrogen and total phosphorus were 71, 98, 97, 86, and 87%, respectively. It was found that using treated effluent for hydroponic cultivation of vegetables could reduce the nitrate content in vegetables.

Lopez et al. (2006) assessed constructed wetlands treating municipal effluents to be reused in agriculture. Recorded average removal efficiencies for suspended solids, five-day biochemical oxygen demand, chemical oxygen demand, total nitrogen and total phosphorus were 85%, 65%, 75%, 42% and 32%, respectively.

1.4. Plant selection

Many vegetables have the potential to grow on recycled wastewater. However, there is the potential of some vegetables such as lettuce and cabbage to become contaminated by microbes, because their edible leaves are too close to the ground receiving the treated wastewater. Therefore, it makes sense to select vegetables where the edible fruit is located far away from the ground. This may include peppers, tomatoes, maize, eggplants, beans, lentils and peas. The next step in selecting suitable vegetables is to decide on easy-to-grow and relatively cost-effective plants with high nutritional value. Many vegetables may fit these conditions in particular geographical settings. However, the authors concentrated on two pepper types in this study, because they can also be grown in greenhouses in the UK (Jones, 2013; Nickels, 2012).

Sweet Pepper (California Wonder; cultivar of *C. annuum* Linnaeus Grossum Group) is described by the supplier B&Q plc (see Section 2) as a high cropping large fruit growing from green to red that can be picked at either stage. The vegetable is usually used for salads and cooking.

Ideal growing conditions for Sweet Pepper are warm soil (21 to 29 °C), which should be kept moist but not waterlogged (Nickels, 2012). According to Haifa Chemicals (2014), the optimal temperature for Sweet Pepper during the germination stage is between 20 and 25 °C, and for the vegetative growth stage, the corresponding range is between 20 and 25 °C through the day, and between 16 and 18 °C through the night. Furthermore, for the flowering and fruiting stage, the recommended temperature should vary between 26 and 28 °C, and between 18 and 20 °C during the day and night, respectively. It follows that commercial growing of Sweet Pepper in the UK is highly fragmented and restricted to greenhouses (or similar) due to low temperatures.

Sweet Pepper is sensitive to an abundance of moisture and excessive temperatures. They are known to be rich in vitamin C (Nickels, 2012) and sensitive to high levels of salinity, requiring salinity conditions below 1280 mg/l (FAO, 2003). Furthermore, the electronic conductivity for irrigation water should be less than 2000 μs/cm (Haifa Chemicals, 2014). Peppers prefer to grow in light and well-drained soil that should be rich in organic matter such as sandy loam or loams with a pH value between 6.5 and 7.5 (Haifa Chemicals, 2014).

Chilli (De Cayenne; *C. annuum* (Linnaeus) Longum Group 'De Cayenne') is described by B&Q as a good crop of slender and hot fruits ideal for growing in pots on the patio, balcony or in a greenhouse. It is also described as a perfect Chilli for general cooking. This type of pepper needs approximately 100 days to mature.

Chillies prefer warm, moist and nutrient-rich soil in a warm climate. The germination time is 5 to 14 days. The sowing to cropping time is approximately 18 weeks. Chillies are mostly perennial (often more than three years) in sub-tropical and tropical regions (Nickels, 2012). However, they are usually grown as annuals in temperate climates such as the UK. Commercial growing of Chillies in the UK is fragmented and also restricted to greenhouses due to low temperatures. Furthermore, Chillies prefer a loamy soil with a pH of between 7.0 and 8.5 (i.e. neutral to weakly alkaline soil) according to Nickels (2012).

1.5. Nutrients and minerals

Jones (2013) discussed the positive and negative impacts on key nutrients and minerals on plants. The major minerals impacting on the growth of plants are nitrogen (predominantly ammonium), phosphorus, potassium, calcium, magnesium and sulphur. Micronutrients that are beneficial in small amounts are (in no particular order) boron, copper, manganese, molybdenum, potassium, iron, zinc, iodine and chlorine. Copper, manganese, molybdenum, iron, zinc and aluminium (in no particular order) are often described as heavy metals. They may be toxic in high inflow water concentrations for peppers. Under acid conditions (soil with a pH of less than 7), heavy metals could be a problem to sensitive plants (FAO, 2003).

Table 1
Comparison of the experimental vertical-flow wetland set-up.

Design and/or operational variable	Unit	Filters 1 and 2	Filters 3 and 4	Filters 5 and 6	Filter 7	Filter 8	Control A	Control B
Aggregate diameter	mm	20	10	10	10	10	10	10
Contact time	h	72	72	72	36	36	72	72
Resting time	h	48	48	48	48	24	48	48
Chemical oxygen demand	mg/l	122.8	122.8	243.8	122.8	122.8	2.3	2.3

Note: Annually treated volumes of wastewater: Filters 1 to 6, 470 l/a; Filter 7, 624 l/a Filter 8, 858 l/a. On 26 September 2013, 130 g of diesel was added to Filters 1, 3 and 5 and Control A.

Metals present in wastewater (cadmium, copper, molybdenum, nickel and zinc) used for irrigation purposes can pose a health risk to humans and animals (Ebrazi Bakhshayesh et al., 2014; FAO, 2003). FAO (2003) states that the most toxic ions in treated wastewater are sodium, chloride and boron. Boron exceeding 0.5 mg/l is toxic to sensitive plants. Sweet Pepper is semi-tolerant (about 2 mg/l) to boron. Pescod (1992) classified boron concentrations in irrigation water according to the degree of restriction on its use: there are no limitations for values of less than 0.7 mg/l, slight to moderate controls for values between 0.7 mg/l and 3.0 mg/l, and severe restrictions for measurements of more than 3.0 mg/l.

Pescod (1992) and FAO (2003) also recommends limits for trace minerals in reclaimed water use for irrigation. Long-term (for water used continuously on all soils) and short-term (for water used for a period of up to 20 years on fine-textured neutral alkaline soils) threshold values for 18 elements have been listed. Recommended maximum concentrations for the trace elements that are often exceeded (see results below) are 5.0 mg/l for iron, 0.2 mg/l for manganese and 2.0 mg/l for potassium.

FAO (1994) classified the suitability of treated wastewater for recycling in terms of pollutants. Acceptable ranges for ammonia–nitrogen, ortho-phosphate-phosphorous and potassium were between 0 and 5, between 0 and 2, and between 0 and 2 mg/l, respectively. Furthermore, Pescod (1992) stated that for irrigation water there is no restriction for its reuse if nitrate–nitrogen values are <5.0 mg/l. Slight to moderate constraints exist for the range between 5 and 30 mg/l. Severe recycling restrictions are usually imposed for values of more than 30.0 mg/l.

Johnson and Decoteau (1996) reported that few reports are available concerning the required limits for nitrogen in soil for good growth of Chillies, which should not be grown when too much nitrogen (>280 kg/ha) is present in the soil, leading to excessive growth of foliage, apparently at the expense of fruit production.

FAO/WHO (2001) recommended the following thresholds for metals in vegetables: cobalt (50.0 mg/kg), chromium (2.3 mg/kg), copper (73.3 mg/kg), nickel (66.9 mg/kg), lead (0.3 mg/kg) and zinc (9.4 mg/kg). In contrast, Chary et al. (2008) recommended a limit for copper in vegetables of 20 mg/kg, while for lead and zinc, the corresponding values were 1 and 50 mg/kg, respectively.

The Ministry of Health of the People's Republic of China (2005) stated the following maximum levels of contaminants in food: arsenic (0.05 mg/kg), chromium (0.5 mg/kg), cadmium (0.05 mg/kg) and lead (0.1 mg/kg). EC (2001) has set maximum levels for certain contaminants in food: copper (20 mg/kg), lead (0.3 mg/kg), zinc (50 mg/kg) and cadmium (0.05 mg/kg).

Ciju (2013a) reports the following nutrition values for 100 g of fresh and raw green Sweet Peppers: phosphorus (20 mg), potassium (175 mg), calcium (10 mg), magnesium (10 mg), iron (0.34 mg) and zinc (0.13 mg). In comparison, Ciju (2013b) reports the following nutrition values for 100 g of sun-dried Chillies: phosphorus (159 mg), potassium (1870 mg), calcium (45 mg), magnesium (88 mg), iron (6.04 mg) and zinc (1.02 mg). Further beneficial elements may include silicon. Other elements such as aluminium should be present in low quantities within the irrigation water.

FAO (2003) stated the nutrient requirements for pepper required for proper canopy formation: nitrogen (90 kg/ha), phosphorus (6 kg/ha), potassium (90 kg/ha), phosphorus pentoxide (14 kg/ha) and potassium oxide (108 kg/ha). The corresponding values for good fruit production are as follows: nitrogen (2.0 kg/t), phosphorus (0.26 kg/t), potassium (1.83 kg/t), phosphorus pentoxide (0.6 kg/t) and potassium oxide (2.2 kg/t).

Haifa Chemicals (2014) stated the required rates of macro-and secondary plant nutrient uptake by pepper plants in greenhouses: nitrogen (390–920 kg/ha), phosphorus pentoxide (200–330 kg/ha),

Fig. 1. Photographs showing an overview of the laboratory set-up for (a) Chilli and (b) Sweet Pepper plants on 4 June 2014.

Table 2
Experimental design in terms of plant number allocations after the second replanting (i.e. final replanting).

Inflow source	Growth media	Sweet Pepper	Chilli
Filter 1 outflow	Compost with bark	P1;P2	C3;C4
Filter 2 outflow	Compost with bark	P5;P6	C6;C8:C9
Filter 3 outflow	Compost with bark	P8;P9;P10	C10;C11;C12
Filter 4 outflow	Compost with bark	P12;P16	C16:C17
Filter 5 outflow	Compost with bark	P18;P19;P20	C18;C19;C20
Filter 6 outflow	Compost with bark	P22;P23	C21
Filter 7 outflow	Compost with bark	P26;P28	C25:C26
Filter 8 outflow	Compost with bark	P31;P32;P33	C27;C28:C29
Control A outflow	Compost with bark	P35	C31;C33
Control B outflow	Compost with bark	P39	C37:C38
Deionised water	Compost with bark	P41	C41
Tap water (100%)	Compost with bark	P44	C42;C43
Tap water with fertiliser (0.7 ml/l)	Compost with bark	P45;P46	C45;C46
Wastewater (20%); tap water (80%)	Compost with bark	P47	C49
Wastewater (100%)	Compost with bark	P51;P54	C52; C54
Filter 1 outflow	Silica sand	P55;P56	C56
Filter 2 outflow	Silica sand	P57	C58
Filter 3 outflow	Silica sand	P59	C61
Filter 4 outflow	Silica sand	P61	C63;C64
Filter 5 outflow	Silica sand	P65	C66
Filter 6 outflow	Silica sand	P66;P67	C68
Filter 7 outflow	Silica sand	P17;P69	C71
Filter 8 outflow	Silica sand	P70;P71	C72;C73
Control A outflow	Silica sand	P73	C74
Control B outflow	Silica sand	P74	C76;C77
Deionised water	Silica sand	P80	C80
Tap water (100%)	Silica sand	P81	C82
Tap water with fertiliser (0.7 ml/l)	Silica sand	P83;P84	C84;C85
Wastewater (20%); tap water (80%)	Silica sand	P86;P87	C87
Wastewater (100%)	Silica sand	P89;P90	C90

Note: Original seed planting reference numbers; Sweet Pepper (P1–P90) and Chilli (C1–C90).

potassium oxide (640–1530 kg/ha), calcium oxide (100–210 kg/ha), magnesium oxide (60–150 kg/ha) and sulphur (40–50 kg/ha).

1.6. Rationale, aims and objectives

Effluent from different types of wetland systems treating domestic wastewater was selected to irrigate vegetables. Some of the wetlands received standard wastewater while the others received wastewater that was subject to a one-off diesel fuel spill. The treated wastewater from all wetland types was recycled for the irrigation of Sweet Peppers and Chillies. This experiment may provide the scientific justification for integrating treatment wetlands into agricultural food production.

The overall aim is to assess if vegetables can be grown successfully on recycled domestic wastewater treated by constructed wetlands. The corresponding key objectives related to the growing of Sweet Pepper and Chilli are to assess (a) the suitability for growth when using recycled wastewater, (b) the impact of different treated wastewaters as a function of the wetland type, (c) the impact of the environmental conditions on growth, (d) the volume of treated wastewater for irrigation, (e) the suitability of different growth media, (f) the effect of a Diesel oil spill on the suitability of the recycled wastewater, and (g) the economic viability of different experimental set-ups.

2. Methodology

2.1. Constructed wetlands set-up and operation

The vertical-flow constructed wetland system is located within a greenhouse (door left open) on top of the roof of the Newton Building of The University of Salford, Greater Manchester, UK (Sani et al., 2013). They were operated between 27 June 2011 and 4 June 2014. The set-up includes two filters that are essentially controls receiving clean dechlorinated water. Table 1 indicates an overview of the statistical experimental set-up used to test the impact of four variables. Filters 1 and 2 compared to Filters 3 and 4 test the influence of a larger aggregate diameter. Filters 5 and 6 compared to Filters 3 and 4 check the impact of a higher loading rate. The application of a lower contact rate is tested if Filter 7 is compared with Filters 3 and 4. Finally, a lower resting time is the difference between Filters 7 and 8.

Ten laboratory-scale vertical-flow constructed wetlands were constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm. The filters were filled with siliceous (minimum of 30%) pea gravel up to a depth of 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). Dead macrophyte plant material was harvested in winter and returned to the corresponding wetland filters by placing it on top of the litter zone (Sani et al., 2013).

The main outlet valve is located at the bottom of each constructed wetland system. Eight further valves (used to test for clogging) are located on the sidewall of each wetland column. The sidewall valves were located at heights of 10, 20, 30, 40, 45, 50, 55 and 60 cm from the bottom of each column (Sani et al., 2013).

Wetland columns received 6.5 l of inflow water during the feeding mode, which was different between several filters (Table 1). Columns 1 to 6 were sampled after 72 h contact time and then left to rest for 48 h, while columns 7 and 8 were sampled after 36 h contact time and left to rest for 48 h and 24 h, respectively. All water quality parameters discussed in this paper were usually determined during or directly after sampling. The preliminary treated urban wastewater used for the inflow water was obtained from the Davyhulme Sewage works, one of the largest waste water treatment plants in Europe (http://en.wikipedia.org/wiki/Davyhulme), operated by the water company United Utilities in Greater Manchester. Fresh wastewater was collected approximately once per week, and was stored and aerated by standard aquarium air pumps in a cold room

Table 3
Comparison of the water quality of the inflow waters received by the vegetable pots.

Parameter	Unit	Overall[a]	FRP[b]	SRPBF[c]	SRPAF[d]
Filter 1 (outflow)					
Total Petroleum Hydrocarbons	μg/l	100	nm	nm	100
Chemical oxygen demand	mg/l	100.2	135.0	nm	69.4
Biochemical oxygen demand	mg/l	24.5	64.0	25.0	21.7
Ammonia-nitrogen	mg/l	6.4	6.6	nm	3.5
Nitrate-nitrogen	mg/l	3.3	0.3	nm	0.5
Ortho-phosphate-phosphorus	mg/l	3.2	3.1	nm	2.3
Suspended solids	mg/l	11.4	25.3	16.5	10.3
Turbidity	NTU[e]	9.6	18.6	10.0	8.8
pH	–	6.4	6.5	6.4	6.4
Filter 2 (outflow)					
Total Petroleum Hydrocarbons	μg/l	0	nm	nm	0
Chemical oxygen demand	mg/l	39.2	79.5	nm	20.3
Biochemical oxygen demand	mg/l	13.9	24.7	14.7	10.8
Ammonia-nitrogen	mg/l	5.9	18.6	nm	3.6
Nitrate-nitrogen	mg/l	5.0	7.0	nm	2.9
Ortho-phosphate-phosphorus	mg/l	3.0	3.1	nm	2.7
Suspended solids	mg/l	7.3	14.3	11.6	7.5
Turbidity	NTU[e]	6.1	11.4	8.2	6.2
pH	–	6.5	6.6	6.4	6.5
Filter 3 (outflow)					
Total Petroleum Hydrocarbons	μg/l	69	nm	nm	69
Chemical oxygen demand	mg/l	106.6	136.0	nm	78.5
Biochemical oxygen demand	mg/l	25.1	67.3	27.5	19.5
Ammonia-nitrogen	mg/l	4.2	7.6	nm	2.3
Nitrate-nitrogen	mg/l	3.4	0.4	nm	0.5
Ortho-phosphate-phosphorus	mg/l	2.9	3.80	nm	2.01
Suspended solids	mg/l	12.2	25.0	16.6	11.8
Turbidity	NTU[e]	10.3	17.0	11.0	10.1
pH	–	6.5	6.5	6.6	6.5
Filter 4 (outflow)					
Total Petroleum Hydrocarbons	μg/l	0	nm	nm	0
Chemical oxygen demand	mg/l	33.1	72.2	nm	13.7
Biochemical oxygen demand	mg/l	12.5	22.7	14.7	7.9
Ammonia-nitrogen	mg/l	4.4	11.0	nm	2.8
Nitrate-nitrogen	mg/l	5.1	8.3	nm	2.8
Ortho-phosphate-phosphorus	mg/l	2.9	2.69	nm	2.7
Suspended solids	mg/l	7.7	20.0	11.8	7.3
Turbidity	NTU[e]	6.5	10.0	7.8	6.6
pH	–	6.5	6.7	6.6	6.5
Filter 5 (outflow)					
Total Petroleum Hydrocarbons	μg/l	14	nm	nm	14
Chemical oxygen demand	mg/l	142.6	360.0	nm	95.2
Biochemical oxygen demand	mg/l	24.2	12.0	44.7	16.2
Ammonia-nitrogen	mg/l	13.6	9.8	nm	9.2
Nitrate-nitrogen	mg/l	4.9	0.7	nm	1.3
Ortho-phosphate-phosphorus	mg/l	4.2	7.7	nm	2.6
Suspended solids	mg/l	13.7	43.0	17.8	10.6
Turbidity	NTU[e]	10.6	22.1	11.7	8.4
pH	–	6.7	6.7	6.7	6.6
Filter 6 (outflow)					
Total Petroleum Hydrocarbons	μg/l	0	nm	nm	0
Chemical oxygen demand	mg/l	47.2	76.1	nm	23.1
Biochemical oxygen demand	mg/l	16.3	44.0	18.4	9.8
Ammonia-nitrogen	mg/l	12.0	10.4	nm	7.9
Nitrate-nitrogen	mg/l	6.8	17.9	nm	2.6
Ortho-phosphate-phosphorus	mg/l	3.9	2.73	nm	2.9
Suspended solids	mg/l	8.5	18.7	11.0	7.4
Turbidity	NTU[e]	6.8	8.1	8.4	5.5
pH	–	6.8	6.8	6.7	6.8
Filter 7 (outflow)					
Total Petroleum Hydrocarbons	μg/l	0	nm	nm	0
Chemical oxygen demand	mg/l	39.5	58.2	nm	19.5
Biochemical oxygen demand	mg/l	13.8	28.0	10.5	11.5
Ammonia-nitrogen	mg/l	4.8	0.3	nm	3.7
Nitrate-nitrogen	mg/l	7.4	4.6	nm	4.3
Ortho-phosphate-phosphorus	mg/l	3.2	2.7	nm	2.5
Suspended solids	mg/l	2.0	3.0	2.4	1.2
Turbidity	NTU[e]	3.1	4.2	2.9	2.5
pH	–	6.5	6.8	6.7	6.5
Filter 8 (outflow)					
Total Petroleum Hydrocarbons	μg/l	116	nm	nm	116
Chemical oxygen demand	mg/l	92.2	61.1	nm	30.5
Biochemical oxygen demand	mg/l	14.5	26.0	12.1	11.6
Ammonia-nitrogen	mg/l	2.9	1.24	nm	1.3
Nitrate-nitrogen	mg/l	6.5	10.7	nm	3.2
Ortho-phosphate-phosphorus	mg/l	3.5	1.92	nm	3.0
Suspended solids	mg/l	3.0	12.7	2.0	2.3

Table 3 (*Continued*)

Parameter	Unit	Overall[a]	FRP[b]	SRPBF[c]	SRPAF[d]
Turbidity	NTU[e]	3.8	4.1	3.6	3.4
pH	–	6.5	6.9	6.6	6.5
Control A (outflow)					
Total Petroleum Hydrocarbons	μg/l	346	nm	nm	346
Chemical oxygen demand	mg/l	68.3	59.5	nm	30.7
Biochemical oxygen demand	mg/l	13.1	17.0	14.7	9.1
Ammonia-nitrogen	mg/l	1.7	0.0	nm	1.7
Nitrate-nitrogen	mg/l	0.6	0.018	nm	0.5
Ortho-phosphate-phosphorus	mg/l	1.9	1.48	nm	1.6
Suspended solids	mg/l	6.3	7.0	11.7	6.3
Turbidity	NTU[e]	4.9	5.7	7.4	4.8
pH	–	6.7	6.9	6.7	6.6
Control B (outflow)					
Total Petroleum Hydrocarbons	μg/l	0	nm	nm	0
Chemical oxygen demand	mg/l	25.8	55.1	nm	7.0
Biochemical oxygen demand	mg/l	8.2	15.0	12.5	5.4
Ammonia-nitrogen	mg/l	1.8	0.1	nm	2.2
Nitrate-nitrogen	mg/l	0.5	0.1	nm	0.5
Ortho-phosphate-phosphorus	mg/l	2.0	1.7	nm	1.8
Suspended solids	mg/l	4.6	9.7	9.4	4.5
Turbidity	NTU[e]	4.9	4.5	7.3	4.8
pH	–	6.6	6.6	6.6	6.5
Deionised water					
Total Petroleum Hydrocarbons	μg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	nm	nm	nm	nm
Biochemical oxygen demand	mg/l	7.1	nm	8.6	6.0
Ammonia-nitrogen	mg/l	nm	nm	nm	nm
Nitrate-nitrogen	mg/l	nm	nm	nm	nm
Ortho-phosphate-phosphorus	mg/l	nm	nm	nm	nm
Suspended solids	mg/l	4.0	nm	0.0	4.0
Turbidity	NTU[e]	1.3	nm	1.5	1.2
pH	–	5.5	nm	5.7	4.4
Tap water (100%)					
Total Petroleum Hydrocarbons	μg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	nm	nm	nm	nm
Biochemical oxygen demand	mg/l	5.1	nm	5.7	4.1
Ammonia-nitrogen	mg/l	nm	nm	nm	nm
Nitrate-nitrogen	mg/l	nm	nm	nm	nm
Ortho-phosphate-phosphorus	mg/l	nm	nm	nm	nm
Suspended solids	mg/l	4.0	nm	0.0	4.0
Turbidity	NTU[e]	3.0	nm	2.6	3.3
pH	–	5.9	nm	5.3	6.8
Tap water with fertiliser (0.7 ml/l)					
Total Petroleum Hydrocarbons	μg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	nm	nm	nm	nm
Biochemical oxygen demand	mg/l	7.3	nm	9.8	6.1
Ammonia-nitrogen	mg/l	nm	nm	nm	nm
Nitrate-nitrogen	mg/l	nm	nm	nm	nm
Ortho-phosphate-phosphorus	mg/l	nm	nm	nm	nm
Suspended solids	mg/l	2.0	nm	1.2	2.0
Turbidity	NTU[e]	2.9	nm	3.2	2.3
pH	–	6.0	nm	6.2	5.8
Wastewater (20%); tap water (80%)					
Total Petroleum Hydrocarbons	μg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	nm	nm	nm	nm
Biochemical oxygen demand	mg/l	50.1	nm	43.8	55.1
Ammonia-nitrogen	mg/l	nm	nm	nm	nm
Nitrate-nitrogen	mg/l	nm	nm	nm	nm
Ortho-phosphate-phosphorus	mg/l	nm	nm	nm	nm
Suspended solids	mg/l	25.8	nm	18.8	38.8
Turbidity	NTU[e]	9.8	nm	6.0	10.8
pH	–	7.1	nm	7.0	7.1
Wastewater (100%)					
Total Petroleum Hydrocarbons	μg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	nm	nm	nm	nm
Biochemical oxygen demand	mg/l	129.2	nm	205.8	105.1
Ammonia-nitrogen	mg/l	nm	nm	nm	nm
Nitrate-nitrogen	mg/l	nm	nm	nm	nm
Ortho-phosphate-phosphorus	mg/l	nm	nm	nm	nm
Suspended solids	mg/l	143.7	nm	37.2	130.4
Turbidity	NTU[e]	83.1	nm	21.5	89.1
pH	–	7.5	nm	7.3	7.4

Note: nm, not measured.

[a] 11/10/13 to 04/06/14.

[b] First replanting period: 11/10/13 to 07/11/13.

[c] Second replanting period before fruiting: 08/11/13 to 19/12/13.

[d] Second replanting period after fruiting:20/12/13 to 04/06/14.

[e] Nephelometric turbidity unit.

before use. The wastewater quality was highly variable, and comprised domestic and industrial wastewater as well as surface water runoff.

In order to simulate a one-off Diesel fuel (100% pure; no additives) spill, 130 g (equivalent to an inflow concentration of 20 g/l) of diesel fuel (100% pure; no additives) were poured into Filters 1, 3and 5, and into one of the two columns (Control A) on 26 September 2013 (Table 1). The fuel was obtained from a petrol station operated Tesco Extra (Pendleton Way, Salford, UK).

Aqua Medic Titan chillers (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were used to maintain the root system and debris layer of all wetland systems at semi-natural below-surface temperatures of about 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located (Sani et al., 2013).

The COD was used as the criterion to differentiate between low and high loads (Table 1). An inflow target COD of about 285 mg/l (usually between 122 and 620 mg/l) was set for wetlands with a high loading rate (Filters 5 and 6). The remaining Filters 1, 2, 3, 4, 7 and 8 received wastewater diluted with de-chlorinated tap water. The target inflow COD for these filters was approximately 138 mg/l (usually between 43 and 350 mg/l).

2.2. *Water quality analysis*

Routine water quality sampling were carried out according to APHA (2005; unless stated otherwise) to monitor clogging evolution, and long-term and seasonal treatment performance, respectively. The spectrophotometer DR 2800 Hach Lange (www.hach.com) was used for standard water quality analysis for variables including COD, ammonia-nitrogen, nitrate-nitrogen, ortho-phosphate-phosphorus and SS.

Total petroleum hydrocarbons were determined by gas chromatography and flame ionisation externally by Exova Health Sciences (70 Montrose Ave, Hillington Park, Glasgow G52 4LA) according to their own TPH in Waters (with Aliphatic/Aromatic Splitting) Method (Exova Health Sciences, 2014), which is accredited to the British Standard (BS) method BS EN ISO IEC 17025 by the United Kingdom Accreditation Service and compatible to the International Organization for Standardization (ISO) standards (e.g., ISO17025), BS method BS DD 220 1994, and American Standard methods (United States Environmental Protection Agency (US EPA) Method 3510C and US EPA SW846 Method 8015).

The five-day BOD was determined in all water samples with the OxiTop IS 12-6 system, a manometric measurement device, supplied by the Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany. Nitrification was suppressed by adding 0.05 ml of 5 g/l N-Allylthiourea (WTW chemical solution No. NTH600) solution per 50 ml of sample water.

Turbidity was measured with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, www.lovibond.com). The pH was measured with a sensION+ benchtop multi-parameter meter (Hach Lange, Düsseldorf, Germany). The redox potential for all water samples was measured using a VARIO PH meter (Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany). This meter was less reliable than the Hach Lange meter for measuring pH. Temperature data for the first year of operation were recorded outside and in the shade at an official weather station in Woodford located South-east of Salford. The raw data were supplied by the UK MetOffice (www.metoffice.gov.uk). Concerning the second year of operation, inside temperature measurements were performed by project team members inside and outside the greenhouse.

Table 4
Overview of environmental boundary conditions associated with the vegetable pots. Note that the sample number n is shown in brackets.

Parameter	Unit	Overall[a]	FPGP[b]	FPAGP[c]	FRP[d]	SRPBF[e]	SRPAF[f]
Illuminance (one-off record during lab visit)	lx	5492 ± 5672.9 (1220)	nm	4208 ± 2560.5 (36)	12316 ± 1823.3 (102)	3682 ± 3246.1 (513)	5877 ± 9262.2 (267)
Temperature (one-off record during lab visit)	°C	25.5 ± 2.72 (247)	20.5 ± 1.25 (13)	24.8 ± 1.17 (48)	25.0 ± 1.89 (102)	26.3 ± 2.32 (204)	25.0 ± 1.83 (236)
Temperature (minimum within a 24 h period)	°C	20.9 ± 2.02 (75)	nm	nm	20.3 ± 1.87 (8)	21.2 ± 2.02 (33)	20.6 ± 2.05 (34)
Temperature (maximum within a 24 h period)	°C	26.6 ± 2.52 (75)	nm	nm	25.3 ± 1.98 (8)	27.0 ± 2.83 (33)	26.6 ± 2.26 (34)
Relative humidity (one-off record during lab visit)	%	48 ± 11.4 (536)	nm	nm	42 ± 5.4 (96)	37 ± 7.6 (156)	57 ± 7.8 (236)
Relative humidity (minimum within a 24 h period)	%	34 ± 7.2 (75)	nm	nm	36 ± 3.7 (8)	30 ± 3.5 (33)	38 ± 8.5 (34)
Relative humidity (maximum within a 24 h period)	%	55 ± 12.1 (75)	nm	nm	46 ± 5.6 (8)	48 ± 10.5 (33)	63 ± 9.8 (34)

[a] 11/10/13 to 04/06/14.
[b] First planting germination period: 17/09/13 to 22/09/13.
[c] First replanting after germination period: 23/09/13 to 10/10/13.
[d] First replanting period: 11/10/13 to 07/11/13.
[e] Second replanting period before fruiting (i.e. development of first fruit): 08/11/13 to 19/01/14.
[f] Second replanting period after fruiting (i.e. development of first fruit): 20/01/14 to 04/06/14; and nm, not measured.

Table 5a

Overview of visual growth problems associated with macronutrient deficiency in old parts of plants (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 13 February 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Stunted growth[a]		Few flowers with poor and deformed fruits[b]		Chlorosis[c]		Burning of leaf margins with midrib remain greeen[d]		Interveinal chlorosis[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4	**P1;P2**	C3;C4	**P1;P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	**C3;C4**
Filter 2 and organic	P5;P6	C6;C8;C9	**P5;P6**	C6;C8;C9	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	**C6;C8;C9**
Filter 3 and organic	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	P8;**P9**;P10	C10;C11;C12	**P8;P9;P10**	**C10;C11;C12**
Filter 4 and organic	P12;P16	C16;C17	**P12;P16**	C16;C17	**P12;P16**	C16;C17	P12;P16	C16;C17	**P12;P16**	**C16;C17**
Filter 5 and organic	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	**P18;P19;P20**	**C18**;C19;**C20**	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	**C18;C19;C20**
Filter 6 and organic	P22;P23	C21	**P22;P23**	C21	**P22;P23**	**C21**	P22;P23	C21	**P22;P23**	**C21**
Filter 7 and organic	P26;P28	C25;C26	**P26;P28**	C25;C26	**P26;P28**	C25;**C26**	P26;P28	C25;C26	**P26;P28**	**C25;C26**
Filter 8 and organic	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	**P31;P32;P33**	**C27;C28;C29**	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	**C27;C28;C29**
Control A and organic	P35	C31;C33	**P35**	C31;C33	**P35**	C31;C33	P35	C31;C33	**P35**	**C31;C33**
Control B and organic	P39	C37;C38	**P39**	C37;C38	**P39**	**C37**;C38	P39	C37;C38	**P39**	**C37;C38**
Deionised water and organic	P41	C41	**P41**	C41	**P41**	C41	P41	C41	**P41**	**C41**
Tap water and organic	P44	C42;C43	**P44**	C42;C43	**P44**	**C42;C43**	P44	C42;C43	**P44**	**C42;C43**
Tap water/fertiliser and organic	P45;P46	C45;C46	**P45;P46**	C45;C46	**P45;P46**	**C45**;C46	P45;P46	C45;C46	**P45;P46**	**C45;C46**
Wastewater/tap water and organic	P47	C49	**P47**	C49	**P47**	**C49**	P47	C49	**P47**	**C49**
Wastewater and organic	P51;P54	C52; C54	**P51;P54**	C52; C54	**P51;P54**	**C52; C54**	P51;**P54**	C52; C54	**P51;P54**	**C52; C54**
Filter 1 and inorganic	**P55;P56**	**C56**	**P55;P56**	**C56**	**P55;P56**	**C56**	P55;P56	C56	**P55;P56**	**C56**
Filter 2 and inorganic	**P57**	**C58**	**P57**	C58	**P57**	**C58**	P57	C58	**P57**	**C58**
Filter 3 and inorganic	**P59**	**C61**	**P59**	**C61**	**P59**	**C61**	P59	C61	**P59**	**C61**
Filter 4 and inorganic	**P61**	**C63;C64**	**P61**	C63;C64	**P61**	**C63;C64**	P61	C63;C64	**P61**	**C63;C64**
Filter 5 and inorganic	**P65**	**C66**	**P65**	C66	**P65**	**C66**	P65	C66	**P65**	**C66**
Filter 6 and inorganic	**P66;P67**	**C68**	**P66;P67**	C68	**P66;P67**	**C68**	P66;P67	C68	**P66;P67**	**C68**
Filter 7 and inorganic	**P17;P69**	**C71**	**P17;P69**	C71	**P17;P69**	C71	P17;P69	C71	**P17;P69**	**C71**
Filter 8 and inorganic	**P70;P71**	**C72;C73**	**P70;P71**	C72;C73	**P70;P71**	**C72**;C73	P70;P71	C72;C73	**P70;P71**	**C72;C73**
Control A and inorganic	**P73**	**C74**	**P73**	C74	**P73**	**C74**	P73	C74	**P73**	**C74**
Control B and inorganic	**P74**	**C76;C77**	**P74**	C76;C77	**P74**	**C76;C77**	P74	C76;C77	**P74**	**C76;C77**
Deionised water and inorganic	**P80**	**C80**	**P80**	C80	**P80**	**C80**	P80	C80	**P80**	**C80**
Tap water and inorganic	**P81**	**C82**	**P81**	C82	**P81**	**C82**	P81	C82	**P81**	**C82**
Tap water/fertiliser and inorganic	P83;P84	C84;C85	**P83;P84**	C84;C85	**P83;P84**	C84;**C85**	P83;P84	C84;C85	**P83;P84**	**C84;C85**
Wastewater/tap water and inorganic	**P86;P87**	**C87**	**P86;P87**	C87	**P86;P87**	**C87**	P86;P87	C87	**P86;P87**	**C87**
Wastewater and inorganic	P89;P90	**C90**	**P89;P90**	C90	**P89;P90**	C90	P89;P90	C90	**P89;P90**	**C90**
Inflow source and growth media	Curly small leaves[f]		Bending of petioles and hang downwards; parallel to stem[g]		Necrosis[h]		Leaf tips brown and necrotic[i]			
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli		
Filter 1 and organic	**P1;P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;**C4**	P1;P2	C3;C4		
Filter 2 and organic	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5**;P6	C6;C8;C9	P5;P6	C6;C8;C9		
Filter 3 and organic	P8;P9;**P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	**C10;C11;C12**	P8;P9;P10	C10;C11;C12		
Filter 4 and organic	P12;**P16**	C16;**C17**	P12;P16	C16;C17	**P12;P16**	**C16**;C17	P12;P16	C16;C17		
Filter 5 and organic	P18;P19;P20	**C18**;C19;**C20**	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	P18;P19;P20	C18;C19;C20		
Filter 6 and organic	**P22;P23**	C21	P22;P23	C21	**P22;P23**	**C21**	P22;P23	C21		
Filter 7 and organic	P26;P28	C25;C26	P26;P28	C25;C26	P26;**P28**	**C25;C26**	P26;P28	**C25;C26**		
Filter 8 and organic	**P31**;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	P31;P32;P33	**C27;C28;C29**		
Control A and organic	P35	C31;**C33**	P35	C31;C33	P35	**C31**;C33	P35	C31;C33		
Control B and organic	**P39**	C37;C38	P39	C37;C38	**P39**	C37;**C38**	P39	C37;C38		
Deionised water and organic	P41	C41	P41	C41	**P41**	C41	P41	C41		
Tap water and organic	**P44**	C42;C43	P44	C42;C43	**P44**	C42;C43	P44	C42;C43		
Tap water/fertiliser and organic	P45;P46	C45;**C46**	P45;P46	C45;C46	**P45;P46**	C45;C46	P45;P46	C45;C46		
Wastewater/tap water and organic	**P47**	**C49**	P47	C49	P47	**C49**	P47	C49		
Wastewater and organic	P51;P54	C52; **C54**	P51;P54	C52; C54	P51;**P54**	**C52; C54**	P51;**P54**	C52; C54		
Filter 1 and inorganic	**P55;P56**	C56	P55;P56	**C56**	**P55**;P56	C56	P55;P56	C56		
Filter 2 and inorganic	**P57**	C58	P57	C58	P57	C58	P57	C58		
Filter 3 and inorganic	P59	C61	P59	**C61**	**P59**	C61	P59	C61		
Filter 4 and inorganic	**P61**	C63;C64	P61	C63;C64	P61	C63;C64	P61	C63;C64		

Table 5a (*Continued*)

Inflow source and growth media	Stunted growth[a]		Few flowers with poor and deformed fruits[b]		Chlorosis[c]		Burning of leaf margins with midrib remain greeen[d]		Interveinal chlorosis[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 5 and inorganic	P65	C66	P65	C66	P65	C66	P65	C66		
Filter 6 and inorganic	**P66;P67**	C68	P66;P67	**C68**	**P66;P67**	C68	P66;P67	C68		
Filter 7 and inorganic	**P17;P69**	C71	P17;P69	C71	**P17;P69**	C71	P17;P69	C71		
Filter 8 and inorganic	**P70**;P71	C72;C73	P70;P71	C72;C73	P70;**P71**	C72;C73	P70;P71	C72;C73		
Control A and inorganic	**P73**	C74	P73	C74	P73	C74	P73	C74		
Control B and inorganic	**P74**	C76;C77	P74	C76;C77	P74	C76;C77	P74	C76;C77		
Deionised water and inorganic	P80	C80	P80	C80	P80	C80	P80	C80		
Tap water and inorganic	P81	C82	P81	C82	**P81**	C82	P81	C82		
Tap water/fertiliser and inorganic	**P83;P84**	C84;C85	P83;P84	C84;C85	**P83;P84**	C84;C85	P83;P84	C84;C85		
Wastewater/tap water and inorganic	**P86;P87**	C87	**P86;P87**	C87	**P86;P87**	C87	P86;P87	C87		
Wastewater and inorganic	**P89;P90**	C90	**P89;P90**	C90	**P89;P90**	C90	P89;P90	C90		

[a] Deficiency in nitrogen and/or phosphorus (Haifa Chemicals, 2014; Kennelly et al., 2012; McCauly et al., 2011; Silva et al., 2000; Wong, 2005) and/or potassium (McCauly et al., 2011; Silva et al., 2000).
[b] Deficiency in nitrogen and/or phosphorus and/or potassium (Haifa Chemicals, 2014; Silva et al., 2000).
[c] Yellowing or whitening of the green plant tissue, because of a decreased amount of chlorophyll due to deficiency in nitrogen (Hosier and Bradley, 1999; Kennelly et al., 2012; McCauly et al., 2011; Silva et al., 2000), and/or potassium (Haifa Chemicals, 2014; McCauly et al., 2011; Silva et al., 2000; Wong, 2005), and/or magnesium (Kennelly et al., 2012; Silva et al., 2000).
[d] Deficiency in potassium (McCauly et al., 2011).
[e] Deficiency in magnesium (Haifa Chemicals, 2014; Kennelly et al., 2012; McCauly et al., 2011; Wong, 2005), and/or potassium (Hosier and Bradley, 1999; Kennelly et al., 2012).
[f] Deficiency in phosphorus (McCauly et al., 2011).
[g] Deficiency in nitrogen (Haifa Chemicals, 2014).
[h] Death of plant tissue due to deficiency in nitrogen and/or potassium (McCauly et al., 2011; Wong, 2005), and/or magnesium (Kennelly et al., 2012).
[i] Deficiency in phosphorus (McCauly et al., 2011), and/or potassium (Kennelly et al., 2012).

2.3. Environmental monitoring

Light measurement readings were undertaken using the LUX meter ATP-DT-1300 for the range between 200 lx and 50,000 lx (TIMSTAR, Road Three,Winsford Industrial Estate, Winsford, Cheshire, UK) just above the top of the plants showed values between 3855 and 12,316 lx (mean of 6921 lx) close the plants.

Typical values for full day light are between approximately 10,000 and 20,000 lx. The humidity and temperature were controlled with the support of a combined Thermometer-Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The humidity measuring range was between 20 and 99%. The corresponding precision was ±4% between 35 and 75%.

The temperature was controlled by the electrical heater Rhino H029400 TQ3 2.8 kW Thermo Quartz Infrared Heater 230 V supplied by Express Tools Ltd., (Alton Road, Bournemouth, UK). The humidity was artificially elevated by a varying number of humidity meters (Challenge 3.0L Ultrasonic Humidifier; Argos, Avebury Boulevard, Central Milton Keynes, England, UK).

2.4. Selected vegetables

Sweet Pepper (California Wonder) and Chilli (De Cayenne) were supplied by B&Q plc (Chandlers Ford, Hants SO53 3LE; www.diy.com) as part of their verve brand. The verve product codes were 311137 and 362387, respectively. All seeds were bought on 14 September 2013.

2.5. Growing the vegetables: First planting

In this experiment, the seeds (90 per plant type (see above), except 72 for Ailsa Craig) were sown thinly in a propagator (verve; B&Q plc) into seed and cutting compost (verve; B&Q plc) and covered with 6 mm of compost on 16 September 2013. Each propagator contained 72 planting cells with an average depth of 5 cm (only planted up to about 4 cm; measured before initial watering) and square sides of approximately 3.5 cm. The compost comprised 58% sustainably sourced (in terms of ecology, archaeology and conservation) *Sphagnum* moss peat and unspecified amounts of composted bark, green compost, wood fibre and coir (natural fibre extracted from the husk (outer shell) of coconuts), and oyster shells (optional), vermiculture (optional), perlite (optional), loam (optional), charcoal (optional), alcosorb (optional), sand (optional), grit (optional), wetting agent (to retain moisture better; between 200 and 400 ml/m^3) and essential nutrients and trace minerals lasting for approximately six weeks. The remaining 42% comprised among other components more than 48% non-peat composted organic material such as a mixture of composted green waste and spent brewery grains. The fertiliser content was between 0 and 3 kg/m^3. The dolomitic limestone content was between 0 and 7 kg/m^3. However, the exact combination of ingredients is commercially sensitive, and was therefore not communicated to the authors.

The propagators were placed within a dark incubation room between 17 and 23 September 2013. The transparent covers of the propagators were kept on top of the propagator bases. The temperature was maintained between 19.5 and 22.5 °C (mean of 20.8 °C). The recommended range according to the supplier is between 18 and 21 °C. The compost was kept moist until the seeds germinated.

After germination of some seeds was noticed on 23 September 2013, all seeds were relocated to a lab fitted with OSRAM HQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights provided by OSRAM (North Industrial Road, Foshan, Guangdong, China) and supported by a H4000 Gear Unit, which was supplied by Philips (London Road, Croydon CR9 3QR). The bulbs were

Table 5b

Overview of visual growth problems associated with macronutrient deficiency in new plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 11 March 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Stunted growth[a]		Spindly small plant with thin stem[b]		Pale green of entire plant[c]		Prematurely falling of buds and blossoms[d]		Necrosis[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4	P1;P2	C3;C4	P1;P2	C3;C4	P1;P2	C3;C4	P1;P2	C3;C4
Filter 2 and organic	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9	P5;**P6**	C6;C8;C9	P5;**P6**	C6;C8;C9
Filter 3 and organic	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12
Filter 4 and organic	P12;P16	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17
Filter 5 and organic	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19;P20**	C18;C19;C20	P18;**P19;P20**	C18;C19;C20
Filter 6 and organic	P22;P23	C21	P22;P23	C21	P22;P23	C21	P22;P23	C21	P22;P23	C21
Filter 7 and organic	P26;P28	C25;C26	P26;P28	C25;C26	P26;P28	C25;C26	P26;**P28**	C25;**C26**	P26;**P28**	C25;**C26**
Filter 8 and organic	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29
Control A and organic	P35	C31;C33	P35	C31;C33	P35	C31;C33	P35	C31;C33	P35	C31;C33
Control B and organic	P39	C37;C38	P39	C37;C38	P39	C37;C38	P39	C37;C38	P39	C37;C38
Deionised water and organic	P41	C41	P41	C41	P41	C41	P41	C41	P41	C41
Tap water and organic	P44	C42;C43	P44	C42;C43	P44	C42;C43	P44	**C42;C43**	P44	**C42;C43**
Tap water/fertiliser and organic	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/tap water and organic	P47	C49	P47	C49	P47	C49	P47	C49	P47	C49
Wastewater and organic	P51;P54	C52; C54	P51;P54	C52; C54	P51;P54	C52; C54	P51;P54	C52; C54	P51;P54	C52; C54
Filter 1 and inorganic	**P55;P56**	**C56**	P55;P56	C56	P55;P56	C56	P55;P56	C56	P55;P56	C56
Filter 2 and inorganic	**P57**	**C58**	P57	**C58**	P57	C58	P57	C58	P57	C58
Filter 3 and inorganic	**P59**	**C61**	P59	C61	P59	C61	P59	C61	P59	C61
Filter 4 and inorganic	**P61**	**C63;C64**	P61	**C63;C64**	P61	C63;C64	P61	C63;C64	P61	C63;C64
Filter 5 and inorganic	**P65**	**C66**	P65	C66	P65	**C66**	P65	C66	P65	C66
Filter 6 and inorganic	**P66;P67**	**C68**	P66;P67	C68	P66;P67	C68	P66;P67	C68	P66;P67	C68
Filter 7 and inorganic	**P17;P69**	**C71**	P17;P69	C71	P17;P69	C71	P17;P69	C71	P17;P69	C71
Filter 8 and inorganic	**P70;P71**	**C72;C73**	P70;P71	**C72;C73**	P70;P71	C72;C73	P70;P71	C72;C73	P70;P71	C72;C73
Control A and inorganic	**P73**	**C74**	P73	C74	P73	**C74**	P73	C74	P73	C74
Control B and inorganic	**P74**	**C76;C77**	P74	C76;C77	P74	**C76;C77**	P74	C76;C77	P74	C76;C77
Deionised water and inorganic	**P80**	**C80**	P80	C80	P80	C80	P80	C80	P80	C80
Tap water and inorganic	**P81**	**C82**	P81	C82	P81	**C82**	P81	C82	P81	C82
Tap water/fertiliser and inorganic	P83;P84	C84;C85	P83;P84	C84;C85	P83;P84	C84;C85	P83;P84	C84;C85	P83;P84	C84;C85
Wastewater/tap water and inorganic	**P86;P87**	**C87**	P86;P87	C87	P86;P87	C87	**P86;P87**	C87	P86;P87	C87
Wastewater and inorganic	**P89;P90**	**C90**	P89;P90	**C90**	P89;P90	C90	**P89;P90**	C90	P89;P90	C90

Inflow source and growth media	Dry or brittle leaf[f]	
	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4
Filter 2 and organic	P5;P6	C6;C8;C9
Filter 3 and organic	P8;P9;P10	C10;C11;C12
Filter 4 and organic	P12;P16	C16;C17
Filter 5 and organic	P18;P19;P20	C18;C19;C20
Filter 6 and organic	P22;P23	C21
Filter 7 and organic	P26;P28	C25;**C26**
Filter 8 and organic	P31;P32;P33	C27;C28;C29
Control A and organic	P35	C31;C33
Control B and organic	P39	C37;C38
Deionised water and organic	P41	C41
Tap water and organic	P44	C42;C43
Tap water/fertiliser and organic	P45;P46	C45;C46
Wastewater/tap water and organic	P47	C49
Wastewater and organic	P51;P54	C52; C54
Filter 1 and inorganic	P55;P56	C56
Filter 2 and inorganic	P57	C58
Filter 3 and inorganic	P59	C61
Filter 4 and inorganic	P61	C63;C64
Filter 5 and inorganic	P65	C66

Table 5b (*Continued*)

Inflow source and growth media	Stunted growth[a]		Spindly small plant with thin stem[b]		Pale green of entire plant[c]		Prematurely falling of buds and blossoms[d]		Necrosis[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 6 and inorganic	P66;P67	C68								
Filter 7 and inorganic	P17;P69	C71								
Filter 8 and inorganic	P70;P71	C72;C73								
Control A and inorganic	P73	C74								
Control B and inorganic	P74	C76;C77								
Deionised water and inorganic	P80	C80								
Tap water and inorganic	P81	C82								
Tap water/fertiliser and inorganic	P83;P84	C84;C85								
Wastewater/tap water and inorganic	P86;P87	C87								
Wastewater and inorganic	P89;P90	C90								

[a] Deficiency in calcium (Kennelly et al., 2012; McCauly et al., 2011; Wong, 2005).
[b] Deficiency in sulfur (McCauly et al., 2011).
[c] Deficiency in sulfur (McCauly et al., 2011).
[d] Deficiency in calcium (Silva et al., 2000).
[e] Deficiency in calcium (Kennelly et al., 2012).
[f] Deficiency in calcium (McCauly et al., 2011).

Table 5c
Overview of visual growth problems associated with micronutrient deficiency in old plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 15 February 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Stunted growth[a]	
	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4
Filter 2 and organic	P5;P6	C6;C8;C9
Filter 3 and organic	P8;P9;P10	C10;C11;C12
Filter 4 and organic	P12;P16	C16;C17
Filter 5 and organic	P18;P19;P20	C18;C19;C20
Filter 6 and organic	P22;P23	C21
Filter 7 and organic	P26;P28	C25;C26
Filter 8 and organic	P31;P32;P33	C27;C28;C29
Control A and organic	P35	C31;C33
Control B and organic	P39	C37;C38
Deionised water and organic	P41	C41
Tap water and organic	P44	C42;C43
Tap water/fertiliser and organic	P45;P46	C45;C46
Wastewater/tap water and organic	P47	C49
Wastewater and organic	P51;P54	C52; C54
Filter 1 and inorganic	**P55;P56**	**C56**
Filter 2 and inorganic	**P57**	**C58**
Filter 3 and inorganic	**P59**	**C61**
Filter 4 and inorganic	**P61**	**C63;C64**
Filter 5 and inorganic	**P65**	**C66**
Filter 6 and inorganic	**P66;P67**	**C68**
Filter 7 and inorganic	**P17;P69**	**C71**
Filter 8 and inorganic	**P70;P71**	**C72;C73**
Control A and inorganic	**P73**	**C74**
Control B and inorganic	**P74**	**C76;C77**
Deionised water and inorganic	**P80**	**C80**
Tap water and inorganic	**P81**	**C82**
Tap water/fertiliser and inorganic	**P83;P84**	**C84;C85**
Wastewater/tap water and inorganic	**P86;P87**	**C87**
Wastewater and inorganic	**P89;P90**	**C90**

[a] Deficiency in molybdenum (Haifa Chemicals, 2014; Silva et al., 2000).

comparable to those used by Boyden and Rababah (1996). The temperature close to the plants ranged between 19.3 and 26.3 °C (mean of 24.2 °C). The lights were set on timers, simulating the sunrise and sunset times in Salford (http://www.timeanddate.com). The transparent covers of the propagators were kept on top of the propagator bases (gap of approximately 6.0 cm) until the first seedlings reached them on 30 September 2013.

2.6. Growing the vegetables: Second planting

Fig. 1 shows and overview of the experimental set-up for both Chillies and Sweet Peppers. The second planting (i.e. first replanting) of the strongest 70 Sweet Pepper and 76 Chilli plants took place when most seedlings had at least two true leaves and were large enough to handle on 11 October 2013. The weakest 10 Sweet Pepper and 1 Chilli plants were not replanted. Moreover, 10 Sweet Pepper and 13 Chilli plants either did not germinate or died before the first replanting.

Seedlings were transplanted into 60-mm diameter (average) pots of moist multipurpose compost and grown on for three to four weeks. Each tray contained 40 pots with a depth of 60 mm each. Multipurpose compost was filled up to a depth of 4.5 cm and covered with a 1.0-cm layer of bark (B&Q verve range). The topping contained small chipped bark from mixed wood (responsibly sourced), and was applied to retain moisture and insulate soil.

Some vegetables were planted in pure sand to assess the impact of the organic grow substrate on plant growth. The product Play Pit Sand (silica), which is described by the supplier Deko-Pak Limited (Deco House, Halifax Road, Hipperholme, Brighouse HX3 8BW) as non-staining, non-toxic, safe and clean, has been used. Sand was filled up to a depth of 5.5 cm.

Table 5d
Overview of visual growth problems associated with micronutrient deficiency in new plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 11 March 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Stunted growth[a]		Death of terminal buds[b]		Thick and curl leaf tips[c]		Necrosis[d]		Poor flowering and seeds[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4	**P1;P2**	C3;C4	**P1**;P2	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;C4
Filter 2 and organic	P5;P6	C6;C8;C9	**P5;P6**	C6;C8;C9	P5;**P6**	C6;C8;C9	P5;**P6**	C6;C8;C9	**P5;P6**	C6;C8;C9
Filter 3 and organic	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;**C11;C12**	P8;P9;P10	C10;C11;C12	**P8;P9;P10**	C10;C11;C12
Filter 4 and organic	P12;P16	C16;C17	**P12;P16**	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	**P12;P16**	C16;C17
Filter 5 and organic	P18;P19;P20	C18;C19;C20	**P18;P19;P20**	C18;C19;C20	P18;P19;**P20**	**C18**;C19;**C20**	P18;**P19;P20**	C18;C19;C20	**P18;P19;P20**	C18;C19;C20
Filter 6 and organic	P22;P23	C21	**P22;P23**	C21	P22;**P23**	C21	P22;P23	C21	**P22;P23**	C21
Filter 7 and organic	P26;P28	C25;C26	**P26;P28**	C25;C26	P26;P28	C25;**C26**	P26;**P28**	C25;**C26**	**P26;P28**	C25;C26
Filter 8 and organic	P31;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29	**P31**;P32;P33	C27;**C28;C29**	**P31**;P32;P33	C27;C28;C29	**P31;P32;P33**	C27;C28;C29
Control A and organic	P35	C31;C33	**P35**	C31;C33	P35	C31;**C33**	P35	C31;C33	**P35**	C31;C33
Control B and organic	P39	C37;C38	**P39**	C37;C38	P39	**C37;C38**	P39	C37;C38	**P39**	C37;C38
Deionised water and organic	P41	C41	**P41**	C41	**P41**	C41	P41	C41	**P41**	C41
Tap water and organic	P44	C42;C43	**P44**	C42;C43	**P44**	C42;**C43**	P44	**C42;C43**	**P44**	C42;C43
Tap water/fertiliser and organic	P45;P46	C45;C46	**P45;P46**	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	**P45;P46**	C45;C46
Wastewater/tap water and organic	P47	C49	**P47**	C49	**P47**	**C49**	P47	C49	**P47**	C49
Wastewater and organic	P51;P54	C52; C54	**P51;P54**	C52; C54	P51;P54	C52; C54	P51;P54	C52; C54	**P51;P54**	C52; C54
Filter 1 and inorganic	**P55;P56**	**C56**	**P55;P56**	C56	**P55**;P56	C56	P55;P56	C56	**P55;P56**	C56
Filter 2 and inorganic	**P57**	**C58**	**P57**	C58	P57	C58	P57	C58	**P57**	C58
Filter 3 and inorganic	**P59**	**C61**	**P59**	C61	P59	C61	P59	C61	**P59**	C61
Filter 4 and inorganic	**P61**	**C63;C64**	**P61**	C63;C64	**P61**	C63;C64	P61	C63;C64	**P61**	C63;C64
Filter 5 and inorganic	**P65**	**C66**	**P65**	C66	**P65**	C66	P65	C66	**P65**	C66
Filter 6 and inorganic	**P66;P67**	**C68**	**P66;P67**	C68	P66;P67	C68	P66;P67	C68	**P66;P67**	C68
Filter 7 and inorganic	**P17;P69**	**C71**	**P17;P69**	C71	P17;P69	C71	P17;P69	C71	**P17;P69**	C71
Filter 8 and inorganic	**P70;P71**	**C72;C73**	**P70;P71**	C72;C73	P70;P71	C72;C73	P70;P71	C72;C73	**P70;P71**	C72;C73
Control A and inorganic	**P73**	**C74**	**P73**	C74	**P73**	C74	P73	C74	**P73**	C74
Control B and inorganic	**P74**	**C76;C77**	**P74**	C76;C77	P74	C76;C77	P74	C76;C77	**P74**	C76;C77
Deionised water and inorganic	**P80**	**C80**	**P80**	C80	P80	C80	P80	C80	**P80**	C80
Tap water and inorganic	**P81**	**C82**	**P81**	C82	P81	C82	P81	C82	**P81**	C82
Tap water/fertiliser and inorganic	P83;P84	C84;C85	**P83;P84**	C84;C85	P83;**P84**	C84;**C85**	P83;P84	C84;C85	**P83;P84**	C84;C85
Wastewater/tap water and inorganic	**P86;P87**	**C87**	**P86;P87**	C87	P86;P87	C87	P86;P87	C87	**P86;P87**	C87
Wastewater and inorganic	**P89;P90**	**C90**	**P89;P90**	C90	P89;**P90**	**C90**	P89;P90	C90	**P89;P90**	C90

[a] Deficiency in boron (McCauly et al., 2011; Silva et al., 2000), and/or iron and/or zinc (McCauly et al., 2011), and/or copper (Hosier and Bradley, 1999; McCauly et al., 2011; Silva et al., 2000).
[b] Deficiency in boron (Haifa Chemicals, 2014; Hosier and Bradley, 1999; McCauly et al., 2011).
[c] Deficiency in boron (McCauly et al., 2011; Silva et al., 2000).
[d] Deficiency in boron and/or iron (McCauly et al., 2011), and/or manganese and/or copper (Silva et al., 2000; Wong, 2005).
[e] Deficiency in zinc (McCauly et al., 2011).

Table 5e
Overview of visual growth problems associated with macronutrient surplus in old plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 15 February 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Dark green and abundant foliage[a]		Stunting and reducing in branches[b]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	**P1;P2**	**C3;C4**	P1;P2	C3;C4
Filter 2 and organic	**P5;P6**	**C6;C8;C9**	P5;P6	C6;C8;C9
Filter 3 and organic	**P8;P9;P10**	**C10;C11;C12**	P8;P9;P10	C10;C11;C12
Filter 4 and organic	**P12;P16**	**C16;C17**	P12;P16	C16;C17
Filter 5 and organic	**P18;P19;P20**	**C18;C19;C20**	P18;P19;P20	C18;C19;C20
Filter 6 and organic	**P22;P23**	**C21**	P22;P23	C21
Filter 7 and organic	**P26;P28**	**C25;C26**	P26;P28	C25;C26
Filter 8 and organic	P31**;P32;P33**	**C27;C28;C29**	P31;P32;P33	C27;C28;C29
Control A and organic	**P35**	**C31;C33**	P35	C31;C33
Control B and organic	**P39**	**C37;C38**	P39	C37;C38
Deionised water and organic	**P41**	**C41**	P41	C41
Tap water and organic	**P44**	**C42;C43**	P44	C42;C43
Tap water/fertiliser and organic	**P45;P46**	C45;C46	P45;P46	C45;C46
Wastewater/tap water and organic	**P47**	C49	P47	C49
Wastewater and organic	**P51;**P54	**C52; C54**	P51;P54	C52; C54
Filter 1 and inorganic	P55;P56	C56	**P55;P56**	**C56**
Filter 2 and inorganic	P57	**C58**	**P57**	**C58**
Filter 3 and inorganic	P59	C61	**P59**	**C61**
Filter 4 and inorganic	P61	**C63;C64**	**P61**	**C63;C64**
Filter 5 and inorganic	P65	C66	**P65**	**C66**
Filter 6 and inorganic	P66;P67	**C68**	**P66;P67**	**C68**
Filter 7 and inorganic	P17;P69	**C71**	**P17;P69**	**C71**
Filter 8 and inorganic	P70;P71	C72;**C73**	**P70;P71**	**C72;C73**
Control A and inorganic	P73	C74	**P73**	**C74**
Control B and inorganic	P74	C76;C77	**P74**	**C76;C77**
Deionised water and inorganic	P80	C80	**P80**	**C80**
Tap water and inorganic	P81	C82	**P81**	**C82**
Tap water/fertiliser and inorganic	**P83;P84**	C84;C85	P83;P84	C84;C85
Wastewater/tap water and inorganic	P86;P87	C87	**P86;P87**	**C87**
Wastewater and inorganic	**P89**;P90	**C90**	**P89;P90**	**C90**

[a] Surplus in nitrogen (Haifa Chemicals, 2014; McCauly et al., 2011; Wong, 2005).
[b] Surplus in nitrogen (Wong, 2005).

All vegetables were kept permanently indoors in the same heated laboratory fixed with grow lights. The environmental boundary conditions were essentially independent of a specific geographical region, but were similar to those of a warm country.

2.7. Growing the vegetables: Third planting

The third planting (i.e. second and final replanting) took place 28 days after the first replanting on 8 November 2013. Table 2 outlines the experimental set-up. The Sweet Pepper and Chilli were planted individually into 10-l plastic and round plant pots provided by scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK). The plant pots dimensions were as follows: height of 22.0 cm, bottom diameter of 22.0 cm and top diameter of 28.5 cm. The top 2 cm were left unplanted. Sand-based plants were planted to a depth of 20.0 cm. In comparison, soil-based plants were planted to a depth of 17.5 cm. and covered by a further 2.5 cm of bark (see above). Sufficient space between plants was always allowed for Sweet Pepper and Gardeners Delight. They remained indoors in the laboratory characterised above.

All plants were initially supported by small bamboo canes (diameter of approximately 0.3 cm; length of up to 30 cm) and later on by bigger bamboo canes (diameter average of 0.8 cm; range between 0.6 and 1.2 cm; length of up to 150 cm) if and when necessary. If and when required, plants were supported using a string, which was loosely tied to the main stem against the cane.

2.8. Growth conditions

Some plants were fed with a liquid and concentrated fruit and vegetable fertiliser from the B&Q verve range. The fertiliser had a nitrogen to phosphorus to potassium ratio of 4 to 4 to 4 according to the EC fertiliser solution for the UK. The total nitrogen component was 4%. Nitric nitrogen and ureic nitrogen parts were 1.1 and 2.1%, respectively. Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) made up 4% each of the solution, but the corresponding P and K content were only 1.7 and 3.3%, respectively. Moreover, the fertiliser contained also trace elements (names not listed) of unspecified quantities.

Domestic cultivars have been selected to maximise self-fertility. In an outside setting, wind or insects provide sufficient motion to produce commercially viable crops (Jones, 2013). Therefore, mechanical movement of the plants and manual pollen transfer between plants was practised in this study. Cross-pollination between Sweet Peppers and Chillies was prevented by separating the growing space with the help of temporary walls.

3. Results and discussion

3.1. Water quality analysis

Table 3 shows the inflow water quality received by the plants. Note that the wetland effluent was used as the influent for the vegetable pots. Highly fluctuating values for TPH were observed in the outflow waters obtained from Filters 1, 3, 5, and 8, and Control A. The TPH concentrations followed this order: Control A > Filter 8 > Filter 1 > Filter 3 > Filter 5. Chemical oxygen demand values were the highest for Filter 5. In contrast, the lowest values were noted for Control B. Filters 1, 3 and 8 had relatively similar COD concentrations. Control A had higher COD values than Filter 6. No differences in COD values were noted for Filters 2, 4 and 7.

The five-day BOD was high for raw wastewater followed by wastewater samples, which were diluted with 80% of tap water. In

Table 5f
Overview of visual growth problems associated with micronutrient surplus in old plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 11March 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Stunting and reducing in branches[a]		Golden yellowish leaves[b]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1;P2	C3;C4	P1;P2	C3;C4
Filter 2 and organic	P5;P6	C6;C8;C9	P5;P6	C6;C8;C9
Filter 3 and organic	P8;P9;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12
Filter 4 and organic	P12;P16	C16;C17	P12;P16	C16;C17
Filter 5 and organic	P18;P19;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20
Filter 6 and organic	P22;P23	C21	P22;P23	C21
Filter 7 and organic	P26;P28	C25;C26	P26;P28	C25;C26
Filter 8 and organic	P31;P32;P33	C27;C28;C29	P31;P32;P33	C27;C28;C29
Control A and organic	P35	C31;C33	P35	C31;C33
Control B and organic	P39	C37;C38	P39	C37;C38
Deionised water and organic	P41	C41	P41	C41
Tap water and organic	P44	C42;C43	P44	C42;C43
Tap water/fertiliser and organic	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/tap water and organic	P47	C49	P47	C49
Wastewater and organic	P51;P54	C52; C54	P51;P54	C52; C54
Filter 1 and inorganic	**P55;P56**	**C56**	P55;P56	C56
Filter 2 and inorganic	**P57**	**C58**	P57	**C58**
Filter 3 and inorganic	**P59**	**C61**	**P59**	C61
Filter 4 and inorganic	**P61**	**C63;C64**	P61	C63;C64
Filter 5 and inorganic	**P65**	**C66**	P65	**C66**
Filter 6 and inorganic	**P66;P67**	**C68**	P66;P67	C68
Filter 7 and inorganic	**P17;P69**	**C71**	P17;P69	C71
Filter 8 and inorganic	**P70;P71**	**C72;C73**	P70;P71	**C72;C73**
Control A and inorganic	**P73**	**C74**	P73	C74
Control B and inorganic	**P74**	**C76;C77**	P74	**C76;C77**
Deionised water and inorganic	**P80**	**C80**	**P80**	C80
Tap water and inorganic	**P81**	**C82**	**P81**	**C82**
Tap water/fertiliser and inorganic	P83;P84	C84;C85	P83;P84	C84;C85
Wastewater/tap water and inorganic	**P86;P87**	**C87**	P86;P87	C87
Wastewater and inorganic	**P89;P90**	**C90**	P89;P90	C90

[a] Surplus in molybdenum (McCauly et al., 2011).

[b] Surplus in molybdenum (McCauly et al., 2011).

comparison, the lowest five-day BOD was observed for tap water with fertiliser, tap water and deionised water.

High concentrations of ammonia-nitrogen, which exceeded the threshold of 5 mg/l (FAO, 1994), were noted for both Filters 5 and 6, followed by those for Filters 1 and 2. Table 3 shows that nitrate-nitrogen for all filters outflow waters was less than the maximum thresholds value of 30 mg/l (Pescod, 1992). Based on the recommended threshold of 2 mg/l for ortho-phosphate-phosphorus (FAO, 1994), the outflow waters from all wetland filters (with the exception of Controls A and B) were associated with too high ortho-phosphate-phosphorus concentrations.

The highest value for SS was noted for raw wastewater followed by that for wastewater, which was diluted with 80% tap water. In contrast, the lowest values were observed for Filter 7 outflow water and tap water with fertiliser. Turbidity was high for raw wastewater. Filter 7 had the lowest turbidity values. The pH values for all filter outflows were within the normal range between 6.0 and 8.5 (Pescod, 1992).

Table 4 shows an overview of the environmental boundary conditions associated with the vegetable pots. According to Haifa Chemicals (2014), temperature measurements for this experiment were within the recommended ranges for different growth stages of Chillies and Sweet Peppers.

3.2. Growth comparisons

The statistical experimental set-up as specified in Table 2 was chosen for the second replanting stage. Table 5a–5g shows visual growth problems and indicates possible reasons. The reference numbers indicating individual plants, which have visual problems of a particular nature, are highlighted in bold (Tables 5a–5g). Table 5a indicates deficiencies for both Sweet Peppers and Chillies for old plant parts regarding magnesium and potassium. Table 5a also highlights phosphorus deficiencies for old Sweet Pepper plant parts. Deficiencies for both Sweet Peppers and Chillies for young plant parts were recorded for calcium and sulphur (Table 5b). A sulphur deficiency was noted for new Chilli plant parts only (Table 5b). Table 5c summarises molybdenum deficiencies. Boron and zinc deficiencies were notes for new Sweet Pepper plant parts (Table 5d). Copper deficiencies were observed for new plant parts for both Sweet Peppers and Chillies (Table 5d).

A surplus of nitrogen was noted for new plant parts of both Sweet Peppers and Chillies (Table 5e). A surplus of molybdenum was noted for the old plant parts for both Sweet Peppers and Chillies (Table 5f). However, this observation is ambiguous, because symptoms for some plants also indicate deficiencies. Table 5g is concerned with surpluses of zinc, manganese, copper and boron.

Table 6 shows an overview of total water volumes for Sweet Pepper and Chilli plants for different planting periods. In countries, where wastewater is seen as a resource, low wastewater consumption by plants is an advantage. However, high wastewater use by plants is seen as an advantage in temperate regions. The productivity of plants in terms of harvest is, however, independent of the wastewater consumption (see Section 3.4). Nevertheless, a higher foliage production requires more water. A high yield is rather related with the most suitable provision of nutrients and trace elements (Table 5a–5g).

Table 7 provides summaries of the bud, flower and fruit development for Sweet Pepper and Chilli plants. The overall growth development of Sweet Peppers was rather disappointing, possibly due to the high concentrations of nutrients and trace minerals, and adverse environmental boundary conditions in the laboratory. In contrast, chillies did reasonably well but the growth of foliage was excessive (Fig. 1a) and the harvest was delayed. The

Table 5g
Overview of visual growth problems associated with micronutrient surplus in new plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 7 March 2014, and possible reasons associated with nutritional disorders.

Inflow source and growth media	Dark green and abundant foliage[a]		Low grow rate[b]		Yellowing and necrosis of leave tip or margins toward midrib[c]		Stunting and reducing in branches[d]		Necrotic lesions on leaves[e]	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	**P1;P2**	C3;C4	P1;P2	C3;C4	P1;**P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;C4
Filter 2 and organic	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	P5;**P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	C6;**C8**;C9
Filter 3 and organic	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12
Filter 4 and organic	**P12;P16**	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;P16	C16;C17	P12;**P16**	C16;C17
Filter 5 and organic	**P18;P19;P20**	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19**;P20	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19;P20**	C18;C19;C20
Filter 6 and organic	**P22;P23**	C21	P22;P23	C21	P22;**P23**	C21	P22;P23	C21	**P22;P23**	C21
Filter 7 and organic	**P26;P28**	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;**C26**
Filter 8 and organic	**P31;P32;P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29
Control A and organic	**P35**	C31;C33	P35	C31;C33	**P35**	C31;C33	P35	C31;C33	**P35**	C31;C33
Control B and organic	**P39**	C37;C38	P39	C37;C38	P39	C37;C38	P39	C37;C38	**P39**	C37;C38
Deionised water and organic	**P41**	C41	P41	C41	P41	C41	P41	C41	**P41**	C41
Tap water and organic	**P44**	**C42;C43**	P44	C42;C43	**P44**	C42;C43	P44	C42;C43	**P44**	C42;**C43**
Tap water/fertiliser and organic	**P45;P46**	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/tap water and organic	**P47**	C49	P47	C49	**P47**	C49	P47	C49	**P47**	C49
Wastewater and organic	**P51;P54**	**C52; C54**	P51;P54	C52; C54	**P51**;P54	C52; C54	P51;P54	C52; C54	P51;P54	C52; **C54**
Filter 1 and inorganic	P55;P56	C56	**P55;P56**	**C56**	P55;P56	C56	**P55;P56**	**C56**	P55;P56	C56
Filter 2 and inorganic	P57	C58	**P57**	**C58**	P57	C58	**P57**	**C58**	P57	C58
Filter 3 and inorganic	P59	C61	**P59**	**C61**	P59	C61	**P59**	**C61**	P59	C61
Filter 4 and inorganic	P61	C63;C64	**P61**	**C63;C64**	P61	C63;C64	**P61**	**C63;C64**	P61	C63;C64
Filter 5 and inorganic	P65	C66	**P65**	**C66**	P65	C66	**P65**	**C66**	P65	C66
Filter 6 and inorganic	P66;P67	C68	**P66;P67**	**C68**	P66;P67	C68	**P66;P67**	**C68**	P66;P67	C68
Filter 7 and inorganic	P17;P69	C71	**P17;P69**	**C71**	P17;P69	C71	**P17;P69**	**C71**	P17;P69	C71
Filter 8 and inorganic	P70;P71	C72;C73	**P70;P71**	**C72;C73**	P70;P71	C72;C73	**P70;P71**	**C72;C73**	P70;P71	C72;C73
Control A and inorganic	P73	C74	**P73**	**C74**	P73	C74	**P73**	**C74**	P73	C74
Control B and inorganic	P74	C76;C77	**P74**	**C76;C77**	P74	C76;C77	**P74**	**C76;C77**	P74	C76;C77
Deionised water and inorganic	P80	C80	**P80**	**C80**	P80	C80	**P80**	**C80**	P80	C80
Tap water and inorganic	P81	C82	**P81**	**C82**	P81	C82	**P81**	**C82**	P81	C82
Tap water/fertiliser and inorganic	P83;P84	C84;C85	**P83;P84**	**C84;C85**	P83;**P84**	C84;C85	**P83;P84**	**C84;C85**	P83;P84	C84;C85
Wastewater/tap water and inorganic	P86;P87	C87	**P86;P87**	**C87**	P86;P87	C87	**P86;P87**	**C87**	P86;P87	C87
Wastewater and inorganic	P89;P90	C90	**P89;P90**	**C90**	P89;P90	C90	**P89;P90**	**C90**	P89;P90	C90

[a] Surplus in zinc (McCauly et al., 2011) and/or iron (Foy et al., 1978).
[b] Surplus in manganese and/or copper (Haifa Chemicals, 2014).
[c] Surplus in boron (Haifa Chemicals, 2014; McCauly et al., 2011; Silva et al., 2000).
[d] Surplus in copper (Haifa Chemicals, 2014; McCauly et al., 2011) and/or manganese (Silva et al., 2000) and/or Iron (Foy et al., 1978).
[e] Surplus in manganese (McCauly et al., 2011; Silva et al., 2000).

Table 6
Overview of total water volumes for Sweet Pepper (P) and Chilli (C) plants for different planting periods.

Plant	Total irrigation water volume (l)			Plant	Total irrigation water volume (l)		
	FRP[a]	SRPBF[b]	SRPAF[c]		FRP[a]	SRPBF[b]	SRPAF[c]
P1	0.290	4.800	13.900	C3	0.290	6.100	17.200
P2	0.290	5.000	20.400	C4	0.290	6.250	18.550
P4	0.290	n/a	n/a	C5	0.290	n/a	n/a
P5	0.290	4.650	14.250	C6	0.290	5.750	17.700
P6	0.290	4.950	13.000	C8	0.290	6.100	15.200
P7	0.290	n/a	n/a	C9	0.280	5.450	15.100
P8	0.280	4.700	12.500	C10	0.280	4.800	10.800
P9	0.280	4.100	11.450	C11	0.280	4.800	13.000
P10	0.280	4.600	9.050	C12	0.280	4.800	14.100
P12	0.244	4.700	12.600	C13	0.280	n/a	n/a
P13	0.244	n/a	n/a	C16	0.280	4.650	13.770
P16	0.280	4.600	13.900	C17	0.280	6.150	18.220
P17	0.280	5.900	10.500	C18	0.280	5.250	12.050
P18	0.280	4.450	10.250	C19	0.280	5.250	12.800
P19	0.280	4.450	11.300	C20	0.280	4.900	12.900
P20	0.280	3.850	10.850	C21	0.280	4.800	21.950
P22	0.280	4.350	10.100	C22	0.280	n/a	n/a
P23	0.280	4.450	11.600	C23	0.280	n/a	n/a
P24	0.280	n/a	n/a	C24	0.280	n/a	n/a
P26	0.280	4.850	14.550	C25	0.280	7.100	18.800
P27	0.280	n/a	n/a	C26	0.280	6.000	17.850
P28	0.280	5.950	15.900	C27	0.280	4.800	15.650
P31	0.280	5.900	13.150	C28	0.280	4.800	14.900
P32	0.280	5.200	9.950	C29	0.280	5.100	16.200
P33	0.280	4.200	10.250	C30	0.280	n/a	n/a
P34	0.280	n/a	n/a	C31	0.280	4.900	22.550
P35	0.280	4.900	16.500	C33	0.280	4.900	22.450
P37	0.280	n/a	n/a	C34	0.280	n/a	n/a
P38	0.280	n/a	n/a	C37	0.280	5.600	18.200
P39	0.280	5.250	12.700	C38	0.280	5.800	16.600
P40	0.280	n/a	n/a	C39	0.280	n/a	n/a
P41	0.280	4.750	14.100	C40	0.280	n/a	n/a
P42	0.280	n/a	n/a	C41	0.280	5.950	18.300
P43	0.280	n/a	n/a	C42	0.280	6.100	28.500
P44	0.280	4.800	20.500	C43	0.280	7.100	23.500
P45	0.280	5.500	20.000	C44	0.280	n/a	n/a
P46	0.280	5.450	24.500	C45	0.280	7.000	30.150
P47	0.280	6.100	17.300	C46	0.280	7.000	28.000
P48	0.280	n/a	n/a	C48	0.280	n/a	n/a
P51	0.280	6.100	24.000	C49	0.280	5.100	20.500
P54	0.280	6.100	23.700	C50	0.280	n/a	n/a
P55	0.270	5.050	10.700	C51	0.280	n/a	n/a
P56	0.270	5.050	10.150	C52	0.280	5.100	20.700
P57	0.270	5.150	9.200	C53	0.280	n/a	n/a
P58	0.270	n/a	n/a	C54	0.280	5.600	21.900
P59	0.270	5.200	5000	C55	0.270	n/a	n/a
P60	0.270	n/a	n/a	C56	0.270	5.900	7.400
P61	0.270	5.450	8.500	C57	0.270	n/a	n/a
P63	0.270	n/a	n/a	C58	0.270	6.050	6.750
P64	0.270	n/a	n/a	C60	0.270	n/a	n/a
P65	0.270	5.050	4.600	C61	0.270	5.350	8.150
P66	0.270	4.600	8.600	C62	0.270	n/a	n/a
P67	0.270	4.450	9.250	C63	0.270	6.550	8.900
P69	0.270	5.800	10.600	C64	0.270	7.150	8.300
P70	0.270	4.350	8.700	C65	0.270	n/a	n/a
P71	0.270	4.250	7.650	C66	0.270	5.250	5.700
P72	0.270	n/a	n/a	C67	0.270	n/a	n/a
P73	0.270	4.350	11.050	C68	0.270	6.400	5.400
P74	0.270	5.450	6.900	C70	0.270	n/a	n/a
P76	0.270	n/a	n/a	C71	0.270	6.100	10.350
P79	0.270	n/a	n/a	C72	0.270	6.700	6.900
P80	0.270	6.000	6.500	C73	0.270	5.900	7.300
P81	0.270	5.450	9.200	C74	0.270	5.250	9.500
P82	0.270	n/a	n/a	C75	0.270	n/a	n/a
P83	0.270	6.050	16.100	C76	0.270	6.550	7.950
P84	0.270	5.950	15.550	C77	0.270	6.550	7.800
P86	0.270	5.800	13.400	C79	0.270	n/a	n/a
P87	0.270	5.400	13.000	C80	0.270	6.350	10.900
P89	0.270	5.500	13.500	C81	0.270	n/a	n/a
P90	0.270	5.400	14.000	C82	0.270	6.200	8.800
				C84	0.270	7.100	22.500
				C85	0.270	7.000	25.500
				C86	0.270	n/a	n/a
				C87	0.270	6.100	11.500
				C88	0.270	n/a	n/a
				C90	0.270	5.900	11.950

n/a, Not applicable.

[a] First replanting period: 11/10/13 to 07/11/13.

[b] Second replanting period before fruiting: 08/11/13 to 19/01/14; and

[c] Second replanting period after fruiting: 20/01/14 to 04/06/14.

Table 7
Overview of total number of flowers (TNF) and total number of fruits (TNF) for Sweet Pepper (P) and Chilli (C) plants after the second replanting period on 4 June 2014.

Inflow source and growth media	Total bud number		Total flower number		Total fruit number		Total harvested fruit number	
	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1 (3); P2 (2)	C3 (0); C4 (26)	P1 (0); P2 (1)	C3 (1); C4 (4)	P1 (0); P2 (3)	C3 (14); C4 (1)	P1 (0); P2 (0)	C3 (0); C4 (0)
Filter 2 and organic	P5 (2); P6 (6)	C6 (8); C8 (0); C9 (0)	P5 (0); P6 (2)	C6 (8); C8 (0); C9 (0)	P5 (0); P6 (3)	C6 (9); C8 (14); C9 (10)	P5 (0); P6 (0)	C6 (0); C8 (4); C9 (2)
Filter 3 and organic	P8 (4); P9 (12); P10 (27)	C10 (16); C11 (43); C12 (8)	P8 (0); P9 (1); P10 (0)	C10 (0); C11 (14); C12 (1)	P8 (0); P9 (0); P10 (0)	C10 (8); C11 (7); C12 (9)	P8 (0); P9 (0); P10 (0)	C10 (0); C11 (0); C12 (1)
Filter 4 and organic	P12 (13); P16 (24)	C16 (4); C17 (2)	P12 (0); P16 (0)	C16 (0); C17 (0)	P12 (0); P16 (0)	C16 (10); C17 (12)	P12 (0); P16 (0)	C16 (3); C17 (1)
Filter 5 and organic	P18 (3); P19 (14); P20 (9)	C18 (3); C19 (12); C20 (15)	P18 (1); P19 (1); P20 (6)	C18 (0); C19 (5); C20 (4)	P18 (0); P19 (0); P20 (0)	C18 (12); C19 (8); C20 (19)	P18 (0); P19 (0); P20 (0)	C18 (3); C19 (2); C20 (0)
Filter 6 and organic	P22 (1); P23 (13)	C21 (8)	P22 (0); P23 (0)	C21 (3)	P22 (0); P23 (0)	C21 (13)	P22 (0); P23 (0)	C21 (0)
Filter 7 and organic	P26 (22); P28 (20)	C25 (6); C26 (0)	P26 (0); P28 (2)	C25 (3); C26 (4)	P26 (0); P28 (2)	C25 (18); C26 (21)	P26 (0); P28 (0)	C25 (3); C26 (2)
Filter 8 and organic	P31 (2); P32 (16); P33 (11)	C27 (2); C28 (13); C29 (2)	P31 (0); P32 (1); P33 (1)	C27 (8); C28 (9); C29 (0)	P31 (0); P32 (1); P33 (0)	C27 (20); C28 (11); C29 (15)	P31 (0); P32 (0); P33 (0)	C27 (4); C28 (0); C29 (1)
Control A and organic	P35 (0)	C31 (3); C33 (0)	P35 (1)	C31 (2); C33 (0)	P35 (3)	C31 (17); C33 (15)	P35 (0)	C31 (0); C33 (2)
Control B and organic	P39 (13)	C37 (11); C38 (7)	P39 (0)	C37 (3); C38 (5)	P39 (0)	C37 (13); C38 (16)	P39 (0)	C37 (1); C38 (3)
Deionised water and organic	P41 (5)	C41 (30)	P41 (0)	C41 (7)	P41 (0)	C41 (13)	P41 (0)	C41 (8)
Tap water and organic	P44 (13)	C42 (0); C43 (0)	P44 (6)	C42 (1); C43 (1)	P44 (6)	C42 (28); C43 (23)	P44 (0)	C42 (13); C43 (10)
Tap water/fertiliser and organic	P45 (20); P46 (47)	C45 (11); C46 (15)	P45 (0); P46 (0)	C45 (6); C46 (21)	P45 (1); P46 (1)	C45 (30); C46 (17)	P45 (0); P46 (0)	C45 (0); C46 (1)
Wastewater/tap water and organic	P47 (0)	C49 (28)	P47 (0)	C49 (11)	P47 (0)	C49 (10)	P47 (0)	C49 (0)
Wastewater and organic	P51 (50); P54 (46)	C52 (6); C54 (2)	P51 (11); P54 (0)	C52 (4); C54 (4)	P51 (0); P54 (5)	C52 (13); C54 (9)	P51 (0); P54 (0)	C52 (1); C54 (0)
Filter 1 and inorganic	P55 (0); P56 (0)	C56 (0)	P55 (0); P56 (0)	C56 (0)	P55 (6); P56 (1)	C56 (3)	P55 (0); P56 (0)	C56 (1)
Filter 2 and inorganic	P57 (4)	C58 (0)	P57 (1)	C58 (2)	P57 (2)	C58 (5)	P57 (0)	C58 (1)
Filter 3 and inorganic	P59 (4)	C61 (0)	P59 (0)	C61 (0)	P59 (0)	C61 (2)	P59 (0)	C61 (1)
Filter 4 and inorganic	P61 (0)	C63 (9); C64 (0)	P61 (1)	C63 (0); C64 (1)	P61 (3)	C63 (1); C64 (2)	P61 (0)	C63 (1); C64 (2)
Filter 5 and inorganic	P65 (0)	C66 (2)	P65 (0)	C66 (0)	P65 (1)	C66 (1)	P65 (0)	C66 (1)
Filter 6 and inorganic	P66 (1); P67 (2)	C68 (0)	P66 (0); P67 (0)	C68 (0)	P66 (1); P67 (1)	C68 (3)	P66 (0); P67 (0)	C68 (1)
Filter 7 and inorganic	P17 (0); P69 (0)	C71 (0)	P17 (0); P69 (0)	C71 (0)	P17 (3); P69 (1)	C71 (3)	P17 (0); P69 (0)	C71 (3)
Filter 8 and inorganic	P70 (0); P71 (0)	C72 (0); C73 (3)	P70 (0); P71 (0)	C72 (0); C73 (3)	P70 (1); P71 (1)	C72 (2); C73 (2)	P70 (0); P71 (1)	C72 (2); C73 (1)
Control A and inorganic	P73 (0)	C74 (?)	P73 (0)	C74 (0)	P73 (1)	C74 (1)	P73 (0)	C74 (0)
Control B and inorganic	P74 (0)	C76 (?); C77 (?)	P74 (0)	C76 (0); C77 (0)	P74 (1)	C76 (1); C77 (1)	P74 (0)	C76 (1); C77 (0)
Deionised water and inorganic	P80 (0)	C80 (0)	P80 (0)	C80 (0)	P80 (0)	C80 (1)	P80 (0)	C80 (0)
Tap water and inorganic	P81 (0)	C82 (0)	P81 (0)	C82 (0)	P81 (1)	C82 (1)	P81 (0)	C82 (1)
Tap water/fertiliser and inorganic	P83 (1); P84 (9)	C84 (9); C85 (25)	P83 (0); P84 (1)	C84 (1); C85 (0)	P83 (2); P84 (2)	C84 (6); C85 (13)	P83 (0); P84 (0)	C84 (3); C85 (8)
Wastewater/tap water and inorganic	P86 (0); P87 (0)	C87 (1)	P86 (0); P87 (1)	C87 (4)	P86 (1); P87 (1)	C87 (2)	P86 (0); P87 (0)	C87 (1)
Wastewater and inorganic	P89 (8); P90 (8)	C90 (0)	P89 (0); P90 (0)	C90 (0)	P89 (1); P90 (0)	C90 (4)	P89 (0); P90 (0)	C90 (0)

Table 8
Overview of the inductively coupled plasma (ICP) optical emission spectrometer analysis for selected elements (mg/l) considerably exceeding common standards for irrigation water (e.g., FAO, 1994, 2003).

Sample name	Sample number	Mean	Standard deviation	Minimum	Maximum
Iron					
Filter 1	3	0.58	0.365	0.32	1.00
Filter 2	3	0.16	0.156	0.06	0.34
Filter 3	3	1.53	1.429	0.42	3.14
Filter 4	3	0.18	0.060	0.12	0.24
Filter 5	3	0.77	0.368	0.43	1.16
Filter 6	3	0.59	0.710	0.17	1.41
Filter 7	3	0.19	0.042	0.14	0.22
Filter 8	3	0.18	0.101	0.12	0.30
Control A	3	0.13	0.046	0.09	0.18
Control B	3	0.07	0.023	0.04	0.08
Wastewater	3	8.23	5.341	2.23	12.46
Tap water with wastewater	2	1.00	0.014	0.99	1.01
Tap water	1	6.89	–	–	–
Fertiliser	1	18.37	–	–	–
Potassium					
Filter 1	3	9.31	2.346	7.87	12.02
Filter 2	3	9.83	2.993	7.07	13.01
Filter 3	3	10.73	2.612	8.39	13.55
Filter 4	3	6.08	1.649	4.92	7.97
Filter 5	3	15.42	3.946	11.28	19.14
Filter 6	3	15.29	0.798	14.68	16.19
Filter 7	3	7.63	0.719	7.11	8.45
Filter 8	3	8.38	3.572	5.72	12.44
Control A	3	1.35	0.367	1.12	1.77
Control B	3	1.22	0.976	0.66	2.35
Wastewater	3	11.25	4.040	7.50	15.53
Tap water with wastewater	2	3.16	1.344	2.21	4.11
Tap water	1	0.59	–	–	–
Fertiliser	1	341.98	–	–	–
Manganese					
Filter 1	3	0.08	0.006	0.07	0.08
Filter 2	3	0.00	0.000	0.00	0.00
Filter 3	3	0.30	0.210	0.14	0.54
Filter 4	3	0.00	0.000	0.00	0.00
Filter 5	3	0.21	0.067	0.13	0.25
Filter 6	3	0.01	0.017	0.00	0.03
Filter 7	3	0.00	0.006	0.00	0.01
Filter 8	3	0.00	0.000	0.00	0.00
Control A	3	0.04	0.012	0.03	0.05
Control B	3	0.00	0.000	0.00	0.00
Waste water	3	0.13	0.078	0.04	0.19
Tap water with wastewater	2	0.01	0.007	0.00	0.01
Tap water	1	0.00	–	–	–
Fertiliser	1	5.65	–	–	–

Note: For all elements; blank, 0.000; standard 1, 0.994; standard 2, 4.973; standard 3, 9.943.

highest number of fruits is associated with tap water and an organic growth medium. Low fruit numbers correlate well with inorganic growth media. Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated waste water are usually too high to produce a good harvest. However, as the compost is depleted of nutrients after about eight months, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost.

3.3. Inductively coupled plasma findings

Table 8 provides an overview of the ICP–OES analysis for selected elements in the irrigation water. High concentrations of iron, which exceeded the threshold of 5 mg/l (FAO, 2003; Pescod, 1992), were noted for both raw wastewater and tap water. Based on the recommended threshold of 2 mg/l for potassium (FAO, 1994), the outflow water from all wetland filters (with the exception of Controls A and B) was associated with too high potassium concentrations. Furthermore, high concentrations were also observed for raw wastewater, and wastewater samples, which were diluted with up to 80% of tap water. Results show for Filters 3 and 5 relatively high manganese concentrations, which exceeded the threshold of 0.2 mg/l (FAO, 2003; Pescod, 1992).

3.4. Brief cost-benefit analysis

Sweet Pepper and Chilli seed packets were purchased from B&Q plc for £1.48 or 148 pence each. The corresponding seed numbers were 45 and 70, respectively. One seed of Sweet Pepper and Chilli costs therefore 3.29 and 2.11 pence, respectively.

Considering the germination success rates of 78 and 74 for Sweet Pepper and Chilli, respectively, each seedling costs 257 and 156 pence in that order. However, only 50 seedlings of Sweet Pepper and Chilli each reached maturity. This corresponds to 165 and 106 pence, respectively.

Sweet Pepper and Chilli can be purchased in the UK for approximately 56 and 16 pence each or 362 and 1040 pence/kg. However, taking into account the costs of watering, fertiliser and maintenance, the calculation becomes more complex.

The potential fear and disgust by consumers of eating microbially contaminated vegetables decreases considerably if vegetables are cooked for a long time at considerable heat. Menegaki et al. (2009) assessed the fear and disgust factors by

Table 9
Chilli (C) harvest classification scheme.

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
Approximate Codex Standard (2013) mapping	"Extra" Class	Class I	Class II	Not applicable	Not applicable
Mean price estimate; pence (Sterling)/g	C: 2.00	C: 1.00	C: 0.50	C: 0.25	C: 0.00
Target market	Top restaurant	National supermarket	Independent Retailer or market	Vegetable industry	Waste company
Product	Fresh vegetable	Fresh vegetable	Fresh vegetable	Powder or canned	Waste
Contamination	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Contaminated
Illnesses	None	None	None	Likely; no harm	Likely; harmful (rotten)
Aesthetics	Fully characteristic; virtually no flaws ($\leq$0.5% of surface area)	Fully characteristic; minor flaws ($\leq$2.0% of surface area)	Essential characteristics only; flaws ($\leq$3.0% of surface area)	Major flaws (>3.0% of surface area); potentially broken, pests and damaged)	Too many major flaws including broken, pests and damaged
Length (L, mm)	Very long ($L \geq 80$)	Long ($60 \leq L < 80$)	Medium ($40 \leq L < 60$)	Short ($20 \leq L < 40$)	Very short ($L < 20$)
Width (W, mm)	Very wide($W \geq 20$)	Wide ($16 \leq W < 20$)	Medium ($12 \leq W < 16$)	Slim ($8 \leq W < 12$)	Very slim ($W < 8$)
Weight (w, g)	Very Large($w \geq 9$)	Large ($7 \leq w < 9$)	Medium ($5 \leq w \leq 7$)	Small ($3 \leq w < 5$)	Very Small ($w < 3$)
Tolerance by weight per plant (%)	5	10	10	10	10
Bending	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Uncharacteristically bend; $L/W < 3.5$	Uncharacteristically bend; $L/W < 3.5$
Colour	Characteristically red	Characteristically red	Characteristically red	Not fully red or unripe	Not fully red or unripe
Pungency (flavour) in Scoville (SHU) units	Strongly characteristic; $SHU \geq 18{,}000$	Characteristic; $8{,}000 \leq SHU < 18{,}000$	Characteristic; $8{,}000 \leq SHU < 18{,}000$	Characteristic; $8{,}000 \leq SHU < 18{,}000$	Poor; $SHU < 8{,}000$
Pungency (flavour) as total capsaicinoids (C; µg/g dry weight)	Strongly characteristic; $C \geq 1{,}200$	Characteristic; $533 \leq C < 1200$	Characteristic; $533 \leq C < 1{,}200$	Characteristic; $533 \leq C < 1200$	Poor; $C < 533$

comparing the effects of descriptive terms on farmers' willingness to use and willingness to pay for recycled water for irrigation and consumers' willingness to use and willingness to pay for products irrigated with recycled water. Treated effluent from wastewater treatment plants was described as "recycled water" for one experimental group and as "treated wastewater" for another. Although the two terms describe the same commodity, willingness to use the water was reliably higher with the "recycled water" descriptor for both farmers and consumers. However, the descriptor affected willingness to pay only in the consumer sample. Both farmers and consumers who were unwilling to use recycled water commodities cited feelings of disgust (32%) as the main cause of their rejection (Menegaki et al., 2009).

Sweet Peppers are often eaten both raw and cooked. However, Chillies are usually cooked, and the risk of microbial contamination is therefore very low. Considering that Sweet Peppers in comparison to Chillies are more likely to be used in a salad than in a cooked dish, it is more difficult to sell, because of the fear and disgust factors discussed above. Therefore, the likelihood of selling the selected plants at a fair price taking the fear of contamination factor into account is likely to be less for Sweet Peppers compared to Chillies.

Table 9 shows a proposed novel harvest classification scheme for Chillies. Only the higher classes are of great commercial interest. However, the estimated prices are dependent of the market. Table 10 indicates the monetary value of the harvest for Chilli plants only. The highest number of fruits categorised as Class A were harvested from plants grown in organic media and watered with tap water. However, tap water was also associated with the highest fruit numbers categorised as Class E. The highest mean price of harvested fruits is also associated with tap water.

3.5. Limitations

This study presents a partly incomplete picture on the reuse of treated wastewater for irrigation, because microbiological parameters were not studied. However, microbial contamination of Chillies and Sweet Peppers is unlikely due to the relatively long distance between the fruits and the potentially contaminated soil (Cirelli et al., 2012). Moreover, vegetable pots receiving wastewater treated with wetlands can be considered as safer than those receiving only preliminary treated wastewater.

Municipal wastewater often lacks the required amount of potassium for growth of commercial crops (Boyden and Rababah, 1996). Therefore, potassium could have been added at an optimal dose for the growth of all tested plants. However, the results in this paper indicate that potassium was sufficient in the outflow waters of most wetlands (Table 8).

The humidity was relatively low throughout the experiment. However, Bakker (1989) found no significant effect of humidity was on the *C. annum* fruit shape, number of cavities per fruit, pericarp thickness, dry matter content and fruit maturation rate. The relative humidity had no effect on the growth of Sweet Peppers between the range of 50 and 80% (Bakker, 1989, 1991).

Table 10
Overview of the outcome of the Chilli (C) harvest (before or on 4 June 2014) classification scheme according to Table 9. Note that the lowest variable class entry for any individual fruit assessment will determine the final class. However, only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, weight, bending. Values shown per plant represent pence (Sterling).

Inflow source and growth media	Class A	Class B	Class C	Class D	Class E	Mean per plant
Filter 1 and organic	C3 (0); C4 (0)	C3 (0); C4 (0)	C3 (0); C4 (0)	C3 (1.2); C4 (0)	C3 (0); C4 (0)	0.6
Filter 2 and organic	C6 (0); C8 (42.1); C9 (0)	C6 (0); C8 (20.5); C9 (10.1)	C6 (0); C8 (0); C9 (0)	C6 (0);C8 (0); C9 (3.0)	C6 (0);C8 (0); C9 (0)	25.1
Filter 3 and organic	C10 (0); C11 (0); C12 (0)	C10 (0); C11(0); C12 (0)	C10 (0);C11 (0); C12(0)	C10 (0); C11 (0); C12 (2.1)	C10 (0); C11 (0); C12 (0)	0.7
Filter 4 and organic	C16 (0); C17 (0)	C16 (7.9); C17 (0)	C16 (9.0); C17 (0)	C16 (0); C17 (1.2)	C16 (0); C17 (0)	9.1
Filter 5 and organic	C18 (0); C19 (0); C20 (0)	C18 (14.6); C19 (9.9); C20 (0)	C18 (3.4); C19 (2.9); C20 (0)	C18 (0); C19 (0); C20 (0)	C18 (0); C19 (0); C20 (0)	10.2
Filter 6 and organic	C21 (0)	C21 (0)	C21 (0)	C21 (0)	C21 (0)	0.0
Filter 7 and organic	C25 (0); C26 (0)	C25 (0); C26 (17.6)	C25 (0); C26 (0)	C25 (1.9); C26 (0)	C25 (0); C26 (0)	9.8
Filter 8 and organic	C27 (0); C28 (0); C29 (0)	C27 (0); C28 (0); C29 (0)	C27 (2.7); C28 (0); C29 (0)	C27 (4.8); C28 (0); C29 (0.8)	C27 (0); C28 (0); C29 (0)	2.8
Control A and organic	C31 (0); C33 (0)	C31 (0); C33 (7.2)	C31 (0); C33 (0)	C31 (0); C33 (2.2)	C31 (0); C33 (0)	4.7
Control B and organic	C37 (0); C38 (20.3)	C37 (15.7); C38 (0)	C37 (0); C38 (2.6)	C37 (0); C38 (0)	C37 (0); C38 (0)	19.3
Deionised water and organic	C41 (0)	C41 (7.6)	C41 (9.1)	C41 (2.0)	C41 (0)	18.7
Tap water and organic	C42 (18.4); C43 (86.8)	C42 (16.8); C43 (8.6)	C42 (5.1); C43 (7.3)	C42 (6.8); C43 (3.5)	C42 (0); C43 (0)	76.7
Tap water/fertiliser and organic	C45 (0); C46 (0)	C45 (0); C46 (0)	C45 (0); C46 (0)	C45 (0); C46 (1.2)	C45 (0); C46(0)	0.0
Wastewater/tap water and organic	C49 (0)	C49 (0)	C49 (0)	C49 (0)	C49 (0)	0.0
Wastewater and organic	C52 (0); C54 (0)	C52 (0); C54 (0)	C52 (0); C54 (0)	C52 (0); C54 (0)	C52 (0); C54 (0)	0.0
Filter 1 and inorganic	C56 (0)	C56 (0)	C56 (0)	C56 (0)	C56 (0)	0.0
Filter 2 and inorganic	C58 (0)	C58 (0)	C58 (3.3)	C58 (0)	C58 (0)	3.3
Filter 3 and inorganic	C61 (0)	C61 (0)	C61 (0)	C61 (0.8)	C61 (0)	0.8
Filter 4 and inorganic	C63 (0); C64 (0)	C63 (7.7); C64 (0)	C63 (0); C64 (0)	C63 (0); C64 (1.7)	C63 (0); C64 (0)	4.7
Filter 5 and inorganic	C66 (0)	C66 (0)	C66 (0)	C66 (0)	C66 (0)	0.0
Filter 6 and inorganic	C68 (0)	C68 (7.1)	C68 (0)	C68 (0)	C68 (0)	7.1
Filter 7 and inorganic	C71 (0)	C71 (7.2)	C71 (0)	C71 (1.3)	C71 (0)	8.5
Filter 8 and inorganic	C72 (0); C73 (0)	C72 (0); C73 (0)	C72 (0); C73 (0)	C72 (1.8); C73 (1.3)	C72 (0); C73 (0)	1.6
Control A and inorganic	C74 (0)	C74 (0)	C74 (0)	C74 (0)	C74 (0)	0.6
Control B and inorganic	C76 (0); C77 (0)	C76 (0); C77 (0)	C76 (0); C77 (0)	C76 (0); C77 (0)	C76 (0); C77 (0)	0.0
Deionised water and inorganic	C80 (0)	C80 (0)	C80 (0)	C80 (0)	C80 (0)	0.0
Tap water and inorganic	C82 (0)	C82 (0)	C82 (0)	C82 (0)	C82 (0)	0.0
Tap water/fertiliser and inorganic	C84 (0); C85 (38.2)	C84 (9.7); C85 (15.7)	C84 (2.6); C85 (3.8)	C84 (2.0); C85 (1.9)	C84 (0); C85 (0)	37.0
Wastewater/tap water and inorganic	C87 (0)	C87 (0)	C87 (2.5)	C87 (0)	C87 (0)	2.5
Wastewater and inorganic	C90 (0)	C90 (0)	C90 (0)	C90 (0)	C90 (0)	0.0

4. Conclusions and recommendations for further research

The experiment shows that Sweet Peppers and Chillies can be grown using wastewater treated by constructed wetlands. However, the yield of Sweet Peppers was insignificant in contrast to that of Chillies, possibly due to the high concentrations of nutrients (particularly nitrogen) and trace minerals, and adverse environmental boundary conditions in the laboratory. Chillies did reasonably well, but the growth of foliage was excessive and the harvest was delayed due to high nitrogen concentrations in the inflow water. The highest number of fruits was associated with tap water and an organic growth medium. In contrast, plants associated with irrigation water contaminated by hydrocarbon were usually associated with a poor harvest. Standard wetland design parameters were only of secondary importance.

Findings also indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated waste water are usually too high to produce a good harvest. A high yield was related with the most suitable provision of nutrients and trace elements. However, as the compost is depleted of nutrients such as nitrogen after about eight months, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost.

The productivity of Chillies in terms of harvest was independent of the wastewater consumption. Nevertheless, higher foliage production due to excess nutrients and trace minerals required more water. The current research will be continued with the same plants to assess if further harvests are economic and determine when the nutrients within the compost are fully depleted. Moreover, the accumulation of metals and their toxicity in the soil as well as microbiological contamination will also be studied. Further research with other vegetables receiving recycled treated wastewater from other wastewater treatment units should also be undertaken to select the best and most cost-effective technology in order to obtain the greatest crop yield.

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Recycling of domestic wastewater treated by vertical-flow wetlands for watering of vegetables

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Abstract

The aim was to assess if domestic wastewater treated by different vertical-flow wetlands can be successfully recycled to water commercially grown crops. The growth of both Sweet Pepper (California Wonder; cultivar of *Capsicum annuum* Linnaeus Grossum Group) and Chilli (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') fed with different treated and untreated wastewater types were assessed. The overall growth development of Sweet Peppers was poor due to the high concentrations of nutrients and trace minerals. In contrast, chilies did reasonably well but the growth of foliage was excessive and the harvest was delayed. High yields were associated with tap water and an organic growth medium, and a wetland with a high aggregate size, leaving sufficient space for biomass. Low fruit numbers correlated well with inorganic growth media and irrigation water contaminated by hydrocarbons. Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated waste water are usually too high to produce a good harvest. However, as the compost is depleted of nutrients after about 8 months, the harvest increased for pots that received pre-treated wastewater. Findings will lead to a better understanding of the effects of different wetland treatment processes.

Key words: agricultural water resources management, ecological sanitation, nitrogen, reed bed, vegetable, water reclamation

INTRODUCTION

Constructed wetlands

Constructed treatment wetlands are engineered wastewater purification systems that encompass biological, chemical and physical processes, which are all similar to processes occurring in natural treatment wetlands. They are implemented for environmental pollution control to treat a variety of wastewaters including industrial effluents, urban and agricultural runoff, animal wastewaters, sludge and mine drainage (Scholz 2010; Sani *et al.* 2013). Recently, some large-scale wetland systems have also been successfully applied to treat domestic wastewater (Dong *et al.* 2011). However, there are few long-term and controlled studies involving domestic wastewater due to health and safety concerns.

Wastewater recycling for crop irrigation

The earliest documented sewage farms, where wastewater has been applied to land for disposal and for agricultural use, were operated in the 16th and 17th centuries in Bunzlau, Germany, and Edinburgh, Scotland (Shuval *et al.* 1986). The scientific basis for the acceptance of wastewater

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reclamation, recycling and reuse in agriculture has evolved from developments in water and wastewater engineering science coupled with an increasing pressure on water resources management. The evaluation of the effects of treated wastewater reuse on crops intended for human consumption is of particular interest (Asano & Levine 1996; Aiello *et al.* 2007; Cirelli *et al.* 2012). Unfavourable concentrations of certain nutrients and trace elements are a challenge to the growth of plants fed by recycled pre-treated wastewater. Moreover, traces of hydrocarbons from diesel spills associated with urban runoff or industrial effluent are a more recent challenge (Scholz 2010; García-Delgado *et al.* 2012).

Cirelli *et al.* (2012) presented the results of a reuse scenario where tertiary-treated municipal wastewater using a constructed wetland was supplied for irrigation of vegetables in Eastern Sicily, Italy. They found elevated levels of *Escherichia coli* in the irrigation water, which were frequently above the Italian limits of 50 colony forming units (CFU)/100 ml for secondary urban effluents.

García-Delgado *et al.* (2012) undertook a greenhouse study in Spain to assess the effect of treated urban waste water on soil and pepper quality. The wastewater application saved fertiliser (37% nitrogen, 66% phosphorus and 12% potassium). Total polyaromatic hydrocarbons and the heavy metals cadmium, lead and arsenic within the pepper fruits were low.

Morari & Giardini (2009) assessed the treatment efficiency of pilot-scale vertical-flow constructed wetlands on municipal wastewaters and their suitability for irrigation reuse in Italy. Only water quality parameters with high removal efficiencies fulfilled the Italian guidelines for irrigation reuse, whereas parameters with lower efficiencies such as suspended solids (SS) and total phosphorus limited the potential water reuse.

Vertical-flow constructed wetlands treating septic tank effluent in Guangzhou (China) achieved removal rates for chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD), SS, total nitrogen and total phosphorus of 60%, 80%, 74%, 49% and 79%, respectively (Cui *et al.* 2003). After that the treated effluent was used for hydroponic cultivation of water spinach and romaine lettuce. It was found that using treated effluent for hydroponic cultivation of vegetables could reduce the nitrate content in vegetables.

Lopez *et al.* (2006) assessed constructed wetlands treating municipal effluents to be reused in agriculture. Recorded average removal efficiencies for SS, five-day BOD, COD, total nitrogen and total phosphorus were 85%, 65%, 75%, 42% and 32%, respectively.

Plant selection

Many vegetables have the potential to grow on recycled wastewater. However, there is the potential of some vegetables such as lettuce and cabbage to become contaminated by microbes, because their edible leaves are too close to the ground receiving the treated wastewater. Therefore, it makes sense to select vegetables where the edible fruit is located far away from the ground (Nickels 2012). This may include peppers, tomatoes, maize, eggplants, beans, lentils and peas. The next step in selecting suitable vegetables is to decide on easy-to-grow and relatively cost-effective plants with high nutritional value. However, the authors concentrated on two pepper types in this study, because they can also be grown in greenhouses in the UK (Nickels 2012; Jones 2013).

Nutrients and minerals

Jones (2013) discussed the positive and negative impacts on key nutrients and minerals on plants. The major minerals impacting on the growth of plants are nitrogen (predominantly ammonium), phosphorus, potassium, calcium, magnesium and sulphur. Micro-nutrients that are beneficial in small amounts are (in no particular order) boron, copper, manganese, molybdenum, potassium, iron, zinc, iodine and chlorine. Copper, manganese, molybdenum, iron, zinc and aluminium (in no

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particular order) are often described as heavy metals. They may be toxic in high inflow water concentrations for peppers. Under acid conditions (soil with a pH of less than 7), heavy metals could be a problem to sensitive plants (FAO 2003).

Pescod (1992) and FAO (2003) also recommended limits for trace minerals in reclaimed water use for irrigation. Long-term (for water used continuously on all soils) and short-term (for water used for a period of up to 20 years on fine-textured neutral alkaline soils) threshold values for 18 elements have been listed. Recommended maximum concentrations for the trace elements that are often exceeded (see results below) are 5.0 mg/l for iron, 0.2 mg/l for manganese and 2.0 mg/l for potassium.

FAO (1994) classified the suitability of treated wastewater for recycling in terms of pollutants. Acceptable ranges for ammonia-nitrogen, ortho-phosphate-phosphorous and potassium were between 0 mg/l and 5 mg/l, between 0 mg/l and 2 mg/l, and between 0 mg/l and 2 mg/l, respectively. Furthermore, Pescod (1992) stated that for irrigation water there is no restriction for its reuse if nitrate-nitrogen values are <5.0 mg/l. Slight to moderate constraints exist for the range between 5 and 30 mg/l. Severe recycling restrictions are usually imposed for values of more than 30 mg/l.

Aim and objectives

The overall aim is to assess if vegetables can be grown successfully on recycled domestic wastewater treated by constructed wetlands. The corresponding key objectives related to the growing of Sweet Pepper and Chilli are to assess (a) the suitability for growth when using recycled wastewater, (b) the impact of different treated wastewaters as a function of the wetland type, (c) the volume of treated wastewater for irrigation, (d) the suitability of different growth media, (e) the effect of a diesel oil spill on the suitability of the recycled wastewater, and (f) the economic viability of different experimental set-ups.

METHODOLOGY

Constructed wetlands set-up and operation

The vertical-flow wetland system is located within a greenhouse (door left open) on top of the roof of the Newton Building, The University of Salford, Greater Manchester, UK (Sani *et al.* 2013). Wetland filters were operated between 27 June 2011 and 4 June 2014. The set-up includes two filters that are essentially controls receiving clean de-chlorinated tap water. Table 1 indicates an overview of the statistical experimental set-up used to test the impact of four variables. Filters 1 and 2 compared to Filters

Table 1 | Comparison of the experimental vertical-flow wetland set-up (after Al-Isawi *et al.* 2015a)

	Design and/or operational variable			
Wetland filters	**Aggregate diameter (mm)**	**Contact time (h)**	**Resting time (h)**	**COD (mg/l)**
Filters 1 and 2	20	72	48	122.8
Filters 3 and 4	10	72	48	122.8
Filters 5 and 6	10	72	48	243.8
Filter 7	10	36	48	122.8
Filter 8	10	36	24	122.8
Control A	10	72	48	2.3
Control B	10	72	48	2.3

Note: annually treated volumes of wastewater: Filters 1–6, 470 l/a; Filter 7, 624 l/a Filter 8, 858 l/a. On 26 September 2013, 130 g of diesel was added to Filters 1, 3 and 5 and Control A.

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3 and 4 test the influence of a larger aggregate diameter. Filters 5 and 6 compared to Filters 3 and 4 check the impact of a higher loading rate. The application of a lower contact rate is tested if Filter 7 is compared with Filters 3 and 4. Finally, a lower resting time is the difference between Filters 7 and 8.

The 10 laboratory-scale wetland filters were constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm. The filters were filled with siliceous (minimum of 30%) pea gravel up to a depth of 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). Dead macrophyte plant material was harvested in winter and returned to the corresponding wetland filters by placing it on top of the litter zone (Sani *et al.* 2013; Al-Isawi *et al.* 2015a).

The main outlet valve is located at the bottom of each constructed wetland system. Eight further valves (used to test for clogging) are located on the sidewall of each wetland column. The sidewall valves were located at heights of 10, 20, 30, 40, 45, 50, 55 and 60 cm from the bottom of each column (Sani *et al.* 2013).

Wetland columns received 6.5 l of inflow water during the feeding mode, which was different between several filters (Table 1). Columns 1– 6 were sampled after 72 h contact time and then left to rest for 48 h, while columns 7 and 8 were sampled after 36 h contact time and left to rest for 48 h and 24 h, respectively. All water quality parameters discussed in this paper were determined during or directly after sampling. The preliminary treated urban wastewater used for the inflow water was obtained from the Davyhulme Sewage works, one of the largest waste water treatment plants in Europe (http://en.wikipedia.org/wiki/Davyhulme), operated by the water utility company United Utilities in Greater Manchester. Fresh wastewater was collected approximately once per week, and was stored and aerated by standard aquarium air pumps in a cold room before use. The wastewater quality was highly variable, and comprised domestic and industrial wastewater as well as surface water runoff.

To simulate a one-off Diesel fuel (100% pure; no additives) spill, 130 g (equivalent to an inflow concentration of 20 g/l) of diesel fuel (100% pure; no additives) were poured into Filters 1, 3 and 5, and into one of the two columns (Control A) on 26 September 2013 (Table 1) as discussed by Al-Isawi *et al.* (2015b). The fuel was obtained from a petrol station operated by Tesco Extra (Pendleton Way, Salford, UK).

Aqua Medic Titan chillers (Aquacadabra, Bexleyheath, UK) were used to maintain the root system and debris layer of all wetland systems at semi-natural below-surface temperatures of about 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located (Sani *et al.* 2013).

The COD was used as an indicator to differentiate between low and high loads (Table 1). An inflow target COD of about 285 mg/l (usually between 122 and 620 mg/l) was set for wetlands with a high loading rate (Filters 5 and 6). The remaining Filters 1, 2, 3, 4, 7 and 8 received wastewater diluted with de-chlorinated tap water. The target inflow COD for these filters was approximately 138 mg/l (usually between 43 and 350 mg/l).

Water quality analysis

Routine water quality sampling was carried out according to APHA (2005), unless stated otherwise, to monitor long-term and seasonal treatment performance. The spectrophotometer DR 2800 Hach Lange (www.hach.com) was used for standard water quality analysis for variables including COD, ammonia-nitrogen, nitrate-nitrogen, ortho-phosphate-phosphorus and SS.

Total petroleum hydrocarbons (TPH) were determined by gas chromatography and flame ionization externally by Exova Health Sciences (Montrose Ave, Hillington Park, Glasgow, UK) according to their own TPH in Waters (with Aliphatic/Aromatic Splitting) Method (Exova Health Sciences 2014), which is accredited to the British Standard (BS) method BS EN ISO IEC 17025 by the United Kingdom Accreditation Service and compatible to the International Organization for

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Standardization (ISO) standards (e.g., ISO17025), BS method BS DD 220 1994, and American Standard methods (United States Environmental Protection Agency (US EPA) Method 3510C and US EPA SW846 Method 8015).

The five-day BOD was determined in all water samples with the OxiTop IS 12-6 system, a manometric measurement device, supplied by the Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany. Nitrification was suppressed by adding 0.05 ml of 5 g/l N-Allylthiourea (WTW chemical solution No. NTH600) solution per 50 ml of sample water.

Growing the vegetables: third planting

The third planting (i.e., second and final replanting) took place 28 days after the first replanting on 8 November 2013. Table 2 outlines the experimental set-up. The Sweet Pepper and Chilli were planted individually into 10-litre round plastic plant pots provided by scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, UK). The plant pot dimensions were as follows: height of 22.0 cm, bottom diameter of

Table 2 | Experimental design in terms of plant number allocations after the second replanting (i.e., final replanting)

Inflow source	Growth media	Sweet Pepper	Chilli
Filter 1 outflow	Compost with bark	P1;P2	C3;C4
Filter 2 outflow	Compost with bark	P5;P6	C6;C8:C9
Filter 3 outflow	Compost with bark	P8;P9;P10	C10;C11;C12
Filter 4 outflow	Compost with bark	P12;P16	C16:C17
Filter 5 outflow	Compost with bark	P18;P19;P20	C18;C19;C20
Filter 6 outflow	Compost with bark	P22;P23	C21
Filter 7 outflow	Compost with bark	P26;P28	C25:C26
Filter 8 outflow	Compost with bark	P31;P32;P33	C27;C28:C29
Control A outflow	Compost with bark	P35	C31;C33
Control B outflow	Compost with bark	P39	C37:C38
Deionized water	Compost with bark	P41	C41
Tap water (100%)	Compost with bark	P44	C42;C43
Tap water with fertilizer (0.7 ml/l)	Compost with bark	P45;P46	C45;C46
Wastewater (20%); tap water (80%)	Compost with bark	P47	C49
Wastewater (100%)	Compost with bark	P51;P54	C52; C54
Filter 1 outflow	Silica sand	P55;P56	C56
Filter 2 outflow	Silica sand	P57	C58
Filter 3 outflow	Silica sand	P59	C61
Filter 4 outflow	Silica sand	P61	C63;C64
Filter 5 outflow	Silica sand	P65	C66
Filter 6 outflow	Silica sand	P66;P67	C68
Filter 7 outflow	Silica sand	P17;P69	C71
Filter 8 outflow	Silica sand	P70;P71	C72;C73
Control A outflow	Silica sand	P73	C74
Control B outflow	Silica sand	P74	C76;C77
Deionized water	Silica sand	P80	C80
Tap water (100%)	Silica sand	P81	C82
Tap water with fertilizer (0.7 ml/l)	Silica sand	P83;P84	C84;C85
Wastewater (20%); tap water (80%)	Silica sand	P86;P87	C87
Wastewater (100%)	Silica sand	P89;P90	C90

Note: original seed planting reference numbers; Sweet Pepper (P1–P90) and Chilli (C1– C90).

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22.0 cm and top diameter of 28.5 cm. All plant pots remained indoors (laboratory) under controlled environmental conditions. The experimental set-up was chosen to allow for the statistical assessment of different types of treatment such as the impact of organic media and nutrients in the wastewater.

Data analysis

Microsoft Excel (www.microsoft.com) was used for general data analysis. IBM SPSS Statistics Version 20 (www.ibm.com) was applied to calculate the correlation between variables and statistical differences between treatments.

RESULTS AND DISCUSSION

Water quality analysis

Findings are shown in Tables 3–6. Table 3 outlines the inflow water quality received by the plants. Note that the wetland effluent was used as the influent for the vegetable pots. Highly fluctuating values for TPH were observed in the outflow waters obtained from Filters 1, 3, 5, and 8, and Control A. The TPH concentrations followed this order: Control A > Filter 8 > Filter 1 > Filter 3 > Filter 5. However, these TPH concentrations were in compliance with the threshold set by the Chinese standard for irrigation water quality (SEPA 2005) highlighting a maximum allowable value of 1 mg/l. Note that the Chinese standard was used, considering that China produces about 54% (estimated in 2008) of the Sweet Peppers in the world (ERS/USDA 2008).

COD values were the highest for raw wastewater followed by those for Filter 5, which was fed with a high inflow load in terms of COD (Table 1). In contrast, the lowest values were noted for Control B. Filters 1, 3 and 8 had relatively similar COD concentrations. Control A had higher COD values than Filter 6. No differences in COD values were noted for Filters 2, 4 and 7. This observation helps to explain why the inflow load of wetland filters directly impacts on the COD values of the outflow water rather than design variables such as aggregate size, contact time and resting time. High rate filters are likely to be overloaded as discussed by Sani *et al.* (2013).

The five-day BOD was high for raw wastewater followed by wastewater samples, which were diluted with 80% of tap water. In comparison, the lowest five-day BOD was observed for tap water with fertiliser, tap water and deionized water.

High concentrations of ammonia-nitrogen, which exceeded the threshold of 5 mg/l (FAO 1994), were noted for both Filters 5 and 6 (filters with a high loads; Table 1), followed by those for Filters 1 and 2 (filters with large aggregate sizes). This confirms results findings by Sani *et al.* (2013) that high rate filters tend to overload. Table 3 shows that nitrate-nitrogen for all filter outflow waters was less than the maximum thresholds value of 30 mg/l (Pescod 1992).

Based on the recommended threshold of 2 mg/l for ortho-phosphate-phosphorus (FAO 1994), the outflow waters from all wetland filters (with the exception of Controls A and B) were associated with too high ortho-phosphate-phosphorus concentrations. In general, phosphorus is one of the most difficult pollutants to be removed by mature constructed wetlands (Pant *et al.* 2001). This can be explained by the fact that phosphorus is usually present in particulate form, and does not dissolve well in filters that are not yet saturated by phosphorus or other compounds competing for adsorption sites (Scholz 2006, 2010).

The highest value for SS was noted for raw wastewater followed by that for wastewater, which was diluted with 80% tap water. In contrast, the lowest values were observed for Filter 7 outflow water and tap water with fertiliser. Turbidity was high for raw wastewater. Filter 7 had the lowest turbidity values. The pH values for all filter outflows were within the normal range; i.e., between 6.0 and 8.5 (Pescod 1992).

Table 3 | Comparison of the water quality of the inflow water received by the vegetable pots (value, sample number (in brackets) and standard deviation, if available)

Outflow	TPH (μg/l)	COD (mg/l)	BOD_5 (mg/l)	NH_4-N (mg/l)	NO_3-N (mg/l)	PO4-P (mg/l)	SS (mg/l)	NTU –	pH –
Filter 1	100	100.2 (12) ± 82.57	24.5 (39) ± 18.14	6.4 (12) ± 7.63	3.3 (11) ± 0.24	3.2 (10) ± 2.74	11.4 (43) ± 11.00	9.6 (42) ± 6.09	6.4 (41) ± 0.25
Filter 2	0	39.2 (8) ± 30.52	13.9 (37) ± 8.69	5.9 (11) ± 6.00	5.0 (10) ± 3.19	3.0 (10) ± 1.22	7.3 (43) ± 10.54	6.1 (41) ± 6.45	6.5 (41) ± 0.22
Filter 3	69	106.6 (12) ± 76.76	25.1 (38) ± 18.47	4.2 (12) ± 4.60	3.4 (11) ± 0.34	2.9 (10) ± 2.47	12.2 (43) ± 10.51	10.3 (41) ± 6.33	6.5 (41) ± 0.19
Filter 4	0	33.1 (8) ± 31.33	12.5 (36) ± 10.26	4.4 (11) ± 4.77	5.1 (10) ± 3.95	2.9 (10) ± 1.29	7.7 (43) ± 11.65	6.5 (41) ± 5.96	6.5 (41) ± 0.16
Filter 5	14	142.6 (12) ± 105.06	24.2 (37) ± 19.24	13.6 (12) ± 15.25	4.9 (11) ± 0.89	4.2 (10) ± 3.80	13.7 (44) ± 14.37	10.6 (41) ± 7.66	6.7 (42) ± 0.23
Filter 6	0	47.2 (8) ± 43.11	16.3 (38) ± 13.73	12.0 (11) ± 15.11	6.8 (10) ± 5.53	3.9 (10) ± 3.46	8.5 (44) ± 9.75	6.8 (41) ± 6.18	6.8 (42) ± 0.23
Filter 7	0	39.5 (7) ± 36.56	13.8 (42) ± 9.72	4.8 (12) ± 7.59	7.4 (11) ± 3.29	3.2 (8) ± 2.05	2.0 (47) ± 3.03	3.1 (44) ± 1.19	6.5 (44) ± 0.18
Filter 8	116	92.2 (7) ± 122.14	14.5 (49) ± 8.53	2.9 (11) ± 4.98	6.5 (11) ± 4.14	3.5 (8) ± 2.14	3.0 (57) ± 4.47	3.8 (58) ± 2.78	6.5 (58) ± 0.21
Control A	346	68.3 (10) ± 90.29	13.1 (37) ± 8.42	1.7 (12) ± 1.71	0.6 (11) ± 0.60	1.9 (10) ± 0.98	6.3 (43) ± 8.94	4.9 (42) ± 4.22	6.7 (42) ± 0.17
Control B	0	25.8 (8) ± 31.48	8.2 (38) ± 10.27	1.8 (11) ± 1.94	0.5 (10) ± 0.42	2.0 (10) ± 0.84	4.6 (43) ± 9.31	4.9 (41) ± 5.31	6.6 (42) ± 0.22
Deionized water	nm	nm	7.1	nm	nm	nm	4.0	1.3	5.5
Tap water	nm	nm	5.1	nm	nm	nm	4.0	3.0	5.9
Tap water with fertiliser (0.7 ml/l)	nm	nm	7.3	nm	nm	nm	2.0	2.9	6.0
Wastewater (20%); tap water (80%)	nm	nm	50.1 (6) ± 28.86	nm	nm	nm	25.8 (9) ± 22.50	9.8 (8) ± 11.47	7.1 (8) ± 0.188
Wastewater (100%)	nm	266.2	129.2	32.2	2.7	14.9	143.7	83.1	7.5
Recommended maximum	1000	–	–	5.0	30.0	2.0	–	–	8.5

TPH – total petroleum hydrocarbons; COD – chemical oxygen demand; BOD_5 – biochemical oxygen demand; NH_4-N – ammonia-nitrogen, NO_3-N – nitrate-nitrogen, PO_4-P – ortho-phosphate-phosphorous; SS – suspended solids; NTU – turbidity; nm – not measured. Note: only Filters 5 and 6 received inflow raw wastewater with the characteristics summarized above. The remaining filters were fed with diluted wastewater (i.e., one part of de-chlorinated tap water and one part of wastewater). The controls received tap water. Filters 1, 3 and 5 as well as Control A were contaminated with diesel.

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Table 4 | Overview of visual growth problems associated with micronutrient surplus in new plant parts (corresponding numbers of plants highlighted in **bold**) observed during the second replanting phase on 7 March 2014, and possible reasons associated with nutritional disorders

	Dark green and abundant foliage[a]		Low grow rate[b]		Stunting and reducing in branches[c]		Necrotic lesions on leaves[d]	
Inflow source and growth media	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	**P1;P2**	C3;C4	P1;P2	C3;C4	**P1;P2**	C3;C4	**P1;P2**	C3;C4
Filter 2 and organic	**P5;P6**	C6;C8;C9	P5;P6	C6;C8;C9	**P5;P6**	C6;**C8**;C9	**P5;P6**	C6;**C8**;C9
Filter 3 and organic	**P8;P9;P10**	C10;C11;C12	P8;P9;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12	P8;**P9**;P10	C10;C11;C12
Filter 4 and organic	**P12;P16**	C16;C17	P12;P16	C16;C17	P12;**P16**	C16;C17	P12;**P16**	C16;C17
Filter 5 and organic	**P18;P19;P20**	C18;C19;C20	P18;P19;P20	C18;C19;C20	P18;**P19;P20**	C18;C19;C20	P18;**P19;P20**	C18;C19;C20
Filter 6 and organic	**P22;P23**	C21	P22;P23	C21	**P22;P23**	C21	**P22;P23**	C21
Filter 7 and organic	**P26;P28**	C25;C26	P26;P28	C25;C26	**P26;P28**	C25;**C26**	**P26;P28**	C25;**C26**
Filter 8 and organic	**P31;P32;P33**	C27;C28;C29	P31;P32;P33	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29	**P31**;P32;**P33**	C27;C28;C29
Control A and organic	**P35**	C31;C33	P35	C31;C33	**P35**	C31;C33	**P35**	C31;C33
Control B and organic	**P39**	C37;C38	P39	C37;C38	**P39**	C37;C38	**P39**	C37;C38
Deionised water and organic	**P41**	C41	P41	C41	**P41**	C41	**P41**	C41
Tap water and organic	**P44**	**C42;C43**	P44	C42;C43	**P44**	C42;**C43**	**P44**	C42;**C43**
Tap water/fertiliser and organic	**P45;P46**	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46	P45;P46	C45;C46
Wastewater/tap water and organic	**P47**	C49	P47	C49	**P47**	C49	**P47**	C49
Wastewater and organic	**P51;P54**	**C52; C54**	P51;P54	C52; C54	P51;P54	C52; **C54**	P51;P54	C52; **C54**
Filter 1 and inorganic	P55;P56	C56	**P55;P56**	**C56**	P55;P56	C56	P55;P56	C56
Filter 2 and inorganic	P57	C58	**P57**	**C58**	P57	C58	P57	C58
Filter 3 and inorganic	P59	C61	**P59**	**C61**	P59	C61	P59	C61
Filter 4 and inorganic	P61	C63;C64	**P61**	**C63;C64**	P61	C63;C64	P61	C63;C64
Filter 5 and inorganic	P65	C66	**P65**	**C66**	P65	C66	P65	C66
Filter 6 and inorganic	P66;P67	C68	**P66;P67**	**C68**	P66;P67	C68	P66;P67	C68
Filter 7 and inorganic	P17;P69	C71	**P17;P69**	**C71**	P17;P69	C71	P17;P69	C71
Filter 8 and inorganic	P70;P71	C72;C73	**P70;P71**	**C72;C73**	P70;P71	C72;C73	P70;P71	C72;C73
Control A and inorganic	P73	C74	**P73**	**C74**	P73	C74	P73	C74
Control B and inorganic	P74	C76;C77	**P74**	**C76;C77**	P74	C76;C77	P74	C76;C77
Deionised water and inorganic	P80	C80	**P80**	**C80**	P80	C80	P80	C80
Tap water and inorganic	P81	C82	**P81**	**C82**	P81	C82	P81	C82

(*Continued.*)

Table 4 | Continued

	Dark green and abundant foliage[a]		Low grow rate[b]		Stunting and reducing in branches[c]		Necrotic lesions on leaves[d]	
Inflow source and growth media	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**
Tap water/fertiliser and inorganic	P83;P84	C84;C85	**P83;P84**	**C84;C85**	P83;P84	C84;C85	P83;P84	C84;C85
Wastewater/tap water and inorganic	P86;P87	C87	**P86;P87**	**C87**	P86;P87	C87	P86;P87	C87
Wastewater and inorganic	P89;P90	C90	**P89;P90**	**C90**	P89;P90	C90	P89;P90	C90

[a]Surplus in iron (Foy *et al.* 1978).
[b]Surplus in manganese (Haifa Chemicals 2014).
[c]Surplus in manganese (Silva *et al.* 2000) and/or Iron (Foy *et al.* 1978).
[d]Surplus in manganese (McCauley *et al.* 2011; Silva *et al.* 2000).

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Table 5 | Overview of the inductively coupled plasma optical emission spectrometer analysis for selected elements (mg/l) considerably exceeding common standards for irrigation water (e.g., FAO (1994, 2003))

		Iron		Potassium		Manganese	
Sample name	**Sample number**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Filter 1	3	0.58	0.365	9.31	2.346	0.08	0.006
Filter 2	3	0.16	0.156	9.83	2.993	0.00	0.000
Filter 3	3	1.53	1.429	10.73	2.612	0.30	0.210
Filter 4	3	0.18	0.060	6.08	1.649	0.00	0.000
Filter 5	3	0.77	0.368	15.42	3.946	0.21	0.067
Filter 6	3	0.59	0.710	15.29	0.798	0.01	0.017
Filter 7	3	0.19	0.042	7.63	0.719	0.00	0.006
Filter 8	3	0.18	0.101	8.38	3.572	0.00	0.000
Control A	3	0.13	0.046	1.35	0.367	0.04	0.012
Control B	3	0.07	0.023	1.22	0.976	0.00	0.000
Wastewater	3	8.23	5.341	11.25	4.040	0.13	0.078
Tap water with wastewater	2	1.00	0.014	3.16	1.344	0.01	0.007
Tap water	1	6.89	–	0.59	–	0.00	–
Fertilizer	1	18.37	–	341.98	–	5.65	–
Recommended maximum (mg/l)	–	5.000	–	2.000	–	0.200	–

Note: for all elements; blank, 0.000; standard 1, 0.994; standard 2, 4.973; standard 3, 9.943. The ICP–OES equipment detection limits for the elements iron, potassium and manganese are 0.10×10^{-3} mg/l, 0.30×10^{-3} mg/l and 0.03×10^{-3} mg/l, respectively. SD, standard deviation.

Table 6 | Chilli (C) harvest classification scheme (after Almuktar *et al.* 2015)

Variable	**Class A**	**Class B**	**Class C**	**Class D**	**Class E**
Length (L, mm)	Very long ($L \geq 80$)	Long ($60 \leq L < 80$)	Medium ($40 \leq L < 60$)	Short ($20 \leq L < 40$)	Very short ($L < 20$)
Width (W, mm)	Very wide($W \geq 20$)	Wide ($16 \leq W < 20$)	Medium ($12 \leq W < 16$)	Slim ($8 \leq W < 12$)	Very slim ($W < 8$)
Weight (w, g)	Very Large($w \geq 9$)	Large ($7 \leq w < 9$)	Medium ($5 \leq w \leq 7$)	Small ($3 \leq w < 5$)	Very Small ($w < 3$)
Bending	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Uncharacteristically bend; $L/W < 3.5$	Uncharacteristically bend; $L/W < 3.5$

Growth comparisons

The statistical experimental set-up as specified in Table 2 was chosen for the second replanting stage. Table 4 shows visual growth problems and indicates possible reasons. Figures 1(a) and 1(b) provide an overview of total irrigation water volume consumed by Sweat Pepper and Chilli plants, respectively, growing in different media. All plants grown in organic media consumed more water than those grown in inorganic media leading to better overall plant development.

In countries, where wastewater is seen as a resource, low wastewater consumption by plants is an advantage. However, high wastewater use by plants is seen as an advantage in temperate regions. The productivity of plants in terms of harvest is, however, independent of the wastewater consumption (Figure 1(b)). Nevertheless, a higher foliage production requires more water.

Correlation analysis results show that the fruit weights were significantly positively correlated with the total water volume used for irrigation ($r = 0.821$, $p = 0.000$). Potential water stress might have

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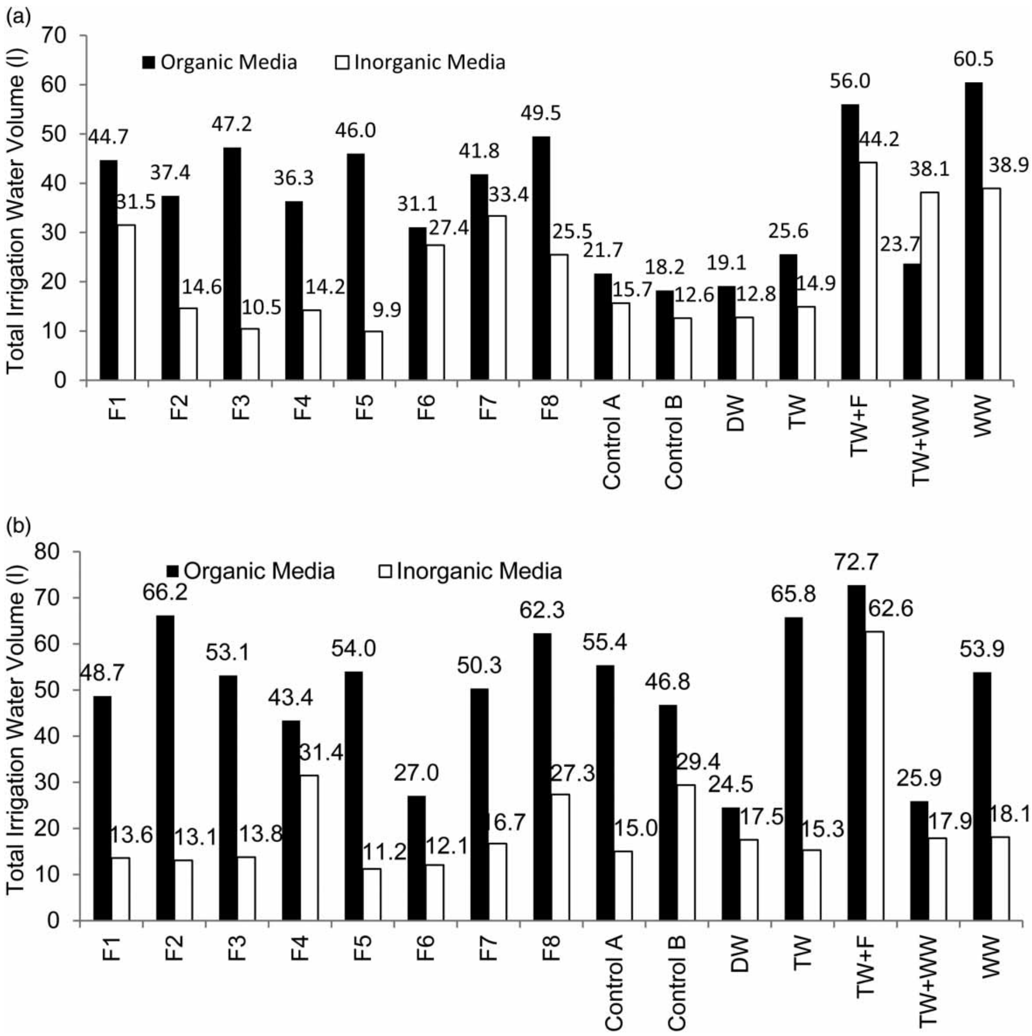

Figure 1 | Overview of total irrigation water volume consumed by (a) Sweet Pepper and (b) Chilli plants growing in different media.

reduced cell division and caused cell enlargement to cease. This could have led to a slowdown of the growth rate and might have been the reason for the relatively low weight, diameter and length of Pepper fruits (Tadesse 1997).

Table 7 provides summaries of the bud, flower and fruit development for Sweet Pepper and Chilli plants. The overall growth development of Sweet Peppers was rather disappointing, possibly due to the high concentrations of nutrients and trace minerals, and adverse environmental boundary conditions in the laboratory (Jones 2013).

Chilies did reasonably well but the growth of foliage was excessive and the harvest was delayed. High numbers of buds were recorded for plants growing in an organic media compared to those growing in an inorganic media. However, most of the buds associated with the organic media fell down before reaching the flowering stage. Most flowers either also fell down or died before producing any fruits, possibly due to the high nutrient concentrations (especially nitrogen) supplied to those plants by rich organic media and irrigation (pre-treated) wastewater (Haifa Chemicals 2014). Plants growing in an inorganic media produced lower numbers of buds compared to those Chillies grown in organic media. Nevertheless, most of these buds successfully reached the flowering and fruiting stages. This is possibly due to a better balance in nutrients supplied to those plants by

Table 7 | Overview of total number of flowers (TNF) and total number of fruits (TNF) for Sweet Pepper (P) and Chilli (C) plants after the second replanting period until 4 June 2014

	Total bud number		Total flower number		Total fruit number		Total harvested fruit number	
Inflow source and growth media	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli	Sweet Pepper	Chilli
Filter 1 and organic	P1(31);P2(39)	C3(32);C4(39)	P1(0);P2(9)	C3(7);C4(4)	P1(0);P2(3)	C3(14);C4(1)	P1(0);P2(0)	C3(0);C4(0)
Filter 2 and organic	P5(34);P6(82)	C6(63);C8(37);C9 (36)	P5(2);P6(9)	C6(31);C8(29);C9 (11)	P5(0);P6(3)	C6(9);C8(14);C9 (10)	P5(0);P6(0)	C6(0);C8(4);C9 (2)
Filter 3 and organic	P8(58);P9(38);P10 (65)	C10(84);C11(99); C12(154)	P8(0);P9(3);P10 (2)	C10(22);C11(22); C12(17)	P8(0); P9(0); P10(0)	C10(8);C11(7); C12(9)	P8(0);P9(0);P10 (0)	C10(0);C11(0); C12(1)
Filter 4 and organic	P12(38);P16(52)	C16(66);C17(53)	P12(0);P16(0)	C16(16);C17(15)	P12(0);P16(0)	C16(10);C17(12)	P12(0);P16(0)	C16(3);C17(1)
Filter 5 and organic	P18(33);P19(33); P20(57)	C18(85);C19(75); C20(61)	P18(7);P19(3); P20(16)	C18(11);C19(12); C20(17)	P18(0);P19(0); P20(0)	C18(12);C19(8); C20(19)	P18(0);P19(0); P20(0)	C18(3);C19(2); C20(0)
Filter 6 and organic	P22(31);P23(66)	C21(62)	P22(1);P23(0)	C21(15)	P22(0);P23(0)	C21(13)	P22(0);P23(0)	C21(0)
Filter 7 and organic	P26(64);P28(57)	C25(100);C26(80)	P26(2);P28(10)	C25(21);C26(39)	P26(0);P28(2)	C25(18);C26(21)	P26(0);P28(0)	C25(3)C26(2)
Filter 8 and organic	P31(46);P32(48); P33(86)	C27(79);C28(79); C29(71)	P31(0);P32(3); P33(4)	C27(37);C28(23); C29(17)	P31(0);P32(1); P33(0)	C27(20);C28(11); C29(15)	P31(0);P32(0); P33(0)	C27(4);C28(0); C29(1)
Control A and organic	P35(33)	C31(47);C33(51)	P35(11)	C31(22);C33(28)	P35(3)	C31(17);C33(15)	P35(0)	C31(0);C33(2)
Control B and organic	P39(38)	C37(127);C38(113)	P39(0)	C37(26);C38(39)	P39(0)	C37(13);C38(16)	P39(0)	C37(1);C38(3)
Deionized water and organic	P41(36)	C41(164)	P41(0)	C41(21)	P41(0)	C41(13)	P41(0)	C41(8)
Tap water and organic	P44(115)	C42(72);C43(38)	P44(17)	C42(37);C43(31)	P44(6)	C42(28);C43(23)	P44(0)	C42(13);C43 (10)
Tap water/fertilizer and organic	P45(61);P46(147)	C45(128);C46(122)	P45(1);P46(2)	C45(44);C46(46)	P45(1);P46(1)	C45(30);C46(17)	P45(0);P46(0)	C45(0);C46(1)
Wastewater/tap water and organic	P47(56)	C49(96)	P47(0)	C49(35)	P47(0)	C49(10)	P47(0)	C49(0)
Wastewater and organic	P51(161);P54 (134)	C52(99); 54(63)	P51(31);P54(23)	C52(25);C54(28)	P51(0);P54(5)	C52(13);C54(9)	P51(0);P54(0)	C52(1);C54(0)
Filter 1 and inorganic	P55(14);P56(6)	C56(8)	P55(7);P56(3)	C56(5)	P55(6);P56(1)	C56(3)	P55(0);P56(0)	C56(1)
Filter 2 and inorganic	P57(28)	C58(18)	P57(8)	C58(6)	P57(2)	C58(5)	P57(0)	C58(1)
Filter 3 and inorganic	P59(12)	C61(10)	P59(1)	C61(6)	P59(0)	C61(2)	P59(0)	C61(1)
Filter 4 and inorganic	P61(13)	C63(11);C64(10)	P61(4)	C63(5);C64(7)	P61(3)	C63(1);C64(2)	P61(0)	C63(1);C64(2)
Filter 5 and inorganic	P65(4)	C66(9)	P65(2)	C66(1)	P65(1)	C66(1)	P65(0)	C66(1)
Filter 6 and inorganic	P66(5);P67(38)	C68(7)	P66(4);P67(3)	C68(5)	P66(1);P67(1)	C68(3)	P66(0);P67(0)	C68(1)

(*Continued.*)

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Table 7 | Continued

	Total bud number		Total flower number		Total fruit number		Total harvested fruit number	
Inflow source and growth media	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**	**Sweet Pepper**	**Chilli**
Filter 7 and inorganic	P17(12);P69(8)	C71(8)	P17(6);P69(2)	C71(5)	P17(3);P69(1)	C71(3)	P17(0);P69(0)	C71(3)
Filter 8 and inorganic	P70(5);P71(7)	C72(10);C73(13)	P70(2);P71(2)	C72(5);C73(4)	P70(1);P71(1)	C72(2);C73(2)	P70(0);P71(1)	C72(2);C73(1)
Control A and inorganic	P73(4)	C74(1)	P73(1)	C74(1)	P73(1)	C74(1)	P73(0)	C74(0)
Control B and inorganic	P74(2)	C76(11);C77(1)	P74(1)	C76(1);C77(1)	P74(1)	C76(1);C77(1)	P74(0)	C76(1);C77(0)
Deionized water and inorganic	P80(2)	C80(7)	P80(0)	C80(4)	P80(0)	C80(1)	P80(0)	C80(0)
Tap water and inorganic	P81(8)	C82(1)	P81(2)	C82(1)	P81(1)	C82(1)	P81(0)	C82(1)
Tap water/fertilizer and inorganic	P83(34);P84(37)	C84(30);C85(68)	P83(5);P84(5)	C84(10)C85(20)	P83(2);P84(2)	C84(6);C85(13)	P83(0);P84(0)	C84(3);C85(8)
Wastewater/tap water and inorganic	P86(3);P87(10)	C87(7)	P86(2);P87(3)	C87(7)	P86(1);P87(1)	C87(2)	P86(0);P87(0)	C87(1)
Wastewater and inorganic	P89(16);P90(18)	C90(10)	P89(1);P90(1)	C90(6)	P89(1);P90(0)	C90(4)	P89(0);P90(0)	C90(0)

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the corresponding irrigation water. Low fruit numbers correlate well with inorganic growth media (Table 7).

Figures 2–4 summarize a comparison of maximum fruit lengths, widths and total weights harvested from plants grown in different media subjected to different irrigation water types. Generally, fruits harvested from plants grown in organic media had diameters, lengths and weights greater than those from plants raised in inorganic media. These results in addition to findings based on other research studies undertaken in greenhouse conditions to assess the effect of different growth media on Pepper growth rates and yields indicated that seedlings benefited from peat moss media (Rahimi *et al.* 2013).

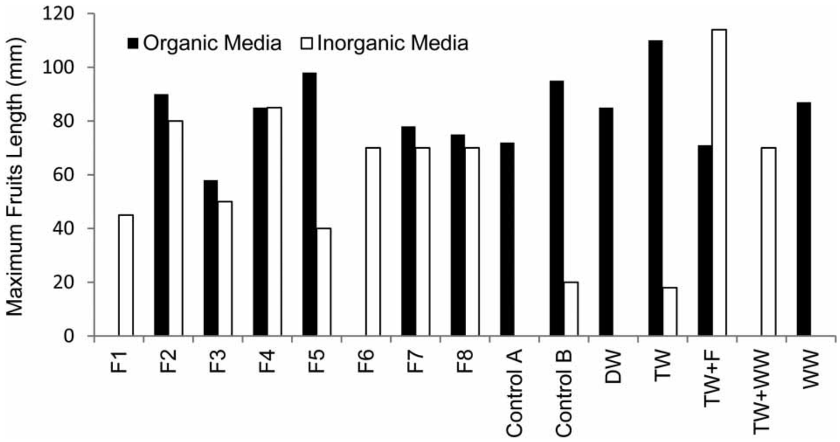

Figure 2 | Comparison of maximum fruit lengths associated with harvested plants grown in different growth media subjected to different irrigation water types.

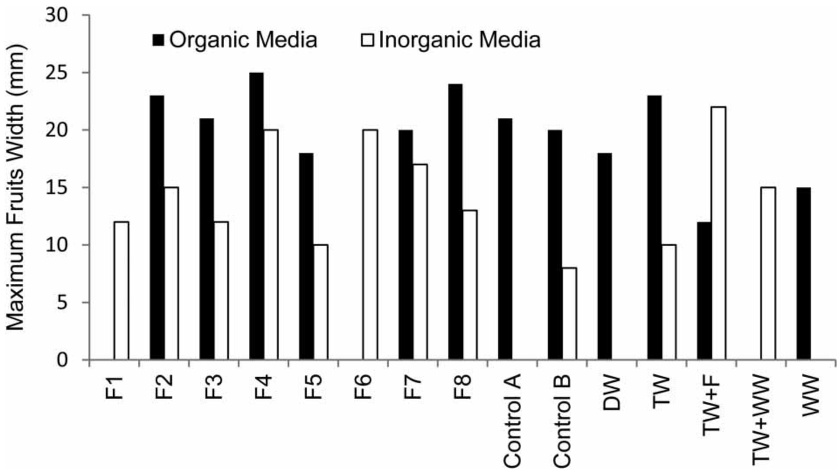

Figure 3 | Comparison of maximum fruit widths linked to harvested plants grown in different growth media subjected to different irrigation water types.

Another study was undertaken to determine the effects of peat and sand on variables such as fruit length, diameter and weight, as well as the total fruit number per plant and yield. Results showed that peat significantly increased length, diameter and weight of fruits in all cultivars grown in comparison to sand (Gungor & Yildirim 2013).

Furthermore, organic substrates decompose over time, and subsequently release nutrients. The rate of decomposition and the physical conditions of the media vary with the parent material. That in turn will enhance crop growth and development. Moreover, better aeration of peat promotes vigorous root growth, which allows rapid development of foliage and therefore increases the whole plant yield (Olle

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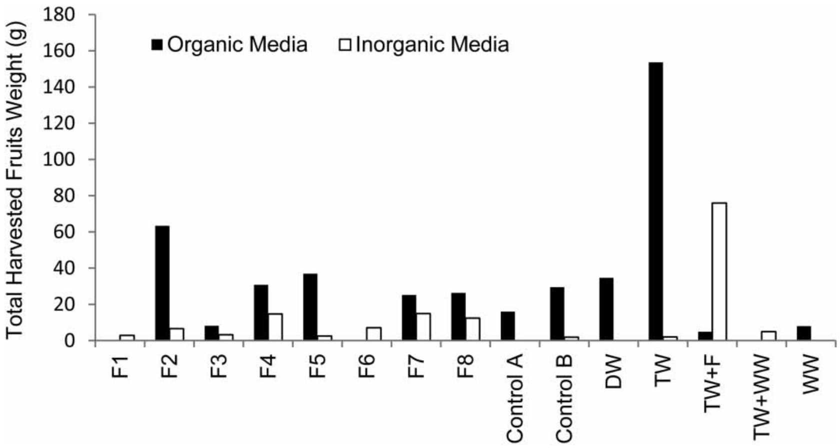

Figure 4 | Comparison of total fruit weights associated with harvested plants growing in different growth media subjected to different irrigation water types.

et al. 2012). In contrast, for inorganic media such as sand, nutrient provision to the crops is limited to the nutrients that are part of the irrigation water resulting in a delay of plant foliage growth with a subsequent poor yield (Olle *et al.* 2012).

Figure 5 summarizes differences in fruit mean diameter, length and weight harvested from plants irrigated with different water types and grown in organic media. Regarding the comparison of mean fruit lengths and widths of plants grown in organic media and irrigated with different wetland outflow waters, statistical results show that harvested fruits were not significant different from each other (*P* value greater than 0.05). However, fruits harvested from plants grown in organic media and irrigated with Filter 2 outflow water were very close to those harvested from Control B outflow water, tap water and deionized water in which plants depended mainly on nutrients provided by the organic growth media, confirming that nutrients provided to plants by the treated wastewater and the compost were very high, produce good yield quality.

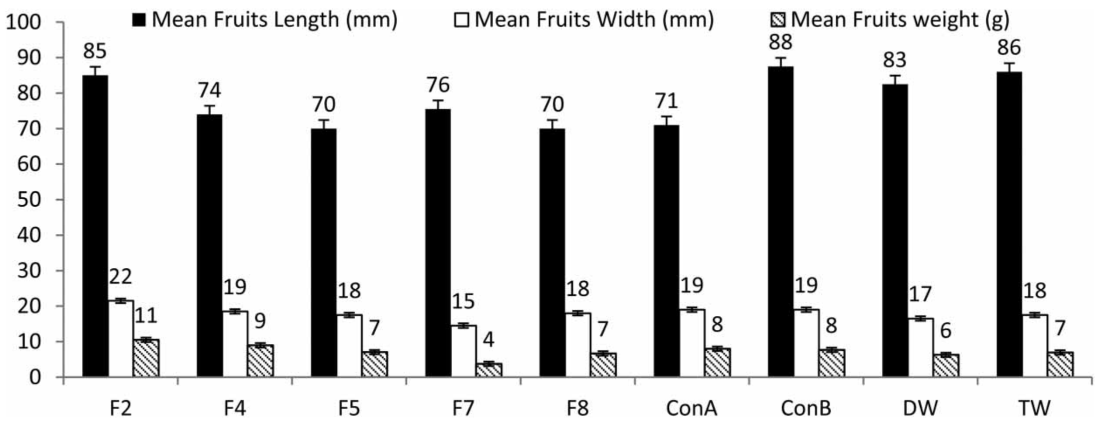

Figure 5 | Differences in fruit mean length, width and weight linked to harvested plants irrigated with different water types and grown in organic media. Note: treatments did not produce enough fruit numbers were not considered in the comparison results at least at this stage of the plant development. Bars indicate standard errors.

Regarding a comparison of mean fruit weights harvested from plants grown in organic media and irrigated with different water types (Figure 5), results showed that plants irrigated with water harvested from Filters 2 and 4 produced fruits of mean weight, which were significantly greater than those harvested from plants irrigated with outflow water from Filter 7 (*P* value of 0.008 and 0.027, respectively). These result can be explained by a higher contact time (Filters 2 and 4 compared to

Filter 7; Table 1) leading to more favourable nutrient quantity distributions (e.g., lower nitrate-nitrogen in the irrigation water for Filters 2 and 4 compared to Filter 7; Table 3) provided by Filters 2 and 4, which subsequently positively impacted on fruit diameters, lengths and weights (Bar-Tal *et al.* 2001). However, since the plants irrigated with tap water and grown in organic media produced the highest number of fruits (23), this explains why the total weight of harvested fruits was linked to plants irrigated with tap water associated with low nutrient loads (Figure 4).

Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated waste water are usually too high to produce a good harvest. However, as the compost is depleted of nutrients after about 8 months, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost. A high yield is rather related with the most suitable provision of nutrients and trace elements.

Inductively coupled plasma findings

Table 5 provides an overview of the ICP–OES analysis for selected elements in the irrigation water. High concentrations of iron, which exceeded the threshold of 5 mg/l (Pescod 1992; FAO 2003), were noted for both raw wastewater and tap water. Based on the recommended threshold of 2 mg/l for potassium (FAO 1994), the outflow water from all wetland filters (with the exception of Controls A and B) was associated with too high potassium concentrations. Furthermore, high concentrations were also observed for raw wastewater, and wastewater samples, which were diluted with 80% of tap water. Results show for Filters 3 and 5 relatively high manganese concentrations, which exceeded the threshold of 0.2 mg/l (Pescod 1992; FAO 2003).

Brief cost-benefit analysis

Table 6 shows the proposed novel harvest classification scheme for Chilli. However, the estimated prices are dependent on the current market value. Only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, weight and bending. The lowest variable class entry for any individual fruit assessment determined the final class. For example, if a fruit is categorized as class A in terms of length, class B in terms of diameter and E in terms of weight, then the final class for this fruit would be class E, and accordingly the corresponding price for this Chilli would be zero pence (Table 8).

The monetary value of the harvest was only calculated for the example plant. A similar assessment could also be undertaken for Sweet Peppers at the end of the harvest. Table 7 showed that the highest number of fruits categorized as Class A were harvested from plants grown in organic media and watered with tap water. However, tap water was also associated with the highest fruit numbers categorized as Class E. The highest mean price of harvested fruits is also associated with tap water (76.7 pence) followed by those harvested from plants growth in organic media and watered with Filter 2 (25.1 pence). A low mean price for harvested fruits is associated with filters contaminated with diesel (Table 1). The lowest mean price is linked to plants growing in inorganic media (Table 8).

In comparison to Chillies, the overall growth development (Table 7) of Sweet Pepper was rather disappointing. This is possibly due to the high concentrations of nutrients and trace minerals, and adverse environmental boundary conditions in the laboratory.

CONCLUSIONS AND RECOMMENDATIONS

The experiment shows that Sweet Peppers and Chillies can be grown successfully using wastewater treated by constructed wetlands. However, the yield of Sweet Peppers was insignificant in contrast

Table 8 | Overview of the outcome of the Chilli (C) harvest (before or on 4 June 2014) classification scheme according to Table 7. Note that the lowest variable class entry for any individual fruit assessment will determine the final class. However, only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, weight, bending. Values shown per plant represent pence (Sterling)

Inflow source and growth media	Class A	Class B	Class C	Class D	Class E	Mean per plant
Filter 1 and organic	C3(0);C4(0)	C3(0);C4(0)	C3(0);C4(0)	C3(1.2);C4(0)	C3(0);C4(0)	0
Filter 2 and organic	C6(0);C8(42.1);C9(0)	C6(0);C8(20.5);C9(10.1)	C6(0);C8(0);C9(0)	C6(0);C8(0);C9(3.0)	C6(0);C8(0);C9(0)	25.1
Filter 3 and organic	C10(0);C11(0);C12(0)	C10(0);C11(0);C12(0)	C10(0);C11(0);C12(0)	C10(0);C11(0);C12(2.1)	C10(0);C11(0);C12(0)	0.7
Filter 4 and organic	C16(0);C17(0)	C16(7.9);C17(0)	C16(9.0);C17(0)	C16(0);C17(1.2)	C16(0);C17(0)	9.1
Filter 5 and organic	C18(0);C19(0);C20(0)	C18(14.6);C19(9.9);C20(0)	C18(3.4);C19(2.9);C20(0)	C18(0);C19(0);C20(0)	C18(0);C19(0);C20(0)	5.4
Filter 6 and organic	C21(0)	C21(0)	C21(0)	C21(0)	C21(0)	0.0
Filter 7 and organic	C25(0);C26(0)	C25(0);C26(17.6)	C25(0);C26(0)	C25(1.9);C26(0)	C25(0);C26(0)	9.8
Filter 8 and organic	C27(0);C28(0);C29(0)	C27(0);C28(0);C29(0)	C27(2.7);C28(0);C29(0)	C27(4.8);C28(0);C29(0.8)	C27(0);C28(0);C29(0)	2.8
Control A and organic	C31(0);C33(0)	C31(0);C33(7.2)	C31(0);C33(0)	C31(0);C33(2.2)	C31(0);C33(0)	4.7
Control B and organic	C37(0);C38(20.3)	C37(15.7);C38(0)	C37(0);C38(2.6)	C37(0);C38(0)	C37(0);C38(0)	19.3
Deionized water and organic	C41(0)	C41(7.6)	C41(9.1)	C41(2.0)	C41(0)	18.7
Tap water and organic	C42(18.4);C43(86.8)	C42(16.8);C43(8.6)	C42(5.1);C43(7.3)	C42(6.8);C43(3.5)	C42(0);C43(0)	76.7
Tap water/fertiliser and organic	C45(0);C46(0)	C45(0);C46(0)	C45(0);C46(0)	C45(0);C46(1.2)	C45(0);C46(0)	0.0
Wastewater/tap water and organic	C49(0)	C49(0)	C49(0)	C49(0)	C49(0)	0.0
Wastewater and organic	C52(0); C54(0)	C52(0); C54(0)	C52(0); C54(0)	C52(0); C54(0)	C52(0); C54(0)	0.0
Filter 1 and inorganic	C56(0)	C56(0)	C56(0)	C56(0)	C56(0)	0.0
Filter 2 and inorganic	C58(0)	C58(0)	C58(3.3)	C58(0)	C58(0)	3.3
Filter 3 and inorganic	C61(0)	C61(0)	C61(0)	C61(0.8)	C61(0)	0.8
Filter 4 and inorganic	C63(0);C64(0)	C63(7.7);C64(0)	C63(0);C64(0)	C63(0);C64(1.7)	C63(0);C64(0)	4.7
Filter 5 and inorganic	C66(0)	C66(0)	C66(0)	C66(0)	C66(0)	0.0
Filter 6 and inorganic	C68(0)	C68(7.1)	C68(0)	C68(0)	C68(0)	7.1
Filter 7 and inorganic	C71(0)	C71(7.2)	C71(0)	C71(1.3)	C71(0)	8.5
Filter 8 and inorganic	C72(0);C73(0)	C72(0);C73(0)	C72(0);C73(0)	C72(1.8);C73(1.3)	C72(0);C73(0)	1.6
Control A and inorganic	C74(0)	C74(0)	C74(0)	C74(0)	C74(0)	0.0
Control B and inorganic	C76(0);C77(0)	C76(0);C77(0)	C76(0);C77(0)	C76(0);C77(0)	C76(0);C77(0)	0.0
Deionized water and inorganic	C80(0)	C80(0)	C80(0)	C80(0)	C80(0)	0.0
Tap water and inorganic	C82(0)	C82(0)	C82(0)	C82(0)	C82(0)	0.0

(*Continued.*)

Table 8 | Continued

Inflow source and growth media	Class A	Class B	Class C	Class D	Class E	Mean per plant
Tap water/fertiliser and inorganic	C84(0);C85(38.2)	C84(9.7);C85(15.7)	C84(2.6);C85(3.8)	C84(2.0);C85(1.9)	C84(0);C85(0)	37.0
Wastewater/tap water and inorganic	C87(0)	C87(0)	C87(2.5)	C87(0)	C87(0)	2.5
Wastewater and inorganic	C90(0)	C90(0)	C90(0)	C90(0)	C90(0)	0.0

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to that of Chillies. The overall growth development of Sweet Peppers was rather disappointing, possibly due to the high concentrations of nutrients (particularly phosphorus and nitrogen) and trace minerals (iron, potassium and manganese). In contrast, chilies did reasonably well but the growth of foliage was excessive due to high nitrogen concentrations in the inflow water and the harvest was delayed.

The highest number of fruits was associated with tap water and an organic growth medium. However, the best fruit quality in terms of length, width and weight was observed for plants grown in organic media and irrigated with outflow water from wetlands with large aggregate size indicating that nutrient concentrations supplied to the Peppers by a combination of compost and treated wastewater were usually too high to produce a good harvest. A high yield was related to the most suitable provision of nutrients and trace elements. However, as the compost got depleted of nutrients such as nitrogen after about 14 months, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost. Filters associated with hydrocarbon contamination were commonly associated with a poor harvest.

The productivity of Chillies in terms of harvest was independent of the wastewater consumption. Nevertheless, higher foliage production due to excess nutrients and trace minerals required more water. A high yield was related with the most suitable provision of nutrients and trace elements.

The current research will be continued with the same plants to assess if further harvests are economic and determine when the nutrients within the compost are fully depleted. Moreover, the accumulation of metals and their toxicity in the soil as well as microbiological contamination will also be studied.

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Microbial contamination of *Capsicum annuum* irrigated with recycled domestic wastewater treated by vertical-flow wetlands

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Streptococcus

ABSTRACT

Due to water scarcity in many arid countries, there is considerable interest in recycling various wastewater streams such as treated urban wastewater for irrigation in the agricultural sector. The aim was therefore to assess if domestic wastewater treated by different wetlands can be successfully recycled to water commercially grown crops. The objectives were (i) to study the effect of irrigation on Chilli (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') with domestic wastewater treated by wetland compared to tap water (fresh water); (ii) to assess the overall quality and particularly the microbiological contamination of Chilli fruits; and (iii) to determine the persistence of microbial contaminants in the soil irrigated by treated wastewater between September 2013 and September 2014. High yields were associated with tap water and an organic growth medium. No bacterial contamination was detected for fruits harvested from plants irrigated by wetland outflow water. In contrast, fruits harvested from those plants irrigated by preliminary treated wastewater showed high contamination by total coliforms, *Streptococcus* spp. and *Salmonella* spp. This was especially the case for fruits, which were located close to the contaminated soil surface. However, findings indicate that vegetable pots receiving wastewater treated with wetlands can be considered as safe compared to those receiving only preliminary treated wastewater.

1. Introduction

1.1. Background

Microbiological and biotechnological solutions to global problems such as water and food shortages as well as water resources pollution are required. As many global issues, for example improving crop quality and productivity as well as public health and wastewater management, have more acute consequences in the developing world, this paper emphasizes the role of biotechnological advances for developing regions where food production using recycled pre-treated wastewater is of interest.

The world is over-populated in many geographical areas causing considerable strain on natural resources such as freshwater. Since water resources are limited, particularly in dry climatic regions, wastewater treatment and subsequent recycling is a viable alternative (Asano, 1994). Treated wastewater can be used for agricultural land irrigation, aquaculture, landscape irrigation, urban and industrial applications, recreational and ecosystem purposes, and artificial recharging of ground water (Asano et al., 2007).

Advantages associated with wastewater recycling include the supply of nutrients and trace minerals to plants, potentially leading to higher yields and a decrease in the demand for inorganic fertilizers (Val-Moraes et al., 2011; Bichai et al., 2012). According to Zavadil (2009), highly microbiologically contaminated wastewater causes a reduction in the overall crop yield and quality. Moreover, these crops are contaminated by pathogens and intestinal helminths. However, high yields can be achieved by using pre-treated wastewater for irrigation of various crops under controlled environmental conditions (Zavadil, 2009).

Materon et al. (2007) identified the agricultural, industrial and human sources of microbial contamination from pre- to post-harvest operations of Cantaloupes (*Cucumis melo* var. *cantalupensis* (Naudin)) grown at ten different farms in southern Texas. The results indicated that irrigation water contained a wide range of micro-organisms that could cause human illnesses and were able to survive on the rind of Cantaloupes before, during and after harvesting.

Wastewater recycling makes particularly sense for popular, relatively expensive and easy-to-grow plants such as chillies, peppers, tomatoes, eggplants, lettuces and cabbages (Almuktar

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et al., 2015). Most of these plants are rich in vitamins and trace minerals. This paper will focus on one example plant only. However, findings should also be relevant for other crops.

1.2. Constructed wetlands

Treatment wetlands are engineered wastewater purification systems that encompass physical, chemical and biological processes that are all similar to developments occurring in natural wetlands. They are implemented for environmental pollution control to treat a variety of wastewaters such as industrial effluents, agricultural and urban runoff, animal farm wastewaters, mine drainage and various types of sludges (Scholz, 2010; Sani et al., 2013). Recently, some large-scale wetland systems have also been successfully applied to treat domestic wastewater (Dong et al., 2011). However, there are few long-term and controlled studies involving domestic wastewater due to health and safety concerns.

1.3. Wastewater recycling for crop irrigation

The earliest reported sewage farms, where wastewater has been applied directly to land for disposal and/or for agricultural use, were operated in the 16th and 17th centuries in Bunzlau (Germany) and Edinburgh (UK) according to Shuval et al. (1986). The scientific basis for the acceptance of wastewater reclamation, reuse and recycling and in agriculture has evolved from developments in water and wastewater engineering science coupled with an increasing pressure on water resources management and development. Treated urban wastewater can be recycled and reused in arid regions, which are subject to increasing water shortages. The assessment of the effects of treated wastewater reuse on crops subsequently intended for human consumption is of particular interest (Asano and Levine, 1996; Aiello et al., 2007; Cirelli et al., 2012). For example, Cirelli et al. (2012) presented the results of a reuse scenario where tertiary-treated municipal wastewater using a constructed wetland was supplied for irrigation of vegetables in Eastern Sicily, Italy. They concluded that the irrigation water contained increased numbers of *Escherichia coli*, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 ml for secondary treated urban wastewater effluents.

In an earlier but related study by the same team, Aiello et al. (2007) assessed the effects of reclaimed municipal wastewater for irrigation on hydrological soil behaviour and vegetable quality. The researchers concluded that the wastewater reuse subsequently resulted in increased microbial contamination (*E. coli*: 3000 most probable number (MPN)/100 ml; faecal *Streptococcus* spp.: 1200 MPN/100 ml) on the soil surface. Morari and Giardini (2009) assessed the treatment efficiency of pilot-scale vertical-flow wetlands on domestic wastewaters and their suitability for irrigation reuse in Italy. Cui et al. (2003) concluded that the removal rates for total heterotrophic bacteria and total coliforms by using vertical-flow bed systems with cinder substrate were between 80 and 90%, and between 85 and 96%, respectively. According to EPA (1992), the typical bacteria survival time in soil, fresh water and crops is less than 70, 60 and 30 days, respectively. The maximum number of total coliforms is 1000 CFU/100 ml for irrigation of crops that are likely to be eaten uncooked (WHO, 1989; FAO, 2003).

García-Delgado et al. (2012) undertook a greenhouse study in Spain to assess the effect of treated urban wastewater on soil and pepper quality. They concluded that the wastewater application saved fertilizer (37% nitrogen, 66% phosphorus and 12% potassium) as well as that the total polyaromatic hydrocarbons and heavy metals (cadmium, lead and arsenic) within the pepper fruits were low. The highest concentration (lower than the proposed threshold concentration for carcinogenicity) was recorded for phenathrene. Moreover, traces of hydrocarbons from diesel spills associated with urban runoff or industrial effluent are a more recent challenge (Scholz, 2010; García-Delgado et al., 2012). Furthermore, Benedek et al. (2013) studied the impact of long-term total petroleum hydrocarbons (TPH), volatile petroleum hydrocarbons, total alkyl benzenes and polycyclic aromatic hydrocarbons on the structure of bacterial communities of four different contaminated soil samples. They concluded that a very high amount of TPH affected positively the diversity of hydrocarbon-degrading bacteria.

1.4. Plant selection

Many vegetables have the potential to grow well on recycled wastewater. However, there is the potential of some vegetables such as lettuce and cabbage to become contaminated by microbes, because their edible leaves are too close to the ground receiving the treated wastewater. Therefore, it makes sense to select vegetables where the edible fruit is located far away from the ground. This may include peppers, tomatoes, maize, eggplants, beans, lentils and peas.

The next step in selecting suitable vegetables is to decide on easy-to-grow and relatively cost-effective plants with high

Fig. 1. Overview of the experimental set-up of wetland filters (15 June 2014).

Table 1
Comparison of the experimental vertical-flow wetland set-up*.

Design and/or operational variable	Unit	Filters 1 and 2	Filters 3 and 4	Filters 5 and 6	Filter 7	Filter 8	Control A	Control B
Aggregate diameter	Mm	20	10	10	10	10	10	10
Contact time	H	72	72	72	36	36	72	72
Resting time	h	48	48	48	48	24	48	48
Chemical oxygen demand	mg/l	122.8	122.8	243.8	122.8	122.8	2.3	2.3

*Annually treated volumes of wastewater: Filters 1 to 6, 470 l/a; Filter 7, 624 l/a Filter 8, 858 l/a. On 26 September 2013, 130 g of diesel was added to Filters 1, 3 and 5 and Control A.

nutritional value. Many vegetables may fit these conditions in particular geographical settings. However, the authors concentrated on Chilli plants in this study, because they can also be grown in greenhouses in the UK (Nickels, 2012; Jones 2013).

A popular and easy-to-obtain Chilli variety was selected from a large supplier. Chilli (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') is described by the retailer B&Q plc as a good crop of slender and hot fruits ideal for growing in pots on the patio, balcony or in a greenhouse. It is also described as a perfect Chilli for general cooking. This type of easy-to-grow Chilli is also known as aleva, Guinea spice, cow-horn pepper, bird pepper and red pepper. This type of pepper requires about 100 days for maturation. Chillies prefer moist, warm and nutrient-rich soil in a predominantly warm overall climate. The germination time is 5–14 days. The plants grow to about 45 cm in height and should be planted about 40 cm apart from each other. The sowing to cropping time is approximately 18 weeks. Chillies are mostly perennial (often more than three years) in sub-tropical and tropical parts of the world (Nickels, 2012). However, they are normally grown as annuals in temperate and oceanic climates such as the UK. Furthermore, Chillies prefer a loamy soil with a pH of between 7.0 and 8.5; i.e., neutral to weakly alkaline soil (Nickels, 2012).

1.5. Rationale, aims and objectives

Effluent from different types of wetland systems treating domestic wastewater was selected to irrigate vegetables. Some of the wetlands received standard wastewater while the others received wastewater that was subject to a one-off diesel fuel spill. The treated wastewater from all wetland types was recycled for the irrigation of Chillies, which are commonly seen as popular, relatively expensive and easy-to-grow vegetables. This experiment may provide the scientific justification for integrating treatment wetlands into agricultural food production.

The overall aim of this paper is to assess the microbial contamination of chilli fruits and soil irrigated by wastewaters treated by different wetland types. The objectives were (i) to study the effect of irrigation on Chillies with domestic wastewater treated by wetlands compared to tap water (fresh water); (ii) to assess the overall quality and particularly the microbiological contamination of Chilli fruits; and (iii) to determine the persistence of key microbial contaminants in the soil irrigated by treated wastewater.

2. Materials and methods

2.1. Constructed wetlands set-up and operation

The experimental vertical-flow constructed wetland systems are located within a greenhouse (door left open) on top of the roof of a building (Fig. 1) located in Greater Manchester, UK (Sani et al., 2013). They were operated between 27 June 2011 and 17 September 2014. The set-up includes two filters that are essentially controls receiving clean de-chlorinated tap water. Table 1 indicates an overview of the statistical experimental set-up used to test the impact of four variables. Filters 1 and 2 compared to Filters 3 and 4 test the influence of a larger aggregate diameter. Filters 5 and 6 compared to Filters 3 and 4 check the impact of a higher loading rate. The application of a lower contact rate is tested, if Filter 7 is compared with Filters 3 and 4. Finally, a lower resting time is the difference between Filters 7 and 8.

The ten laboratory-scale vertical-flow constructed wetlands were constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm. The filters were filled with siliceous (minimum of 30%) pea gravel up to a depth of 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). Dead macrophyte plant material was harvested in winter and returned to the corresponding wetland filters by placing it on top of the litter zone (Sani et al., 2013). The main outlet valve is located at the bottom of each constructed wetland system. Wetland columns received about 6.5l of inflow water during the operational feeding mode that was different between several filters as shown in Table 1. Columns 1–6 were sampled after 72 h contact time and then left to rest for 48 h, while columns 7 and 8 were sampled after 36 h contact time and left to rest for 48 h and 24 h, respectively. All water quality variables discussed in this paper were usually determined during or directly after sampling.

Table 2
Chilli (C) harvest classification scheme (after Almuktar et al., 2015).

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
Mean price estimate; pence (Sterling)/gram	C: 2.00	C: 1.00	C: 0.50	C: 0.25	C: 0.00
Length (L, mm)	Very long ($L \geq 80$)	Long ($60 \leq L < 80$)	Medium ($40 \leq L < 60$)	Short ($20 \leq L < 40$)	Very short ($L < 20$)
Width (W, mm)	Very wide($W \geq 20$)	Wide ($16 \leq W < 20$)	Medium ($12 \leq W < 16$)	Slim ($8 \leq W < 12$)	Very slim ($W < 8$)
Weight (w, g)	Very Large($w \geq 9$)	Large ($7 \leq w < 9$)	Medium ($5 \leq w \leq 7$)	Small ($3 \leq w < 5$)	Very small ($w < 3$)
Bending	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Uncharacteristically bend; $L/W < 3.5$	Uncharacteristically bend; $L/W < 3.5$

The preliminary treated (screens) urban wastewater used for the inflow water was obtained from the Davyhulme Sewage works, which is one of the largest wastewater treatment works in Europe (http://en.wikipedia.org/wiki/Davyhulme), operated by the water company United Utilities in Greater Manchester. Fresh wastewater was collected approximately once per week, and was subsequently aerated by standard aquarium air pumps and stored in a cold room before recycling. The wastewater quality was highly variable, and comprised domestic and industrial wastewater as well as surface water runoff.

In order to simulate a one-off diesel fuel (100% pure; no additives) spill, 130 g (equivalent to an inflow concentration of 20 g/l) of diesel fuel (100% pure; no additives) were poured into Filters 1, 3 and 5, and into one of the two columns (Control A) on 26 September 2013 (Table 1). The fuel was obtained from a petrol station operated by Tesco Extra (Pendleton Way Salford, UK).

Aqua Medic Titan chillers (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were used to maintain the root system and debris layer of all wetland systems at semi-natural below-surface temperatures of about 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located (Sani et al., 2013).

The COD was used as the criterion to differentiate between low and high loads (Table 1). An inflow target COD of about 285 mg/l (usually between 122 and 620 mg/l) was set for wetlands with a high loading rate (Filters 5 and 6). The remaining Filters 1–4, 7 and 8 received wastewater diluted with de-chlorinated tap water. The target inflow COD for these filters was approximately 138 mg/l (usually between 43 and 350 mg/l).

2.2. Water quality analysis

Routine water quality sampling was carried out according to APHA (2005) to monitor the main physical and chemical characteristics of irrigation water. The spectrophotometer DR 2800Hach Lange (www.hach.com) was used for standard water quality analysis for variables including COD, ammonia–nitrogen, nitrate–nitrogen, ortho-phosphate–phosphorus and SS.

Total petroleum hydrocarbons were determined by gas chromatography and flame ionization externally by Exova Health Sciences (70 Montrose Ave Hillington Park, Glasgow G52 4LA) according to their own internationally accredited TPH in Waters (with aliphatic/aromatic splitting) method (Exova Health Sciences, 2014). The five-day BOD was determined in all water samples with the OxiTop IS 12-6 system, a manometric measurement device, which was supplied by the Wissenschaftlich-Technische Werkstätten (WTW) located in Weilheim, Germany. Nitrification within the bottle was suppressed by adding 0.05 ml of 5 g/l *N*-Allylthiourea (WTW chemical solution No. NTH600) solution per 50 ml of sample water.

The analysis of water samples for trace elements were performed using a Varian 720-ES Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK, Ltd., Wharfedale Road, Wokingham, Berkshire, UK) analysis was undertaken to determine nutrient and trace element concentrations. Water samples of 50 ml were preserved in glassware bottles at 4 °C. The samples were then acidified, if appropriate, by adding 1 ml of 70% concentrated nitric acid to dissolve any suspended material in order to extract heavy metals and to reduce the pH to below 2, which was required for analysis. The samples were then filtered through a filter paper with a diameter of 0.45 μm before analyses by ICP–OES.

Turbidity was measured with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, www.lovibond.com). The pH was measured with a sensION + Benchtop Multi-Parameter Meter (Hach Lange, Düsseldorf, Germany). The electronic conductivity was determined by the conductivity meter METTLER TOLEDO Five Go™ (Keison Products, Chelmsford, Essex, England, UK).

Total coliforms, faecal coliforms, *E. coli*, faecal *Streptococcus* spp. and *Salmonella* spp. were estimated by using aseptic pour plate techniques according to standard methods (APHA, 1998). The agars used for growing bacteria colonies are characterised below. Chromocult Coliform Agar (Central of Merck Group, Darmstadt, Germany) was used for estimating coliforms and *E. coli* (Manafi and Kneifel, 1989; Ossmer et al., 1999). Following the manufacturer's instructions, the agar was prepared by dispersing 26.5 g of powder in 1 l of deionised water. The mixture was allowed to soak for 10 min, swirled to mix and then sterilised during boiling for 30 min. The agar was subsequently cooled to between 45 and 50 °C, and subsequently thoroughly mixed. About 12.5 ml of the prepared agar was dispensed into Petri dishes with a diameter of 90 mm and a height of 16.2 mm (Fisher Scientific UK Ltd., Loughborough, UK). Chromocult Coliform agar is composed of peptone (3 g/l), sodium chloride (5 g/l), sodium hydrogen phosphate (2.7 g/l), sodium dihydrogen phosphate (2.2 g/l), sodium pyruvate (1 g/l), tryptophane (1 g/l), tergitol-7 (0.15 g/l), sorbitol (1 g/l), chromogenic mixture (0.4 g/l) and agar–agar (10 g/l). The pH of the agar was 6.8 ± 0.1. According to the manufacturer's instructions, typical *E. coli* colonies may appear as dark blue to violet convex colonies, which could be entirely glossy with a size between 0.1 and 2.0 mm. Coliform colonies are coloured rose-pink. The convex may be entirely glossy with a size of between 1.5 and 2.5 mm in the agar plats after the incubation period.

Kanamycin Aesculin Azide Agar (LABM limited, Lancashire, UK) is a selective isolation and enumeration medium for *Streptococcus* spp. Sodium azide and kanamycin provide the selective inhibition required, whilst iron salts and aesculin form an indicator system for the presumptive identification of *Enterococci* (Mossel et al., 1978). This agar was prepared by dispersing 43 g of powder in one

Table 3
Experimental design (after Almuktar et al., 2015) in terms of plant number allocations after the third planting).

Inflow source	Growth media	Chilli[a]
Filter 1 outflow	Compost with bark	C3;C4
Filter 2 outflow	Compost with bark	C6;C8:C9
Filter 3 outflow	Compost with bark	C10;C11;C12
Filter 4 outflow	Compost with bark	C16:C17
Filter 5 outflow	Compost with bark	C18;C19;C20
Filter 6 outflow	Compost with bark	C21
Filter 7 outflow	Compost with bark	C25:C26
Filter 8 outflow	Compost with bark	C27;C28:C29
Control A outflow	Compost with bark	C31;C33
Control B outflow	Compost with bark	C37:C38
Deionised water	Compost with bark	C41
Tap water (100%)	Compost with bark	C42;C43
Tap water with fertiliser (0.7 ml/l)	Compost with bark	C45;C46
Wastewater[b] (20%); tap water (80%)	Compost with bark	C49
Wastewater[b] (100%)	Compost with bark	C52; C54
Filter 1 outflow	Silica sand	C56
Filter 2 outflow	Silica sand	C58
Filter 3 outflow	Silica sand	C61
Filter 4 outflow	Silica sand	C63;C64
Filter 5 outflow	Silica sand	C66
Filter 6 outflow	Silica sand	C68
Filter 7 outflow	Silica sand	C71
Filter 8 outflow	Silica sand	C72;C73
Control A outflow	Silica sand	C74
Control B outflow	Silica sand	C76;C77
Deionised water	Silica sand	C80
Tap water (100%)	Silica sand	C82
Tap water with fertiliser (0.7 ml/l)	Silica sand	C84;C85
Wastewater[b] (20%); tap water (80%)	Silica sand	C87
Wastewater[b] (100%)	Silica sand	C90

[a] Original seed planting reference numbers; Chilli (C1–C90).
[b] Pre-treated using screens.

litre of deionised water, allowing it to soak for 10 min, mixing it and subsequently sterilising it by autoclaving at 121 °C for 15 min. The gar was then allowed to cool to 47 °C before dispensing it into Petri dishes, following the manufacturer's instructions. Kanamycin Aesculin Azide Agar is composed of tryptone (20 g/l), yeast extract (5 g/l), sodium chloride (5 g/l), sodium citrate (1 g/l), aesculin (1 g/l), ferric ammonium citrate (0.5 g/l), sodium azide (0.15 g/l) and kanamycin sulphate (0.02 g/l). According to manufacturer's instructions, white and/or grey colonies with a diameter of approximately 2 mm surrounded by a black halo can be expected in the agar plates after the incubation period.

Harlequin™ *Salmonella* ABC (LABM limited, Heywood, Lancashire, UK) medium has been developed for the isolation of *Salmonella* spp. The agar utilises a dual chromogenic system to visualise enzymatic activities. Sodium desoxycholate and sodium citrate function as inhibitors. The ABC medium reduces the requirement for 'false positive' screening, which saves labour and reduces consumable costs (Perry et al., 1999). The agar was prepared by dispersing 36.5 g of powder in 1 l of deionised water. The mixture was allowed to soak for 10 min, and was subsequently mixed and then sterilised by boiling. The medium was allowed to cool to 47 °C, mixed well and dispensed into Petri dishes, following the manufacturer's instructions. Harlequin™ *Salmonella* ABC composes of beef extract (5 g/l), peptone (5 g/l), sodium citrate (8.5 g/l), sodium desoxycholate (5 g/l), agar (12 g/l), ferric ammonium citrate (0.5 g/l), X-a-Gal (0.08 g/l), CHE-ß-Gal (0.3 g/l), Isopropyl β-D-1-thiogalactopyranoside (IPTG; 0.03 g/l). The pH was 7 ± 0.2. According to manufacturer's instructions, black colonies with a diameter of 1 to 2 mm diameter are formed on the agar plates after the incubation period, if *Salmonellae* are present. Buffered Peptone Water (LABM limited, Heywood, Lancashire, UK) was applied as a pre-enrichment medium designed to support sub-lethally damaged *Salmonellae* to recover before introducing the bacteria into a selective medium (Poemla and Silliker 1976). This nutrient medium is free from inhibitors and is well buffered to maintain the pH at 7.2 during the incubation period. Following manufacturer's guidelines, this medium was prepared by weighing 20 g of powder and dispersing it into one litre of deionised water. The solution was subsequently distributed into tubes and bottles, and sterilised by autoclaving at 121 °C for 15 min. Buffered Peptone Water comprises peptone (10 g/l), sodium chloride (5 g/l), disodium hydrogen phosphate (3.7 g/l) and potassium dihydrogen phosphate (1.5 g/l). The pH is 7 ± 0.2.

The initial water samples were prepared as a 1:10 dilution using buffer peptone water. Subsequently, ten-fold dilutions were conducted with the same medium (APHA, 1998). The prepared agar media were poured into Petri dishes with a diameter of 90 mm and a depth of 16.2 mm (Fisher Science Ltd., Loughborough, UK). Each dish contained about 20 ml of agar. According to standard methods, 100 µl from each dilution was plated into a petri dish in duplicates by gently swirling clockwise and anti-clockwise on the surface of the media using a sterilised spreader (Fisher Science Ltd., Loughborough, UK). The prepared dishes were incubated at 37° C for 24 h. Following the incubation period, bacteria colonies were counted on dishes containing between 30 and 300 colonies.

2.3. Fruit quality and analysis

Harvested chilli fruits from each plant were categorised according to the chilli classification scheme in Table 2, which has been adapted from Almuktar et al. (2015). The variables length, width, weight and bending were used for classifying the harvested fruits. The monetary value of the harvest for chilli plants were calculated according to estimated prices on the UK market in 2014.

Chilli fruits were harvested at different distances from the soil: 0–50 cm, 50–100 cm and more than 100 cm. All fruits were washed

Table 4
Comparison of the water quality of the inflow waters received by the vegetable pots.

Water type	TPH^a (µg/l)	COD^b (mg/l)	BOD^c (mg/l)	NH_4-N^d (mg/l)	NO_3-N^e (mg/l)	PO_4-P^f (mg/l)	SS^g (mg/l)	Turb^h (NTU)	pH (–)	EC^i (µs/cm)	Fe^j (mg/l)	K^k (mg/l)	Mn^l (mg/l)	SAR^m (me/l)^n
Filter1 outflow	100	107.9 ± 67.69	23.6 ± 3.58	7.4 ± 4.31	1.1 ± 1.16	3.5 ± 1.75	9.7 ± 4.50	12.6 ± 6.38	6.3 ± 0.34	432 ± 93.8	2.1 ± 2.00	9.6 ± 1.96	0.23 ± 0.157	2.4 ± 1.07
Filter2 outflow	0	44.8 ± 30.35	13.6 ± 3.68	6.3 ± 3.99	3.2 ± 0.39	3.2 ± 0.69	7.1 ± 1.81	7.1 ± 2.27	6.4 ± 0.18	395 ± 73.9	0.3 ± 0.30	8.0 ± 3.22	0.07 ± 0.081	1.8 ± 0.60
Filter3 outflow	69	75.9 ± 34.92	24.0 ± 5.60	5.1 ± 3.08	1.2 ± 1.28	3.2 ± 1.76	11.4 ± 3.36	10.6 ± 4.13	6.4 ± 0.27	453 ± 111.6	2.2 ± 1.59	10.0 ± 2.15	0.37 ± 0.154	1.7 ± 0.53
Filter4 outflow	0	50.4 ± 30.24	12.2 ± 4.32	4.7 ± 2.60	2.9 ± 1.94	3.1 ± 0.61	7.9 ± 1.53	6.9 ± 1.93	6.4 ± 0.33	477 ± 96.3	0.3 ± 0.16	3.9 ± 2.88	0.07 ± 0.085	2.4 ± 0.39
Filter5 outflow	14	154.9 ± 104.24	24.6 ± 7.81	15.3 ± 6.73	2.2 ± 1.86	4.8 ± 2.97	13.4 ± 4.26	12.1 ± 5.12	6.6 ± 0.45	654 ± 97.0	2.0 ± 1.35	14.2 ± 3.23	0.38 ± 0.198	2.2 ± 0.85
Filter6 outflow	0	53.0 ± 34.59	16.5 ± 7.23	12.6 ± 7.03	5.5 ± 2.78	4.4 ± 2.16	8.4 ± 1.07	7.2 ± 1.15	6.7 ± 0.15	768 ± 215.0	1.0 ± 0.81	13.4 ± 3.32	0.12 ± 0.157	3.1 ± 0.61
Filter7 outflow	0	43.8 ± 24.60	13.9 ± 2.61	5.1 ± 2.17	4.4 ± 1.70	3.1 ± 1.12	3.4 ± 2.96	5.5 ± 5.63	6.5	387 ± 152.8	0.9 ± 1.44	7.2 ± 1.10	0.16 ± 0.277	2.4 ± 0.47
Filter8 outflow	116	71.9 ± 55.72	14.1 ± 3.23	3.8 ± 2.65	4.3 ± 1.42	3.3 ± 0.85	3.7 ± 2.07	4.0 ± 0.62	6.5 ± 0.28	482 ± 120.2	0.5 ± 0.71	5.7 ± 4.18	0.09 ± 0.122	2.1 ± 0.44
Control A outflow	346	57.7 ± 18.73	13.4 ± 2.89	1.3 ± 0.87	0.4 ± 0.24	1.9 ± 0.73	7.6 ± 2.33	5.5 ± 1.10	6.6 ± 0.34	138 ± 60.1	0.2 ± 0.14	1.0 ± 0.52	0.11 ± 0.064	0.5 ± 0.15
Control B outflow	0	30.8 ± 20.00	9.8 ± 3.84	1.5 ± 0.96	0.4 ± 0.22	2.3 ± 0.84	6.2 ± 3.17	4.8 ± 1.72	7.2 ± 2.07	159 ± 22.9	0.1 ± 0.02	0.8 ± 0.85	0.04 ± 0.044	0.5 ± 0.14
Deionised water	nm^o	nm^o	7.6 ± 3.04	nm^o	nm^o	nm^o	1.3 ± 2.07	1.8 ± 0.64	5.4 ± 0.70	1.4 ± 0.36	0.1 ± 0.01	0.0 ± 0.00	0.08 ± 0.000	0.1 ± 0.15
Tap water (100%)	nm^o	nm^o	5.2 ± 1.43	nm^o	nm^o	nm^o	1.3 ± 2.07	3.1 ± 0.32	6.3 ± 0.65	89 ± 14.0	3.4 ± 4.72	0.4 ± 0.14	0.06 ± 0.040	0.8 ± 0.15
Tap water with fertiliser (0.7 ml/l)	nm^o	nm^o	7.8 ± 3.78	nm^o	nm^o	nm^o	0.9 ± 0.99	2.9 ± 0.80	6.1 ± 0.15	248 ± 60.1	0.1 ± 0.00	19.2 ± 0.00	0.09 ± 0.000	0.8
Wastewater (20%); tap water (80%)	nm^o	nm^o	36.5 ± 18.01	nm^o	nm^o	nm^o	33.5 ± 9.04	9.8 ± 8.65	7.2 ± 0.26	163 ± 91.9	0.5 ± 0.49	3.4 ± 0.87	0.07 ± 0.056	1.7 ± 0.59
Wastewater (100%)	nm^o	266.0 ± 56.88	144.4 ± 88.02	40.4 ± 15.19	3.0 ± 2.12	14.3 ± 2.63	103.1 ± 52.80	70.5 ± 56.69	7.5 ± 0.28	647 ± 224.0	3.8 ± 5.13	14.1 ± 4.25	0.17 ± 0.141	2.8 ± 0.62

Table 5
Overview of environmental boundary conditions associated with the vegetable pots.

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number of records
Illuminance (one-off record during lab visit)	lux	5587	5501.1	640	97500	1350
Temperature (one-off record during lab visit)	°C	25.4	2.12	18.0	32.2	582
Temperature (minimum within a 24 h period)	°C	20.8	1.97	17.3	25.1	81
Temperature (maximum within a 24 h period)	°C	26.8	2.59	18.4	33.3	81
Relative humidity (one-off record during lab visit)	%	49	11.7	27	73	576
Relative humidity (minimum within a 24 h period)	%	35	7.1	32	90	81
Relative humidity (maximum within a 24 h period)	%	55	12.5	25	72	81

with 50 ml of distilled water using an ultrasonic Sonicor Table Top Cleaner machine (Sonicor Inc.-New York, NY, USA). Collected washing solutions were analysed for bacterial contamination according to standard methods (APHA, 1998). For fruits, which were directly harvested from any position of the plant, skin was manually separated from the fruit flesh by using a scalpel (Lustig et al., 2014). The two proportions of the fruits were analysed for their microbiological contamination. One gram of fruit skin or flesh was homogenised with 9 ml of buffer peptone water into a Stomacher Lab-Blender 80 (Gemini BV, Apeldoorn, The Netherlands), and then mixed for one minute. The mixture was allowed to settle for two to three minutes. A series of dilution was subsequently carried out for the agar plates according to APHA (1998).

2.4. Soil microbiological testing

Undisturbed soil samples were collected with sterile equipment and consumables for subsequent bacterial testing (Lopez et al., 2006). Samples were collected from the top 10 cm of soil, and 1 g of soil sample was added to 9 ml of buffer peptone water for subsequent bacteria extraction in a sterile blender jar. The mixture was then blended for two minutes at low speed (8000 rpm) according to APHA (1998). The appropriate decimal dilutions of the homogenized slurry were prepared quickly to minimize settling. The solution was subsequently poured into the prepared agar located within the Petri dishes. Counting of the developed colonies was undertaken according to APHA (1998).

2.5. Environmental monitoring

Light measurement readings were undertaken using the lux meter ATP-DT-1300 for the range between 200 lx and 50,000 lx (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, Cheshire, England, UK) just above the top of the plants showed values between 3855 and 12,316 lx (mean of 6921 lx) close the plants. The humidity and temperature were controlled with the support of a combined Thermometer-Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The humidity measuring range was between 20 and 99%. The corresponding precision was ±4% between 35 and 75%.

The temperature was controlled by the electrical heater Rhino H029400 TQ3 2.8 kW Thermo Quartz Infrared Heater 230 V supplied by Express Tools Ltd. (Alton Road, Bournemouth, England, UK). The humidity was artificially increased by a varying number of humidity meters (Challenge 3.0 L Ultrasonic Humidifier; Argos, Avebury Boulevard, Central Milton Keynes, England, UK).

2.6. Selected fruiting vegetables

Chilli (De Cayenne) was supplied by B&Q plc (Chandlers Ford, Hampshire, England, UK) as part of their verve brand. The verve product code was 362387. All seeds were bought on 14 September 2013.

2.7. Growing the Chillies

The Chilli seeds were first planted in shallow seed trays for about one week, and subsequently replanted (second planting) in larger nursery pots. The third and final planting took place 28 days after the second planting on 8 November 2013. Table 3 outlines the experimental set-up. The Chilli was planted individually into 10 l plastic and round plant pots provided by scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK). The plant pots dimensions were as follows: height of 22.0 cm, bottom diameter of 22.0 cm and top diameter of 28.5 cm. The top 2 cm were left unplanted for both sand-based and soil-based pots. However, sand-based plants were planted to a depth of 20.0 cm. In comparison, soil-based plants were planted to a depth of 17.5 cm. and covered by a further 2.5 cm of bark (see above). The plant pots remained indoors in the laboratory characterised above.

2.8. Growing conditions

Some plants were fed with a liquid and concentrated fruit and vegetable fertiliser from the B&Q plc verve range. The fertiliser had a nitrogen to phosphorus to potassium ratio of 4:4:4 according to the EC fertiliser solution for the UK. The total nitrogen component was 4%. Nitric nitrogen and ureic nitrogen parts were 1.1 and 2.1%, respectively. Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) made up 4% each of the solution, but the corresponding P and K content were only 1.7 and 3.3%, respectively. Moreover, the fertiliser contained also trace elements (names not listed) of unspecified quantities. Liquid fertiliser was added to the inflow water as specified.

3. Results

Table 4 shows the inflow water quality received by the plants. Note that the wetland effluent was used as the influent for the vegetable pots. Table 5 provides an overview of the environmental boundary conditions. The microbiological analysis results of irrigation water samples are outlined in Table 6.

Fig. 2 indicates the monetary value of the harvest for Chilli plants. Moreover, Figs. 3 and 4 provide an overview of fruits and classes A–C, and D–E, respectively. Water consumption data associated with Chillies are summarised in Fig. 5. The bacteriological results for those Chilli fruits, which were harvested at a plant height of below 50 cm, are shown in Fig. 6. In comparison, Table 7 outlines the bacteriological results for the soil analysis.

4. Discussion

4.1. Water quality analysis

Highly fluctuating values for TPH were observed in the outflow waters obtained from Filters 1, 3, 5, and 8, and Control A (Table 4). The TPH concentrations followed this order: Control A > Filter 8 > Filter 1 > Filter 3 > Filter 5. Chemical oxygen demand values

Table 6
Microbiological examination for the irrigation water (colony forming units (CFU)/100 ml)*.

Filter number	Mean	Standard deviation	Minimum	Maximum
Filter 1 outflow				
Total coliforms	13 500	119 582.2	19 000	284 000
Escherichia coli	1833	2994.4	0	7000
Streptococci spp.	114 833	178 628.6	0	370 000
Salmonella spp.	161 500	137 004.7	25 000	366 000
Filter 2 outflow				
Total coliforms	3833	1472.0	2000	6000
Escherichia coli	0	0.0	0	0
Streptococci spp.	1333	3266.0	0	8000
Salmonella spp.	3000	1673.3	0	5000
Filter 3 outflow				
Total coliforms	118 167	108 147.0	0	243 000
Escherichia coli	1167	1834.8	0	4000
Streptococci spp.	70 333	119 869.4	0	290 000
Salmonella spp.	114 167	113 520.8	0	266 000
Filter 4 outflow				
Total coliforms	1333	816.5	0	2000
Escherichia coli	0	0.0	0	0
Streptococci spp.	4833	7139.1	0	15 000
Salmonella spp.	3167	1940.8	2000	7000
Filter 5 outflow				
Total coliforms	194 833	131 099.1	21 000	387 000
Escherichia coli	5667	8140.4	0	18 000
Streptococci spp.	24 667	42 949.6	0	105 000
Salmonella spp.	232 500	78 025.0	123 000	317 000
Filter 6 outflow				
Total coliforms	19 667	16 033.3	4000	48 000
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	25 167	17 971.3	6000	58 000
Filter 7 outflow				
Total coliforms	4667	4366.5	0	12 000
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	12 000	15 126.1	1000	32 000
Filter 8 outflow				
Total coliforms	5167	5492.4	0	15 000
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	6833	5419.1	1000	16 000
Control A outflow				
Total coliforms	47 167	60 832.3	0	148 000
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	71 833	74 831.6	0	181 000
Control B outflow				
Total coliforms	5000	10 353.7	0	26 000
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	11 500	17 952.7	0	38 000
Wastewater (20%); tap water (80%)				
Total coliforms	121 250	17 876.9	106 000	147 000
Escherichia coli	3000	2582.0	0	6000
Streptococci spp.	750	500.0	0	1000
Salmonella spp.	131 000	45 526.5	81 000	173 000
Wastewater (100%)				
Total coliforms	113 167	84 383.5	3000	205 000
Escherichia coli	8000	10 526.2	0	22 000
Streptococci spp.	2667	2658.3	0	7000
Salmonella spp.	202 167	171 352.8	18 000	467 000

*0 entries indicate absolutely no growth on the plate after incubation.

were the highest for Filter 5. Chemical oxygen demand values were the highest for preliminary treated wastewater followed by Filter 5 and Filter 1. In contrast, the lowest values were noted for Control B. Filters 3 and 8 had relatively similar COD concentrations. Control A had higher COD values than Filter 6. No differences in COD values were noted for Filters 2, 4 and 7.

The five-day BOD (Table 4) was more than the maximum thresholds value of 30 mg/l (FAO, 2003) in preliminary treated wastewater followed by wastewater samples, which were diluted with 80% of tap water. In comparison, the lowest five-day BOD was observed for tap water deionized water and Control B. High concentrations of ammonia–nitrogen, which exceeded the threshold of 5 mg/l (FAO, 1994), were noted for preliminary treated wastewater followed by those for Filters 5, 6, 1 and 2.

Table 4 shows that nitrate–nitrogen for all filters outflow waters was less than the maximum thresholds value of 30 mg/l (Pescod, 1992). Based on the recommended threshold of 2 mg/l for ortho-phosphate–phosphorus (FAO, 1994), the preliminary treated wastewater and outflow waters from all wetland filters (with the exception of Controls A and B) were associated with too high ortho-phosphate–phosphorus concentrations.

The highest value for SS (Table 4) was noted for preliminary treated wastewater and wastewater (diluted with 80% of tap) samples, followed by outflow water obtained from Filters 5 and 3. In contrast, the lowest values were observed for tap water with fertilizer and deionised water followed by outflow water from Filter 7. Turbidity was high for preliminary treated wastewater followed by the outflow waters obtained from Filters 1 and 5. Filter 8 had the lowest turbidity values.

The pH values for all filter outflows (Table 4) were within the normal range between 6.0 and 8.5 (Pescod, 1992). The electronic conductivity for all outflow waters was less than the maximum threshold value of 3000 μs/cm (FAO, 2003).

Furthermore, the sodium adsorption ratio (SAR) values for all outflows were within the normal range between 0 milliequivalent per litre (me/l) and 15 me/l (FAO, 1994). Table 4 also provides an overview of the ICP–OES analysis for selected elements in the irrigation water. Based on the recommended threshold of 2 mg/l for potassium (FAO, 1994), the outflow water from all wetland filters (with the exception of Controls A and B) were associated with too high potassium concentrations. Furthermore, high concentrations were also observed for preliminary treated wastewater and wastewater samples, which were diluted with up to 80% of tap water. Results for Filters 1, 3 and 5 show relatively high manganese concentrations, which exceeded the threshold of 0.2 mg/l (Pescod, 1992; FAO, 2003).

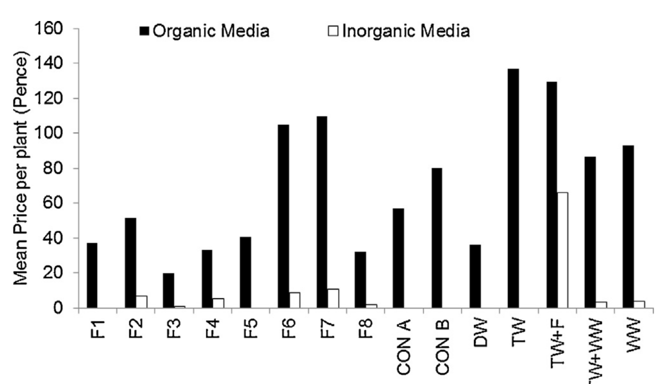

Fig. 2. Overview of the outcome of the Chilli harvest (17 September 2014) classification scheme according to Table 2. The lowest variable class entry for any individual fruit assessment determined the final class. Only the following variables were used to classify fruits: length, width, weight and bending.

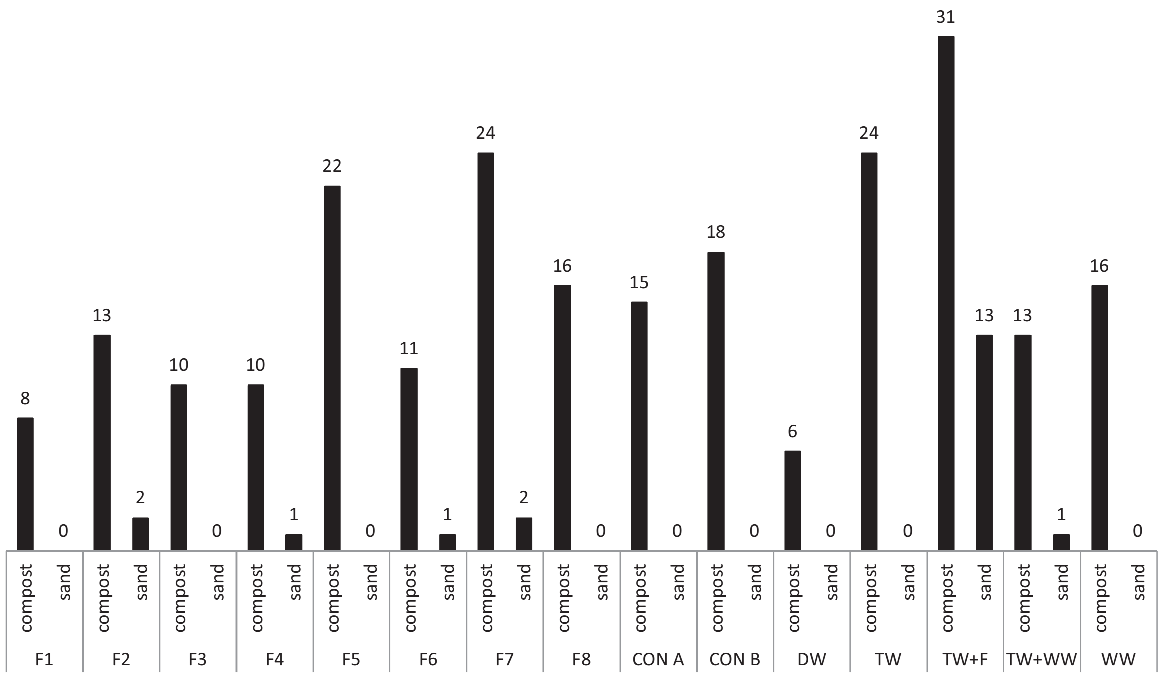

Fig. 3. Overview of harvested Chilli fruits for classes A– C combined.

According to Nickels (2012), temperature measurements (Table 5) for this experiment were within the recommended ranges for different growth stages of Chillies. High temperature has a direct impact on the growth of potentially pathogenic organisms.

Based on the maximum value for total coliforms (1000 CFU/100 ml) for the irrigation of crops, which are often eaten uncooked (WHO, 1989; FAO, 2003), the outflow waters from all wetland filters were associated with too high contamination by total coliforms (Table 6). Furthermore, high contamination by total coliforms was also observed for preliminary treated wastewater and wastewater samples, which were diluted with up to 80% of tap water.

Total coliforms were removed well from the outflow water of standard wetland filters, which were not contaminated with hydrocarbons. This finding confirmed research undertaken by Cui et al. (2003), reporting that the removal rates for total heterotrophic bacteria and total coliforms when using vertical-flow bed systems were between 80 and 90%, and between 85 and 96%, respectively.

Preliminary treated wastewater was associated with the highest contamination by *E. coli* (8000 CFU/100 ml) followed by outflow water from Filter 5 and wastewater samples, which were diluted with up to 80% of tap water. Outflow waters from Filters 1 and 3 were associated with a similar numbers of *E. coli*

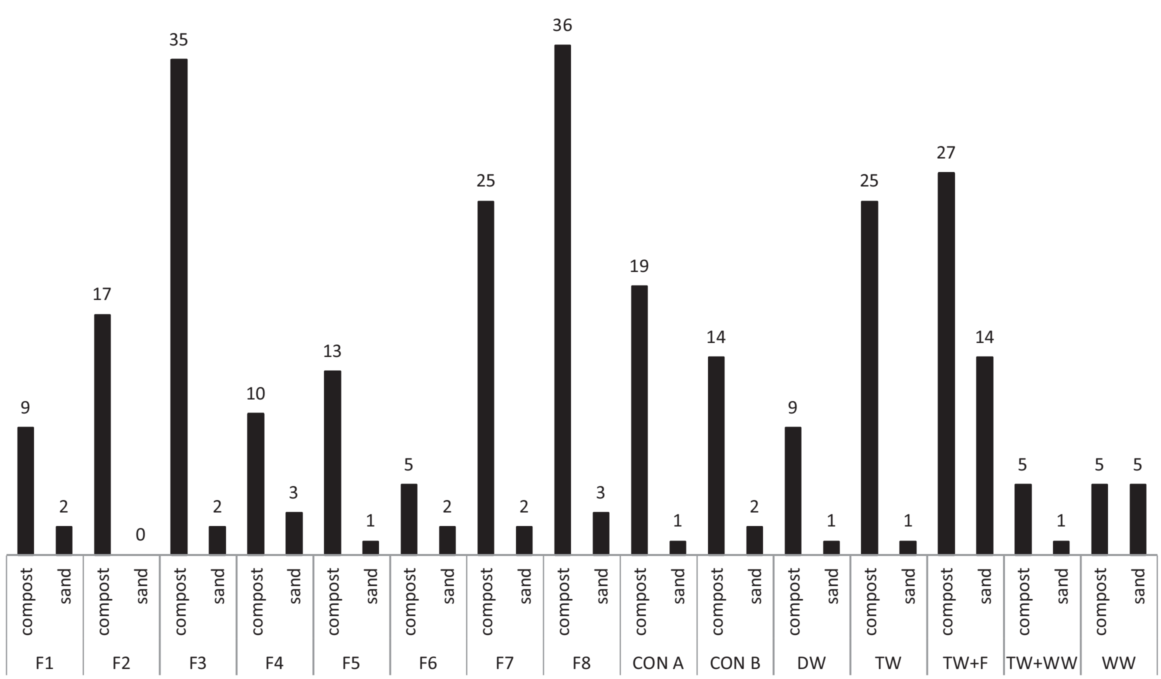

Fig. 4. Overview of harvested Chilli fruits for classes D and E combined.

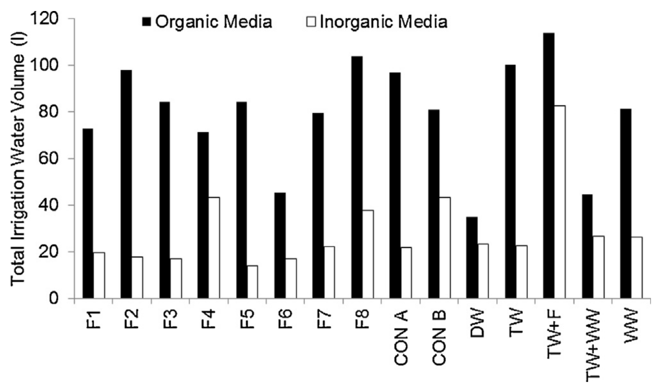

Fig. 5. Overview of the total water volumes for the Chilli plants.

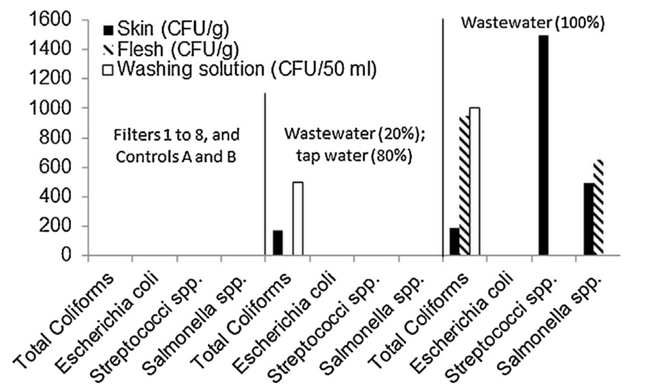

Fig. 6. Contamination of fruits (only detected for locations below a plant height of 50 cm) by bacteria as a function of water sources.

contamination. No contamination by *E. coli* was detected for outflow waters from other wetland filters. However, this result contradicted findings reported by Cirelli et al. (2012), who presented results of a reuse scenario where municipal wastewater was treated by constructed wetlands (tertiary treatment step), and reused for the supply of irrigation water for vegetables in Eastern Sicily, Italy. They found increased numbers of *E. coli* in the irrigation water, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 ml for secondary treated urban wastewater effluents.

The highest contamination by *Streptococcus* spp. was associated with Filter 1 outflow water followed by those for Filters 3 and 5 (Table 6). Filter 4 had a higher *Streptococci* contamination than Filter 2. Furthermore, preliminary treated wastewater was observed with higher contamination by *Streptococci* than wastewater samples, which were diluted with up to 80% of tap water.

The highest *Salmonellae* counting was observed in the outflow water from Filter 5 followed by preliminary treated wastewater (Table 6). Filter 1 outflow water was associated with higher *Salmonellae* contamination than the water from Filter 3. Furthermore, *Salmonella* spp. contamination in Control A outflow water was higher than that associated with Control B. *Salmonellae* were removed well from the outflow water of the wetland systems. This confirms good bacteria removal efficiency of vertical-flow bed systems as reported by Cui et al. (2003).

Table 6 shows that the microbial contamination of outflow water from wetland filters contaminated with hydrocarbons was higher than those from standard filters (uncontaminated). This confirms findings by Benedek et al. (2013), who studied the impact of long-term TPH, volatile petroleum hydrocarbons, total alkyl benzenes and polycyclic aromatic hydrocarbons on the structure of

Table 7
Microbiological results for soil irrigated by different water types (colony forming units (CFU)/g).

Filter No.	Mean	Standard deviation	Minimum	Maximum
Filter 1 outflow				
Total coliforms	648	101.5	570	790
Escherichia coli	0	0.0	0	0
Streptococci spp.	0	0.0	0	0
Salmonella spp.	270	80.4	190	380
Filter 2 outflow				
Total coliforms	845	333.3	490	1270
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	405	159.3	260	630
Filter 3 outflow				
Total coliforms	663	418.8	80	1030
Escherichia coli	5	10.0	0	20
Streptococci	0	0.0	0	0
Salmonella	405	165.0	290	640
Filter 4 outflow				
Total coliforms	3763	1320.4	2210	5400
Escherichia coli	0	0.0	0	0
Streptococci	23	22.2	0	50
Salmonella	2248	1661.2	220	4280
Filter 5 outflow				
Total coliforms	473	292.6	90	730
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	180	126.2	70	350
Filter 6 outflow				
Total coliforms	910	706.3	210	1720
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	1520	171.5	1280	1680
Filter 7 outflow				
Total coliforms	1503	1160.5	320	2810
Escherichia coli	5	5.8	0	10
Streptococci	0	0.0	0	0
Salmonella	963	492.10	560	1610
Filter 8 outflow				
Total coliforms	1113	590.1	450	1620
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	713	495.7	240	1230
Control A outflow				
Total coliforms	1988	939.9	1180	2940
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	2190	1114.6	1130	3530
Control B outflow				
Total coliforms	1293	645.9	670	2150
Escherichia coli	0	0.0	0	0
Streptococci	0	0.0	0	0
Salmonella	320	73.5	220	380
Wastewater (20%); tap water (80%)				
Total coliforms	983	267.3	610	1200
Escherichia coli	3	5.0	0	10
Streptococci	20	33.7	0	70
Salmonella	763	26.3	740	800
Wastewater (100%)				
Total coliforms	1118	293.9	890	1540
Escherichia coli	10	8.2	0	20
Streptococci	3	5.0	0	10
Salmonella	1760	756.6	1130	2860

*0 entries indicate absolutely no growth on the plate after incubation.

bacterial communities. The results indicated that a very high concentration of TPH affected positively the diversity of hydrocarbon-degrading bacteria. Furthermore, wetland filters fed with undiluted inflow water (Table 1) showed higher microbial contamination levels than those fed with diluted inflow. This confirms results by Sani et al. (2013) that high rate filters tend to be overloaded.

Finally, the impacts of aggregate size, contact time and resting time on outflow water quality parameters were statistically insignificant. However, the duration of the experiment (growth period of Chillies until harvest) might have been too short.

4.2. Fear of consuming contaminated fruits

The potential fear and disgust by consumers of eating microbiologically contaminated vegetables decreases considerably if vegetables are cooked for a long time at considerable heat. Menegaki et al. (2009) assessed the fear and disgust factors by comparing the effects of descriptive terms on farmers' willingness to use and pay for recycled water for irrigation and consumers' willingness to use and pay for products irrigated with recycled pre-treated wastewater. Treated effluent from wastewater treatment works was described as 'recycled water' for one experimental test group and as 'treated wastewater' for another fraction. Although both terms describe the same commodity, the willingness to use the water was reliably higher with the 'recycled water' descriptor for consumers and farmers. However, the descriptor affected the willingness to pay only in the consumer cohort. Both farmers and consumers who were unwilling to use recycled water referred to feelings of disgust (32%) as the predominant cause of their rejection (Menekaki et al., 2009). However, Chillies are usually cooked, and the risk of microbial contamination is therefore very low.

Table 2 shows a novel harvest classification scheme for Chillies. Only the higher classes are of great commercial interest. However, the estimated prices are dependent on global commodity market developments.

The highest number of fruits categorised as Class A were harvested from plants grown in organic media and watered with tap water followed by those grown in organic media and watered by Filter 7 (Figs. 3 and 4). However, Filter 3 was associated with the highest fruit numbers, which received low category classifications (i.e., $C = 10$, $D = 26$, and $E = 9$). The highest mean price of harvested fruits is also associated with tap water followed by Filter 7. Findings showed that the productivity of Chillies in terms of harvest was independent of the wastewater consumption (see Fig. 5).

No fruits harvested at a plant height of equal or above 50 cm were associated with microbial contamination. Fig. 6 indicates that microbial contamination of Chillies was overall insignificant. Findings also show that no microbiological contamination was recorded for skin, flesh and washing solutions for the fruits harvested from plants irrigated with outflow water obtained from wetlands filters. In contrast, the fruits harvested from plants irrigated with wastewater which was diluted by 80% with tap water, and with preliminary treated wastewater showed high contamination numbers with total coliforms. Furthermore, high contamination with *Streptococcus* spp. and *Salmonella* spp. were recorded for Chilli fruits harvested from plants irrigated by preliminary treated wastewater.

The approximate number of Chillies harvested below 50 cm was only about 5% of the total harvest for most plants. The results showed that there is no microbial contamination of Chillies located higher up on the plant branches. This can be explained by the relatively long distance between the fruits and the potentially contaminated soil (Cirelli et al., 2012). Moreover, vegetable pots receiving wastewater treated with wetlands acting as a biological filter bed can be considered as safer than those receiving only preliminary treated wastewater.

4.3. Microbiological results of the soil analysis

Findings indicate that the highest contamination by total coliforms was associated with the soil irrigated by outflow water obtained from Filter 4, followed by those for Control A and Filters 7 (Table 7). In contrast, the lowest soil contamination by total coliforms was found in soil irrigated by outflow water associated with Filter 5. Soil irrigated by water treated by Filter 2 was more contaminated by total coliforms compared to Filter 1. Furthermore, soil irrigated by harvested water from Filter 8 had similar contamination in terms of total coliforms compared to preliminary treated wastewater. The contamination by *E. coli* in soil irrigated with outflow water from wetland filters was not observed; with the exception of those waters associated with Filters 3 and 7, which were similarly contaminated. The highest contamination by *E. coli* was recorded for soil irrigated with preliminary treated wastewater. In contrast, the lowest contamination by *E. coli* was observed in soil irrigated with wastewater, which was diluted by 80% with tap water. Contamination by *Streptococci* was not observed for soil irrigated with treated wastewater obtained from all wetland filters; with the exception of Filter 4. Soil irrigated with wastewater, which was diluted by 80% with tap water, was reported to have higher contamination recordings by *Streptococci* than those soils irrigated by preliminary treated wastewater.

The highest contamination recordings by *Salmonellae* were observed in soil irrigated with water harvested from Filter 4 followed by those soils irrigated by waters associated with Control A and Filter 6. In contrast, the lowest contamination by *Salmonella* spp. was observed in soil irrigated with outflow water that came from Filter 5. Furthermore, soils irrigated with waters from Filters 7 and 2 were more contaminated by *Salmonella* spp. compared to those irrigated by waters harvested from Filters 8 and 1, respectively.

5. Conclusions and recommendations

The experiment shows that Chillies can be grown successfully using wastewater treated by constructed wetlands. The highest number of fruits at class A is associated with tap water and an organic growth medium. Low fruit numbers correlate well with inorganic growth media. Findings indicate those nutrients concentrations supplied to the Chillies by a combination of compost and treated wastewater are usually too high to produce a good harvest. However, as the compost is depleted of nutrients such as nitrogen after about one year, the harvest increased for pots that received pre-treated wastewater in comparison to those pots depending only on the nutrients associated with the compost. Filters associated with hydrocarbon contamination were usually associated with a poor harvest. The productivity of Chillies in terms of harvest was independent of the wastewater consumption. Nevertheless, higher foliage production due to excess nutrients and trace minerals required more water. A high yield was related with the most suitable provision of nutrients and trace elements. Chillies harvested at a plant height below 50 cm were often contaminated by potentially pathogenic microbes. However, the overall proportion of contaminated harvest was less than 5%. No bacteriological contamination was detected for any Chilli fruits harvested from a plant height of equal or above 50 cm.

Further research with other vegetables receiving recycled treated wastewater from other wastewater treatment units should also be undertaken to select the best and most cost-effective technology in order to obtain the greatest crop yield. The long-term impact of soil enrichment with contaminants such as heavy metals is also worth consideration.

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Mineral and biological contamination of soil and *Capsicum annuum* irrigated with recycled domestic wastewater

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ABSTRACT

Due to water scarcity in many arid countries, there is considerable interest in recycling wastewater streams such as treated urban wastewater for irrigation in the agricultural sector. The aim of this study is to assess the contamination of soil and *Capsicum annuum* (grown in pots) irrigated by domestic wastewaters treated by different wetland types between September 2013 and September 2014. The objectives were to assess (a) the suitability of the irrigation water for growth when using recycled wastewater contaminated by trace minerals and pathogens, (b) the impact of differently treated wastewaters on soil and fruits as a function of the wetland type, and (c) the marketable yield of the harvest as a function of mineral and biological contamination risk. Ortho-phosphate-phosphorus, ammonia–nitrogen, potassium and manganese concentrations in the irrigation water considerably exceeded the thresholds. High contamination levels by total coliforms, *Salmonella* spp. and *Streptococcus* spp. were detected. No mineral contamination was observed in the soils due to irrigation with treated wastewater. Results showed that slight to moderate zinc contamination was detected in some vegetables. Potassium accumulation in the yield showed the highest values followed by zinc. In contrast, the lowest mineral accumulation of the yield was observed for iron. No bacterial contamination was detected for fruits harvested from plants irrigated by wetland outflow water. In contrast, fruits harvested from those plants irrigated by preliminary treated wastewater showed high contamination by total coliforms, *Streptococcus* spp. and *Salmonella* spp. especially for fruits, which were located close to the contaminated soil surface. However, findings indicate that vegetables receiving wastewater treated with wetlands can be considered as safe compared to those receiving only preliminary treated wastewater. High yields in terms of economic return were associated with tap water and an organic growth medium, and a wetland with a small aggregate size and a low contact time.

1. Introduction

Microbiological and biotechnological solutions to global problems such as water scarcity, food shortages and water resources pollution are required. This paper emphasizes the role of biotechnological advances for developing regions where food production using recycled pre-treated wastewater is of interest. Advantages associated with wastewater recycling include the supply of nutrients and trace minerals to plants, potentially leading to higher yields and a decrease in the demand for inorganic fertilizers. However, there are many expected problems associated with recycling of wastewater for irrigation, such assoil salinization and reduced permeability, accumulation of nutrients and potential toxic elements in soil and plants irrigated with wastewater (FAO, 2003; Pinto et al., 2010). Moreover, the highly microbiologically contaminated wastewater causes a reduction in the overall crop yield and quality in addition to the contamination of crops by pathogens and intestinal helminths. However, high yields can be achieved by using pre-treated wastewater for irrigation of various crops under controlled environmental conditions (Zavadil, 2009).

Constructed treatment wetlands are engineered wastewater purification systems that encompass biological, chemical and physical processes, which are all similar to processes occurring in natural treatment wetlands. They are implemented for environmental pollution control to treat a variety of wastewaters including industrial effluents, urban and agricultural runoff, animal wastewaters as well as sludge and mine drainage (Sani et al., 2013; Scholz, 2010).

The evaluation of the effects of treated wastewater reuse on crops intended for human consumption is of particular interest. For example, Cirelli et al. (2012) presented the results of a reuse scenario where tertiary-treated municipal wastewater using

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a constructed wetland was supplied for irrigation of vegetables in Eastern Sicily, Italy. They concluded that the irrigation water contained increased numbers of *Escherichia coli*, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 ml for secondary treated urban wastewater effluents. Chary et al. (2008) assessed the heavy metal pollution of soil irrigated by sewage and wastewater. The results showed that the partitioning patterns of soil revealed high levels of zinc, chromium and copper associated with labile fractions making them more mobile and plant available. Furthermore, Benedek et al. (2013) studied the impact of long-term total petroleum hydrocarbons (TPH) on the structure of bacterial communities of four different contaminated soil samples. They concluded that a very high amount of TPH affected positively the diversity of hydrocarbon-degrading bacteria.

This article fills gaps in knowledge and understanding by assessing the impact of different wetland (some contaminated with diesel) system designs in terms of their suitability in providing irrigation water for an example crop, which should be safe for human consumption, lead to a good economic return and its corresponding water management should not result in soil contamination.

The findings of this paper are based on a follow-up study of work previously reported by Almuktar et al. (2015a,b). Almuktar et al. (2015a) focused on the visual appearance of Chillies and Sweet Pepper when irrigated by outflow waters from different wetland systems. Mineral surpluses and deficiencies could be identified by eye. The follow-up paper by Almuktar et al. (2015b) covered the development stages of Chillies and Sweet Peppers (buds, flowers and fruits) and the subsequent harvest of a fraction of the fruits as a function of general boundary conditions. In contrast, this paper focuses only on Chillies and their marketable yield as a function of specified mineral and biological (selected pathogens) contamination of both the soil and fruits.

Effluent from different types of wetland systems treating domestic wastewater was selected to irrigate an example crop. Some of the wetlands received standard wastewater while the others received wastewater that was subject to a one-off diesel fuel spill. The treated wastewater from all wetland types was recycled for the irrigation of Chillies (*Capsicum annuum*), which are commonly seen as popular, relatively expensive and easy-to-grow vegetables. The authors concentrated on Chilli in this study, because it is an easy-to-grow and relatively cost-effective plant with high nutritional value; also, Chilli can be grown in greenhouses in the UK (Nickels, 2012; Almuktar et al., 2015a,b). This experiment may provide the scientific justification for integrating treatment wetlands into agricultural food production.

The aim of this study is to assess the mineral and biological contamination of soil and Chilli fruits irrigated by recycled wastewaters treated by different wetland types. The objectives were to assess (a) the suitability of the irrigation water for long-term growth when using recycled wastewater contaminated by trace minerals and pathogens, (b) the impact of differently treated wastewaters on soil and fruits as a function of the wetland type as well as its operation and management, and (c) the marketable yield of the harvest as a function of mineral and biological contamination risk.

2. Methodology

2.1. Experimental set-up and operation

The experimental set-up of the wetland system and the planting regime have previously been discussed by Almuktar et al. (2015a,b). For the purpose of this paper, the vertical-flow constructed wetland system was operated between 27 June 2011 and 25 September 2014. The set-up includes two filters that are essentially controls receiving clean de-chlorinated tap water (Fig. 1). Table 1 indicates an overview of the statistical experimental set-up (complete randomised design) used to test the impact of four variables: aggregate size and inflow loading rate as well as contact and resting times. Filters 1 and 2 compared to Filters 3 and 4 test the influence of a larger aggregate diameter. Filters 5 and 6 compared to Filters 3 and 4 check the impact of a higher loading rate. The application of a lower contact rate is tested if Filter 7 is compared with Filters 3 and 4. Finally, a lower resting time is the difference between Filters 7 and 8; the ten laboratory-scale vertical-flow constructed wetlands were constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm. The filters were filled with siliceous (minimum of 30%) pea gravel up to a depth of 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). The main outlet valve is located at the bottom of each constructed wetland system (Sani et al., 2013).

Fig. 1. Experimental set-up of wetland filters as outlined in Table 1.

The preliminary treated urban wastewater used for the inflow water was obtained from the Davyhulme Sewage works, one of the largest wastewater treatment plants in Europe, operated by the water company United Utilities in Greater Manchester. In order to simulate diesel fuel (100% pure; no additives) spills, 130 g of diesel were poured into Filters 1, 3 and 5, and into Control A on 26 September 2013. The fuel was obtained from a petrol station operated by Tesco Extra (Pendleton Way, Salford, UK). Aqua Medic Titan chillers (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were used to maintain the root system and debris layer of all wetland systems at semi-natural below-surface temperatures of about 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located (Sani et al., 2013).

2.2. Water, soil and vegetables quality analysis

Table 2 shows the inflow water quality received by the plants between September 2013 and September 2014. Note that the wetland effluent was used as the influent for the vegetable pots. The plants were visually monitored to check the soil moisture content empirically on a daily basis. If the top soil was dry, sufficient irrigation water was carefully added (without splashing) to the pots until a few drops of water drained out of the pot into a saucer, which was directly located below the pot to capture drainage. The volumes of irrigation water required were recorded.

The total petroleum hydrocarbon concentrations followed this order: Control A > Filter 8 > Filter 1 > Filter 3 > Filter 5. Routine water quality sampling was carried out according to the standard methods for examination of water and wastewater of the American Public Health Association (APHA, 2005). The analysis of water samples for nutrients and trace elements were performed using a Varian 720-ESInductively Coupled Plasma–Optical Emission Spec-

Table 1
Comparison of the experimental vertical-flow wetland set-up (after Almuktar et al. (2015a,b)).

Filters	Design variables					
	Aggregate diameter (mm)	Contact time (h)	Resting time (h)	Chemical oxygen demand (mg/L)	Annually treated volumes of wastewater (L/a)	Diesel spill[a]
Filter 1	20	72	48	123.3	470	Yes
Filter 2	20	72	48	123.3	470	No
Filter 3	10	72	48	123.3	470	Yes
Filter 4	10	72	48	123.3	470	No
Filter 5	10	72	48	244.7	470	Yes
Filter 6	10	72	48	244.7	470	No
Filter 7	10	36	48	123.3	624	No
Filter 8	10	36	24	123.3	858	No
Control A	10	72	48	2.3	470	Yes
Control B	10	72	48	2.3	470	No

[a] On 26 September 2013, 130 g of diesel (equivalent to an inflow concentration of 20 g/L) have been added.

trometer (ICP–OES; Agilent Technologies UK Ltd., Wharfedale Road, Wokingham, Berkshire, UK) according to EPA (1994). At the end of the experiment, soil samples were taken by a soil auger until a depth of 20 cm was reached. Soil mineral content analysis was undertaken by using a Varian 720-ESInductively Coupled Plasma–Optical Emission Spectrometer according to Chary et al. (2008). After finishing the harvest, fruits were selected randomly from each treatment and analysed for mineral content using a Varian 720-ESInductively Coupled Plasma–Optical Emission according to Plank (1992).

The microbial test (total coliforms, faecal coliforms, *E. coli*, faecal *Streptococcus* spp. and *Salmonella* spp.) for water, soil and vegetables (skin, flesh and washing solution harvested at different distances from the soil: 0 to 50 cm, 50 to 100 cm and more than 100 cm) was undertaken using aseptic pour plate techniques according to standard methods (APHA, 2005).

2.3. Environmental monitoring

As previously reported by Almuktar et al. (2015a,b), light conditions were controlled with the help of the LUX meter ATP-DT-1300 (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, Cheshire, UK). Humidity and temperature were controlled with the support of a combined Thermometer–Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The humidity in the laboratory was artificially elevated by a varying number of humidity meters (Challenge 3.0 L Ultrasonic Humidifier; Argos, Avebury Boulevard, Central Milton Keynes, England, UK) to create more realistic growing conditions. The mean, minimum and maximum relative humidity values were 49%, 27% and 73%, respectively. Laboratory temperature records (average of 25.4 °C, minimum of 18.0 °C and maximum of 32.2 °C) were within the recommended range for growing Chillies (Haifa Chemicals, 2014).

2.4. Growing the vegetables

Chilli (De Cayenne) was supplied by B&Q plc (Chandlers Ford, Hampshire, England, UK) as part of their verve brand (Almuktar et al., 2015a,b). The Chilli seeds were first planted in shallow seed trays for about one week, and subsequently replanted (second planting) in larger nursery pots. The third and final planting took place 28 days after the second planting on 8 November 2013, following the supplier instructions. Table 3 outlines the experimental set-up according to complete randomised design principles; e.g., treatments 1 and 2 in Table 3 are different by only one variable (presence or absence of a diesel spill; Table 1). Three out of ninety plants were randomly chosen for each of the two treatments. In addition to the irrigation waters obtained from the wetland systems (Table 1), some plants were irrigated with other water types for comparison. For example, deionised water and tap water were used to monitor the depletion of nutrients and trace elements supplied by the organic media. Tap water spiked with fertiliser was used to assess the impact of artificial fertiliser on growth. Furthermore, real and diluted wastewaters were used to study the impact of high nutrients and trace elements on plant growth and production.

The Chilli was planted individually into 10 L plastic and round plant pots (Table 3) provided by scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK). The plant pots dimensions were as follows: height of 22.0 cm, bottom diameter of 22.0 cm and top diameter of 28.5 cm. The top 2 cm were left unplanted for both sand-based and soil-based pots. Sand-based plants were planted to a depth of 20.0 cm. In comparison, soil-based plants were planted to a depth of 17.5 cm. and covered by a further 2.5 cm of bark. The plant pots remained indoors in the laboratory characterised above for running an experiment under controlled conditions.

Some plants (Table 3) were fed with a liquid and concentrated fruit and vegetable fertiliser from the B&Q plc verve range. The fertiliser had a nitrogen to phosphorus to potassium ratio of 4:4:4 according to the EC fertiliser solution for the UK. The total nitrogen component was 4%. Nitric nitrogen and ureic nitrogen parts were 1.1 and 2.1%, respectively. Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) made up 4% each of the solution, but the corresponding *P* and *K* content were only 1.7 and 3.3%, respectively. Moreover, the fertiliser contained also trace elements (names not listed) of unspecified quantities. Liquid fertiliser was added to the inflow water as specified.

Harvested chilli fruits from each plant were categorised according to the Chilli classification scheme (Section 3.3.3), which has been adapted from Almuktar et al. (2015a). The variables length, width, weight and bending were used for classifying the harvested fruits. The monetary value of the harvest for chilli plants was calculated according to estimated prices on the UK market in 2014. The lowest variable class entry for any individual fruit assessment determined the final class. For example, if a fruit is categorised as class A in terms of length, class B in terms of width and E in terms of weight, then the final class for this fruit is class E, and accordingly the corresponding price for this fruit will be zero pence.

2.5. Data analysis

Microsoft Excel (www.microsoft.com) was used for descriptive statistics. IBM SPSS Statistics Version 20 (www.ibm.com) was applied to compute the non-parametric Mann–Whitney *U*-test for comparing the medians of two (unmatched) independent water

Table 2
Comparison of the water quality of the inflow waters received by the vegetable pots (mean ± standard deviation and sample numbers).

Water type	TPH[a] (µg/L)	COD[b] (mg/L)	BOD[c] (mg/L)	NH_4-N[d] (mg/L)	NO_3-N[e] (mg/L)	PO_4-P[f] (mg/L)	SS[g] (mg/L)	Turb[h] (NTU)	pH (−)	EC[i] (µs/cm)	DO[j] (mg/L)	SAR[k](meq/L)[l]
Filter1 outflow	100.0 (1)	77.7 ± 23.35 (18)	25.8 ± 16.74 (53)	4.8 ± 2.83 (22)	0.4 ± 0.22 (19)	4.0 ± 2.48 (18)	11.3 ± 10.42 (56)	9.0 ± 5.65 (54)	6.4 ± 0.26 (54)	345.9 ± 67.12 (22)	1.5 ± 1.00 (15)	2.4 ± 1.07 (5)
Filter2 outflow	<10.0 (1)	34.9 ± 19.21 (15)	13.6 ± 8.11 (51)	6.2 ± 5.84 (20)	2.2 ± 2.72 (18)	3.3 ± 1.33 (18)	6.7 ± 9.49 (56)	5.4 ± 5.75 (53)	6.5 ± 0.21 (54)	338.3 ± 70.08 (22)	1.9 ± 1.38 (15)	1.8 ± 0.60 (5)
Filter3 outflow	69.0 (1)	87.5 ± 26.00 (18)	22.8 ± 16.42 (51)	3.7 ± 2.53 (22)	0.4 ± 0.28 (19)	3.3 ± 2.04 (18)	11.7 ± 10.79 (56)	8.7 ± 6.09 (53)	6.5 ± 0.18 (54)	403.5 ± 81.63 (22)	1.6 ± 1.15 (15)	1.7 ± 0.53 (5)
Filter4 outflow	<10.0 (1)	34.9 ± 23.77 (15)	12.8 ± 8.86 (50)	5.0 ± 10.53 (20)	1.8 ± 3.27 (18)	2.9 ± 1.06 (18)	7.4 ± 10.57 (56)	5.7 ± 5.46 (53)	6.5 ± 0.19 (54)	361.3 ± 78.13 (22)	2.0 ± 1.60 (15)	2.4 ± 0.39 (5)
Filter5 outflow	14.0 (1)	100.8 ± 67.90 (18)	22.5 ± 16.35 (51)	9.7 ± 3.20 (21)	0.9 ± 0.86 (19)	4.4 ± 2.07 (18)	11.3 ± 12.76 (57)	8.6 ± 6.22 (53)	6.6 ± 0.19 (54)	583.5 ± 185.02(22)	1.5 ± 0.81 (15)	2.2 ± 0.85 (5)
Filter6 outflow	<10.0 (1)	35.6 ± 22.46 (14)	15.9 ± 12.68 (52)	9.0 ± 7.28 (20)	3.6 ± 4.68 (18)	4.6 ± 3.16 (18)	6.9 ± 8.68 (57)	5.4 ± 4.41 (53)	6.8 ± 0.19 (55)	545.4 ± 180.34 (22)	1.6 ± 1.09 (15)	3.1 ± 0.61 (5)
Filter7 outflow	<10.0 (1)	32.5 ± 20.40 (14)	11.9 ± 8.01 (61)	3.6 ± 5.52 (24)	2.8 ± 2.98 (18)	3.6 ± 2.23 (17)	2.6 ± 3.86 (66)	3.4 ± 2.24 (62)	6.6 ± 0.18 (62)	375.7 ± 116.35 (28)	1.8 ± 1.36 (25)	2.4 ± 0.47 (5)
Filter8 outflow	116.0 (1)	55.9 ± 86.05 (15)	13.9 ± 7.50 (69)	1.4 ± 1.35 (22)	2.8 ± 3.51 (16)	3.3 ± 1.90 (16)	2.9 ± 4.31 (76)	3.6 ± 2.48 (76)	6.5 ± 0.20 (78)	347.9 ± 110.83 (25)	2.1 ± 1.50 (22)	2.1 ± 0.44 (5)
Control A outflow	346.0 (1)	66.4 ± 44.32 (17)	12.0 ± 7.58 (51)	1.3 ± 1.79 (22)	0.4 ± 0.44 (19)	1.8 ± 0.56 (18)	9.0 ± 10.25 (56)	5.7 ± 4.31 (53)	6.7 ± 0.17 (55)	149.2 ± 32.47 (22)	1.3 ± 0.90 (15)	0.5 ± 0.15 (5)
Control B outflow	<10.0 (1)	16.0 ± 15.12 (15)	8.8 ± 7.58 (52)	1.3 ± 1.77 (21)	0.3 ± 0.35 (18)	1.9 ± 0.33 (18)	3.6 ± 8.18 (56)	4.1 ± 4.54 (53)	6.5 ± 0.20 (54)	153.9 ± 29.87 (22)	2.1 ± 1.73 (15)	0.5 ± 0.14 (5)
Deionised water	nm	nm	7.2 ± 1.31 (5)	nm	nm	nm	2.0 ± 18.58 (10)	1.3 ± 0.14 (10)	5.1 ± 0.58 (10)	1.5 ± 0.72 (10)	nm	0.1 ± 0.15 (5)
Tap water (100%)	nm	nm	5.0 ± 0.81 (5)	nm	nm	nm	2.0 ± 20.88 (10)	3.1 ± 0.39 (10)	6.3 ± 0.84 (10)	95.8 ± 15.20 (10)	nm	0.8 ± 0.15 (5)
Tap water with fertiliser	nm	nm	7.7 ± 1.89 (55)	nm	nm	nm	1.6 ± 16.98 (10)	2.5 ± 0.79 (10)	6.1 ± 0.20 (10)	204.0 ± 5.66 (10)	nm	0.8 ± 0.00 (5)
Wastewater (20%); tap water (80%)	nm	47.6 ± 15.39 (17)	49.7 ± 5.66 (55)	6.7 ± 3.69 (22)	0.5 ± 0.64 (21)	3.0 ± 1.43 (21)	17.8 ± 12.66 (63)	13.7 ± 9.84 (56)	7.1 ± 0.08 (55)	264.0 ± 66.47 (22)	nm	1.7 ± 0.59 (5)
Wastewater (100%)	nm	237.9 ± 76.96 (17)	105.3 ± 75.98	33.6 ± 18.46 (22)	2.4 ± 3.22 #(21)	14.9 ± 7.15 (21)	131.9 ± 92.64963)	80.4 ± 75.97 (56)	7.5 ± 0.42 (55)	943.7 ± 146.94 (22)	4.9 ± 3.73 (16)	2.8 ± 0.62 (5)

[a] total petroleum hydrocarbon.
[b] chemical oxygen demand.
[c] biochemical oxygen demand.
[d] ammonia-nitrogen.
[e] nitrate-nitrogen.
[f] ortho-phosphate–phosphorus.
[g] suspended solids.
[h] turbidity.
[i] electrical conductivity.
[j] dissolved oxygen.
[k] sodium adsorption ratio (sodium/(calcium + magnesium)/2) 0.5).
[l] milliequivalent per litre; nm, not measured.

Table 3
Experimental design in terms of plant number allocations for the third and final planting stage (after Almuktar et al. (2015a,b)).

Treatmentnumber	Inflow source	Growth media	Surviving Chilli plant label	Remaining sample number
1	Filter 1 outflow	Compost with bark	C3;C4	2
2	Filter 2 outflow	Compost with bark	C6;C8:C9	3
3	Filter 3 outflow	Compost with bark	C10;C11;C12	3
4	Filter 4 outflow	Compost with bark	C16:C17	2
5	Filter 5 outflow	Compost with bark	C18;C19;C20	3
6	Filter 6 outflow	Compost with bark	C21	1
7	Filter 7 outflow	Compost with bark	C25:C26	2
8	Filter 8 outflow	Compost with bark	C27;C28:C29	3
9	Control A outflow	Compost with bark	C31;C33	2
10	Control B outflow	Compost with bark	C37:C38	2
11	Deionised water	Compost with bark	C41	1
12	Tap water (100%)	Compost with bark	C42;C43	2
13	Tap water with fertiliser (0.7 ml/l)	Compost with bark	C45;C46	2
14	Wastewater (20%); tap water (80%)	Compost with bark	C49	1
15	Wastewater (100%)	Compost with bark	C52; C54	2
16	Filter 1 outflow	Silica sand	C56	1
17	Filter 2 outflow	Silica sand	C58	1
18	Filter 3 outflow	Silica sand	C61	1
19	Filter 4 outflow	Silica sand	C63;C64	2
20	Filter 5 outflow	Silica sand	C66	1
21	Filter 6 outflow	Silica sand	C68	1
22	Filter 7 outflow	Silica sand	C71	1
23	Filter 8 outflow	Silica sand	C72;C73	2
24	Control A outflow	Silica sand	C74	1
25	Control B outflow	Silica sand	C76;C77	2
26	Deionised water	Silica sand	C80	1
27	Tap water (100%)	Silica sand	C82	1
28	Tap water with fertiliser (0.7 ml/l)	Silica sand	C84;C85	2
29	Wastewater (20%); tap water (80%)	Silica sand	C87	1
30	Wastewater (100%)	Silica sand	C90	1

Note: Initial seed planting reference numbers during the first planting stage; Chilli (C1–C90). Three plants were allocated at random to each of the 30 treatments. Note that 40 plants did not survive the first and second planting stage.

samples. This was required, because virtually all sample data were not normally distributed, so that an analysis of variance could not be applied (Sani et al., 2013). The Spearman's test was used to calculate the correlation coefficients of different variables. The Duncan's multiple range test was applied to determine significant differences among treatments at a significant level of $p < 0.05$.

3. Results and discussion

3.1. Comparison of irrigation water qualities

Regarding filters contaminated by diesel (Filters 1, 3, 5 and Control A), the total petroleum hydrocarbon concentrations in effluent were 100 μg/L, 69 μg/L, 14 μg/L and 346 μg/L, respectively (Table 2). These concentrations were in compliance with the Chinese standards for irrigation water quality (SEPA, 2005) highlighting a maximum allowable threshold value of 1000 μg/L. Note that Chinese standards were used, considering that China produces about 54% (estimated in 2008) of Peppers (including Chilli) in the world (ERS/USDA, 2008). Control A, which lacks mature biomass, showed high total petroleum hydrocarbon concentration values compared with those values for the other filters. This can be explained by diesel toxicity to microorganisms due to the absence of sufficient nutrients in tap water. Although Filter 8 lacked diesel spill contamination, the total petroleum hydrocarbon concentration was 116 μg/L. This can be explained by the elevated loading rate for this filter, resulting in the accumulation of hydrocarbons originating from the petroleum background concentration in wastewater. Moreover, total petroleum hydrocarbon values for Filter 1 outflow water were higher than that for Filter 3, explaining the impact of aggregate size on the diesel removal process. However, the diesel concentration in Filter 5 outflow water was lower than those concentrations in Filters 1 and 3 due to the strong and mature biomass available in Filter 5 as a result of a high inflow loading rate (Table 1) supporting the growth of microorganisms and subsequently enhancing the hydrocarbon biodegradation. However, correlation analysis results showed that total petroleum hydrocarbon was significantly ($p < 0.05$) positively correlated with selected micro-organisms (total coliforms: $r = 0.860$, $P = 0.001$; *E. coli*: $r = 0.724$, $P = 0.018$; Salmonella spp.: $r = 0.782$, $P = 0.007$). This observation confirms previous studies (Al-Baldawi et al., 2014).

Table 2 shows that chemical oxygen demand values were the highest for raw wastewater (domestic wastewater) followed by Filters contaminated with diesel following the order of F5 > F3 > F1 > Control A. There is no statistically significant difference in chemical oxygen demand mean values between Filter 1 and 2 outflow waters compared to those concentrations of Filters 3 and 4, indicating that aggregate size may not matter. Filter 8 outflow water had average chemical oxygen demand values higher than that for Filter 7, indicating the impact of long resting time on chemical oxygen demand removal efficiency (Table 2). In contrast, the lowest mean values were noted for Control B.

The five-day BOD was the highest for raw wastewater and wastewater samples diluted with tap water followed by filters contaminated with diesel (Filters 1, 3 and 5). The mean five-day BOD values for Filter 8 (short resting time) outflow water were higher than those for Filter 7 (long resting time). The mean five-day BOD values for Control A (contaminated with diesel) were higher than those for Control B. Tap water had the lowest five-day BOD values (Table 2).

Dissolved oxygen mean values were higher for those filters without diesel (Table 2). Correlation analysis results indicated that dissolved oxygen was significantly ($p < 0.05$) negatively correlated with micro-organisms (e.g., total coliforms; $r = -0.688$; $P = 0.019$), total petroleum hydrocarbon ($r = -0.914$, $P = 0.000$) and COD ($r = -0.809$, $P = 0.005$). This negative correlation can be explained by an improvement of the COD and total petroleum hydrocarbon removal efficiencies as micro-organisms responsible for biodegradation acclimatised, resulting in a reduction of the amount of available dissolved oxygen in the system (Scholz, 2010).

Table 2 shows that high average concentrations of ammonia-nitrogen, which exceeded the threshold of 5 mg/L (FAO, 2003), were noted for raw wastewater, wastewater samples diluted with 80% of tap water, and outflow waters from Filters 5 and 6, which were fed with high inflow loads, followed by that from Filter 2, which had a large aggregate size (Table 1). Moreover, Table 2 shows that nitrate–nitrogen for all filter outflow waters was less than the maximum irrigation threshold value of 30 mg/L (FAO, 2003). Based on the recommended irrigation threshold of 2 mg/L for ortho-phosphate–phosphorus (FAO, 2003), the outflow waters from all wetlands were associated with too high ortho-phosphate–phosphorus concentrations (Table 2). However, phosphorus is one of the most difficult pollutants to be removed by mature constructed wetlands (Smith et al., 2006). This can be explained by the fact that phosphorus is usually present in particulate form, and does not dissolve well in filters that are not saturated by phosphorus or other compounds competing for adsorption sites (Scholz, 2010). High phosphorus levels are known to interfere with plants normal metabolism. Also, it is known to promote manganese uptake by plants (FAO, 1972).

Fig. 2 provides an overview of the ICP–OES analysis for selected elements determined in the irrigation water. Based on the recommended irrigation threshold of 2 mg/l for potassium (FAO, 1994, 2003), the outflow water from all wetland filters (with the exception of Controls A and B) was associated with too high potassium concentrations. Furthermore, high potassium concentrations were also observed for raw wastewater and wastewater samples, which were diluted with up to 80% of tap water (Fig. 2d). However, Cakmak (2005) reported that increasing potassium concentration in irrigation water provided important protection against stem damage from low night temperatures in plants. Moreover, decreases in yield and increases in leaf damage induced by frost under field conditions could be alleviated by high application of potassium fertilizer. Fig. 2f shows that results for Filters 1, 3 and 5, which were contaminated with diesel, show relatively high manganese concentrations, which exceeded the irrigation threshold of 0.2 mg/l (FAO, 1994, 2003). Manganese is an essential trace element for most plants, intervening in several metabolic processes, mainly in photosynthesis. Nevertheless, an excess of this micronutrient is often toxic for plants. Manganese phyto-toxicity is exhibited in a reduction of biomass and photosynthesis, and biochemical disorders including oxidative stress (FAO, 1972).

Table 2 shows that the highest value for suspended solids was noted for raw wastewater and wastewater samples diluted with 80% of tap water followed by those for outflow water from Filters contaminated with diesel (Filters 1, 3, 5 and Control A).

Turbidity had the highest mean values for raw wastewater and raw wastewater diluted with tap water followed by outflow waters obtained from Filters 1, 3 and 5, which were contaminated with diesel. Filters 7 and 8 had the lowest mean turbidity values in spite of different resting times. Correlation analysis results showed that turbidity was significantly ($p < 0.05$) positively correlated with suspended solids ($r = 0.702$, $P = 0.004$) and micro-organisms (total coliforms: $r = 0.864$, $P = 0.000$; *E. coli*: $r = 0.802$, $P = 0.000$; *Streptococcus* spp.: $r = 0.798$, $P = 0.000$ and *Salmonella* spp.: $r = 0.871$, $P = 0.000$) in treatment systems indicating a good relationship between turbidity and indicator micro-organisms activity due to degradation of organic matter and a subsequent increase in suspended solids (Sani et al., 2013). Moreover, Table 2 shows that the pH values for all filter outflows were within the normal range of between 6.0 and 8.5 (FAO, 2003).

Electrical conductivity is the most important indirect measure of salinity, posing a great hazard to crops and determining the suitability of water for irrigation use. High levels of electrical conductivity in water create saline soil. Salts negatively impact on the growth of plants. The electronic conductivity values for all filter outflow waters complied with the threshold standards of 3000 μs/cm (FAO, 1994, 2003). Furthermore, the sodium adsorption ration (SAR) values for all outflows were within the normal range between 0 milliequivalent per litre (meq/L) and 15 meq/L (FAO, 1994).

Microbial characteristics of irrigation water are summarised in Fig. 3. Based on the maximum value for total coliforms (1000CFU/100 mL) for the irrigation of crops (USEPA, 2004), which are often eaten uncooked, the outflow waters from all wetland filters were associated with too high contamination by total coliforms. Furthermore, high contamination by total coliforms was also observed for raw wastewater and wastewater samples, which were diluted with up to 80% of tap water. Total coliforms were removed well from the outflow waters of standard wetland filters, which were not contaminated by hydrocarbons. This finding confirmed research undertaken by Cui et al. (2003), reporting that the removal rates for total heterotrophic bacteria and total coliforms when using vertical-flow bed systems were between 80 and 90%, and between 85 and 96%, respectively.

Raw wastewater was associated with the highest contamination by mean *E. coli* (8000CFU/100 mL) counts followed by outflow water from Filter 5 and wastewater samples, which were diluted with up to 80% of tap water. Outflow waters from Filters 1 and 3 (both contaminated by diesel; Table 1) had similar mean numbers of *E. coli* contamination. No contamination by *E. coli* was detected for outflow waters from other wetland filters. However, these results agreed with those reported by Cirelli et al. (2012) who undertook the reuse scenario of tertiary-treated municipal wastewater using a constructed wetland to supply irrigation water for vegetables in Eastern Sicily, Italy. They concluded that the irrigation water contained increased numbers of *E. coli*, which were frequently above the Italian threshold of 50 colony forming units (CFU)/100 mL

The highest contamination by mean *Streptococcus* spp. counts was associated with Filter 1 outflow water followed by those for Filters 3 and 5 which were contaminated with hydrocarbons (Fig. 3). Filter 4 had a higher *Streptococcus* spp. contamination than Filter 2 indicating the effect of aggregate size when comparing those filters with each other (Table 1). A larger aggregate size allows for more microorganisms to colonise the empty spaces between the filter media. Furthermore, raw wastewater contained higher *Streptococcus* spp. than wastewater samples, which were diluted with up to 80% of tap water. The highest mean *Salmonella* spp. number was observed in the outflow water from Filter 5 followed by raw wastewater (Fig. 3). Filter 1 outflow water was associated with higher *Salmonella* spp. contamination than the water from Filter 3 explaining the impact of aggregate size (Table 1). Furthermore, *Salmonella* spp. contamination in Control A outflow water was higher than that associated with Control B explaining the effect of hydrocarbons contamination.

Fig. 3 showed that the microbial contamination of outflow water from wetland filters contaminated with hydrocarbons was higher than that from uncontaminated filters. This confirms findings by Benedek et al. (2013), who studied the impact of long-term total petroleum hydrocarbons (TPH) on the structure of bacterial communities. The results indicated that a very high concentration of TPH affected positively the diversity of hydrocarbon-degrading bacteria. Furthermore, wetland filters fed with undiluted inflow water showed higher microbial contamination levels than those fed with diluted inflow. This confirms results by Sani et al. (2013) that high rate filters tend to be overloaded.

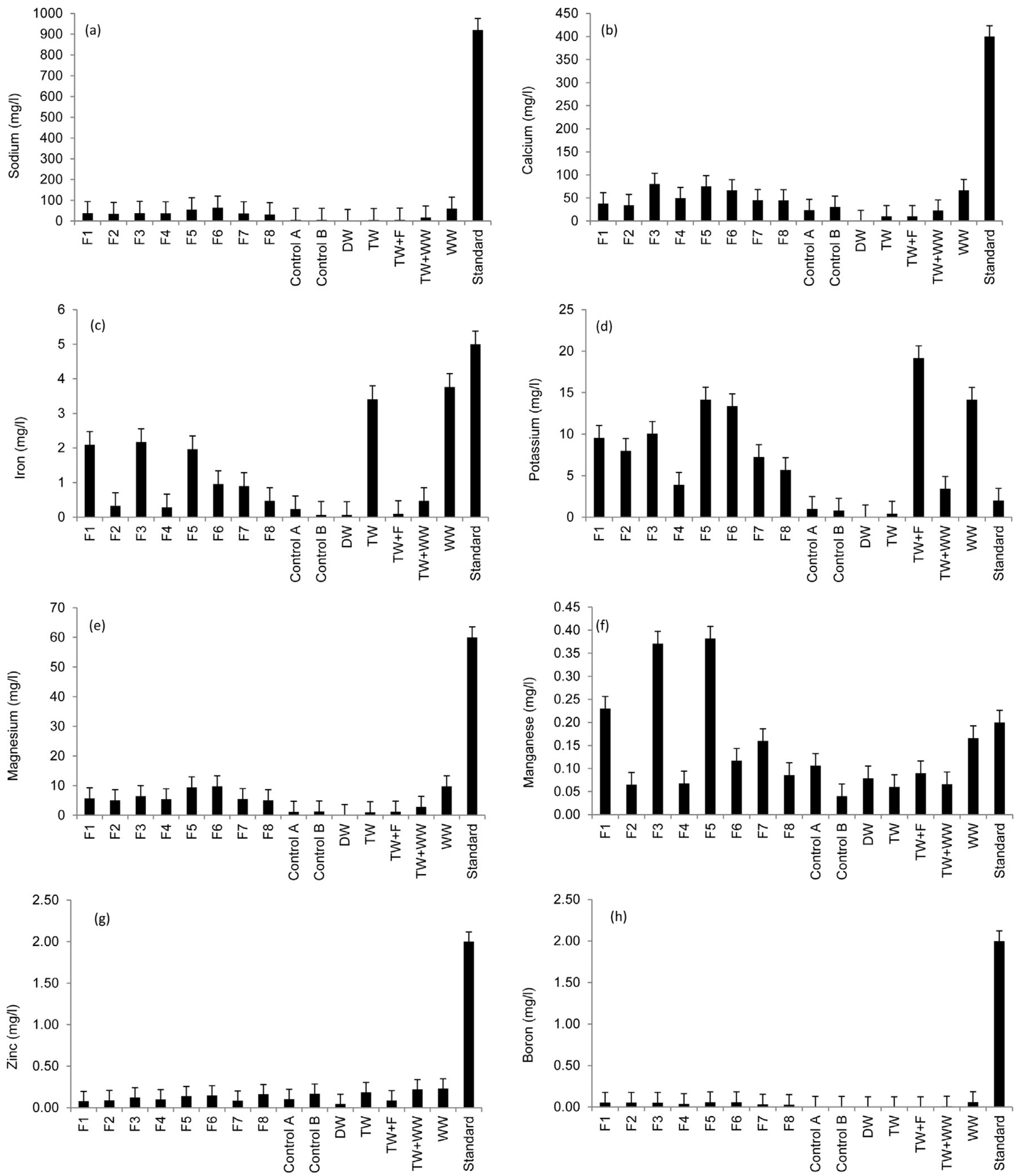

Fig. 2. Overview of the Inductively Coupled Plasma (ICP) Optical Emission Spectrometer analysis for selected elements compared with common standards for irrigation water (e.g., FAO (1994, 2003)): (a) sodium; (b) calcium; (c) iron; (d) potassium; (e) magnesium; (f) manganese; (g) zinc; and (h) boron.
Note: elements not shown (i.e., arsenic, barium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, strontium and titanium) were not detected.

3.2. Soil quality analysis

3.2.1. Comparison of soil pH and redox potential

Table 4 shows pH, redox potential and electrical conductivity for organic and inorganic growth media irrigated with different water types. All pH values of organic media indicated acidic conditions (pH value < 7). In comparison, pH values for inorganic media were alkaline (pH value > 7). The soil pH can markedly affect the availability and consequently the plant uptake of trace elements (FAO, 1972, 2003). The ability of plants to utilize trace elements decreases with decreasing acidity (increase in pH), while the utilization at higher pH values remained constant (FAO, 1972). Table 4 lists the redox potential of organic and inorganic media. According to Husson (2013), soil could be classified as moderately reduced soil (redox potential values between +100 and +400 mV), reduced soil (redox potential values between −100 and +100 mV) and highly reduced soil (redox potential values between −100 and −300 mV). Based on this classification, Table 4 indicates that organic media irrigated with different water types could be considered as reduced soil. In comparison, inorganic media irrigated with outflow water from Filters 3, 4, 6–8 as well as Control B could be classified as highly reduced soils, while others may be classified as reduced soils (Husson, 2013). The redox potential and pH are major drivers for change in soil, plant and microorganism systems. High levels of redox potentials can impact on system functioning as well as on plant health and production (Husson, 2013). However, climate

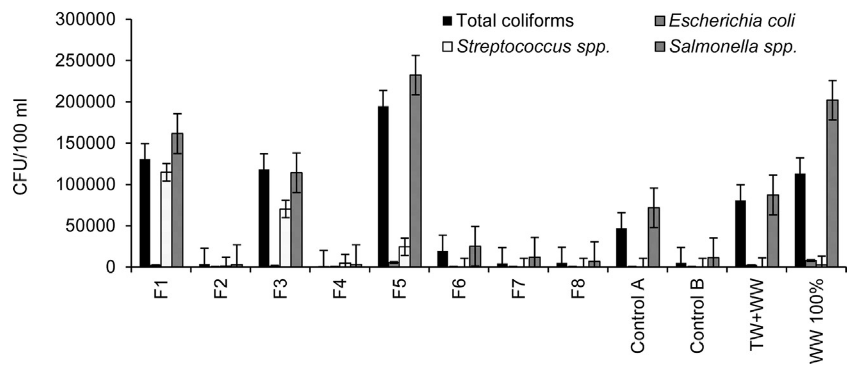

Fig. 3. Microbiological characteristics of irrigation water.

Table 4
Soil properties for pots irrigated with different water sources.

Inflow source and growth media	pH	Eh (mV)	EC (μs/cm)
Filter 1 and organic	6.36	66.3	2259.3
Filter 2 and organic	5.84	93.6	2374.5
Filter 3 and organic	6.18	76.0	1153.5
Filter 4 and organic	6.26	71.8	1764.0
Filter 5 and organic	6.49	59.8	800.0
Filter 6 and organic	6.82	45.5	2338.7
Filter 7 and organic	6.60	53.9	522.0
Filter 8 and organic	6.57	55.6	490.0
Control A and organic	6.44	62.2	976.5
Control B and organic	6.38	65.4	473.5
Deionised water and organic	6.16	77.1	1477.3
Tap water and organic	6.01	84.5	752.8
Tap water/fertiliser and organic	5.49	111.8	1378.0
Wastewater/tap water and organic	6.26	71.8	1032.0
Wastewater and organic	6.24	72.2	1611.0
Raw organic growth media	6.43	62.6	2438.5
Filter 1 and inorganic	8.13	−19.6	474.0
Filter 2 and inorganic	9.74	−95.1	374.0
Filter 3 and inorganic	11.01	−154.8	511.0
Filter 4 and inorganic	10.69	−139.4	581.0
Filter 5 and inorganic	8.91	−56.4	783.6
Filter 6 and inorganic	10.77	−143.4	874.2
Filter 7 and inorganic	10.99	−153.5	817.5
Filter 8 and inorganic	10.47	−129.2	528.8
Control A and inorganic	7.78	−3.3	835.3
Control B and inorganic	10.72	−141.2	370.0
Deionised water and inorganic	9.34	−76.3	996.4
Tap water and inorganic	9.47	−82.6	606.2
Tap water/fertiliser and inorganic	7.83	−5.8	404.5
Wastewater/tap water and inorganic	9.40	−79.1	598.7
Wastewater and inorganic	10.57	−134.1	2081.7
Raw inorganic growth media	9.40	−79.2	116.0

Note: Eh, redox potential; EC, Electrical conductivity; pH, Eh and EC entries are mean values of three samples.

conditions and soil moisture could directly affect pH and redox potential values, especially in organic soil.

3.2.2. *Comparison of soil salinity*

Generally, the electrical conductivity values (Table 4) of the organic media were higher than those for the inorganic ones. This can be explained by the acidic conditions of the organic media, which increase the dissolution of sodium, potassium, calcium and magnesium, and subsequently increase the salinity of the soil (FAO, 1972, 2003). However, irrigation with treated wastewater did not increase the salinity of organic media compared to the compost. In comparison, inorganic media showed higher salinity after irrigation with treated wastewater compared to sand. This can be explaining by the pH values of different media and their relationship with the salinity as discussed above. Furthermore, nutrient imbalances could result from excessive soil salinity leading to high accumulations of toxic elements, reducing water infiltration and subsequently limiting the growth of plants (FAO, 1972, 2003).

3.2.3. *Soil mineral content*

Fig. 4 shows the concentrations of elements detected by ICP–OES analysis in the organic and inorganic media irrigated with different water types. The mineral content in the studied soils seems to be greater in the organic media than the inorganic ones as reported by FAO (1972).

3.2.3.1. Soil aluminium. Aluminium solubility is mainly governed by soil pH, and by soil organic matter and clay contents. Exchangeable aluminium rapidly increases when pH decreases. However, irrigation of organic media with wetland filter outflow waters may cause increases in aluminium concentrations compared to the raw organic media (Fig. 4a) with the exception of those plants irrigated with outflow waters from Filters 2, 3 and 5, possibly due to the irrigation water volumes applied on those soils (Fig. 5). In com-

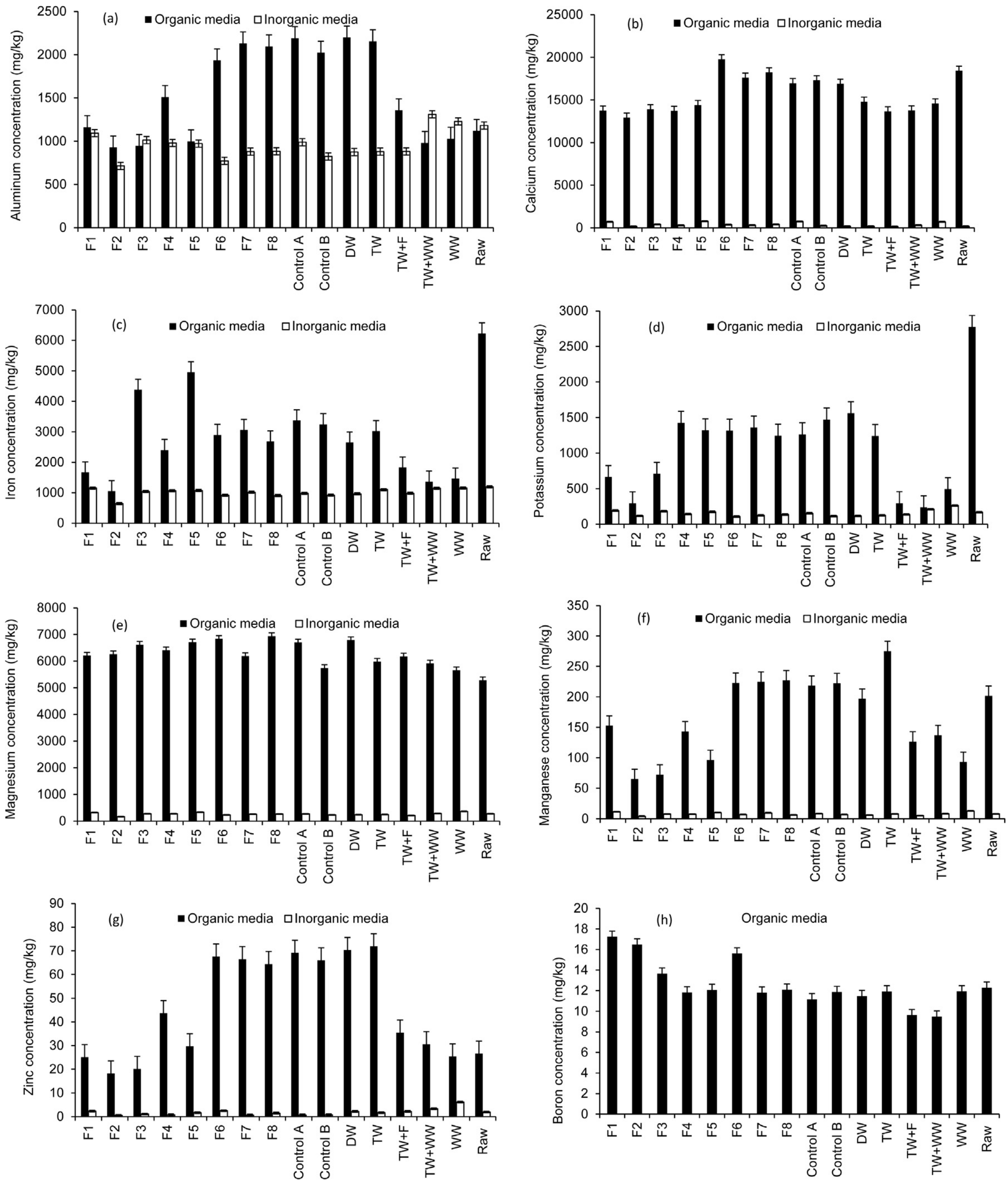

Fig. 4. Inductively Coupled Plasma (ICP) Optical Emission Spectrometer analysis for selected elements in different growth media: (a) aluminium; (b) calcium; (c) iron; (d) potassium; (e) magnesium; (f) manganese; (g) zinc; and (h) boron. Samples number = 3. Bars indicate standard errors. Note: Since arsenic, barium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, strontium and titanium were not detected, they are not shown.

parison, no increase in aluminium concentration was observed for irrigated inorganic media compared to the raw sand. Statistical analysis showed that mean aluminium concentrations in soils irrigated with outflow water from Filter 4 were significantly ($p < 0.05$) greater than those irrigated with Filter 2 drain water, explaining the impact of aggregate size of wetland filters on aluminium concentrations of irrigated soils. Moreover, soils irrigated with water harvested from Filters 7 had aluminium mean concentrations significantly ($p < 0.05$) greater than those irrigated with Filters 3 and 4 outflow waters indicating the impact of contact time variable of wetlands design (Table 1). Significant ($p < 0.05$) differences were also observed in mean aluminium concentrations of soils irrigated with Filters 4 and 6 compared to those irrigated with Filters 3 and 5, respectively due to differences in irrigation water volumes applied on those soils (Fig. 5) and impact of diesel contamination (Table 1).

3.2.3.2. Soil calcium. Compared to the raw organic media, irrigation with Filter 6 outflow water caused an increase in the calcium concentration of the compost (Fig. 4b). However, statistical analysis showed that the mean calcium concentration of soil irrigated with outflow water from Filter 6 was greater than the other concentrations due to the highest irrigation water volume applied on the soil irrigated with Filter 6 outflow, resulting in a high amount of calcium application (Fig. 5). However, calcium is an important ele-

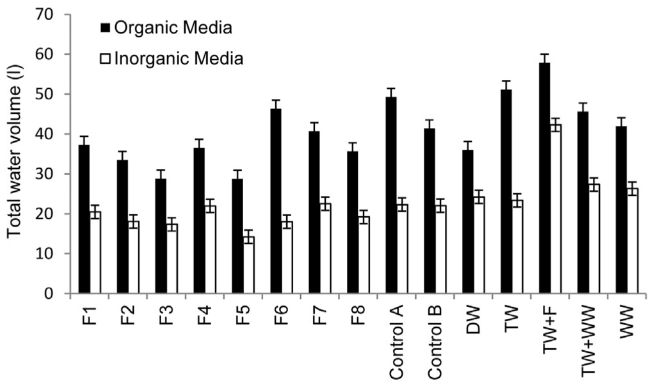

Fig. 5. Overview of total irrigation water volumes (l) for Chilli plants per each water source.

ment required for the growth and development of plants, especially their roots and shoot tips (Haifa Chemicals, 2014). Furthermore, the availability of high calcium levels will improve the effects of uptake of toxic cations like aluminium and sodium from the soil, while the presence of high levels of potassium and magnesium may reduce calcium uptake (FAO, 1972).

3.2.3.3. Soil iron. The solubility of iron is strongly influenced by both redox potential and pH. Iron toxicity is frequently observed at low redox potentials and pH values, (FAO, 1972). Statistically, soils irrigated with outflow water from Filters 1, 3 and 5 as well as Control A had mean iron concentrations, which were significantly ($p < 0.05$) different from those of Filters 2, 4 and 6 as well as Control B, explaining the impact of diesel contamination on iron concentrations of the outflow waters and the subsequent impact of the amount of iron applied on irrigated soils (Fig. 2). The high iron concentrations observed in irrigated soil can be explained by the already high iron concentration in the compost and the iron present in the irrigation water. Iron has a low bioavailability in terms of uptake by plants (FAO, 1972). This leads to the accumulation of iron in the irrigated soil.

No increase of the iron concentrations in the irrigated soils was observed compared to the raw media. Moreover, iron concentrations in the irrigated soils did not exceed the corresponding metal threshold of 50000 mg/kg (FAO/WHO, 2001).

3.2.3.4. Soil potassium. Potassium availability is mainly related to soil pH, and to clay content and type. An increase in pH increases potassium fixation to the soil (FAO, 1972). Irrigation with wetland filter outflow waters did not cause any increase of potassium concentration compared to the raw compost (Fig. 4d). Statistically, there are significant ($p < 0.05$) differences in mean potassium concentrations of soils irrigated with outflow water from Filter 4 compared to Filter 2, and 5 compared to Filter 3, explaining the impacts of aggregate size and inflow loading rate, respectively, of wetland systems on potassium concentrations in the outflow waters (Fig. 2), and subsequently their impacts on the distribution of potassium concentrations applied to the irrigated soils (Table 1). Moreover, significant ($p < 0.05$) differences in mean potassium concentrations were observed between soils irrigated with Filter 1 compared to Filter 2 and 3 compared to Filter 4 due to the impact of irrigation water volume (Fig. 5) and diesel contamination (Table 1) applied on those soils.

3.2.3.5. Soil magnesium. Irrigation of organic media with all water types caused increases in the magnesium concentrations compared to the raw compost (Fig. 4e) due to significant ($p < 0.05$) differences in irrigation volumes applied to organic soils compared to those for inorganic soils (Fig. 2). No increase in magnesium concentrations were observed in the irrigated inorganic media compared to the raw sand. However, sandy soils often have a low cation exchange capacity and may not contain adequate levels of magnesium (FAO, 1972).

Statistically, soils irrigated with Filter 4 outflow waters had mean magnesium concentrations, which were significantly ($p < 0.05$) different from those irrigated with Filter 6 waters, showing the impact of the inflow loading rate of wetlands (Table 1) on the magnesium concentration of the outflow water (Fig. 2), impacting on the distribution of magnesium applied on to the irrigated soil.

3.2.3.6. Soil manganese. The availability of manganese is strongly influenced by the soil redox potential and pH (Husson, 2013). Manganese toxicity is quite common in association with a low soil pH (FAO, 1972). Statistical analysis results showed that there are significant ($p < 0.05$) differences in mean manganese concentrations of soil irrigated with Filters 1, 3 and 5 compared to those irrigated with Filters 2, 4 and 6, explaining the impact of diesel contamination on manganese concentration values in the outflow waters (Fig. 2) and resulting in significant ($p < 0.05$) differences in manganese load applied to the corresponding irrigated soils. Wetland aggregate size impacted on the manganese concentration variation of outflow waters (Fig. 2), which led to significant ($p < 0.05$) differences in soil manganese concentrations distribution, when comparing soil irrigated with Filters 1 and 2 to those soils irrigated by Filter 3 and 4 outflow waters, respectively. Moreover, soil irrigated with Filter 4 outflow water had manganese concentrations, which were significantly ($p < 0.05$) different from those irrigated with Filter 6 outflow water, explaining the impact of the inflow loading rate of the wetland system on manganese concentrations in the outflow waters (Fig. 5) and the subsequent impact on the manganese distribution in the irrigated soils. However, for irrigated organic and inorganic media, manganese concentrations did not exceed the corresponding metal threshold of 2000 mg/kg (FAO/WHO, 2001).

3.2.3.7. Soil zinc. Organic media irrigated with most water types had zinc concentrations higher than those of the raw compost. Statistically, mean zinc concentrations in soil irrigated with Filter 2 drain water were significantly ($p < 0.05$) different from those irrigated with Filter 4, irrigation with Filter 7 water was significantly different from irrigation with Filter 4, and soil irrigated with Filter 4 water was significantly ($p < 0.05$) different from that irrigated with Filter 6 outflow waters (Fig. 4g), explaining the impact of aggregate size, contact time and inflow loading rate of the wetland systems on the zinc concentrations of the outflow waters (Fig. 2), which subsequently impact on the zinc concentrations applied on the corresponding irrigated soils. Moreover, soil irrigated with waters from Filter 5 had mean zinc concentrations, which were significantly ($p < 0.05$) different from those of Filter 6, indicating the impact of diesel contamination on outflow water zinc concentrations (Fig. 2) and the subsequent impact of the distribution of zinc values on the corresponding irrigated soils. However, zinc concentrations in irrigated soil did not exceed the corresponding metal threshold of 300 mg/kg (FAO/WHO, 2001).

3.2.3.8. Soil boron. Fig. 4h shows that boron was detected only in the organic media. However, the bioavailability of boron in the soil is affected by many factors such as soil parent material, texture, nature of minerals in the soil, content of organic matter, soil pH, irrigation water source, interrelationship with other elements and the environmental conditions (especially dry weather) and high light intensity (FAO, 1972). Statistically, results showed that there are significant ($p < 0.05$) differences of mean boron concentrations in soil irrigated with outflow waters of Filters 1 and 2 (large aggregate

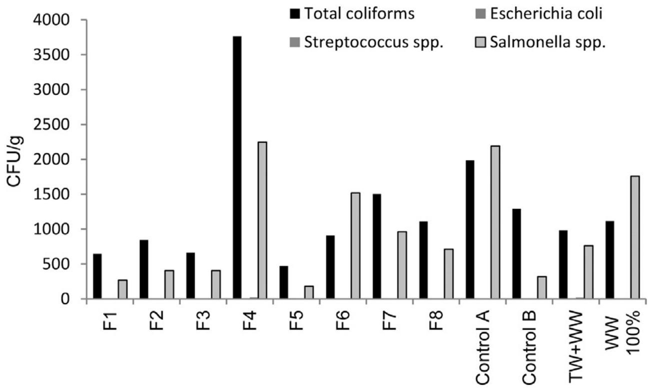

Fig. 6. Microbiological results for soil irrigated by different water types.

size) compare to those irrigated with Filters 3 and 4 (small aggregate size), Filters 3 and 4 (low inflow loads) compared to Filters 5 and 6 (high inflow load), explaining the impact of wetland design variables on boron concentrations of outflow waters, resulting in differences on boron distributions in the irrigated soils. Moreover, soils irrigated with water from Filters 1, 3 and 5 had boron mean concentrations significantly ($p < 0.05$) different from those of Filters 2, 4 and 6, explaining the impact of diesel contamination on boron values of the outflow water, resulting in differences in boron concentrations in the corresponding irrigated soils.

3.2.4. Soil microbial content

Findings indicate that the highest contamination by total coliforms was associated with soil irrigated by outflow water obtained from Filter 4 (not contaminated by diesel and low COD load; Table 1), followed by those for Control A and Filters 7 (Fig. 6). In contrast, the lowest soil contamination by total coliforms was found in soil irrigated by outflow water associated with Filter 5 (contaminated by diesel and high COD load; Table 1) due to the low corresponding irrigation water volume compare to those for the other systems (Fig. 5). Soil irrigated by water treated by Filter 2 was more contaminated by total coliforms compared to Filter 1. Soil irrigated by harvested water from Filter 8 had similar contamination in terms of total coliforms compared to raw wastewater. Furthermore, the contamination by *E. coli* in soil irrigated with outflow water from wetland filters was not observed; with the exception of those waters associated with Filters 3 and 7, which were similarly contaminated. The highest contamination by *E. coli* was recorded for soil irrigated with raw wastewater. In contrast, the lowest contamination by *E. coli* was observed in soil irrigated with wastewater, which was diluted by 80% with tap water. Contamination by *Streptococci* was not observed for soil irrigated with treated wastewater obtained from all wetland filters; with the exception of Filter 4. Soil irrigated with wastewater, which was diluted by 80% with tap water, was reported to have higher contamination recordings by *Streptococci* than those soils irrigated by raw wastewater. Finally, the highest contamination recordings by *Salmonellae* were observed in soil irrigated with water harvested from Filter 4 followed by those soils irrigated by waters associated with Control A and Filter 6 (Fig. 6). In contrast, the lowest contamination by *Salmonella* spp. was observed in soil irrigated with outflow water that came from Filter 5. Furthermore, soils irrigated with waters from Filters 7 and 2 were more contaminated by *Salmonella* spp. compared to those irrigated by waters harvested from Filters 8 and 1, respectively. However, the typical bacteria survival time in soil, fresh water and crops is less than 70, 60 and 30 days, respectively, according to EPA (1992).

3.3. Chilli quality and analysis

3.3.1. Chillies mineral content

Table 5 shows the concentrations of elements detected in Chilli harvested from plants grown in organic media. However, none of these elements were detected in fruits harvested from plants grown in inorganic media. This can be explained by the alkaline media condition, which limited most of the elements availability to be absorbed by the plant root systems (FAO, 1972, 2003). The high pH of the sand limited the uptake of nutrients by plants, explaining the poor fruit quality productions (Section 3.3.3). However, some element concentrations in fruits linked to sand media were too low to be detected by ICP-OES. Furthermore, compared to organic media, it is difficult to study the impact of inorganic media on the chemical composition of vegetables. This is due to the low cation exchange capacity of sandy soil, which may lead to the development of deficiencies regarding most elements in terms of their available for plants. Moreover, the low cation exchange capacity of sandy soil causes high leaching of elements as reported by Olle et al. (2012).

3.3.1.1. Comparison of potassium. Potassium concentrations in all tested fruits were very high compared to those reported by Ciju (2003) who recommended the potassium value of 1870 mg per 100 g of sun dried chillies. However, the highest potassium concentrations were observed in fruits harvested from plants irrigated with raw wastewater followed by those irrigated with Filters 8 and 7 outflow waters, while the lowest values were recorded for fruits harvested from plants irrigated with Filter 3 drain water (Table 5). Moreover, statistical analysis showed significant ($p < 0.05$) differences in the mean potassium concentration of fruits harvested from plants irrigated with water obtained from Filter 3 compared to those of Filter 4, explaining the impact of irrigation water volume (Fig. 5) and diesel contamination (Table 1), and compared to Filter 5, explaining the impact of inflow loading rate of the wetland system (Table 1) on average potassium concentrations of the yield. The differences of potassium values in harvested Chillies can be explained by the significant ($p < 0.05$) differences of potassium concentrations in the corresponding growth media irrigated by various irrigation water types (see also Section 3.2.3.4). However, from the human health point of view, potassium is an important mineral that can maintain the water balance and blood pressure within human bodies (FAO/WHO, 2001).

3.3.1.2. Comparison of calcium. Table 5 shows that calcium concentrations in tested fruits were higher than that reported by Ciju (2003) of 45 mg per 100 g of sun dried Chillies. However, results showed that calcium was detected in high values in all harvested fruits due to high calcium availability in the organic growth media (Fig. 4b). Calcium is an essential mineral for human health, especially important for metabolism processes, bone structure, muscle and nerve function control, and managing the balance of blood stream. This explains how food, which is rich in calcium, can play an important role in human health (Zhu and Prince, 2012).

3.3.1.3. Comparison of magnesium. Table 5 indicates that magnesium concentrations in tested fruits were higher than that reported by Ciju (2003) of 88 mg per 100 g of dried Chillies. The highest magnesium concentrations were observed in fruits harvested from plants irrigated with Filter 7 outflow water followed by those of Filter 4 and 2, while the lowest concentration values were recorded for fruits of plants irrigated with Filter 3 outflow water. Furthermore, statistical analysis showed that there are significant ($p < 0.05$) differences in mean fruit magnesium concentrations of plants irrigated with Filter 7 water compared to those of Filter 8, explaining the impact of resting time (Table 1). Moreover, significant ($p < 0.05$) difference was observed between fruit magnesium mean concen-

Table 5
Overview of the Inductively Coupled Plasma (ICP) Optical Emission Spectrometer analysis for selected elements (mean ± standard deviation) compared with common standards for vegetables (e.g., EC (2001) and FAO/WHO (2001)). Note: Ten fruit samples per treatment were analysed.

Inflow source and organic growth media	Detected element (mg/kg)						
	K	Ca	Mg	Al	Mn	Zn	Fe
Filter 1	35943.86 ± 16018.584	2620.61 ± 1259.112	2234.65 ± 268.675	393.41 ± 70.431	112.23 ± 8.852	93.87 ± 43.046	176.65 ± 3.395
Filter 2	36969.28 ± 8610.192	3985.47 ± 2434.226	2621.71 ± 799.430	145.92 ± 45.279	99.92 ± 4.850	78.91 ± 34.477	189.14 ± 7.383
Filter 3	26115.69 ± 6191.515	1110.33 ± 269.040	1940.32 ± 635.952	n.d.	n.d.	53.35 ± 28.157	161.97 ± 29.143
Filter 4	49235.75 ± 7015.649	2006.76 ± 769.763	2692.50 ± 689.897	n.d.	66.41 ± 3.145	61.66 ± 26.257	186.96 ± 6.137
Filter 5	50012.58 ± 14679.311	1754.69 ± 73.403	2032.50 ± 193.319	n.d.	52.92 ± 4.147	91.45 ± 11.325	117.94 ± 4.674
Filter 6	44898.53 ± 8389.098	970.55 ± 664.798	2062.83 ± 514.969	256.15 ± 33.476	36.83 ± 8.642	47.72 ± 7.084	138.04 ± 17.109
Filter 7	57836.97 ± 2653.866	2053.91 ± 974.201	3078.40 ± 594.100	n.d.	56.48 ± 3.362	111.41 ± 32.405	159.51 ± 7.406
Filter 8	58314.42 ± 4264.132	1388.97 ± 112.485	2233.43 ± 250.491	n.d.	39.76 ± 3.452	94.28 ± 20.855	156.30 ± 34.141
Control A	35559.51 ± 9245.399	1207.45 ± 964.379	2451.85 ± 63.758	n.d.	56.68 ± 5.920	56.00 ± 0.907	111.63 ± 13.099
Control B	52524.86 ± 2606.509	1520.74 ± 764.821	2231.78 ± 107.822	n.d.	57.22 ± 10.061	71.73 ± 4.356	118.41 ± 9.343
Deionised water	35567.81 ± 2483.283	1169.69 ± 705.679	1613.21 ± 355.003	n.d.	45.90 ± 7.322	61.77 ± 2.042	51.11 ± 6.161
Tap water	28083.94 ± 2009.930	1028.79 ± 25.198	2336.40 ± 192.372	n.d.	39.294 ± 3.831	27.83 ± 0.420	74.05 ± 1.414
Tap water/fertiliser	31250.03 ± 2967.701	579.46 ± 454.494	1918.14 ± 429.386	n.d.	n.d.	52.48 ± 7.022	98.19 ± 22.573
Wastewater/tap water	27664.65 ± 4808.340	1499.39 ± 322.552	2105.04 ± 408.256	n.d.	47.18 ± 7.913	70.24 ± 12.712	74.37 ± 19.037
Wastewater	64087.97 ± 8934.644	1558.09 ± 144.109	3028.46 ± 156.971	n.d.	50.77 ± 8.898	69.09 ± 11.720	109.13 ± 8.754
Recommended maximum	–	–	–	–	500.00	50.00	425.00

Note: Elements not listed in this Table (i.e. arsenic, boron, barium, bismuth, cadmium, cobalt, chromium, copper, lithium, nickel, lead, strontium and titanium) were either below (or close to) the detection limits or could not be measured via the ICP-OES technology. n.d., not detected.

trations of Filters 3 and 4 due to impact of irrigation water volume (Fig. 5) and diesel contamination (Table 1). Considering human health, magnesium plays a role in the structural development of bones, and the active transport of calcium and potassium ions across cell membranes, which is important to nerve impulse conduction, muscle contraction, and a normal heart rhythm. Moreover, too much magnesium from food does not pose a health risk for healthy individuals (Musso, 2009).

3.3.1.4. Comparison of aluminium. Table 5 shows that aluminium was detected only in fruits harvested from plants irrigated with outflow waters from Filters 1, 2 and 6. However, aluminium was found in abundancy in growth media (Fig. 4a), since its solubility is mainly governed by the soil pH, and by soil organic matter and clay content. Exchangeable aluminium rapidly increases when pH decreases (Husson, 2013). In spite of that, aluminium was limited in terms of its transfer into fruit tissue. This can be explained by the high abundance of calcium in the growth media (compost) leading to the limited transport of aluminium to the plants (FAO, 1972). However, aluminium was not considered harmful to human health, because of its relatively low bioavailability (Stahl et al., 2011).

In acid mineral soils (pH < 7.0), aluminium buffers the soil pH at around 4, and is thus available to plants in the toxic form Al^{3+}. However, plant populations present in these soils normally evolve some degree of tolerance to aluminium in the soil solution and any aluminium present in these soils is likely to be as non-toxic organo-aluminium complexes (Kidd and Proctor, 2000).

3.3.1.5. Comparison of manganese. Manganese concentrations in tested fruits are shown in Table 5. The highest manganese concentrations were observed in fruit of plants irrigated with Filter 1 followed by Filter 2 outflow waters, while the lowest values were observed in those fruits of plants irrigated with Filter 6 drain water. Statistical analysis showed that fruit mean manganese concentrations of plants irrigated with Filters 2, 4 and 7 outflow waters were significantly ($p < 0.05$) different from those of plants irrigated with Filter 4, 6 and 8 outflow waters indicating the impact of aggregate size, inflow loading rate and resting time of wetlands on corresponding growth media (see Section 3.2.3.6). Furthermore, fruits of plants irrigated with Filters 1 and 5 had average magnesium concentrations significantly ($p < 0.05$) different from those of Filters 2 and 6, respectively, explaining the impact of diesel contamination. However, the differences of manganese concentration in the harvested fruits can be explained by the differences in manganese values regarding the corresponding growth media as shown in Section 3.2.3.6. Manganese concentrations in tested fruits did not exceed the corresponding metal threshold of 500 mg/kg (EC, 2001; FAO/WHO, 2001).

3.3.1.6. Comparison of zinc. Table 5 shows that the highest zinc concentrations were observed in fruit harvested from plants watered with Filter 7 outflow water followed by those of Filters 8 and 1, while the lowest concentrations were observed on fruits of plants irrigated with Filter 6 outflow water. However, detected zinc concentrations exceeded those reported by Ciju (2003) of 1.02 mg for 100 g of dried Chillies. Statistical analysis showed that mean zinc concentrations of fruits from Filter 1 plants were significantly ($p < 0.05$) different from those of Filter 3, because of a difference in aggregate size (Table 1). Average zinc concentrations in fruits of Filter 4 irrigated plants were significantly ($p < 0.05$) different from those of Filter 7, explaining the impact of the contact time variable on the corresponding zinc concentrations of the growth media (Section 3.2.3.7). Inflow loading rate impact was observed due to the significant ($p < 0.05$) differences in mean zinc concentrations of Filter 3 plants compared to those of Filter 5 (Table 1). Moreover, the impact of diesel spill filter contamination (Table 1) on mean zinc concentration of harvested fruits was observed due to the significant ($p < 0.05$) differences when comparing fruits from Filter 5 plants with those of Filter 6. Differences in zinc concentrations in harvested fruits are likely due to the differences in zinc values in the corresponding growth media as shown in section 3.2.3.7. However, zinc concentration in fruits from all irrigated plants (except for those irrigated with Filter 6 outflow water and tap water) exceed the corresponding metal threshold of 50 mg/kg (EC, 2001; FAO/WHO, 2001). Lacatusu (1998) assessed the levels of heavy metals in vegetables by evaluating the contamination/pollution index (C/P). This index is based on the metal concentration in vegetables (or water or soil) divided by the corresponding maximum permissible concentration levels (thresholds). Lacatusu (1998) listed the significance intervals of the C/P index. Based on that, all fruits tested for zinc were slightly polluted (C/P value between 1.1 and 2.0) with the exception of those harvested from plants irrigated with Filter 7 outflow water, which was moderately polluted (C/P values between 2.1 and 4.0). Considering human health, zinc is an essential micronutrient in the body and can be used in numerous pharmaceutics. Nevertheless, zinc is toxic, when taken long-term in high doses (FAO/WHO, 2001).

3.3.1.7. Comparison of iron. Table 5 shows that the highest iron concentrations were observed in fruits harvested from plants irrigated with outflow water obtained from Filter 2, followed by those fruits irrigated with outflow waters from Filters 4 and 1. In comparison, the lowest iron concentrations were recorded for fruits of plants irrigated with outflow water received from Filter 5. However the recorded iron concentrations in fruits harvested from all treatments (except fruits from deionised water plants) were exceeding those reported by Ciju (2003) of 6.04 mg per 100 g dried Chillies.

The impact of the wetland inflow loading rate was significant ($p < 0.05$) for a comparison between the iron mean concentrations in fruits harvested from plants of Filters 3 and 4 with those of Filters 5 and 6, respectively. Moreover, iron concentrations in tested fruits did not exceed the threshold of 425 mg/kg (EC, 2001; FAO/WHO, 2001). Although iron is present at high quantities in the soil (Fig. 4c), its availability to plants is usually very low (FAO, 1972). Although iron is an essential element for human health, excessive iron amount can lead to tissue damage (Abbaspour et al., 2014).

3.3.1.8. Comparison of boron. In spite of boron availability in growth media (Fig. 4h), results showed that boron was not detected in the tested fruits harvested from all treatments. Boron can be available in the soil at different concentrations and compositions, but only a relatively small proportion is obtainable by plants (Diana, 2006). Regarding human health, boron is considered as an essential mineral that can positively affect bone growth and reduce the risk of some cancer types (Nielsen, 2014).

3.3.2. Chillies microbial content

Fig. 3 shows that the bacterial contamination of water, while Fig. 7 indicates the bacterial contamination of fruits. No fruits harvested at a plant height of equal or above 50 cm were associated with microbial contamination. Fig. 7 indicates that no microbial contamination of Chillies irrigated by wastewaters (treated by wetlands) was detected. Findings also show that no microbiological contamination was recorded for skin, flesh and washing solutions for the fruits harvested from plants irrigated with outflow water obtained from wetland filters. In contrast, the fruits harvested from plants irrigated with wastewater, which was diluted by 80% with tap water, and with raw wastewater showed high contamination by total coliforms. Furthermore, high contamination levels with *Streptococcus* spp. and *Salmonella* spp. were recorded for Chilli fruits harvested from plants irrigated by raw wastewater. However, the fruits linked to wastewater and wastewater plus tap water treat-

Table 6
Chilli harvest classification scheme (after Almuktar et al. (2015a)).

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
Mean price estimate; pence (Sterling)/gram	2.00	1.00	0.50	0.25	0.00
Length (L, mm)	Very long ($L \geq 80$)	Long ($60 \leq L < 80$)	Medium ($40 \leq L < 60$)	Short ($20 \leq L < 40$)	Very short ($L < 20$)
Width (W, mm)	Very wide($W \geq 20$)	Wide ($16 \leq W < 20$)	Medium ($12 \leq W < 16$)	Slim ($8 \leq W < 12$)	Very slim ($W < 8$)
Weight (w, g)	Very Large($w \geq 9$)	Large ($7 \leq w < 9$)	Medium ($5 \leq w \leq 7$)	Small ($3 \leq w < 5$)	Very Small ($w < 3$)
Bending	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Characteristically bend; $L/W \geq 3.5$	Uncharacteristically bend; $L/W < 3.5$	Uncharacteristically bend; $L/W < 3.5$

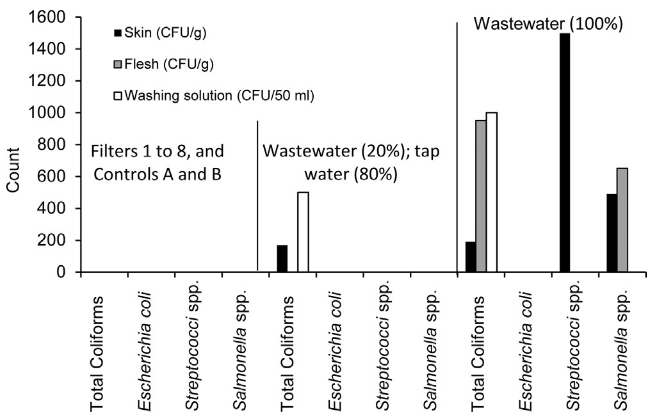

Fig. 7. Contamination of Chilli fruits (only detected for locations below a plant height of 50 cm) by bacteria as a function of water sources.

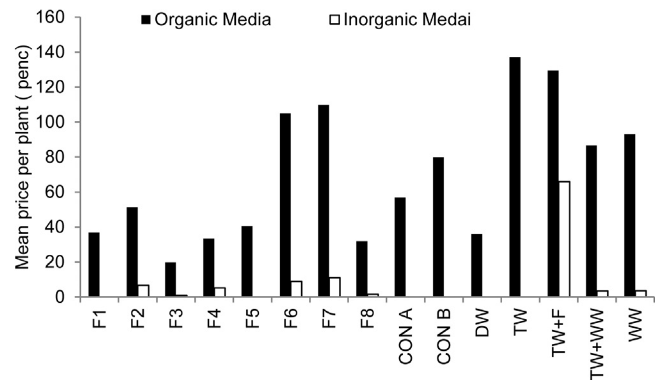

Fig. 8. Overview of the outcome of the Chilli harvest classification scheme according to Table 6. The lowest variable class entry for any individual fruit assessment determined the final class. Length, width, weight and bending were used to classify fruits.

ments were contaminated due to the contact with contaminated soil, while other fruits, which were located far away from the soil, did not show any bacterial contamination.

The approximate number of Chillies harvested below 50 cm was only about 5% of the total harvest for most plants. The results showed that there is no microbial contamination of Chillies located higher up on the plant branches. This can be explained by the relatively long distance between the fruits and the potentially contaminated soil (Cirelli et al., 2012). Moreover, vegetable pots receiving wastewater treated with wetlands acting as a biological filter bed can be considered safer than those receiving only preliminary treated wastewater.

3.3.3. Chilli production practice and marketable yield assessment

Table 6 shows a harvest classification scheme (after Almuktar et al. (2015a)) for Chillies. Only the higher classes are of great commercial interest. However, the estimated prices are dependent on global commodity market developments. The highest number of fruits categorised as Class A were harvested from plants grown in organic media and watered with tap water followed by those grown in organic media and watered by Filter 7 (Fig. 8). However, Filter 3 was associated with the highest fruit numbers, which received low category classifications (i.e. $C = 10$, $D = 26$, and $E = 9$). The highest mean price of harvested fruits is also associated with tap water followed by Filter 7. Findings showed that the productivity of Chillies in terms of marketable yield was independent of wastewater consumption volume (Fig. 5), but may depend on the water quality (e.g., nutrients and trace mineral availability).

4. Conclusions and recommendations for further research

The experiment shows that Chillies can be grown successfully in organic media using wastewater treated by some constructed wetland types despite of irrigation water contaminated by metals and pathogens. The major findings are as follows:

(1) Phosphorus, ammonia-nitrogen, manganese and potassium significantly ($p < 0.05$) exceeded the thresholds set for irrigation purposes.
(2) The mineral content of organic soil was significantly ($p < 0.05$) higher than that for inorganic soil before and after irrigation with treated wastewater.
(3) Compared to the raw growth media, irrigation with treated wastewater led to concentration increases of some elements such as magnesium, aluminium, zinc and boron.
(4) No substantial mineral contamination was observed in the soils due to irrigation with treated wastewater.
(5) Slight to moderate zinc contamination was detected in harvested fruits based on common standards for vegetables.
(6) No bacteriological contamination was detected for any Chilli fruits harvested from a plant height of ≥50 cm
(7) High Chilli yields in terms of economic return were associated with tap water and an organic growth medium, and a wetland with a small aggregate size and a low contact time.
(8) Findings indicate that nutrient concentrations supplied to the Chillies by a combination of compost and treated wastewater are usually too high to produce a good harvest.

Further research by the authors will be undertaken on a new generation of Chilli plants grown in organic media to study the effect of irrigation with recycled wastewater treated by constructed wetlands and to obtain a new cultivar adapted to urban wastewater. Additional work on regenerating organic soil used for growing Chillies is currently been carried out. Considering that the soil got depleted of nitrogen, Alfalfa and Red Cover are being grown on the same soil to reintroduce nitrogen.

Recycling of domestic wastewater treated by wetlands for irrigation purposes seems to be a viable alternative to the use of drinking water and fertiliser application. However, the authors recommend undertaking long-term field trials by the agricultural industry to assess changes in the soil properties due to different irrigation management schemes. Considering the fact that field trial results are very variable, because each field is an uncontrolled open system, the findings of this paper do not necessarily extrapolate well to field conditions.

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Experimental Assessment of Recycled Diesel Spill-Contaminated Domestic Wastewater Treated by Reed Beds for Irrigation of Sweet Peppers

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Abstract: The aim of this experimental study is to assess if urban wastewater treated by ten different greenhouse-based sustainable wetland systems can be recycled to irrigate *Capsicum annuum* L. (Sweet Pepper; California Wonder) commercially grown either in compost or sand within a laboratory environment. The design variables were aggregate diameter, contact time, resting time and chemical oxygen demand. The key objectives were to assess: (i) the suitability of different treated (recycled) wastewaters for irrigation; (ii) response of peppers in terms of growth when using recycled wastewater subject to different growth media and hydrocarbon contamination; and (iii) the economic viability of different experimental set-ups in terms of marketable yield. Ortho-phosphate-phosphorus, ammonia-nitrogen, potassium and manganese concentrations in the irrigation water considerably exceeded the corresponding water quality thresholds. A high yield in terms of economic return (marketable yield expressed in monetary value) was linked to raw wastewater and an organic growth medium, while the plants grown in organic medium and wetlands of large aggregate size, high contact and resting times, diesel-spill contamination and low inflow loading rate produced the best fruits in terms of their dimensions and fresh weights, indicating the role of diesel in reducing too high nitrogen concentrations.

Keywords: *Capsicum annuum*; hydrocarbon; marketable yield; nutrient; sustainable agricultural water resource; water reclamation; wetland; vegetable

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1. Introduction

1.1. Background and Motivation

Since water resources are limited in dry climates, sustainable wastewater treatment and the recycling of the corresponding effluent is a good alternative to using potable water for irrigation [1]. Treated urban water can be recycled for irrigation in agriculture, urban landscape management, industry and ground water recharge [2]. Around 20M ha of land is irrigated by untreated and treated wastewater [3]. Advantages linked to urban wastewater recycling include the abundant supply of nutrients to crops, which could lead to higher marketable yields as well as a decrease in the need for commercial fertilizers [4].

1.2. Wastewater Recycling for Irrigation

Wastewaters can be treated with a wide range of standard (e.g., activated sludge process or trickling filters) and alternative (e.g., wetlands) technologies that can be selected based on criteria such as reliability, simplicity, efficiency, land requirement, affordability, social acceptability and sustainability [5]. Various practical and academic tools have been developed to assist decision-makers in selecting the most appropriate technology including expert opinion [6,7].

Treated wastewater can be recycled to irrigate crops in arid regions that are confronted by considerable water shortages, supporting renewable agriculture and food systems. However, pre-treated wastewaters might require disinfection before application to fields [6]. Irrigation by recycled wastewater can increase the productivity of farming by between 100% and 400%, allowing some crops to be grown in regions with unfavorable conditions [8].

The assessment of the impacts of wastewater reuse on agricultural products intended for consumption is important [9–11]. Either too high or too low concentrations of nutrients in the reused water are a potential problem to crops. Hydrocarbons within road runoff are a new challenge [12,13].

Researchers [12] studied the impact of treated urban wastewater on soil and Sweet Pepper. Recycling saved fertilizer. Moreover, hydrocarbons and heavy metals were low in the harvested fruits. The reuse of nutrients from settled primary urban wastewater on peppers was researched, previously [14]. The crops grown removed both nitrogen and phosphorous, and were healthy compared to the control using standard fertilizer. Nutrients such as ammonia have a negative effect on fruit, leaf and stem developments [15]. Moreover, the total yield increases as the nitrate-nitrogen to ammonia-nitrogen ratio increases. This can be explained by a reduction in fruit physiological disorders, which usually reduce fruit mean weight [16].

Research was undertaken to evaluate the impact of various growth media on Sweet Pepper yields, indicating that peat moss media benefited seedlings. Moreover, peat moss and coco-peat alone or mixed with sand led to a better harvest than other media [17]. The yield response of peppers to mineral and organic fertilization has been assessed, previously [18]. Findings indicate that no differences in yield were noted between organic and conventional farming practices. Another study was undertaken to assess the effects of only peat, and a mixture of peat, perlite and sand (volume ratio of 1:1:1) on yield-related parameters. Results indicated that mixed media increased length, diameter and weight of fruits in all cultivars in comparison to only peat media [19].

Wetland systems can be applied to treat urban wastewater well [13,20]. The treatment efficiency of constructed wetlands on wastewaters and their suitability for reuse in agriculture was also assessed elsewhere [21]. Only water quality variables with high removal efficiencies fulfilled the guidelines for recycling. However, parameters with rather low efficiencies such as solids and phosphorus limited the water reuse potential. Wetlands treating urban effluents to be reused in the agricultural sector were assessed previously [22]. Mean removal efficiencies for suspended solids, biochemical oxygen demand, chemical oxygen demand, total nitrogen and total phosphorus were 85%, 65%, 75%, 42% and 32%, respectively, indicating the possibility for ecological sanitation.

1.3. Nutrient and Mineral Requirements

The authors of this article focused on Sweet Pepper, because it is an easy-to-grow and cost-effective plant with good nutritional benefits [23]. The major elements influencing the growth of crops are nitrogen, phosphorus, potassium, calcium, magnesium and sulphur [24]. Heavy metals may be toxic for peppers.

Maximum concentrations for iron, manganese and potassium are 5.0 mg/L, 0.2 mg/L and 2.0 mg/L, respectively [25,26]. The application of various treated wastewaters for recycling has been classified [27]. Suitable ranges for ammonia-nitrogen and ortho-phosphate-phosphorous are 0 mg/L to 5 mg/L, and 0 mg/L to 2 mg/L in that order. Furthermore, no restriction for the reuse of pre-treated waters with a nitrate-nitrogen value below 5 mg/L has been proposed. Slight to moderate constraints exist for the range between 5 mg/L and 30 mg/L. However, severe restrictions are imposed for measurements greater than 30.0 mg/L [26].

1.4. Rationale, Aim and Objectives

Effluents from different wetlands treating urban wastewater were recycled to irrigate Sweet Pepper. Some wetlands received wastewater contaminated by diesel. The aim of this study is to evaluate if Sweet Pepper can be grown successfully using recycled urban wastewater treated by wetlands to obtain a high marketable yield.

The specific and measurable objectives related to the growing of peppers are to assess: (a) the appropriateness of treated (recycled) wastewater for irrigation compared to corresponding standards; (b) the impact of various waters as a function of the type of wetland; (c) the response of peppers in terms of growth when using recycled (pre-treated) wastewater streams (some contaminated by a diesel spill) subject to different growth media; (d) the effect of environmental boundary conditions on the yield; and (e) the economic return of various experimental systems in terms of marketable yield.

2. Materials and Methods

2.1. Wetlands Set-Up and Operational Arrangements

Ten vertical-flow wetland filters were operated between 27 June 2011 and 25 September 2014. The design variables were aggregate diameter, contact time, resting time and chemical oxygen demand. Contact time is defined as the period of time when the inflow water stays within the wetland. In comparison, resting time indicates the duration when the wetland is drained. The set-up includes two controls receiving clean de-chlorinated tap water (Table 1).

Table 1. Comparison of the experimental vertical-flow wetland set-up.

Filters [a]	Design variables			
	Aggregate Diameter (mm)	**Contact Time (h)**	**Resting Time (h)**	**Chemical Oxygen Demand (mg/L)**
Filter 1 [b]	20	72	48	123.3
Filter 2	20	72	48	123.3
Filter 3 [b]	10	72	48	123.3
Filter 4	10	72	48	123.3
Filter 5 [b]	10	72	48	244.7
Filter 6	10	72	48	244.7
Filter 7	10	36	48	123.3
Filter 8	10	36	24	123.3
Control A [b]	10	72	48	2.3
Control B	10	72	48	2.3

[a] Annually treated volumes of wastewater: Filters 1 to 6, 470 L/a; Filter 7, 624 L/a; Filter 8, 858 L/a; Control A and B, 470 L/a. [b] On 26 September 2013, 130 g of diesel (equivalent to an inflow concentration of 20 g/L) have been added to Filters 1, 3 and 5, and Control A.

The wetland filters were constructed from Pyrex tubes (inner diameter of 19.5 cm and height of 120 cm). The filters were filled with pea gravel up to 60 cm and planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed)(*P. australis*). The outlet valve is located at the bottom of each filter [28].

Preliminary treated wastewater was obtained from the Davyhulme Sewage works in Manchester. In order to simulate diesel fuel spills, 130 gram of diesel were poured into Filters 1, 3 and 5, and into Control A on 26 September 2013. Chillers (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were used to maintain the root system at 12 °C. All wetland columns received approximately 6.5 L of inflow [28]. All water quality parameters were recorded during or directly after harvesting the wastewater from the wetland filter.

2.2. Water, Soil and Pepper Quality Analysis

Routine water quality sampling was carried out according to standard methods [29]. The analysis of water samples for nutrients and trace element concentrations was undertaken using Varian 720-ES

Inductively Coupled Plasma—Optical Emission Spectrometer technology (ICP–OES [30]) manufactured by Agilent Technologies UK (Wharfedale Road, Wokingham, Berkshire, UK).

Soil quality analysis was undertaken [31]. Pepper marketable yield was assessed according to Table 2 [32–34]. Microbial tests (total coliforms, *Escherichia coli*, fecal *Streptococcus* spp. and *Salmonella* spp.) for water and vegetables (skin, flesh and washing solution harvested at different distances from the soil: 0 to 50 cm, 50 to 100 cm and more than 100 cm) were performed using aseptic pour plate techniques according to standard methods [29]. Where relevant, sample numbers, sampling periods and replicate numbers for water, soil and plant samples have been identified in the illustrations shown in section 3.

2.3. Light, Humidity and Temperature

Light measurements were undertaken using the LUX meter ATP-DT-1300 (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, Cheshire, UK). Humidity and temperature were recorded using wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The humidity was controlled using Challenge 3.0L Ultrasonic Humidifiers (Argos, Avebury Boulevard, Central Milton Keynes, England, UK).

2.4. Sweet Pepper Growing

Sweet Pepper seeds were obtained from B&Q plc (Chandlers Ford, Hants SO53 3LE;) on 14 September 2013. The seeds were first planted in shallow seed trays for about one week, and subsequently replanted (second planting) in larger nursery pots. The third and final planting was undertaken 28 days after the second planting on 8 November 2013, following supplier instructions. The peppers were planted into 10-litre plastic and round plant pots sourced from scotplants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK).

Plant pots dimensions were: height of 22.0 cm, bottom diameter of 22.0 cm and top diameter of 28.5 cm. Compost and pure sand were used. The compost was supplied by B&Q plc as part of their verve brand (product code: 03717644). The sand (Play Pit Sand (silica), product code: 5060096123309) was provided by Deko-Pak Limited (Deco House, Halifax Road, Hipperholme, Brighouse HX3 8BW).

The basic soil properties are listed in Table 3. The top 2 cm were left unplanted for both sand- and soil-based pots. However, sand-based plants were planted to a depth of 20.0 cm, and soil-based plants were planted to a depth of 17.5 cm, and covered by 2.5 cm of bark (B&Q verve range, product code: 5397007188110), which was described by B&Q as ideal for pots, beds and borders to control weeds, retain moisture and insulate soil. The plant pots remained indoors under laboratory conditions characterized in section 2.3.

Some peppers received fertilizer sourced from the B&Q plc verve range (product code: 5397007068245). The fertilizer had a nitrogen to phosphorus to potassium ratio of 4:4:4. Liquid fertilizer was added to the inflow water as specified.

2.5. Data Analysis

Microsoft Excel and IBM SPSS Statistics Version 20 were used. Significant findings have been highlighted, where appropriate.

Table 2. Sweet Pepper harvest classification scheme (partly adopted from elsewhere [32–34].

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
European Union classification equivalent	"Extra" Class	Class I	Class II	Not applicable	Not applicable
Mean price estimate; pence (Sterling)/gram	0.28	0.22	0.16	0.10	0.00
Target market	Top restaurant	National supermarket	Independent retailer or market	Vegetable industry	Waste company
Product	Fresh vegetable	Fresh vegetable	Fresh vegetable	Frozen or canned	Waste
Contamination	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Contaminated
Illnesses	None	None	None	Likely; no harm	Likely; harmful (rotten)
Length (L, mm)	Jumbo ($L \geq 110$)	Extra-large ($90 \leq L < 110$)	Large ($70 \leq L < 90$)	Medium ($40 \leq L < 70$)	Small ($L < 40$)
Diameter (D, mm)	Jumbo ($D \geq 90$)	Extra-large ($70 \leq D < 90$)	Large ($50 \leq D < 70$)	Medium ($30 \leq D < 50$)	Small ($D < 30$)
Weight (w, g)	Very Large ($w \geq 190$)	Large ($120 \leq w < 190$)	Medium ($70 \leq w \leq 120$)	Small ($20 \leq w < 70$)	Very Small ($w < 20$)
Tolerance by weight or number per plant (%)	5	10	10	10	10
Defect in shape (Damage (%) of surface area)	Damage ≤ 10	10 ≤ Damage < 20	20 ≤ Damage < 30	30 ≤ Damage < 60	Too many damages (>60)
Defect of the skin (Damage (%) of surface area)	Damage ≤ 3	3 ≤ Damage < 4	4 ≤ Damage < 5	5 ≤ Damage < 20	Too many damages (>20)

Table 3. Basic soil properties based on three replicates each (14 September 2013).

Parameter	Soil Type		Total Per Pot (mg)	
	Compost	Sand	Compost	Sand
pH	6.43	9.40	-	-
Redox potential (mV)	62.60	−79.20	-	-
Electrical conductivity (μs/cm)	2438.50	116.00	-	-
Total nitrogen (mg/kg)	998.75	7.60	3495.63	114.00
Total phosphor (mg/kg)	367.50	0.85	1286.25	12.75
Aluminium (mg/kg)	1118.38	1180.43	3914.33	17,706.45
Calcium (mg/kg)	18,421.96	174.16	64,476.86	2612.40
Iron (mg/kg)	6233.15	1196.48	21,816.03	17,947.20
Potassium (mg/kg)	2776.02	168.57	9716.07	2528.55
Magnesium (mg/kg)	5287.67	279.53	18,506.85	4192.95
Manganese (mg/kg)	201.59	8.09	705.57	121.35
Zinc (mg/kg)	26.59	1.95	93.07	29.25
Boron (mg/kg)	12.29	<0.0001	43.02	0.0015
Organic matter (%)	89.00	0.03	-	-
Bulk density(g/L)	350	1522	-	-

3. Results and Discussion

3.1. Comparison of Irrigation Water Qualities

3.1.1. Comparison of Hydrocarbon Values

Table 4 shows the inflow water quality. Total petroleum hydrocarbon values followed this order: Control A > Filter 8 > Filter 1 > Filter 3> Filter 5. Regarding filters contaminated by diesel (Filters 1, 3, 5 and Control A), the total petroleum hydrocarbon concentrations were 0.100 mg/L, 0.069 mg/L, 0.014 mg/L and 0.346 mg/L, respectively. These concentrations were in compliance with Chinese standards [35], indicating a maximum threshold of 1.0 mg/L. Note that Chinese standards were used, considering that China produces about 54% (estimated in 2008) of peppers in the world [36].

3.1.2. Comparison of Oxygen Demand Variables

Table 4 shows that chemical oxygen demand values were the highest for raw urban wastewater followed by filters contaminated with diesel following the order of F5 > F3 > F1 > Control A. Chemical oxygen demand concentrations were highly variable due to seasonal changes. Moreover, the standard deviation for Filter 8 was particularly high due to the low resting time (Table 1) resulting in insufficient biodegradation in some seasons. Statistically, no significant difference ($p > 0.05$) in chemical oxygen demand values of Filters 2 and 4 were found, indicating that aggregate size may not matter (Table 1). Filter 8 outflow water had chemical oxygen demand values, which were higher than those of Filter 7, highlighting the impact of long resting time on outflow water chemical oxygen demand (Table 1). In comparison, the lowest chemical oxygen demand values were recorded for Control B (no diesel contamination; Table 1).

The biochemical oxygen demand was the highest for raw urban wastewater and corresponding samples diluted with dechlorinated potable water followed by filters contaminated with diesel (Filters 1, 3 and 5). The biochemical oxygen demand values for Filter 8 (short resting time) outflow water were higher than those for Filter 7 (long resting time). The corresponding values for Control A (contaminated with diesel) were higher than those for Control B. Tap water had the lowest biochemical oxygen demand (Table 4).

Table 4. Comparison of the water quality of the inflow waters received by the vegetable pots (mean ± standard deviation (number of samples)) between 11 October 2013 and 25 September 2014.

Water Type	TPH [a] (μg/L)	COD [b] (mg/L)	BOD [c] (mg/L)	NH_4-N [d] (mg/L)	NO_3-N [e] (mg/L)	PO_4-P [f] (mg/L)	SS [g] (mg/L)	Turbidity (NTU) [h]	Ph (–)	EC [i] (μS/cm)	DO [j] (mg/L)	SAR [k] (me/L) [l]
Filter 1 outflow	100	77.7 ± 23.35 (18)	25.8 ± 16.74 (53)	4.8 ± 2.83 (22)	0.4 ± 0.22 (19)	4.0 ± 2.48 (18)	11.3 ± 10.42 (56)	9.0 ± 5.65 (54)	6.4 ± 0.26 (54)	336.5 ± 50.82 (22)	1.5 ± 1.03 (15)	2.4 ± 1.07 (5)
Filter 2 outflow	<10	34.9 ± 19.21 (15)	13.6 ± 8.11 (51)	6.2 ± 5.84 (20)	2.2 ± 2.72 (18)	3.3 ± 1.33 (18)	6.7 ± 9.49 (56)	5.4 ± 5.75 (53)	6.5 ± 0.21 (54)	328.6 ± 53.37 (22)	1.7 ± 1.10 (15)	1.8 ± 0.60 (5)
Filter 3 outflow	69	87.5 ± 26.00 (18)	22.8 ± 16.42 (51)	3.7 ± 2.53 (22)	0.4 ± 0.28 (19)	3.3 ± 2.04 (18)	11.7 ± 10.79 (56)	8.7 ± 6.09 (53)	6.5 ± 0.18 (54)	396.7 ± 76.59 (22)	1.7 ± 1.18 (15)	1.7 ± 0.53 (5)
Filter 4 outflow	<10	34.9 ± 23.77 (15)	12.8 ± .8.86 (50)	5.0 ± 10.53 (20)	1.8 ± 3.27 (18)	2.9 ± 1.06 (18)	7.4 ± 10.57 (56)	5.7 ± 5.46 (53)	6.5 ± 0.19 (54)	352.6 ± 67.56 (22)	2.0 ± 1.60 (15)	2.4 ± 0.39 (5)
Filter 5 outflow	14	100.8 ± 67.90 (18)	22.5 ± 16.35 (51)	9.7 ± 3.20 (21)	0.9 ± 0.86 (19)	4.4 ± 2.07 (18)	11.3 ± 12.76 (57)	8.6 ± 6.22 (53)	6.6 ± 0.19 (54)	564.1 ± 163.66 (22)	1.5 ± 0.81 (15)	2.2 ± 0.85 (5)
Filter 6 outflow	<10	35.6 ± 22.46 (14)	15.9 ± 12.68 (52)	9.0 ± 7.28 (20)	3.6 ± 4.68 (18)	4.6 ± 3.16 (18)	6.9 ± 8.68 (57)	5.4 ± 4.41 (53)	6.8 ± 0.19 (55)	524.3 ± 152.66 (22)	1.6 ± 1.09 (15)	3.1 ± 0.61 (5)
Filter 7 outflow	<10	32.5 ± 20.40 (14)	11.9 ± 8.01 (61)	3.6 ± 5.52 (24)	2.8 ± 2.98 (18)	3.6 ± 2.23 (17)	2.6 ± 3.86 (66)	3.4 ± 2.24 (62)	6.6 ± 0.18 (62)	355.0 ± 83.11 (28)	1.7 ± 0.86 (25)	2.4 ± 0.47 (5)
Filter 8 outflow	116	55.9 ± 86.05 (15)	13.9 ± 7.50 (69)	1.4 ± 1.35 (22)	2.8 ± 3.51 (16)	3.3 ± 1.90 (16)	2.9 ± 4.31 (76)	3.6 ± 2.48 (76)	6.5 ± 0.20 (78)	339.7 ± 104.74 (25)	1.9 ± 1.15 (22)	2.1 ± 0.44 (5)
Control A outflow	346	66.4 ± 44.32 (17)	12.0 ± 7.58 (51)	1.3 ± 1.79 (22)	0.4 ± 0.44 (19)	1.8 ± 0.56 (18)	9.0 ± 10.25 (56)	5.7 ± 4.31 (53)	6.7 ± 0.17 (55)	149.2 ± 32.47 (22)	1.4 ± 0.93 (15)	0.5 ± 0.15 (5)
Control B outflow	<10	16.0 ± 15.12 (15)	8.8 ± 7.58 (52)	1.3 ± 1.77 (21)	0.3 ± 0.35 (18)	1.9 ± 0.33 (18)	3.6 ± 8.18 (56)	4.1 ± 4.54 (53)	6.5 ± 0.20 (54)	153.9 ± 29.87 (22)	1.8 ± 1.04 (15)	0.5 ± 0.14 (5)
Deionised water	Nm [m]	3.5 ± 0.08 (3)	7.3 ± 1.84 (3)	0.1 ± 0.13 (3)	0.0 ± 0.00 (3)	0.0 ± 0.00 (3)	2.0 ± 2.31 (10)	1.3 ± 0.14 (?)	5.1 ± 0.58 (10)	1.5 ± 0.72 (10)	nm	0.1 ± 0.15 (5)
Tap water (100%)	Nm	6.2 ± 0.33 (3)	4.9 ± 1.13 (3)	0.1 ± 0.00 (3)	0.2 ± 0.00 (3)	0.8 ± 0.00 (3)	2.0 ± 2.31 (10)	1.4 ± 0.21 (10)	6.1 ± 1.06 (10)	95.8 ± 15.20 (10)	nm	0.8 ± 0.15 (5)
Tap water with fertiliser	Nm	8.6 ± 0.22 (3)	8.0 ± 2.62 (3)	16.0 ± 0.01 (3)	8.9 ± 0.38 (3)	14.9 ± 0.07 (3)	1.6 ± 0.46 (10)	3.0 ± 0.49 (10)	6.0 ± 0.28 (10)	204.0 ± 5.66 (10)	nm	0.8 ± 0.10 (5)
Wastewater (20%); tap water (80%)	Nm	47.6 ± 15.39 (17)	21.8 ± 15.99 (55)	6.7 ± 3.69 (22)	0.5 ± 0.64 (21)	3.0 ± 1.43 (21)	26.4 ± 18.48 (63)	16.2 ± 15.18 (56)	7.1 ± 0.07 (55)	122.1 ± 55.98 (22)	nm	1.7 ± 0.59 (5)
Wastewater (100%)	Nm	237.9 ± 76.96 (17)	105.3 ± 75.98 (55)	33.6 ± 18.46 (22)	2.4 ± 3.22 (21)	14.9 ± 7.15 (21)	131.9 ± 92.64 (63)	80.4 ± 75.97 (56)	7.5 ± 0.42 (55)	575.5 ± 181.66 (22)	5.2 ± 3.72 (16)	2.8 ± 0.62 (5)
Standard	1000	-	-	5	30	2			6.0–8.5	3000		≤15

[a] TPH: total petroleum hydrocarbon; [b] COD: chemical oxygen demand; [c] BOD: five-day biochemical oxygen demand; [d] NH_4-N: ammonia-nitrogen; [e] NO_3-N: nitrate-nitrogen; [f] PO_4-P: ortho-phosphate-phosphorus; [g] SS: suspended solids; [h] NTU: turbidity; [i] EC: electrical conductivity; [j] DO: dissolved oxygen; [k] SAR: sodium adsorption ratio (sodium (calcium+magnesium)$^{-2}$)$^{-0.5}$); [l] me/L: milliequivalent per litre; [m] nm: not measured.

Dissolved oxygen values were higher for those filters without diesel (Table 4). Correlation analysis results indicated that dissolved oxygen was significantly negatively correlated with micro-organisms, total petroleum hydrocarbon and chemical oxygen demand in the treatment system. This negative correlation can be explained by an improvement of the chemical oxygen demand and the total petroleum hydrocarbon removal efficiencies as micro-organisms responsible for biodegradation acclimatized, resulting in a reduction of the amount of available dissolved oxygen [13].

3.1.3. Comparison of Nitrogen Compounds

The inflow water quality was highly variable for both nitrogen species, because of seasonal water quality variations in the wetland systems (Table 4). The inflow waters with a high chemical oxygen demand resulted in statistically significant ($p < 0.05$) differences between the ammonia-nitrogen concentrations of Filters 3 and 4 compared to those of Filters 5 and 6, respectively (Table 1). Elevated concentrations of ammonia-nitrogen exceeding the threshold of 5 mg/L [25,26] were recorded for raw wastewater, wastewater diluted with 80% of potable water, and outflow waters from Filters 5 and 6, which were fed with high inflow loads, followed by that from Filter 2, which had a large aggregate size (Table 4).

Moreover, the mean nitrate-nitrogen values of Filter 4 compared to those of Filters 7, and the concentrations for Filters 3 and 4 compared to those of Filters 5 and 6 were statistically significantly different from each other ($p < 0.05$), indicating the impact of contact time and inflow loading rate of wetland systems on outflow water nitrate-nitrogen values (Table 1). However, nitrate-nitrogen concentrations for all outflows (Table 4) were below 4 mg/L, which is considerably less than the threshold of 30 mg/L [25,26].

3.1.4. Comparison of Ortho-Phosphate-Phosphorus

Considering the threshold of 2 mg/L for ortho-phosphate-phosphorus [25], the outflow waters from all wetlands were associated with too high ortho-phosphate-phosphorus concentrations (Table 4). Statistical results did not show any significant differences in ortho-phosphate-phosphorus values of the outflow waters indicating that wetland aggregate diameter, contact and resting times as well as inflow loading rate do not matter (Table 1). However, phosphorus is difficult to remove by mature wetlands [37], because it is often present in particulate form [13].

3.1.5. Comparison of Trace Elements

Figure 1 shows an overview of the ICP–OES findings for selected elements determined in the irrigation water compared to standards. Figure 1a shows that sodium concentration for all irrigation waters did not exceed the standard for irrigation water of 920 mg/L [25,27]. However, statistical analysis showed that the sodium values of Filters 3 and 4 compared to those of Filters 5 and 6 outflow waters were significantly different from each other indicating the impact of the inflow loading rate of wetland systems on outflow water sodium concentrations (Table 1).

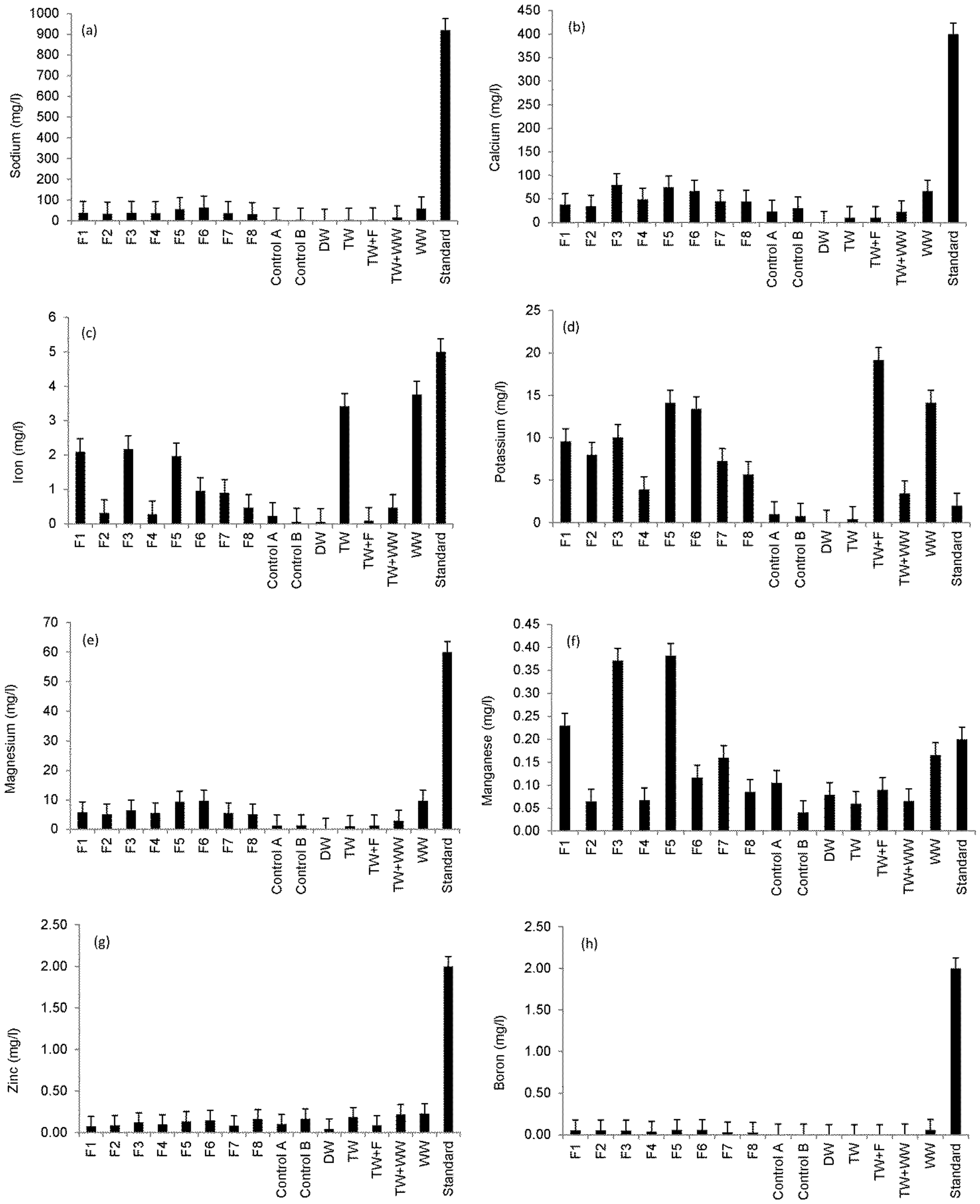

Figure 1. Overview of the Inductively Coupled Plasma—Optical Emission Spectrometer (ICP–OES) analysis (sample number: 10; 11 October 2013 to 25 September 2014) for detected elements compared with common standards for irrigation water [25,27].

Figure 1b,c shows that the calcium and iron concentrations of all irrigation waters did not exceed the standards of 400 mg/L and 5 mg/L, respectively. Regarding the threshold of 2 mg/L for potassium [25,27], all irrigation water types (except for Controls A and B) had too high potassium concentrations. Statistical results showed that outflow water from Filter 4 had potassium concentrations, which were significantly different from those associated with Filter 7, indicating the impact of contact time on outflow water potassium concentrations (Table 1).

Moreover, a high inflow loading rate of the wetland system resulted in significant differences ($p < 0.05$) in outflow water potassium concentrations for Filter 4 compared with Filter 6 (Figure 1d). No magnesium concentrations exceeded the threshold of 60 mg/L [25,27] as shown in Figure 1e. However, statistical results showed that outflow waters from Filters 3 and 4 compared to those of Filters 5 and 6 had magnesium concentrations, which were significantly ($p < 0.05$) different from each other, explaining the impact of inflow loading rate of wetland systems on outflow water magnesium concentrations (Table 1). Figure 1f shows that results for Filters 1, 3 and 5, which were contaminated with diesel, have elevated manganese concentrations exceeding the threshold of 0.2 mg/L [25,27]. Figure 1g,h show that zinc and boron concentrations in all irrigation water types did not exceed the threshold of 2 mg/L [25,27].

3.1.6. Comparison of Particles

The inflow water quality was highly variable for particles indicated by suspended solids and turbidity (Table 4). This can also be explained by the seasonal water quality variations in the wetland systems. For example, as above-ground *P. australis* plant parts decay in winter and early spring, more particles are created as by-products of the biodegradation process. Furthermore, the standard deviations for very clean waters such as tap water are high due to the random presence of larger particles.

Table 4 shows that the highest value for suspended solids was noted for raw wastewater and diluted wastewater followed by those for outflow waters from filters contaminated with diesel (Filters 1, 3 and 5, and Control A). Turbidity had the highest values for raw wastewater and diluted wastewater followed by outflow waters received from Filters 1, 3 and 5, which were contaminated with diesel. Filters 7 and 8 had the lowest turbidity values in spite of different resting times. Correlation analysis results showed that turbidity was significantly positively correlated with suspended solids and micro-organisms in treatment systems indicating a good relationship between turbidity and indicator micro-organisms activity due to degradation of organic matter and a subsequent increase in particles [28]. However, high values of suspended solids and turbidity associated with irrigation water will considerably increase the development of hydrophobicity in the soils, and subsequently affect plant growth.

3.1.7. Comparison of pH and Salinity

Table 4 shows that the pH values were normal [25]. Conductivity is the most important indirect measure of salinity, posing a great hazard to crops and determining the suitability of water for irrigation use. Salts negatively impact on the growth of plants, and the soil structure and permeability, indirectly affecting plant growth as well. However, the conductivity values for all filter outflow waters complied with the threshold of 3000 μs/cm [25,27]. Furthermore, the sodium adsorption ratio concentrations for all outflows were normal; *i.e.* between 0 milliequivalents per liter (me/L) and 15 me/L [27].

3.1.8. Comparison of Microbial Content

Microbial characteristics of irrigation water are summarized in Figure 2. Based on the maximum value for total coliforms (1000 CFU per 100 mL) regarding the irrigation of crops [38], the outflow waters from all wetlands were associated with too high contamination by total coliforms. Furthermore, high contamination by total coliforms was also observed for raw wastewater and diluted wastewater.

Raw wastewater was associated with the highest contamination by *Escherichia coli* (8000 CFU per 100 mL) followed by outflow water from Filter 5 and wastewater. Outflow waters from Filters 1 and 3 had similar numbers of *Escherichia coli*. No contamination by *Escherichia coli* was detected for outflow waters from other wetlands.

The highest contamination by *Streptococcus* spp. was associated with Filter 1 outflow water followed by those for Filters 3 and 5, which were contaminated with hydrocarbons (Figure 2). Filter 4 had a higher *Streptococcus* spp. contamination than Filter 2, indicating the effect of aggregate size when comparing these filters with each other (Table 1). Furthermore, raw wastewater was more contaminated by *Streptococcus* spp. than diluted wastewater. The highest *Salmonella* spp. counting was observed in the outflow water from Filter 5 followed by raw wastewater (Figure 2). Filter 1 outflow water was associated with higher *Salmonella* spp. contamination than the water from Filter 3, highlighting the impact of aggregate size (Table 1). Furthermore, *Salmonella* spp. contamination in Control A outflow water was higher than that associated with Control B, explaining the effect of hydrocarbon contamination.

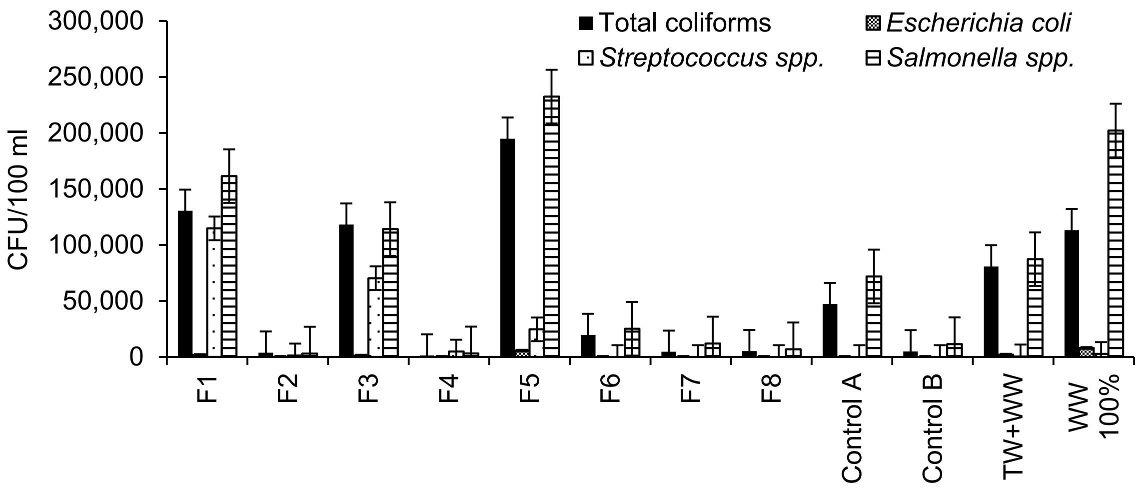

Figure 2. Microbiological characteristics of irrigation water (sample number: 20; 11 October 2013 to 25 September 2014).

Figure 2 showed that the microbial contamination of outflow water from wetland filters polluted with hydrocarbon was higher than those from standard filters (uncontaminated). This confirms previous findings [39], assessing the impact of long-term total petroleum hydrocarbon (TPH) on the structure of bacterial communities. The results indicated that a high concentration of TPH positively impacted on the diversity of hydrocarbon-degrading bacteria. Furthermore, wetland filters fed with undiluted inflow water showed higher microbial contamination levels than those fed with diluted inflow, confirming other findings [28], indicating that high-rate filters tend to be overloaded.

3.2. Environment Boundary Conditions

Table 5 shows environmental boundary conditions. The light intensity records for this experiment during the flowering and fruiting stage were below the proposed range from about 8600 lux to 17200 lux [40]. Low light intensity may lead to flower inhibition or cause flower abscission [41]. Moreover, low light intensity applied to plants will produce leggy plants growing toward light, which is necessary for photosynthesis [42].

For the germination stage (Table 5), the temperature records complied with the optimal temperature for peppers during this phase [43]. Concerning the vegetative growth stage, the temperature records (Table 5) for this experiment were higher than the recommended optimum values of between 21 °C and 23 °C [44]. However, temperature records for this stage complied with the values associated with the highest photosynthesis rate, which can be achieved at temperatures between 24 °C and 29 °C [45,46].

Table 5 shows that the relative humidity before and after fruiting was low (37 ± 7.6% and 57 ± 7.8%, respectively). Humidity values below 50% could have a negative impact on the fruit development as humid atmosphere is necessary for flowers to successfully pollinate; otherwise, the unfertilized flowers will drop off as reported elsewhere [46].

3.3. Sweet Pepper Growth Comparisons

Figure 3a,f shows a growth comparison between pepper plants growing in organic and inorganic media in terms of plant overall height, number of leaves, buds, flowers and total weight of fruits harvested from each treatment. Findings indicate that compost compared to sand is associated with considerably greater plant growth and productivity. This is due to the elevated nutrient availability in the basic compost [17] compared to sand (Table 3). Furthermore, organic substrate decomposes, releasing nutrients [47,48].

Sweet Pepper prefers light and well-drained soil, which is rich in organic substances with a pH value from 6.5 to 7.5 (Table 3) [43]. However, under acid soil conditions (soil pH < 7), heavy metals could be a challenge to Sweet Pepper [49]. Figure 4 shows that plants grown in compost consume more water than those grown in sand and subsequently increase the nutrient load applied to plants via irrigation water, leading to higher foliage and yield production.

Regarding the overall height of plants growing in organic media, Figure 3a shows that the maximum height was associated with plants irrigated with raw wastewater followed by those irrigated with tap water spiked with fertilizer. This can be explained by the high nutrient load (Table 6) applied via irrigation water. Results were statistically significantly different ($p < 0.05$) for the overall height of plants irrigated with water harvested from Filter 7 and Control B.

Regarding the total number of leaves (Figure 3b) linked to peppers grown in organic media, findings indicated that peppers irrigated with tap water spiked by fertilizer produced the highest leave number followed by those plants irrigated with water harvested from Filter 4 and raw wastewater, while the lowest leave numbers were recorded for plants irrigated with deionized water followed by tap water and Controls A and B.

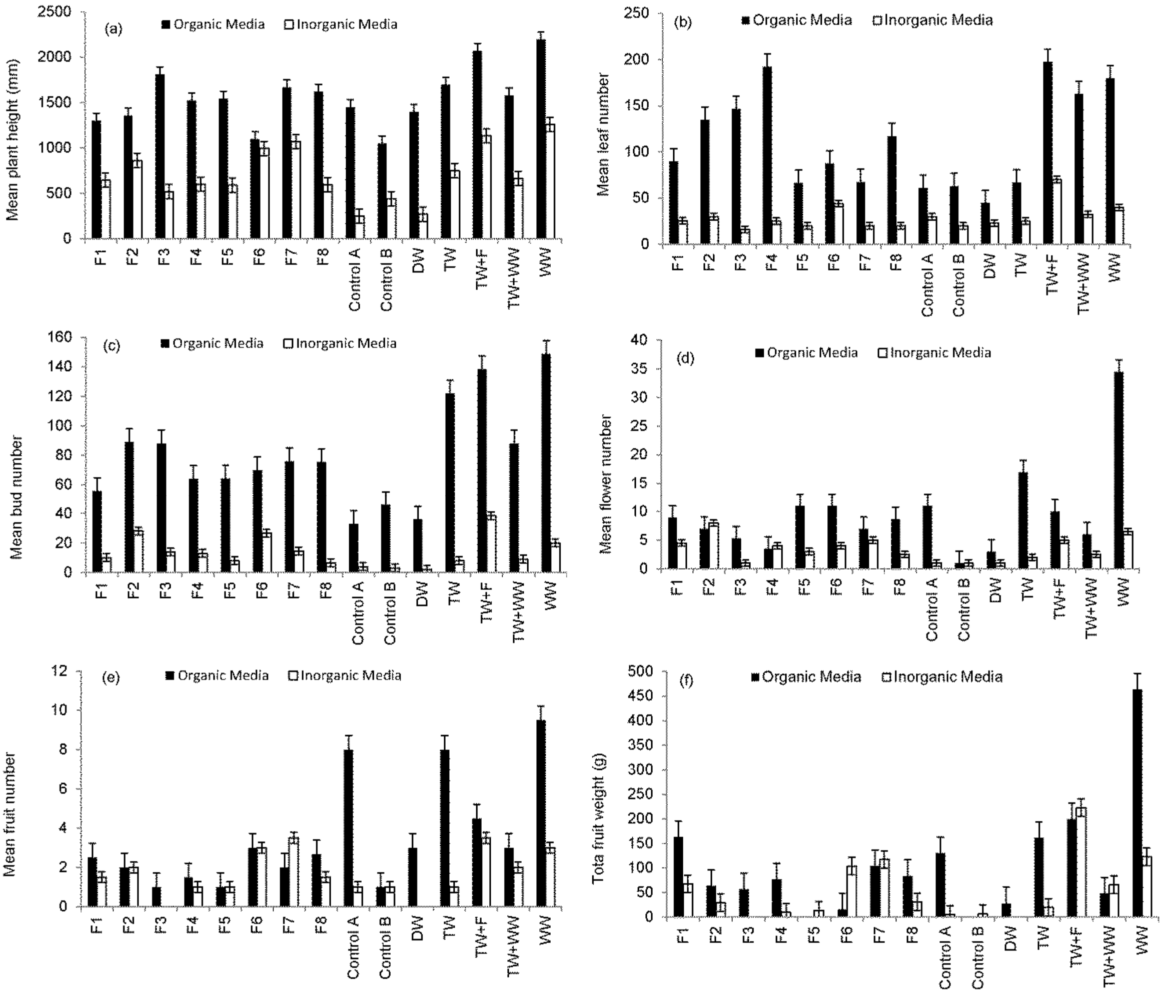

Figure 3. Comparison in growth of plants grown in different media and subjected to different irrigation water types (harvest between 20 January and 25 September 2014): (**a**) mean plant height; (**b**) mean leaf number; (**c**) mean bud number; (**d**) mean flower number; (**e**) mean fruit number; and (**f**) mean fruit weight.

Table 5. Overview of environmental boundary conditions associated with the vegetable pots (mean ± standard deviation (number of records)).

Parameter	Unit	A [a]	B [b]	C [c]	D [d]	E [e]	F [f]
Illuminance (one-off record during lab visit)	lux	5587 ± 5501.1 (918)	nm	4208 ± 2560.5 (36)	12316 ± 1823.3 (102)	3682 ± 3246.1 (513)	5877 ± 9262.2 (267)
Temperature (one-off record during lab visit)	°C	25.4 ± 2.12 (603)	20.5 ± 1.25 (13)	24.8 ± 1.17 (48)	25.0 ± 1.89 (102)	26.3 ± 2.32 (204)	25.0 ± 1.83 (236)
Temperature (minimum within a 24-hour period)	°C	20.8 ± 1.97 (75)	nm	nm	20.3 ± 1.87 (8)	21.2 ± 2.02 (33)	20.6 ± 2.05 (34)
Temperature (maximum within a 24-hour period)	°C	26.8 ± 2.59 (75)	nm	nm	25.3 ± 1.98 (8)	27.0 ± 2.83 (33)	26.6 ± 2.26 (34)
Relative humidity (one-off record during lab visit)	%	49 ± 11.7 (488)	nm	nm	42 ± 5.4 (96)	37 ± 7.6 (156)	57 ± 7.8 (236)
Relative humidity (minimum within a 24-hour period)	%	35 ± 7.1 (75)	nm	nm	36 ± 3.7 (8)	30 ± 3.5 (33)	38 ± 8.5 (34)
Relative humidity (maximum within a 24-hour period)	%	55 ± 12.5 (75)	nm	nm	46 ± 5.6 (8)	48 ± 10.5 (33)	63 ± 9.8 (34)

[a] A: Overall period (11 October 2013 to 25 September 2014); [b] B: Germination period (17 September 2013 to 22 September 2013); [c] C: First Planting period (23 September 2-13 to 10 October 2013); [d] D: Second planting period (11 October 2013 to 07 November 2013); [e] E: Final planting period before fruiting (8 November 2013 to 19 January 2014); [f] F: Final planting period after fruiting (20 January 2014 to 25 September 2014); nm: not measured.

Figure 3c–f provides summaries of plant developments. Very high numbers of buds were recorded for peppers grown in organic media. Most flowers died before producing any fruits due to the elevated ammonia-nitrogen concentrations supplied to those plants grown in organic media and irrigated by wastewater [43].

Sweet Peppers grown in sand had less buds compared to those peppers grown in organic media. Most buds reached the fruiting stage (Figure 3e), because of a better balance in nutrients supplied to those plants by the irrigation water. The potential of a rather moderate diesel spill to function as stimulation for plant growth in clean water becomes apparent when comparing both controls with each other (Table 1).

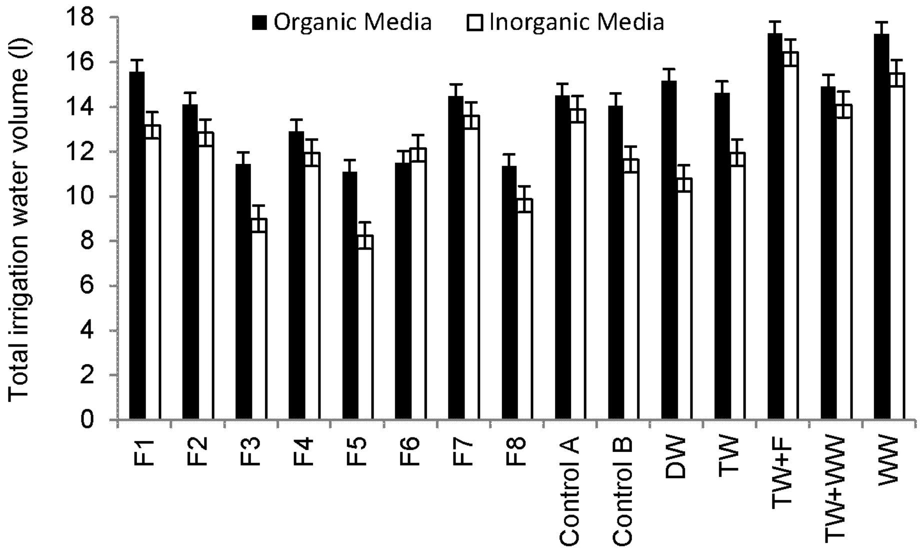

Figure 4. Overview of total irrigation water volumes for Sweet Pepper plants during the whole experiment period (11 October 2013 to 25 September 2014).

Figure 3f summaries a comparison of total weight. Fruits harvested from plants grown in organic media were heavier than those from plants raised in inorganic media. This confirms results obtained by other researchers [17,19] showing the impact of various growth media on pepper harvests.

Figure 5 summaries differences in fruit characteristics. Findings show that fruits harvested from plants irrigated with Filter 1 outflow water were greater than those obtained from peppers irrigated with waters from Filter 2 due to high element loads applied to plants associated with Filter 2 compared to Filter 1 (Table 6). Moreover, fruits belonging to Filter 1 had diameters, which were greater than others indicating the impact of nutrient (mainly nitrogen) and trace element loads provided by irrigation water obtained from Filter 1 compared to the other filters (Table 6).

Figure 5 shows that there is no statistically significant difference in mean fruit length harvested from plants irrigated with different irrigation water types. However, fruits harvested from plants irrigated with Filter 7 (low contact time) outflow water were the longest followed by those irrigated with water obtained from Filters 1 (large aggregate size) and 3 (small aggregate size), which were contaminated with hydrocarbons. The shortest fruit lengths were observed for those harvested from plants irrigated with Filter 6 (high inflow rate) outflow water.

Regarding mean fruit weight, statistical analysis showed that there are significant differences ($p < 0.05$) in fruit mean weight for plants irrigated with water harvested from Filter 6, tap water spiked with fertilizer and raw wastewater. Figure 5 indicates that Sweet Peppers irrigated with water harvested from Filter 1 produced fruits of the highest mean weight (54 g) followed by those harvested from plants irrigated with Filter 7 outflow water, which produced fruits of 52 g mean weight, while the lowest mean fruit weight was recorded for those plants irrigated with Filter 6 outflow water (16 g), explaining the negative impact of high nutrients and trace elements applied to plant fruit weight (Table 6).

Table 6. Overview of element mass applied on plants grown in organic media subjected to different irrigation water types (11 October 2013 to 25 September 2014).

Water Type	Total Applied Mass (mg)									
	NH_4-N [a]	NO_3-N [b]	PO_4-P [c]	Ca [d]	Fe [e]	K [f]	Mg [g]	Mn [h]	Zn [i]	B [j]
Filter 1 outflow	74.8	6.2	62.3	591.9	32.6	149.0	89.0	3.6	1.2	0.8
Filter 2 outflow	87.5	31.0	46.5	484.3	4.6	112.7	71.1	0.9	1.3	0.7
Filter 3 outflow	42.4	4.6	37.8	919.3	24.9	115.1	74.1	4.2	1.4	0.6
Filter 4 outflow	64.5	23.2	37.4	638.7	3.7	50.6	69.8	0.9	1.3	0.5
Filter 5 outflow	107.7	10.0	48.9	834.9	21.8	157.2	104.2	4.2	1.5	0.6
Filter 6 outflow	103.7	41.5	53.0	765.8	11.0	154.1	112.5	1.3	1.7	0.7
Filter 7 outflow	52.2	40.6	52.2	650.8	13.1	105.0	78.9	2.3	1.2	0.4
Filter 8 outflow	15.9	31.8	37.5	506.8	5.4	69.4	57.6	1.0	1.8	0.3
Control A outflow	18.9	5.8	26.1	342.7	3.4	14.5	17.2	1.5	1.5	0.1
Control B outflow	18.3	4.2	26.7	429.8	1.0	11.1	17.8	0.6	2.3	0.1
Deionised water	1.5	<0.1	<0.1	<0.1	1.0	<0.1	1.4	1.2	0.7	<0.1
Tap water (100%)	1.5	10.2	29.7	151.8	49.9	6.2	14.6	0.9	2.7	<0.1
Tap water with fertiliser	276.7	153.9	257.7	179.8	1.6	331.4	20.9	1.6	1.5	<0.1
Wastewater (20%); tap water (80%)	100.0	7.5	44.8	336.6	7.0	51.2	42.1	1.0	3.3	0.1
Wastewater (100%)	580.3	41.4	257.3	1149.9	65.0	244.4	169.1	2.9	4.0	1.0

[a] NH_4-N: ammonia-nitrogen; [b] NO_3-N: nitrate-nitrogen; [c] PO_4-P: ortho-phosphate-phosphorus; [d] Ca: calcium; [e] Fe: iron; [f] K: potassium; [g] Mg: magnesium. [h] Mn: manganese; [i] Zn: zinc; [j] B: boron.

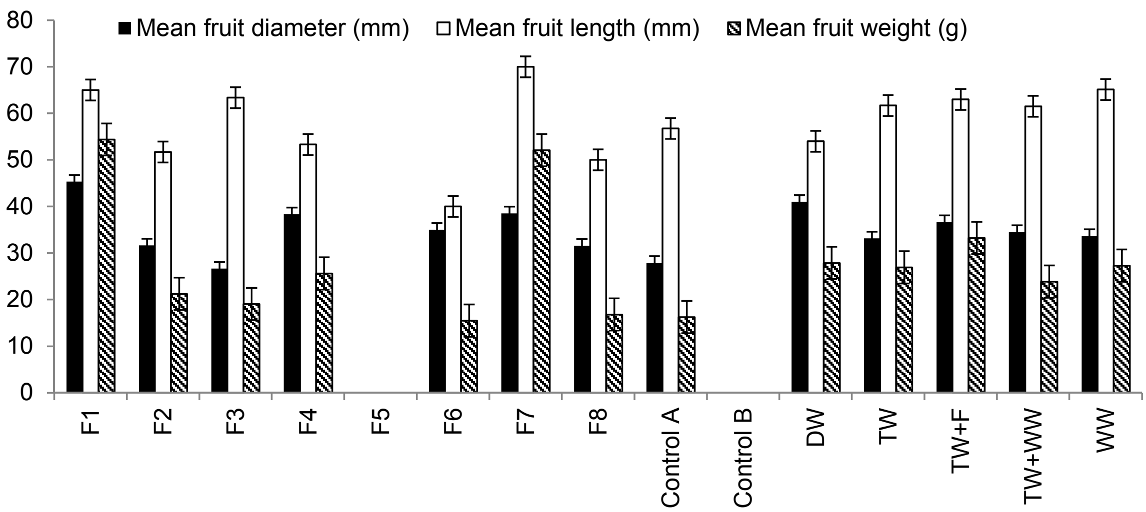

Figure 5. Differences in mean fruit diameter, mean fruit length and mean fruit weight linked to harvested plants (20 January to 25 September 2014) irrigated with different water types and grown in organic media. Notes: No fruit harvest has been noted for plants associated with Filter 5 and Control B.

Ammonia-nitrogen has a negative effect on plant fruit, leaf and stem developments [15,16]. However, the total yield increases as the nitrate-nitrogen to ammonia-nitrogen ratio increases. This can be explained by a reduction in fruit physiological disorders, which usually reduce fruit mean weight [15,16]. Moreover, high phosphorus levels are known to interfere with the normal metabolism of peppers. Also, it is known to promote manganese uptake by plants [50]. However, researchers [51] reported that high potassium concentration in irrigation water provides protection against stem damage from low night temperatures. Manganese is an essential trace element for most plants, intervening in several metabolic processes (mainly in photosynthesis). Nevertheless, an excess of this micronutrient is often toxic for plants. Manganese phyto-toxicity is exhibited in a reduction of biomass and photosynthesis, and biochemical disorders including oxidative stress [50].

Correlation analysis findings indicated that fruit weights were significantly positively correlated with total water volumes used for irrigation (R = 0.821, $p < 0.001$). Since the peppers irrigated with raw wastewater and grown in organic media had the highest number of fruits (Figure 3e), this helps to explain why the total weight of harvested fruits was associated with plants irrigated with raw urban wastewater (Figure 3g). The provision of plants with high nutrient and trace element loads leads to increases in the quantity at the expense of quality of yield.

3.4. Sweet Pepper Quality

Table 2 proposes a novel but conservative harvest classification scheme for Sweet Peppers. The lowest variable class entry for any individual pepper fruit assessment determined the final class. If a fruit is categorized, for example, as class A with respect to length, class B in terms of diameter and E regarding weight, then the final class for this fruit is class E. It follows that the corresponding price for this pepper sample will be zero pence (Table 2).

Figure 6 indicates the monetary value of the pepper harvest. No fruits from any plant were categorized as Class A, B or C. The highest number of fruits categorized as Class D was harvested from peppers grown in organic media and irrigated with raw wastewater followed by those irrigated with tap water, Control A and Filter 1 outflow waters. The highest number of fruits categorized as Class E was harvested also from plants grown in organic media and watered with raw wastewater. No microbial contamination was detected in fruits (skin, flesh and washing solution) harvested from any treatments. However, microbial contamination of peppers is rather unlikely due to the relatively long distance between the fruits and the contaminated soil.

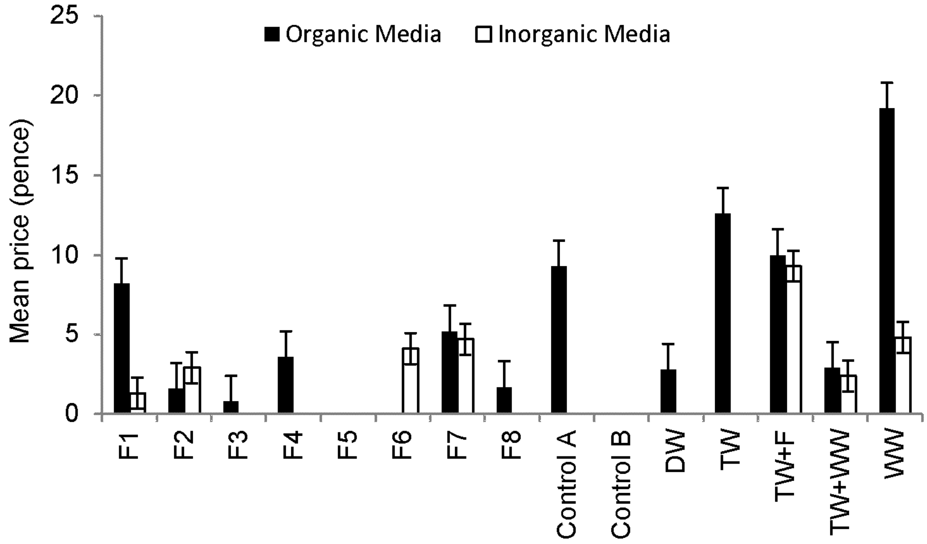

Figure 6. Comparison of the Sweet Pepper harvest outcome linked to plants grown in different media (after classification scheme (Table 2) application).

4. Conclusions and Further Research

The key research findings have been compared to what was promised in Section 1.4 stating the objectives. Sweet Peppers can be grown using wastewater treated by constructed wetlands. However, the marketable yield was too low to make a decent profit (addressing objective (a)).

The highest number of fruits was linked to raw wastewater and an organic growth medium, while the highest fruit quality indicated by diameter, length and weight was observed for peppers grown in organic media and irrigated with outflow water from wetlands with large aggregates, long contact and resting times, and low inflow loading rate. These results correspond to objectives (b) and (c).

As the nutrients within the degraded compost got depleted about ten months after the start of the experiment, the harvest increased for pots that received pre-treated wastewater in comparison to those depending only on the remaining nutrients obtained from the almost exhausted compost. These findings correspond to objectives (c) and (d).

Results show that nutrient concentrations supplied to the peppers by biodegrading compost and nutrient-rich wastewater were too high to produce a reasonable harvest, because Sweet Peppers are sensitive to too high nutrient concentrations leading to plant development challenges. A high marketable yield (harvest of Sweet Peppers, which are of good quality) related to the most suitable provision of nutrients to the peppers, addressing objectives (c) to (e)).

A good pepper harvest was linked to a wetland system with a large aggregate diameter and diesel spill. This can be explained by the fact that the degradation of hydrocarbon requires the presence of considerable nitrogen resources. In the absence of diesel, too much nitrogen increases leave and decreases fruit developments (addressing objectives (c) to (e)).

Considering that findings indicate the unsuitability for Sweet Peppers to be grown in the tested manner, further research will be undertaken with a mixture of different growth media, and different dilutions of outflow water from wetland filters to optimize the concentrations of nutrients applied to plants supporting the production of the highest yield and best quality. An assessment of the long-term impact of soil and fruit enrichment with minerals will be undertaken in the future. Finally, the authors recommend that field studies should complement experimental studies.

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