AZO TEXTILE DYES WASTEWATER TREATMENT WITH CONSTRUCTED WETLANDS

Design and operation of experimental vertical-flow constructed wetlands applied for the treatment of azo textile dyes (with/without artificial wastewater)

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ACRONYMS AND ABBREVIATIONS

AAFA	American Apparel and Footwear Association
AB113	Acid Blue 113
ABSA	3-AminoBenzeneSulfonic Acid
ADMI	American Dye Manufactures Institute
Al	Aluminium
AMD	Acid Mine Drainage
ANOVA	Analysis of Variance
ANSA	5-Amino-8-(phenylamino) Naphthalene-1-Sulfonic Acid
AO7	Acid Orange 7
As	Arsenic
AY	Acid Yellow
BB	Basic Blue
BOD	Biochemical Oxygen Demand
BR46	Basic Red 46
Cd	Cadmium
cm	Centimetre
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
CWs	Constructed Wetlands
DAN	1,4-DiAminoNaphthalene
DO	Dissolved Oxygen
DR81	Direct Red 81
DY	Disperse Yellow
EC	Electrical Conductivity
Fe	Iron
FTIR	Fourier Transform Infrared Spectroscopy
FWS	Free Water Surface
FWSF	Free Water Surface Flow
HF	Horizontal Flow
Hg	Mercury

HLR	Hydraulic Loading Rate
HPLC	High-Performance Liquid Chromatography
HRT	Hydraulic Retention Time
HSSF	Horizontal Sub-Surface Flow
IBM SPSS	International Business Machine Statistical Package for Social Science
IVF	Integrated Vertical Flow
IWA	International Water Association
K_s	K factor at saturated conditions
ƛmax	Maximum Absorbance Wavelength
MLR	Mass Loading Rate
mm	Millimetre
Mn	Manganese
Mol.	Mole
N	Nitrogen
N/A	Not Applicable
N=N	Nitrogen Atom
N_2	Gaseous Nitrogen
NBNMA	*N*-Benzyl-*N*-Methyl Aniline
NH_4-N	Ammonia-Nitrogen
Ni	Nickel
NBNMD	*N*-Benzyl-*N*-Methylbenzene-Diamine
NO_2-N	Nitrate-Nitrogen
NO_3-N	Nitrate-Nitrogen
NTU	Nephelometric Turbidity unit
Pb	Lead
PE	Person Equivalent
pH	Potential of Hydrogen
PO_4-P	Ortho-phosphate-phosphorus
POD	Plant Peroxidase
Pt Co.	Platinum-Cobalt
RB*	Reactive Blue
RB**	Reactive Black
Redox	Reduction-Oxidation Reaction

RZM	Root Zone Method
SD	Standard Deviation
SE	Standard Error
SF	Surface Flow
SS	Suspended Solids
SSF	Subsurface Flow
STWI	Sweden Textile Water Initiative
TDS	Total Dissolved Solids
TN	Total Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UK	United Kingdom
USA	United States of America
USEPA	United State Environmental Protection Agency
VF	Vertical Flow
VFCW	Vertical-Flow Constructed Wetland
VSSF	Vertical Subsurface Flow
VY	Vat Yellow
WHO	World Health Organization
WRc	Water Research council
Zn	Zinc

ABSTRACT

Wetlands have long played a significant role as natural purification systems. Textile industry processes are among the most environmentally unsustainable industrial processes, because they produce coloured effluents in large quantities polluting water. The aim of this study is to assess the performance of VFCWs to treat two different azo textile dyes with and without artificial wastewater for long periods of time through different operation modes such as contact and resting times, and loading rates, which has rarely been considered in previous research works. The corresponding key objectives are (a) to assess the role of gravel (as a control wetland) and plants on dye reduction and other pollutants; (b) to determine the influence of two groups of dyes (acid (AB113) and basic (BR46)), each dye having a different molecular weight and chemical structure, at two different concentrations (7 mg/l and 215 mg/l); (c) to evaluate the impact of the mixture of both dyes on the performance of vertical-flow constructed wetlands in terms of dye reduction with/without artificial wastewater; (d) to determine the annual and seasonal reduction; and (e) to assess the influence of operational parameters such as contact time (48h and 84h), resting time and mass loading rate on dye reduction and other pollutants for a long period.

The first phase dealt with treating the two azo textile dyes only during the period between 1 June 2015 and 31 May 2016, while the second phase dealt with artificial wastewater containing the two azo textile dyes during the period between 1 June 2016 and 31 May 2017. According to the first phase, for the low concentration of BR46, there was no significant ($p \geq 0.05$) difference between the wetlands in terms of dye reductions. However, for chemical oxygen demand (COD), the reduction percentages were 50%, 59% and 67% for the control and for the wetlands with short and long contact times, respectively. All reductions were statistically significant ($p<0.05$). For the high

concentration of BR46, the reduction percentages for the dyes were 94% and 82%, and for COD, they were 89% and 74% for the long and short contact times, correspondingly. A good reduction was noted for total suspended solids for long and short contact times. For the low concentration of AB113, the percentage reductions for the dye were 71%, 68% and 80%, and for COD, they were 5%, 7% and 16% for the control, and the short and long contact times, respectively. For the high concentration of AB113, the percentage reductions for the dye were 72% and 73%, and for COD, they were 54% and 55% for the 48 h and 96 h contact times in this order. Regarding ortho-phosphate-phosphorous for the low concentrations of BR46 and AB113, the reduction percentages for wetlands, which have high contact times, were significantly ($p<0.05$) better than those of the control wetlands, as well as wetlands, which have low contact times. In the case of high concentration regarding BR46, the reduction percentages of wetlands with low loading rates were significantly ($p<0.05$) better than wetlands with high loading rates, while for AB113, the reduction percentages of wetlands with high loading rate were significantly ($p<0.05$) better than those for wetlands with low loading rates. In the case of ammonia-nitrogen for the high concentration of dyes, there were no significant ($p\geq0.05$) differences between wetlands. Regarding nitrate-nitrogen reduction for low and high concentration of BR46 and AB113, the reduction percentages for wetlands with long contact times were better than those for wetlands having short contact times.

In the case of phase two, the presence of plants had no effect on the dye and COD reductions. For the low concentration of BR46, the percentage reductions for the dye were 92%, 89% and 91%, and for COD, they were 69%, 82% and 70% for the control, and the short and long contact times, respectively. All reductions were statistically significant ($p<0.05$). For the high concentration of BR46, the reduction percentages for the dyes were 73% and 33%, and for COD, they were 56% and 39% for the long and short contact times, respectively. For the low concentration of AB113, the percentage reductions for the dye were 85%, 77% and 82%, and for COD, they were 76%, 81% and 62% for the control, and the short and long contact times in this order. For the high concentration of AB113, the percentage reductions for the dye were 44% and 54%, and for COD, they were 40% and 56% for the 48 h and 96 h contact times, correspondingly. Regarding ortho-phosphate-phosphorous for the low concentrations in the case of AB113 and BR46 and the mixture of both dyes, the reduction percentage in wetlands with high contact time was significantly ($p<0.05$) better than those of the control wetlands and wetlands with low contact time. For the high concentration of BR46, AB113 and the mixture of both of them, wetlands with high resting and contact times had lower ortho-phosphate-phosphorous effluent concentrations when compared with wetlands with low resting and contact times. Regarding ammonia-nitrogen reduction percentages for low concentrations of BR46 and AB113 and the mixture of both dyes, wetlands with high resting times had better reduction percentages ($p<0.05$) when compared with the control wetlands as well as wetlands with low resting times. In the case of high concentrations for BR46, AB113 and the mixture of both of them, wetlands with low loading rates had a better reduction percentage when compared with wetlands with a high loading rate. Regarding nitrate-nitrogen reductions for low and high concentrations of BR46, AB113 and the mixture of both of them, the reduction percentages for all wetlands were in the range from 75 to 100%. Regarding aromatic amine compound reductions, wetlands with long contact times showed significant ($p<0.05$) differences when compared with the control and wetlands with short contact times for the low concentrations of BR46 and AB113. For the high concentration of BR46 and AB113, wetlands with low loading rates showed a significant difference ($p<0.05$) when compared with wetlands with a high loading rate. The researcher recommended that using HPLC combined with FTIR to investigate the reduction in aromatic amines and working on modelling of the results should help the designer in improving the construction of wetlands on an industrial scale.

1. CHAPTER ONE: INTRODUCTION

1.1 Overview

The purpose of this chapter is to present the concept of wetlands, including definition, foundation, attributes, significance, types, procedures and standard of their application. Moreover, the chapter is further partitioned into sections. Section 1.2 presents the background of the research. The problem statement, aim of the study and its objectives are presented in sections 1.3 and 1.4, respectively. Finally, the thesis structure is presented in section 1.5.

1.2 Background of the Research

The environment is composed of water, earth, space and atmosphere. Without pollution, it remains perfect and charming. The complex nature of the environment is changing due to different human activities, including transportation, construction, industrialization and so on. Such activities, although desirable for human development and welfare, lead to environmental degradation and the release of objectionable materials into the environment, leading to loss of quality of life.

Over half of the world's population faces water shortage. Almost 900 million people in the world still do not have access to safe water and somewhere in the range of 2.6 billion, a large portion of the number of inhabitants in the developing world, do not have access to satisfactory sanitation. No less than 1.8 million children under five years old die every year, consistently, due to water-related diseases. Furthermore, 1.2 billion people (one fifth of the total populace) live in areas of water shortage (Corcoran, 2010; Sultana, 2014; Connor, 2015). Figure 1.1 shows the worldwide renewable water resources distribution.

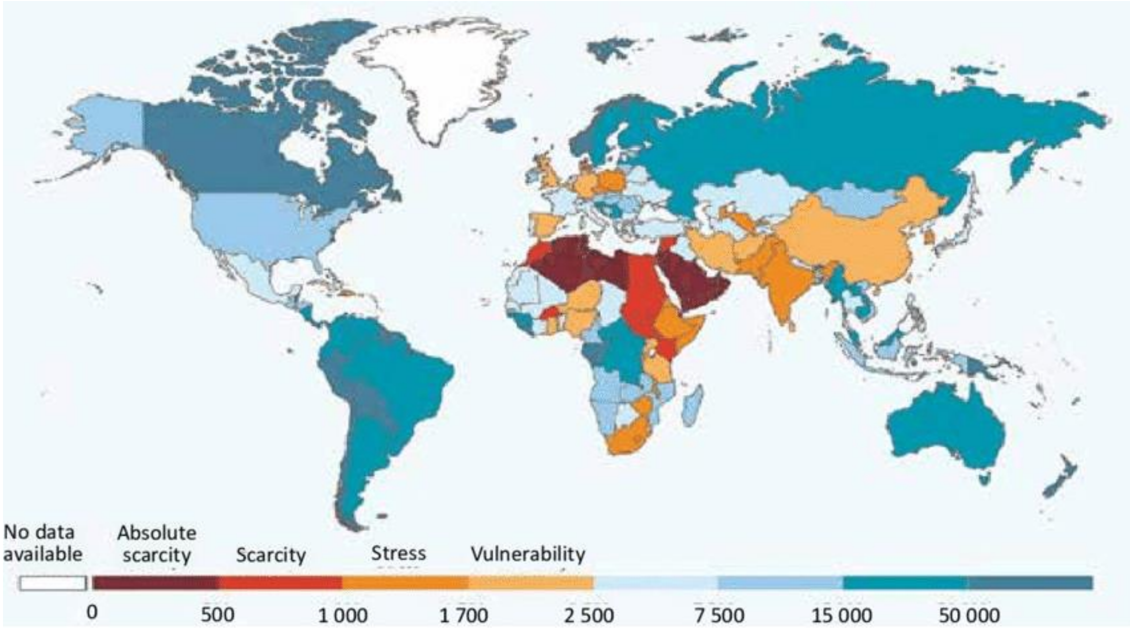


Figure 1.1 Worldwide renewable water resources (m^3 per capita per year, adapted from Sultana, 2014)

Extreme use and pollution of freshwater resources are nowadays worldwide ecological issues that apply pressure on the supportability of worldwide ecosystems. Most of the activities which renewable freshwater is used for – industrial, agricultural and domestic purposes – lead to pollution of freshwater systems worldwide by thousands of natural and industrial chemical compounds. These chemical compounds accumulate in ground waters, surface waters, plants and substrate, degrading the water quality and causing a negative impact on the receiving streams (Kanu and Achi, 2011; Robles-Molina et al., 2014). Furthermore, Schwarzenbach et al. (2006) stated that around two million tons of waste, derived from all human activities, are released into receiving streams everyday with incomplete treatment or no treatment at all, and this has been demonstrated by Corcoran (2010) as well.

Any increase in population will be accompanied by an increase in industrial, agricultural and urbanization land. These increases lead to increases in the effluent wastewater, which contains a wide diversity of pollutants including azo dyes, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS) nitrogen compounds, turbidity toxic metals (such as Cr, Ni, Cd and Pb), and faecal coliform. These pollutants have harmful effects on the water environment and make the water unsuitable for irrigation, drinking and aquatic life. All these negative impacts of pollutants have forced governments at different levels to assess guidelines and regulations with regard to treatment of effluent wastewater before disposal to receiving streams (Helmer et al., 1997; Spiegel and Maystre, 1998).

Textile dyeing processes are the most environmentally unfriendly industrial processes, because the chemical reagents used are very rich in chemical compounds ranging in both inorganic and organic products (Juang et al., 1996; Robinson et al., 2001). Furthermore, the presence of colour in the effluent textile wastewater is one of the most important problems. Sultana (2014) stated that coloured wastewaters, produced from dyeing processes, are heavily polluted with chemicals, textile auxiliaries and dyes. The properties of textile wastewater depend on the production, technology and chemicals used (Wang et al., 2011). Textile industries devour gigantic amounts of water and generate vast volumes of wastewater through different steps in dyeing and finishing processes, and the discharged wastewater (Figures 1.2 and 1.3) is an overwhelming blend of various polluting substances such as organic, inorganic, elemental and polymeric products (Babu et al., 2007; Kant, 2012). Dye wastes are the most dominating materials in textile wastewater and these materials are toxic to the biological world as well as the dark colour of these materials blocking sunlight which causes acute problems in biological communities (Ratna and Padhi, 2012; Dey and Islam, 2015). Some examples of the effects of azo dyes on human health and the environment are shown in Table 1.1.

Figure 1.2 The effluent of textile industry- China (Wood, 2010)

Figure 1.3 The effluent of textile industry- India (Ramesh, 2011)

Table 1.1 Some examples of azo dye (listed in order of date) effects on human health and the environment

Authors (Year)	Human	Environmental
(Hatch and Maibach, 1995)	Dermatitis	
(Specht and Platzek, 1995)	Eczema and dermatitis	
(Greene and Baughman, 1996)		Algae growth inhibited
(Rizzo et al., 2010)	Alzheimer	
(Gudelj et al., 2011)	Allergenic, carcinogenic, mutagenic and teratogenic properties	Bioaccumulation
(Gopinathan and Nair, 2011)		River pollution
(Kant, 2012)		Depletion of dissolved oxygen
(Pereira and Alves, 2012)	Bladder cancer, splenic sarcomas and hepatocarcinomas	Hindering photosynthesis
(Akarslan and Demiralay, 2015)	Cancer and allergenic	

The receiving of azo dye effluent wastewater by the river without any degradation or reduction, will lead later to azo dye degradation by the intestinal tract and release of aromatic amine compounds, which are absorbed by the intestine with carcinogenic effects, as demonstrated by Brown and De Vito (1993). For instance, in April 2013 more than 1100 people were killed in Bangladesh due to untreated textile wastewater that came from garment factories output. Bangladesh's garment and textile industries have contributed heavily to what experts describe as a water pollution disaster. Many rice paddies are inundated with toxic wastewater and fish stocks are dying in the nearby water bodies (Yardley, 2013; Belal et al., 2015). In China, a 2003 survey found that since an industrial zone opened in 1992, almost 60 of the 1,500 residents in the two neighbouring villages have died of cancer – about 3% of the population – accounting for 80% of all deaths (Wei, 2003; Zheng et al., 2017). Polluted water causes 75% of diseases and over

100,000 deaths annually, according to the World Health organization (WHO). Meanwhile, cancer rates among villagers who live along polluted waterways are much higher than the national average (China Water Risk, 2011)

Textile manufacture can be classified into three categories viz., cotton, woollen, and synthetic fibres depending upon the raw materials used (Wang et al., 2011). Pure water in large quantities will be consumed and a large volume of wastewater will be produced by the textile dyeing industry from different steps in the dyeing and finishing processes (Allegre et al., 2004; Parshetti et al., 2006; Ojstršek et al., 2007), with an average 200 l/kg of fibre (Pereira and Alves, 2012). An extensive variety of chemicals and dyestuffs, are required for the operation of dyeing and finishing. These materials are organic substances of complex structure, are found in the effluent wastewater, and cause disposal difficulty (Joshi et al., 2004; Ji et al., 2008).

The main pollutants in textile wastewater are shown in Table 1.2 and Figure 1.4. Furthermore, more than 10,000 different textile dyes with an expected annual production of $7x10^5$ metric tonnes are commercially available around the world; 30% of these dyes are used in excess of 1000 tonnes per annum (Baban et al., 2010). Azo dyes represent 65-75% of the total textile dyes. In the dyeing process, 10-25% of textile dyes are lost and 2-20% are specifically discharged as fluid effluents in different environmental components (Carmen and Daniela , 2012).

Table 1.2 Textile processing categories (adopted from Correia et al., 1994) related with Figure 1.4

Parameters	Categories						
	1	2	3	4	5	6	7
BOD_5/COD	0.2	0.29	0.35	0.54	0.35	0.3	0.31
BOD_5 (mg/l)	6000	300	350	650	350	300	250
TSS (mg/l)	8000	130	200	300	300	120	75
COD (mg/l)	30000	1040	1000	1200	1000	1000	800
Oil and grease (mg/l)	5500	N/A	N/A	14	53	N/A	N/A
Total chrome (mg/l)	0.05	4	0.014	0.04	0.05	0.42	0.27
Phenol (mg/l)	1.5	0.5	N/A	0.04	0.24	0.13	0.12
Sulphide (mg/l)	0.2	0.1	8.0	3.0	0.2	0.14	0.09
Colour (ADMI)	2000	1000	N/A	325	400	600	600
pH	8.0	7.0	10	10	8.0	8.0	11
Temp. (˚C)	28	62	21	37	39	20	38
Water usage (l/kg)	36	33	13	113	150	69	150

Note: BOD_5, biochemical oxygen demand after five days; COD, chemical oxygen demand; TSS, total suspended solids; ADMI, American dye manufacture institute; ˚C, degrees Celsius; N/A, not applicable; categories descriptions: 1, Raw wool scouring; 2, Yarn and fabric manufacturing; 3, Wool finishing; 4, Woven fabric finishing; 5, Knitted fabric finishing; 6, Carpet manufacturing; 7, Stock and yarn dyeing and finishing.

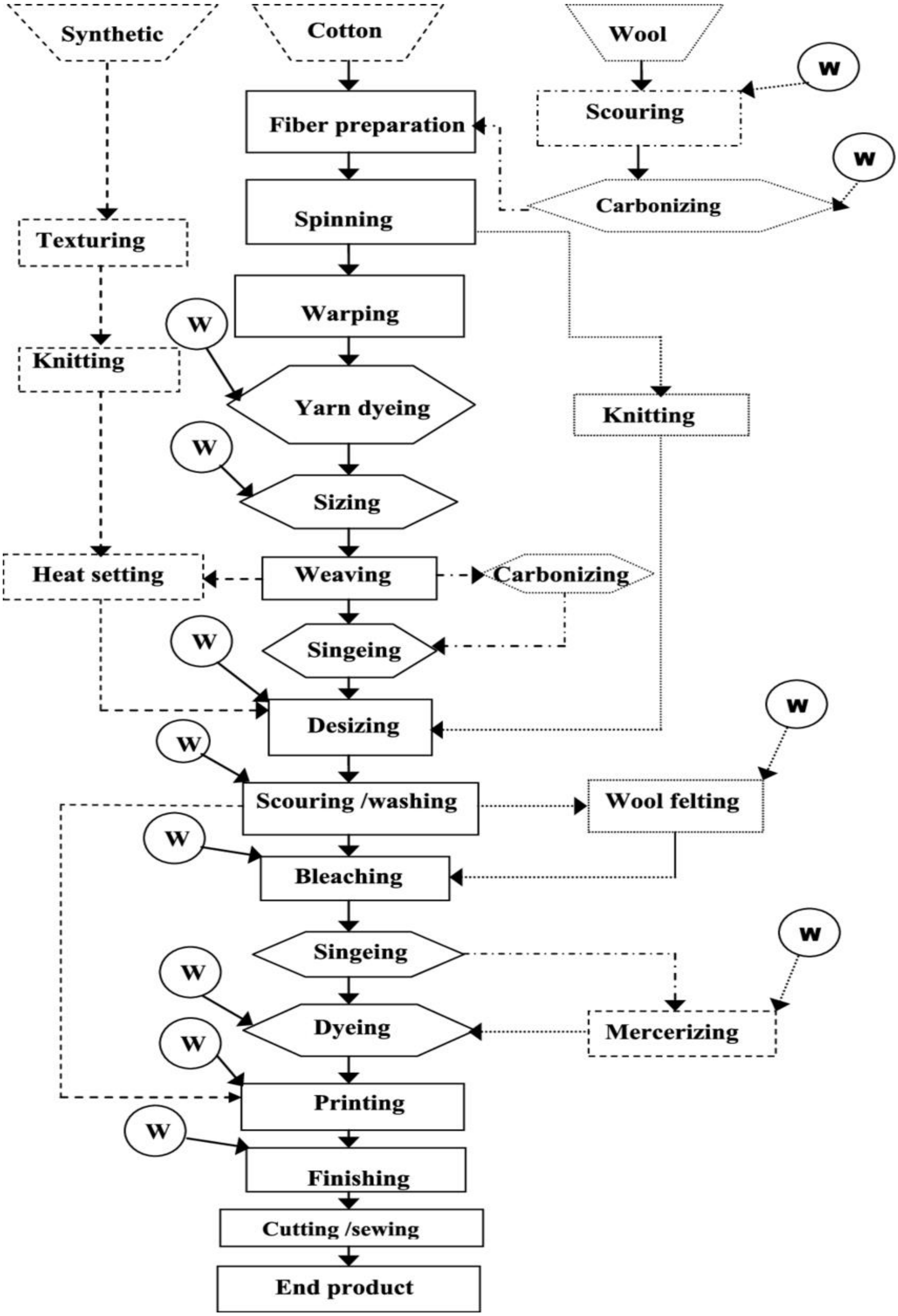


Figure 1.4 Flowchart for the general steps in textile industry Adopted from Dasgupta et al. (2015).

Wastewater from printing and dyeing units is rich in colour, containing residues of dyes and chemical materials, such as complex components, high Chroma, and high COD and BOD concentration, as well as materials which are difficult to degrade, leading to their concentrations exceeding standard limits and causing ecological and/or health problems (Scholz, 2006, 2011). The toxic effects on the public of dyestuffs and other organic compounds, as well as acidic and alkaline contaminants, from industrial establishments are widely accepted. At present, the dyes are mainly aromatic and heterocyclic compounds, with colour-display groups and polar groups. Their structure is more complicated and stable, resulting in greater difficulty to degrade printing and dyeing wastewater (Ding et al., 2010).

Textile printing and dyeing processes include pre-treatment, dyeing/printing, finishing and other technologies. Pre-treatment includes de-sizing, scouring, washing, and other processes. Dyeing mainly aims at dissolving the dye in water, which will be transferred to the fabric to produce coloured fabric under certain conditions. Printing is a branch of dyeing which generally is defined as ‘localized dyeing’, i.e. dyeing that is confined to a certain portion of the fabric that constitutes the design. It is really a form of dyeing in which the essential reactions involved are the same as those in dyeing. In dyeing, colour is applied in the form of solutions, whereas colour is applied in the form of a thick paste of the dye in printing. Both natural and synthetic textiles are subjected to a variety of finishing processes. This is done to improve specific properties in the finished fabric and involves the use of a large number of finishing agents for softening, crosslinking, and waterproofing. All of the finishing processes contribute to water pollution. In addition, in different circumstances, the singeing, mercerizing, base reduction, and other processes may have been carried out before dyeing/printing (Ding et al., 2010).

Azo dyes play an important role as colouring agents in the textile, food, and pharmaceutical industry (Parshetti et al., 2006; Zhang et al., 2010; Senthilvelan et al., 2014; Tee et al., 2015). Due to the toxicity, mutagenicity and carcinogenicity of azo dyes and their breakdown products, their reduction in industrial wastewaters has been an urgent challenge (Robinson et al., 2001; Erkurt, 2010; Zhang et al., 2010).

The era of intensifying environmental crises such as pollution, climatic changes, water shortages (Alavian et al., 2009; Ward et al., 2010), rapid population growth and several compelling factors such as pipe vandalization by saboteurs, and underground tank leakages, justify the need for sustainable wastewater treatment technology that is environmentally friendly, easy to operate, less energy-intensive, and cost-effective. Many technology solutions used by the textile industry, such as coagulation-flocculation (Golob et al., 2005) and the advanced oxidation process (Hassaan et al., 2016), to treat the effluent are not feasible in practice, because of too high costs and the complex processes involved (Sivakumar et al., 2013), making their use in field conditions difficult (Ji et al., 2007). Furthermore, some developing countries, such as China (Chen et al., 2007), Bangladesh (Islam et al., 2011) and India (Roy, 1998), which have a strong textile industry, but unreliable energy sources, may benefit from less-energy demanding methods of wastewater treatment. Therefore, it is very important to find an alternative system, which is simple, natural and cost-effective to treat such wastewater, such as constructed wetlands (Knight et al., 2000; Scholz and Lee, 2005; Vymazal, 2010a).

For reasons such as this, it is important that a method be used to clean textile effluents to avoid further pollution and the risk of poor health to the local area. Many solutions which have been developed to treat these dyes are not feasible due to the difficulty of degradation (Parshetti et al., 2006), and high cost and technological complexity of the processes involved. For countries which have an unreliable energy source, it is necessary for other methods of treatment to be used.

Natural systems such as constructed wetlands (CWs) are engineered systems and commonly used because of their properties such as convenience, mechanical simplicity, low energy requirement, environmental friendliness and low cost of operation. Constructed wetlands have been used to attain wastewater treatment goals by using natural components and processes that significantly reduce the use of energy intensive mechanical devices and technical complexity. Furthermore, constructed wetlands involve natural processes resulting in the efficient conversion of hazardous compounds. Many publications have reported the efficiency of constructed wetlands in treatment of various types of wastewaters, including animal wastewater, urban runoff, and mine drainage (Hunt and Poach, 2001; Mays and Edwards, 2001; Rozema et al., 2016), textile industry wastewater (Davies and Cottingham, 1994; Davies et al., 2009), petroleum wastewater (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b) and domestic wastewater (Sani et al., 2013; Paing et al., 2015).

The values of wetlands are multiple, and they have played a necessary role in the history of humankind from the earliest civilizations, such as those in Mesopotamia and Egypt, who used to be near to the wetlands areas that provided a variety of economic and essential resources to them. Many studies have assessed the economic values of constructed wetlands in different regions of the world (UK, Klein and Bateman, 1998; India, Verma et al., 2001; Greece, Ragkos et al., 2006; China, Yang et al., 2008). Wetlands as a water body include various animals and plant types, provide artificial impoundments for water storage, which can be used for other purposes such as irrigation, and constructed wetlands can be used to provide flood protection by using them as areas for flood storage (Ghermandi et al., 2010). Furthermore, other values include the absorption of carbon dioxide leading to reduce global warming, and supporting the food chain, indirectly, by fish production and other related edible water animals.

Constructed wetlands (CWs) are considered an alternative efficient method to treat many types of wastewater, including that from the textile industry, due to their green qualities, low investment requirement, ease of operation, low maintenance cost, and low or zero energy input, besides landscape aesthetics (Scholz and Xu, 2002a; Guimaraes, 2016; Sehar et al., 2016). CWs are engineered ecosystems for reduction of pollutants from water by wastewater treatment that resembles the hydraulic conditions and habitat occurring in a swamp. Constructed or artificial wetland systems mimic the treatment that occurs in natural wetlands by relying on heterotrophic micro-organisms and aquatic plants and a combination of naturally occurring biological, chemical and physical processes. Textiles and dyeing wastewater causes significant environmental pollution, the main problems being high concentrations of organic matter and colorants (dyes) that have to be resistant to the effects of sweat, soap, water, light and oxidants (Olejnik and Wojciechowski, 2012).

The performance abilities of constructed wetlands are dependent on the natural environment, microbial communities, plants and the weather of the region (Scholz, 2011; Hernández, 2013; Mahmood et al., 2013). The two major types of CWs are Surface flow (SF) and subsurface flow (SSF) (Kadlec and Knight, 1996; Jørgensen, 2009; Vymazal, 2013; Albalawneh et al., 2016). The main difference between SSF and SF CWs is the water level; SF CWs have an uncovered water surface, while SSF CWs have no clear water surface. Furthermore, SSF CWs can be divided into vertical flow (VF) and horizontal flow (HF) regarding the direction of water movement into the constructed wetlands system (Vymazal, 2005, 2008b). In general, media in HFCWs is submerged in water, unlike the media in VFCWs, which is ponded and drained due to the type of operation (Stefanakis et al., 2014; Hou et al., 2017).

VFCWs are the state of the art in constructed wetlands technology around the world (Olsson, 2011; Stefanakis et al., 2014). Many publications (Vymazal, 2002, 2005; Saeed and Sun, 2012; Shi et al., 2012; Paing et al., 2015; Butterworth et al., 2016) have reported the ability of this type of constructed wetland to transfer oxygen at a higher rate. Furthermore, the wastewater enters the constructed wetland from the top and drains vertically by gravity through the porous media, and air from the atmosphere follows in its place (Maier et al., 2009; Stefanakis et al., 2014), enhancing the aeration and microbial activity. Many authors (Korkusuz et al., 2004; Scholz, 2011; Sousa et al., 2011) have reported that VFCWs have performed well in reduction of COD, BOD and phosphorus. In addition, regrading nitrification processes, vertical-flow constructed wetlands can achieve a satisfactory level (Platzer, 1999; Song et al., 2015).

1.3 Problem Statement

The release of dye textile wastewater into receiving streams is unacceptable not only for aesthetic reasons and the effect on aquatic life, but also since many of these dyes are toxic and carcinogenic (Lourenço et al., 2003; dos Santos et al., 2007; Dey and Islam, 2015). Current strategies used for treatment of textile wastewaters have technical and economic restrictions. The majority of the physico-chemical methods, which are used to treat this type of wastewater, are costly, produce large amounts of sludge and are wasteful of some soluble dyes (Van der Zee et al., 2001; Sivakumar et al., 2013; Costa et al., 2012). In contrast, biological treatment such as constructed wetlands is much cheaper than the previous methods, environmentally friendly and does not produce large amounts of sludge (Knight et al., 2000; Scholz and Lee, 2005; Vymazal, 2010a; Tee et al., 2015). Furthermore, the highlights in the above introduction suggest that the sustainable and prospective treatment of textile wastewater is with subsurface flow constructed wetlands to reduce azo dye and other pollutants of different types and concentrations (Davies et al., 2005; Ong et al., 2011; Shehzadi et al., 2014).

Two azo textile dyes (AB113 and BR46) were selected in this research with two different concentrations (low with a target concentration 7mg/l and high with a target concentration 215 mg/l). Typically, textile industry-processing effluents contain dyes in the range between 10 and 200 mg/l (Lavanya et al., 2014; Yaseen and Scholz, 2016). Most textile dyes at a rather low concentration of even < 1 mg/l can be detected by the human eye (Chung, 1983; Lavanya et al., 2014; Pandey et al., 2007). Furthermore, Van der Zee (2002) stated that algal growth was not inhibited at dye concentrations < 1 mg/l. Both of which are commercial dyes extensively used in the textile industry (Chung et al., 1992; Riu et al., 1997; Pervez et al., 1999; Olgun and Atar, 2009; Ong et al., 2010; Deniz and Karaman, 2011; Deniz and Saygideger, 2011). AB113 is an acid dye and BR46 is a basic dye. An acid dye is defined as a negatively charged dye at a chemical level, which contains one or more acidic groups such as a sulphonic group (Akbari et al., 2002; Martínez-Huitle and Brillas, 2009). A basic dye is defined as a positively charged stain at a chemical level (Martínez-Huitle and Brillas, 2009; Brillas and Martínez-Huitle, 2015) which means it reacts well with negatively charged materials (Sun and Yang, 2003).

1.4 The novelty of the work

Most of the previous studies on treating azo dye textile wastewater by subsurface flow constructed wetlands focus on studying the role of plant activity on dye degradation,

degradation of dyes under aerobic and anaerobic conditions, and using different plants and media or enhancing the media by adding extra material (Pervez et al., 1999; Davies et al., 2005; Mbuligwe, 2005; Davies et al., 2006; Bulc and Ojtrsek, 2008; Ong et al., 2011; Yalcuk and Dogdu, 2014).

Despite the numerous articles published on constructed wetlands during recent decades, there is a notable gap in the literature regarding research on the long-term CW treatment performance under outdoor weather conditions affected by season, and its relationship with other pollutants of vertical-flow constructed wetlands treating azo textile dye wastewaters. It is rarely to find a researcher worked on treatment azo textile dyes with CWs for long term period. Furthermore, working on different contact and resting times, and different hydraulic loading on two different groups of azo dyes with low and high concentration, all together.

1.5 Aim of the study and objectives

Constructed wetlands are already widely used to treat wastewaters polluted with various compounds (Scholz, 2006, 2010, 2015). The aim of the project is to assess the efficiency of vertical-flow constructed wetlands in reducing dyes (mixed of them with/without artificial wastewater), aromatic amines compounds, COD, PO_4-P and other nutrients for long term.

In order to achieve the aim, the research involved in depth investigation covering the following objectives:

a. Undertake critical review of relevant literatures on role of temperature, hydrology and other components of the wetland in the treating efficiency of dye reduction, COD, nutrients (PO_4-P, NH_4-N and NO_3-N) and other water quality parameters such as TSS, pH, etc. in the textile wastewater.

b. To assess the role of gravel and plants on dye reduction and other pollutants.

c. To assess the influence of two groups of dyes (acid and basic), each dye having a different molecular weight and chemical structure, at two different concentrations (low and high), and the mixture of both dyes, on the performance of vertical-flow constructed wetlands in dyes reduction, with/without artificial wastewater to evaluate the annual and seasonal reduction.

d. To assess the influence of operational parameters such as contact time (also known as hydraulic retention time), resting time and mass loading rate on dye reduction and other pollutants for long term. Contact time is defined as the duration the wastewater is in contact with the wetland filter content. In comparison, resting time is the duration when the wetland filter is empty (i.e. no wastewater input). All these operational parameters are linked with the pervious objectives to make the novelty of my work.

1.6 Thesis Structure

This investigation began by reviewing the existing information on CWs treatment of textile wastewater. This study then investigated the performance of VFCWs in a greenhouse applied for dye reduction.

Chapter 1 describes the background of the research, problem statement, aim and objectives, and finally lists the thesis chapters.

Chapter 2 presents the impact of the textile wastewater, an overview of the historical development of the constructed treatment wetland system and components of it (water, media, vegetation and micro-organisms). An overview is also given of the constructed wetlands classification, and the reduction mechanisms of the azo dyes and other pollutants. Furthermore, the choice of vertical-flow over horizontal-flow constructed wetlands is stated in this chapter which also discusses amine compounds and their classification. Finally, an overview is given regarding treating textile wastewater with vertical-flow constructed wetlands.

Chapter 3 describes the materials, the experimental set-up, environmental conditions and operation methods applied for the experimental work. It explains the design of the experimental constructed wetlands, their media and the type of plant. In addition, as an influent, the compositions of the artificial wastewater, the two azo textile dyes and the nutrients for the plant are explained. Furthermore, it includes determinations of water quality parameters and calibration of equipment used for the measuring of water quality parameters. Finally, the type of statistical methods, used for the normality test and the other functions such as significant effect, are described in this chapter.

Chapter 4 presents the results and discussions of the performance efficiency of the vertical-flow constructed wetlands to treat two azo textile dyes. In addition, the other water quality parameters are discussed including pH, redox potential, total suspended solids, turbidity, dissolved oxygen, electrical conductivity, chemical oxygen demand and nutrients (ammonia nitrogen, nitrate nitrogen and ortho-phosphate-phosphorus).

Chapter 5 talks about the performance efficiency of the vertical-flow constructed wetlands in treatment of artificial wastewater containing two azo textile dyes with other water quality parameters such as COD and nutrients. In addition, other parameters, such as colour and amines compounds reduction, are discussed.

Chapter 6 presents the conclusions and recommendations for further work.

2. CHAPTER TWO: CRITICAL LITERATURE REVIEW

2.1 Overview

In this chapter, the historical development of wetlands is described in section 2.2 and the components of constructed wetlands are presented in section 2.3. The classification of constructed wetlands is demonstrated in section 2.4. Section 2.5 includes the reduction mechanisms of pollutants in constructed wetlands. The choice of VSSF over HSSF CW is justified in section 2.6. Amine compounds are documented in section 2.7. Finally, the use of constructed wetland systems for the treatment of textile wastewater is presented in section 2.8 and a concluded remarks of the chapter is given in section 2.9. This chapter addresses the impact of textile wastewater on humans and the environment. The historical development of constructed wetland systems is presented, as well as the early principles of the technology. In addition, this chapter covers water, media, vegetation and micro-organisms as key components of constructed wetlands. After that, a classification of constructed wetlands is presented, and applications of intermittent loading of vertical-flow treatment systems for different types of wastewater are shown. The mechanisms of pollutant reduction are covered with focus on azo dye reduction mechanisms. Finally, the choice of vertical-flow over horizontal-flow constructed wetlands and types of amine compounds is justified.

2.2 Historical Development of Constructed Treatment Wetlands

Natural wetlands, in general, are a transitive area between land and water. The boundaries between wetlands and uplands or deep water are therefore not always distinct (Stefanakis et al., 2014). Wetlands are an ecosystem whose formation and characteristics are mainly controlled by water (Barbour and Billings, 2000). The water saturation level is the most important factor, which determines the nature of the soil and the types of animal species that live in wetlands as well as the kind of plants (Novitzki et al., 2002; Stefanakis et al., 2014). Scholz (2006) reported that wetlands have been appreciated as an important natural resource during human history. Their value has been recognized in their natural state by such people as the Marsh Arabs around the junction of the rivers Euphrates and Tigris in the south of Iraq as well as in managed forms, for example rice paddies, in the south of Asia. Furthermore, wetlands serve as a wildlife conservation resource and can be seen as natural recreational areas for the local community. *Typha spp*., *Phragmites spp.* and other macrophytes typical of swamps are widely used in North America and Europe (Knight et al., 2001; Scholz, 2011), and can be found at any place except Antarctica. In most wetlands, the hydrology of the wetlands is either generally one of slow flows and shallow waters or saturated substrates. The slow flows and shallow water depths allow sediments to settle as the water passes through the wetland. The slow flows also provide prolonged contact times between the water and the surfaces within the wetland. The complex mass of organic and inorganic materials and the diverse opportunities for gas/water interchanges foster a diverse community of micro-organisms that breakdown or transform a wide variety of substances. This vegetation slows the water, creates microenvironments within the water column, and provides attachment sites for the microbial community. The litter that accumulates as plants die back in the fall creates additional material and exchange sites, and provides a source of carbon, nitrogen, and phosphorous to fuel microbial processes.

The best definition for natural wetlands was provided by the Ramsar Convention on Wetlands, which was held in Iran (Ramsar, 1971). In this Convention, wetlands are defined as "*areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters.*" This definition was founded on a wide approach to best describe the main characteristics of wetlands (Mustafa, 2010; Stefanakis et al., 2014). Many publications reported the benefits of natural wetlands to human life (Turner, 1991; Breaux et al., 1995; Knight, 1997; Kirsten and Brander, 2004; Ghermandi et al., 2009; Costanza et al., 2016). This identification of the wide range of economic and ecological benefits has indicated increasing interest in the construction wetlands ecosystems that mimic the functions of natural wetlands in order to support human use (Hammer and Bastian, 1989; Stefanakis et al., 2014).

Constructed wetlands are engineered to optimize the treatment conditions found in natural wetlands by using a complex mixture of water, substrate, plants and a variety of micro-organisms (Langergraber, 2008). The fundamental idea of building constructed wetlands is to duplicate the various natural wetland processes in a way that is more advantageous for humans and under controlled environmental conditions such as temperature and humidity (Eke, 2008). It has more advantages, such as low cost, minimization of biological and chemical sludge and high efficiency (Bulc, 2006; Sivakumar et al., 2013).

Kadlec and Knight (1996) documented that natural wetlands were used for wastewater disposal in 1912. On the other hand, Brix (1994c) and Wallace (2004) documented that the first use of constructed wetlands was in Australia in 1904. The first use of constructed wetlands with plants as a wastewater treatment was begun in Germany in the 1950s by Seidel (1965a) as reported by Eke (2008), Vymazal (2010a, 2014) and (Stefanakis et al., 2014). Seidel carried out much experimental work to study the effect of the presence of plants on the treatment of different types of wastewater such as dairy wastewater treatment with plant *S. lacustris*, phenol wastewater treatment with plant *Scirpus lacustris*, and livestock wastewater (Seidel, 1955, 1961, 1965a, 1966, 1976) as documented by Vymazal (2005). She noticed that growth of plants in pollutant water (wastewater) is better than in uncontaminated water and the plants, which she used in the experimental works (bulrush; kind of reed), have an ability to reduce various pollutants from wastewater (Stefanakis et al., 2014). Moreover, Seidel worked very hard, in the early 1960s, using wetland plants in sludge with different types and wastewater, and improve the performance of rural wastewater treatment, and tried to decentralized the treatment of wastewater by using either pond systems or septic tanks (Vymazal, 2005). After that, she tried to improve her early system by using sandy soil as a media with high hydraulic conductivity with different types of plants. Due to the anaerobic conditions of septic tanks, she worked on improving the system by using a stage of primary sludge as an infiltration bed with a vertical flow and the wastewater flowed as an elimination bed with a horizontal flow, and planting with *Phragmites australis* (Seidel, 1965b). This system was the foundation for hybrid systems that were re-established at the end of the 20^{th} century, as reviewed by Vymazal (2005). Kickuth, one of Seidel's students, in collaboration with her, invented a wetland treatment process known as the Root Zone Method (RZM) (Kickuth, 1977, 1978, 1981; Vymazal, 2009), which was first used at Othfresen, Germany in 1974 (Brix, 1987). The design of the RZM consists of a rectangular bed planted with *Phragmites australis*, and the media in this system differed from Seidel's design in the use of cohesive soil with a high content of clay (Vymazal, 2005). Water flows horizontally through the roots of the plants as a subsurface horizontal flow (Brix, 1994c). Kickuth carried out his experimental work and made this concept popular with his team in Europe, resulting in about 200 industrial and municipal wastewater treatment systems, and attention in the growth of these subsurface (RZM) flow constructed wetlands led to the spread of their use in Europe by the mid-1980s (Bastian and Hammer, 1993).

The first use of CWs in the Netherlands was in 1967 and it was FWSF (Veenstra, 1998; Vymazal, 2010b). In addition, in Hungary, a FWSF CW was built in 1968 near Keszthely to treat the effluent wastewater of the town and set in the place of an existing natural wetland (Lakatos, 1998).

In the USA, research studies on the use of FWSF CWs to treat wastewater started in the late 1960s (Eke, 2008; Sani, 2015). Gessner et al. (2005) reported that the first constructed wetland for engineering purposes was constructed in New York, North America between 1973 and 1976. Nevertheless, in 1901, a constructed wetland as a purposely engineered treatment vessel was used (Monjeau, 1901, Wallace, 2004) as documented by Eke (2008). The research studies became more effective throughout the 1970s and 1980s, and between 1980 and 1990 (USEPA, 2000a). For instance, in 1975, a constructed wetland/pond system was built in North Dakota to treat industrial wastewater (Litchfield and Schatz, 1989) and FWSF CWs were established to treat urban storm water in California in the early 1980s (Chan et al., 1982). In North America, most CWs are used as large-scale facilities for municipal wastewater treatment (Stefanakis et al., 2014). Knight et al. (1993) and Brix (1994c) pointed out that FWSF CWs are the most common type of constructed wetlands used for wastewater treatment. Use of constructed wetlands has been increased widely in the USA after the publication, in 1993 by the Tennessee

Valley Authority, of a design manual aimed primarily for separate houses (Wallace, 2004).

Constructed wetlands were introduced to the UK in the mid-1980s after the UK WRc (Water Research council) visited Kickuth, Seidel's student who was an early advocate of the technology (Boon, 1986). Later, the WRc and UK Water Services Associated established the Reed Bed Treatment System Coordinating Group, which combined the results of the initial technology trials carried out by water utilities with the plan of accelerating development (Cooper and Green, 1998; Murphy and Cooper, 2010; Knowles, 2012). Severn Trent, a member of UK Water Services Associated, confirmed that HSSF CWs have an ability to treat domestic wastewater effluent for communities of <2000 people as a tertiary treatment and as a secondary treatment for communities of <50 people (Green and Upton, 1993). Green and Martin (1996) reported that HSSF CWs were used in 1990 to treat storm water overflow. Therefore, using this type of CWs became more common in small communities. Later, as a result of many problems being found with this type of CW (Cooper et al. 1996), many researchers turned to the use of VSSF CWs as an alternative solution.

In China, the first constructed wetland system was built in 1987 (Ding and Shen, 2006; Liu et al., 2009) and by 2006 there were more than 200 CW systems, almost all of which were established to treat domestic, industrial and agricultural wastewater, and 54.3% of which were VSSF or combined integrated vertical flow (IVF). On the other hand, the volume of wastewater effluent has also increased rapidly. For example, industrial wastewater effluent increased between 2000 and 2005 from 19.4 to 24.3 billion tons and domestic wastewater increased from 15.1 to 28.1 billion tons in the same period (NBS, 1995-2005) as reviewed by Liu et al. (2009). Furthermore, China has the largest industry of aquaculture in the world (Liu and Diamond, 2005) and that's another challenge, especially as the application of CWs to treat this type of wastewater is largely absent (Liu et al., 2009). Furthermore, some researchers have been published in other countries. For example, Kenya (Bojcevska and Tonderski, 2007; Kimani et al., 2012), Tanzania (Haule et al., 2000), Thailand (Klomjek and Nitisoravut, 2005), India (Juwarkar et al., 1995; Sharma and Brighu, 2014) and Egypt (Abou-Elela and Hellal, 2012; Abou-Elela et al., 2013; ElZein et al., 2016).

In the case of textile wastewater, a root zone method (RZM) was used, in 1983 in Germany, for the elimination of sulphur compounds from the textile finishing industry. The system consisted of sand as a media, planted with *Phragmites Communis Trin*. (Winter and Kickuth, 1989). In 1992, HSSF CWs, with gravel as a media and planted with *Phragmites australis* were used in Australia to treat a complex wastewaters, which were the effluents of different processes of textile and dyeing facilities (Davies and Cottingham, 1994). In the USA in 1999, VSSF CWs with gravel as a media and planted with *Phragmites australis* were used to reduce two different dyes from a synthetic wastewater simulating textile wastewater (Pervez et al., 1999). A combination of VSSF and HSSF CWs (hybrid system), with sand as a media and planted with *Phragmites australis*, was used in 2006 in Slovenia to treat real textile wastewater (Bulc et al., 2006).

2.3 Components of Constructed Wetlands

A constructed wetland consists of a properly designed basin that contains water, a media, plants and micro-organisms. These components can be manipulated in constructing a wetland as shown in Figure 2.1.

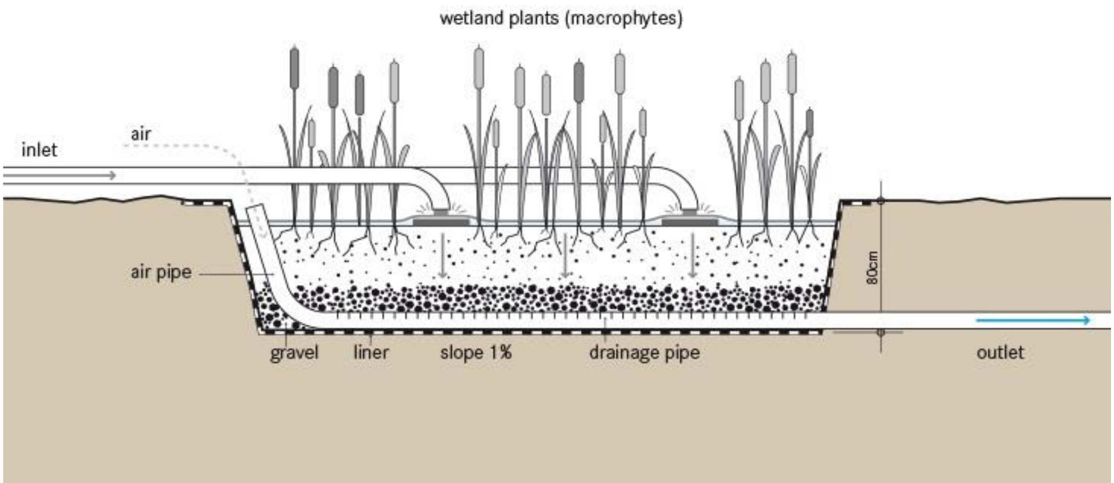


Figure 2.1 Schematic representation of vertical-flow constructed wetlands (Tilley et al., 2014)

2.3.1 The Water

In general, wetland hydrology refers to influent and effluent of water through the wetland and the communication with the other functions of the wetland: media, vegetation and micro-organisms. Actually, it is the one of most important factors in the design of wetlands because all of the other functions are linking with each other through it (Luise et al., 1993; Carter, 1996). It is often the primary factor in the success or failure of a constructed wetland due to it is role in determining the duration of water biota interactions (Kadlec and Wallace, 2008). Any changes in hydrology can have an effect on a wetland and its treatment performance (Kadlec and Knight, 1996; Okurut, 2000). For instance, Lim et al. (2001) found an increase in pollutant concentration as a result of losses in water.

In constructed wetlands, hydrology engages with the anaerobic condition. Under this condition, constructed wetlands can reduce the pollutant (Scholz, 2011). For example, denitrification, which is a process to reduce nitrogen, takes place under anaerobic conditions (Merino-Solís et al., 2015). The time for which water remains in constructed wetlands, named hydraulic retention time (HRT) or hydraulic contact time, is an important variable in designing and evaluating the treatment performance of a constructed wetlands system (Ghosh and Gopal, 2010; Merino-Solís et al., 2015). Toet et al. (2005) evaluated the effect of four different hydraulic retention times (0.3, 0.8, 2.3 and 9.3 days) on the pollutant reduction by surface flow wetlands. The results showed the reduction of nitrogen and faecal coliform was enhanced by increasing the HRT.

2.3.2 The Media

The media used in constructed wetlands is the substrate. The selection of substrate type is very important to the successful performance of wastewater treatment by the wetland system (Crites et al., 2014). Substrates used to construct wetlands include soil, sand, gravel, rock, and organic materials such as compost (Dordio and Carvalho, 2013). The media in subsurface flow constructed wetlands supports the growth of plant macrophytes and provides a direction for wastewater to pass and at the same time provides surfaces for micro-organisms to live, enabling the reduction of wastewater pollutants via consumption by micro-organisms (Stefanakis et al., 2014; Zidan et al., 2015). The characteristics of the filter media, which define its permeability, should be selected carefully (Stefanakis et al., 2014).

Many researchers have investigated enhancing the constructed wetland ability to reduce pollutants by adding special materials, which are supported by the media (Kadlec and Wallace, 2008; Ballantine and Tanner, 2010; Meng et al., 2014; Austin and Yu, 2016). Zurayk et al. (1997), Shilton et al. (2005), Xu et al. (2006) and Park and Polprasert (2008) enhanced the reduction of phosphorus by adding limestone, seashells, slag and fly ash to wetland media. García-Pérez et al. (2015) demonstrated that using recycled shredded-tire chips could enhance the reduction efficiency of typical wastewater contaminants, while Prochaska and Zouboulis (2006) found the use of dolomite with sand as a filter media had no significant on the phosphorus reduction rate when they used a synthetic wastewater treated by vertical-flow constructed wetlands. Gorra et al. (2007) used zeolite to enhance the reduction of ammonia-nitrogen from wastewater, the results showed that this material is appropriate for ammonia-oxidizing bacteria and that it led to increased reduction of ammonia-nitrogen, while using zeolite and bauxite together has no significant effect on the reduction of wastewater pollutants by vertical-flow constructed wetlands as pointed out by Stefanakis and Tsihrintzis (2012). Furthermore, Scholz and Xu (2002) used a granular activated carbon as the adsorption media to increase filtration performance of constructed wetlands, the results showed no improvement in the media adsorption capacity. Lavrova and Koumanova (2014) and Shen et al. (2015) used a solid carbon source (methanol and starch blends, respectively) to achieve a higher denitrification in vertical-flow constructed wetlands. Drizo et al. (1997) used shale as a media to enhance the reduction of phosphorus and ammonium by horizontal subsurface constructed wetlands. The results showed an extremely high reduction for both of them.

The size of media, distribution of particle size and the particle shape also affect the treatment performance of constructed wetlands by affecting the hydraulic conductivity (Knowles et al., 2011), Table 2.1. Dordio and Carvalho (2013) and Meng et al. (2014) pointed out that biofilm growth is better established in media of smaller particle size compared with that of excessively larger size, giving microbes a higher biodegradation ability. Furthermore, Austin and Yu (2016) stated that biomass for media of particle size

3.5 mm is three times greater than when the particle size is 10 mm, while the possibility of clogging increases with fine-pore media (Wallace and Knight, 2006; Song et al., 2015). Nevertheless, Sani et al. (2013) and Al-Isawi et al. (2015b) found that COD and BOD results of vertical-flow constructed wetlands with large and small aggregate were similar during the whole period of their experimental work.

Until recently, soil was only utilized as a growth medium for the plants in subsurface flow constructed wetlands (Arias et al., 2001). This practice, however, often resulted in clogging problems due to the relatively low hydraulic conductivity of this material. Therefore, most wetland engineers advise using a fine gravel instead of soil, which reduces the possibility of clogging (Ávila et al., 2014). Many researchers have suggested using a coarser material as a bottom layer in VFCW beds to avoid clogging (Weedon, 2003; Brix and Arias, 2005; Masi et al., 2007; Öövel et al., 2007;Molle et al., 2008) as reviewed by Ávila Martín (2013). Kalyani Kenkar and Kadlag (2016) pointed out that where gravel is used as the growth medium for the microbes, it works as a sieve and determines the hydraulic contact time.

Table 2.1 Wetland media characteristics (Strickland and Korleski, 2007)

Type	Effective Size (mm)	Porosity (n) %	Hydraulic Conductivity (Ks; ft/day)
Fine Gravel	16	38	7500
Medium Gravel	32	40	10000
Coarse Rock	128	45	100000

2.3.3 The Plants

Today, the significance of plant presence in CWs is recognized and the positive effects on the system operation and performance have been – more or less – proved (Stottmeister et al., 2003; Scholz, 2006, 2010; Gargi Sharma et al., 2014; Villa et al., 2014). Although it was a controversial issue in the past, today it is generally accepted that constructed wetland plants provide a series of benefits and contribute to the creation of the necessary conditions which directly or indirectly affect the system efficiency (Stefanakis et al., 2014). Brix (1994b) documented that plants have an ability to release oxygen into the rhizosphere from their root. This process is very important to help subsurface flow constructed wetlands in aerobic degradation and nitrification. Among the most common plant species used in CWs (*Phragmites spp., Scirpus spp., Typha spp., Carex sp.*), reported values for plant-mediated oxygen transfer are within the range of 0.005-12 g $O_2/m^2/d$ (Armstrong et al., 1990; Nivala et al., 2012; Stefanakis and Tsihrintzis, 2012). Furthermore, many researchers have demonstrated that plants play an important role in nutrients uptake (Brix, 1994b; Tanner, 1996; Bachand and Horne, 1999; Stottmeister et al., 2003;Vymazal, 2007; Hussein and Scholz, 2017).

The density of vegetation of a constructed wetland strongly affects its hydrology; firstly, by obstructing flow paths as the water finds its sinuous way through the network of stems, leaves, roots, and rhizomes, and secondly, by blocking exposure to wind and sun as stated by Luise et al. (1993). Furthermore, the presence of plants, especially in VFCWs, prevents clogging by dissolving organic matter through the roots, and this process leads to the pervasion of the water through the media of an intermittent or tidal loading vertical-flow system (Brix, 2003). Hua et al. (2014) studied the effect of plants on prevention of clogging between unplanted and planted wetlands, by measuring the coefficient of permeability and effective porosity of the media within different operational modes. The results of their experimental work suggested that the coefficient of permeability and effective porosity increased in the plant root zone. Moreover, Münch et al. (2005) reported a higher microbial density in planted constructed wetlands.

Many publications have investigated the effect of plant presence on the organic matter reduction by constructed wetlands. For example, Kaseva (2004) reported the reduction percentage rate of COD of domestic wastewater was 56-61% in planted wetlands, while 33% in unplanted wetlands. Karathanasis et al. (2003) found the reduction percentage rate for BOD and TSS was >75% and >88%, respectively, in planted wetlands, and 63% and 46%, respectively, in unplanted wetland, when treating domestic wastewater. However, Akratos and Tsihrintzis (2007) found a slight increase in COD reduction percentage rate in wetland planted with *T. latifolia* (89.3%) when compared with unplanted wetland (87.2%).

. Ong et al. (2009) found a slight increase in COD reduction percentage rate with the presence of plants, when they used UFCWs to treat azo dye AO7 (contact time 3 d). The influent COD was 383.8±13.7 mg/l, the reduction percentage rate was 78% and 79% for the unplanted and planted wetlands, respectively. For the dye reduction, there was no difference in dye reduction rate between planted and unplanted wetlands; the influent of dye AO7 was 50.5±2.0 mg/l and the reduction percentage rate was 96% for planted and unplanted wetlands. Furthermore, Yalcuk and Dogdu (2014) treated azo dye Acid yellow 2G by vertical-flow intermittent-feeding constructed wetlands with 3.74 d as a contact time. The influent COD was 75 mg/l, the reduction percentage rate was 59% and 61-65% for unplanted and planted wetlands, respectively. In the same study, the influent colour was 22900 Pt Co. and the reduction percentage rate was 87% and 98% for the unplanted and planted wetlands, respectively.

2.3.4 Micro-organisms

A micro-organism, also known as a microbe, is defined as a life organism which is difficult to see with the naked eye, such as fungi, bacteria and protozoa, but is visible under a microscope (Madigan et al., 2008). Many researchers have pointed out that micro-organism communities exist in wetlands of both aerobic and anaerobic conditions (Kadlec and Knight, 1996; Ottova et al., 1997; Scholz et al., 2001; Meng et al., 2014). Micro-organisms are one of the key factors on which functions of constructed wetlands are largely dependent to treat wastewater (Wetzel, 1993; Brix, 2003), and constructed wetlands provide an ideal environmental to support micro-organism growth (Saeed and Sun, 2012). Micro-organisms play a major role in reduction of the organic contaminants of wastewater, which are present because of interaction of the processes (physical, chemical and biological) which occur in constructed wetlands in the purification of wastewater and transformation of nitrogen and phosphorus (Kayombo et al., 2005). For instance, photosynthesis is one of the biological processes, which is performed by the wetland plant and algae (one species of micro-organism), with the process adding carbon and oxygen to the wetlands, both of which are very important in the nitrification process (Stottmeister et al., 2003; Kayombo et al., 2005). Richardson and Craft (1993) and Cooper and Findlater (2013) demonstrated that microbial uptake is one of the major mechanisms in the reduction of phosphorus. Furthermore, Kosolapov et al. (2004) and Sheoran and Sheoran (2006) stated that microbial uptake is one of the reduction mechanisms of heavy metals.

In textile wastewater treatment by constructed wetlands, micro-organisms play an important function in decolourization of azo dyes under aerobic, anaerobic and anoxic conditions (Kodam et al., 2005; Pandey et al., 2007). Under aerobic conditions, a type of microorganism known as *P. aeruginosa* works on decolourization of azo dyes due to its ability to depend on azo groups as a solo carbon source (Edwards, 2000; Pandey et al., 2007), while under anaerobic conditions, many types of micro-organisms work on decolourization of azo dyes with external carbon as an energy source (Şen and Demirer, 2003; Haroun and Idris, 2009). Under anoxic conditions, other types of micro-organisms, such as *Pseudomonas luteola*, work on this process (Grekova-Vasileva et al., 2009) and those need a complex organic carbon for reduction of azo dyes (Khehra et al., 2005b).

2.4 Classification of Constructed Wetlands for Wastewater Treatment

2.4.1 Overall Classification

The purpose of these engineered wetlands is to receive and purify wastewater of various types, based on the naturally occurring treatment processes (Stefanakis et al., 2014; Scholz, 2015). Constructed wetlands for wastewater treatment can be further divided into other categories. Knight (1997), Fonder and Headley (2010) and Vymazal (2010a, 2014) reported three categories of constructed wetlands. The first category depends on the water level on the bed, the second depends on the type of wetland plants and the third depends on the direction of the water movement in the constructed wetland as shown in Figure 2.2. In terms of the water level, the constructed wetlands are classified into free water surface flow (FWSF CWs) and subsurface flow (SSF CWs). Depending on the direction of water movement, the constructed wetlands are classified into horizontal flow (HFCWs) and vertical flow (VFCWs). The hybrid system is a combination of horizontal-flow and vertical-flow constructed wetlands to achieve the maximum benefits from both flows in the reduction of the wastewater pollutants (Vymazal, 2013).

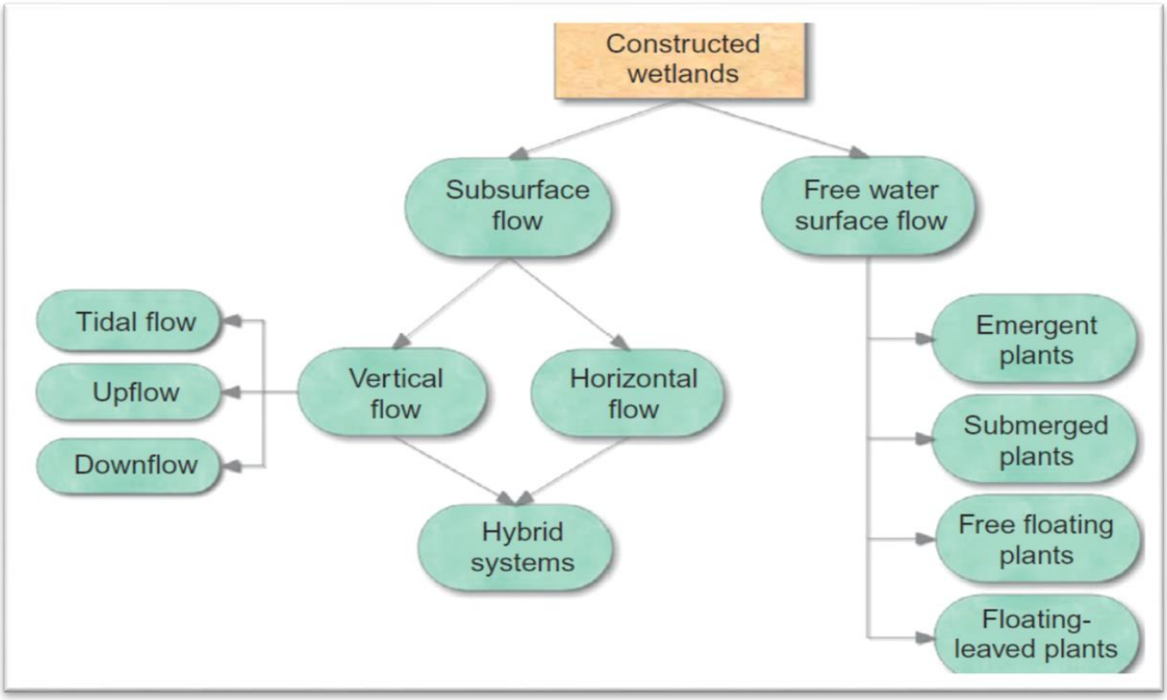


Figure 2.2 Classification of constructed wetlands (Stefanakis et al., 2014)

2.4.2 Free Water Surface Flow Constructed Wetlands

FWSF CWs are defined as constructed wetlands where the water surface is exposed to the atmosphere (USEPA, 2000b), the wastewater enters at the top and moves horizontally on the top of the constructed wetland media as shown in Figure 2.3, so the wastewater infiltrates the media or is evaporated to the atmosphere (Bendoricchio et al., 2000). FWSF CWs are similar to natural wetlands in that they are basins planted with emergent, submerging and/or floating wetland plants as shown in Figure 2.4 (Jadhav and Buchberger, 1995; Lim et al., 2001; Ellis et al., 2003b; Stefanakis et al., 2014). The influent passes as free surface flow (and/or at shallow depths) and at low velocities above the supporting substrates, which provide a promoting environment for biological, chemical and physical reduction processes to take place (Hamilton et al., 1993; Xianfa and Chuncai, 1995; Stefanakis et al., 2014). FWSF CWs are very effective for reduction of nitrogen, biochemical oxygen demand, pathogenic organisms, suspended solids and heavy metals (DeBusk, 1999; Choudhary et al., 2011; Midhun et al., 2016), while they are not effective for phosphorus reduction as wastewater does not tend to come into contact with the media, which precipitates or adsorbs phosphorus (Taylor et al., 2006). Use of FWSF CWs is more common in North America (Tousignant et al., 1999) where they are applied for municipal wastewater treatment only, as stated by Sani (2015). However, although they have advantages of simple technology and low cost of operation (Economopoulou and Tsihrintzis, 2004), FWSF CWs require more land than SSF CWs, carry increased risk of human exposure to surface flowing wastewater, and odour and insects might be a problem because of the free water surface (Halverson, 2004; Stefanakis et al., 2014).

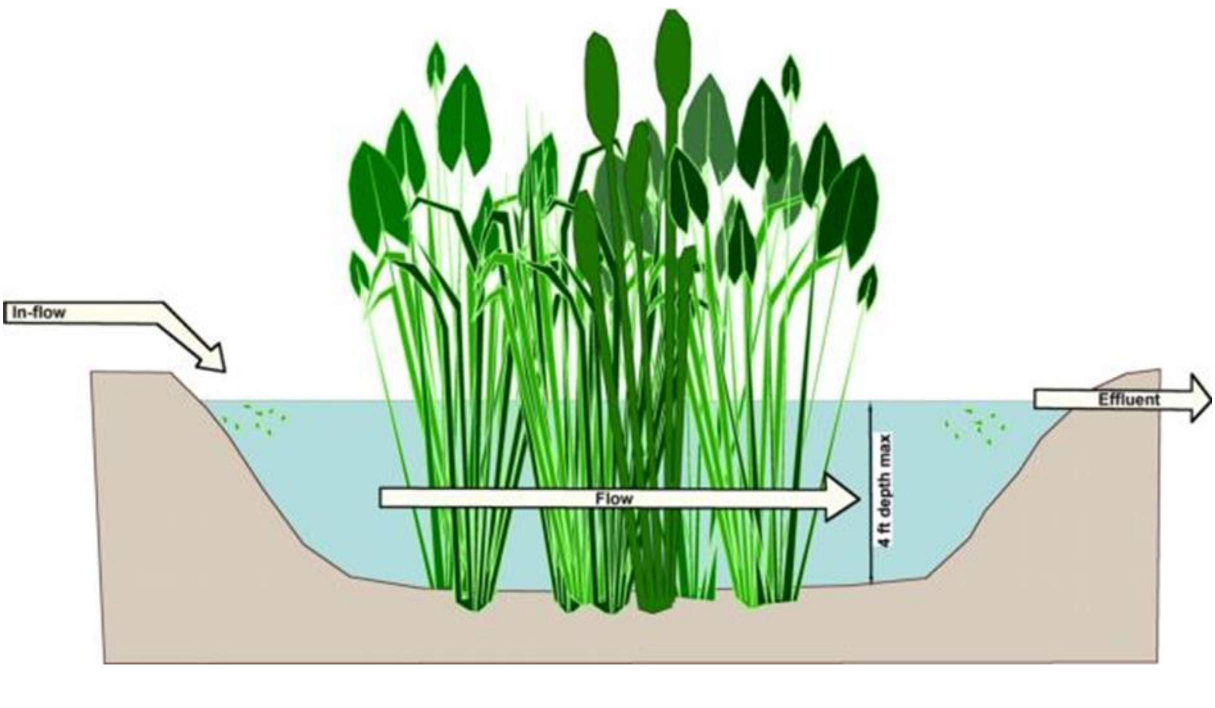


Figure 2.3 Schematic representation of free water surface flow constructed wetland (Oki and White, 2011)

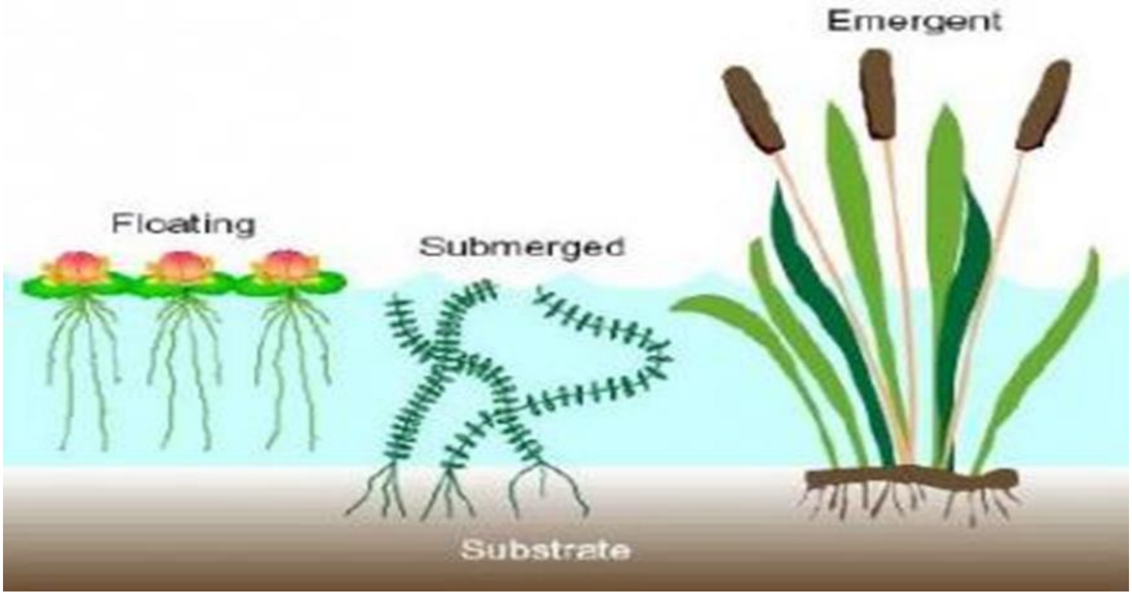


Figure 2.4 Plants for free water surface flow constructed wetlands (Kamariah, 2006)

2.4.3 Subsurface Flow

Subsurface flow systems operate with the influent flowing below the surface of the soil or gravel substrate. Purification occurs during contact with the plant roots and substrate surfaces, which are water-saturated and can be considered oxygen-limited. The overlying vegetation and litter layer thermally insulates the substrate in these systems and so the wetland performance is insignificantly reduced during the winter. There are three types, horizontal flow, vertical flow and hybrid flow (Vymazal, 2010a).

2.4.3.1 Horizontal Subsurface Flow Constructed Wetlands

The wastewater enters HSSF CWs (Figure 2.5) through an inlet and flows slowly through the substrate under the surface of the bed, through the pores of the porous media and the roots of the plants, in a horizontal path until it reaches the outlet where it is collected (Vymazal et al., 1998a). This means there is no water surface exposed to the atmosphere, as in FWSF CWs, the surface flow is invisible and the water level is about 5-15 cm below the surface of the media (Vymazal et al., 2006). Moreover, mosquito breeding is not favoured and the health risk for humans and wildlife habitat is minimized (Kadlec and Wallace, 2008). Usually, gravel or a mixture of sand and gravel is used as a media with a depth between 30 and 80 cm. This media supports the growth of the plants (Vymazal et al., 2006; Akratos and Tsihrintzis, 2007). The value of the media depth depends on the depth of the plant roots, and an impermeable geo-membrane covers the bottom of the media bed. The slope of the bottom varies from 1-3%, meaning gravitational wastewater flow is promoted (Kadlec and Wallace, 2008). During this passage, the wastewater is exposed to a network of aerobic, anoxic and anaerobic zones. The aerobic zones occur around roots and rhizomes that leak oxygen into the substrate (Cooper et al., 1996; Vymazal, 2014).

Many publications have demonstrated that the presence of plant roots and porous media in this type of subsurface flow constructed wetland supports the development of the biofilm, which enhances the reduction of suspended solids and organic matter, while nitrogen and phosphorus reduction is low (Brix, 1994a; Vymazal et al., 1998a; Rousseau et al., 2004b; Kadlec and Wallace, 2008; Gikas and Tsihrintzis, 2010; Jegatheesan, 2011; Vymazal, 2013).

HSSF CWs are commonly used in the USA and Europe (Vymazal et al., 2006; Vymazal, 2008, 2011) and occupy a similar area when compared with FWSF CWs but the cost is higher (Tsihrintzis et al., 2007; Kadlec and Wallace, 2008).

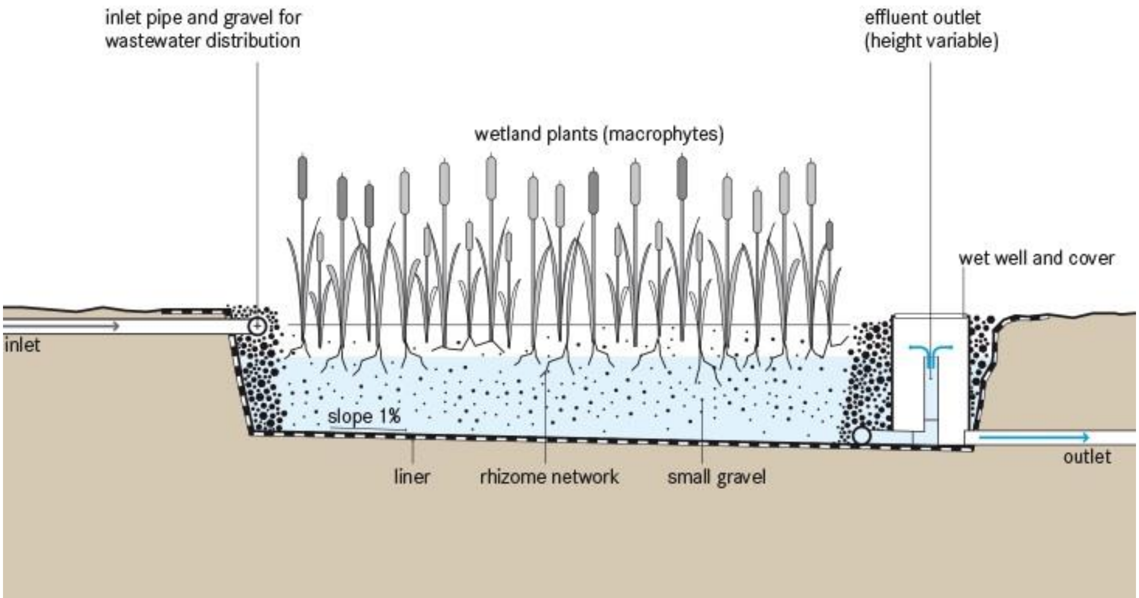


Figure 2.5 Schematic of the horizontal subsurface flow constructed wetland (Tilley et al., 2014)

2.4.3.2 Vertical Subsurface Flow Constructed Wetlands

VSSF CWs have a structure made from several layers of aggregates (gravel and sand) as a media and planted with macrophytes with increasing gradation with depth (Cooper et al., 1996; Vymazal et al., 2006). The wastewater is dosed over the media surface and collected by an underdrain at the bottom of the media surface, so the wastewater is forced to flow perpendicular to the length of the wetland (Tousignant et al., 1999). The depth of the media varies from 45-120 cm and the slope of the bottom varies from 1-2%, which helps to collect the effluent (Vymazal et al., 2006) as shown in Figure 2.6.

In VSSF CWs, the wastewater disperses on the surface of the wetland media, thereby creating an aerobic environment (Scholz, 2006) by driving out the trapped air and sucking fresh air into the bottom of the media (Stefanakis et al., 2014), this enhances the nitrification process and decomposition of the organic matter, when compared to the horizontal subsurface flow system (Vymazal et al., 2006; Kadlec and Wallace, 2008; Garcia et al., 2010; Abou-Elela et al., 2013; Kantawanichkul and Wannasri, 2013). Ye et al. (2012) found that 50% of atmospheric oxygenation was supplied to the upper layer of the media. Furthermore, many researchers have demonstrated that VSSF CWs have a good ability for the reduction of wastewater pollutants such as BOD, COD and suspended solids (Brix and Arias, 2005; Mimis and Gaganis, 2007; Bulc and Ojtrsek, 2008; Ong et al., 2010; S Wu et al., 2015). On the other hand, this mode of operation does not support the denitrification process and the reduction of phosphorus is limited when compared to other pollutants, because the contact time between the media and the wastewater is insufficient, as founded by Rustige et al. (2003), Prochaska and Zouboulis (2006) and Stefanakis and Tsihrintzis (2012). Vymazal and Kröpfelová (2008) related the reduction of phosphorus with sorption capacity of the media. However, VSSF CWs with tidal loading can achieve a higher phosphorus reduction, as proved by Hussein and Scholz (2017) when they treated azo textile dyes wastewater without any modification or adding any extra materials to the gravel, which was used as a media in their study. Furthermore, some researchers have stated that vertical-flow constructed wetlands with an intermittent operation mode can denitrify well with amendment (Gross et al., 2007; Weedon, 2010; Song et al., 2015).

VFCWs are mainly used in Europe, especially in Denmark, Austria, Germany, France, and the United Kingdom, but are also used in the USA (USEPA, 2000a; Kadlec and Wallace, 2008), as stated by Stefanakis et al. (2014) and Brazil (Philippi et al., 2006). Another countries in Asia and Africa have started to use this type of subsurface constructed wetland (Kivaisi, 2001; Hoffmann et al., 2011; Von Muench and Winker, 2011; Gargi Sharma et al., 2014).

VSSF CWs are mainly used to treat municipal and domestic wastewater, when the effluent limits are set for ammonia-nitrogen (Vymazal and Kröpfelová, 2008). However, many publications have reported on the use of VSSF CWs in treatment of other types of wastewater such as textile (Davies et al., 2005; Ong et al., 2011), domestic greywater (Pillai and Vijayan, 2012), artificial (Białowiec et al., 2011), refinery (Sharma and Brighu, 2014; Mustapha et al., 2015), dairy (Dąbrowski et al., 2017), combined raw wastewater and storm-water (Lopez, 2014) and mari-culture (Sousa et al., 2011).

VSSF CWs can be classified into further variants depending on flow direction, level of saturation and the duration of saturation (Stefanakis et al., 2014), and also VSSF CWs with intermittent loading (down-flow), recirculating VSSF CWs, tidal flow, saturated vertical up-flow, saturated vertical down-flow and integrated VSSF CWs. VSSF CWs with tidal loading are widely used, especially in Europe. The main advantage of this operation mode is the creation of a range from 3-5 cm of temporary wastewater ponding at the top of the media surface, this condition is known as a saturated (anaerobic) mode (Stefanakis et al., 2014) and the time for which this anaerobic mode takes place is known as the contact or retention time. The trapped air moves downward because of the wastewater force, then the wastewater drains vertically by gravity through the porous media and air from the atmosphere follows in its place, this condition is known as an unsaturated (aerobic) mode (Maier et al., 2009) and the time for which this aerobic mode takes place is known as the resting time. In this type of operation, the bed aeration increases and accelerates the growth of micro-organisms (Du et al., 2016), improving the nitrification process and oxidation of the organic matter to prevent the clogging (Kadlec and Wallace, 2008; Hua et al., 2013; Stefanakis et al., 2014).

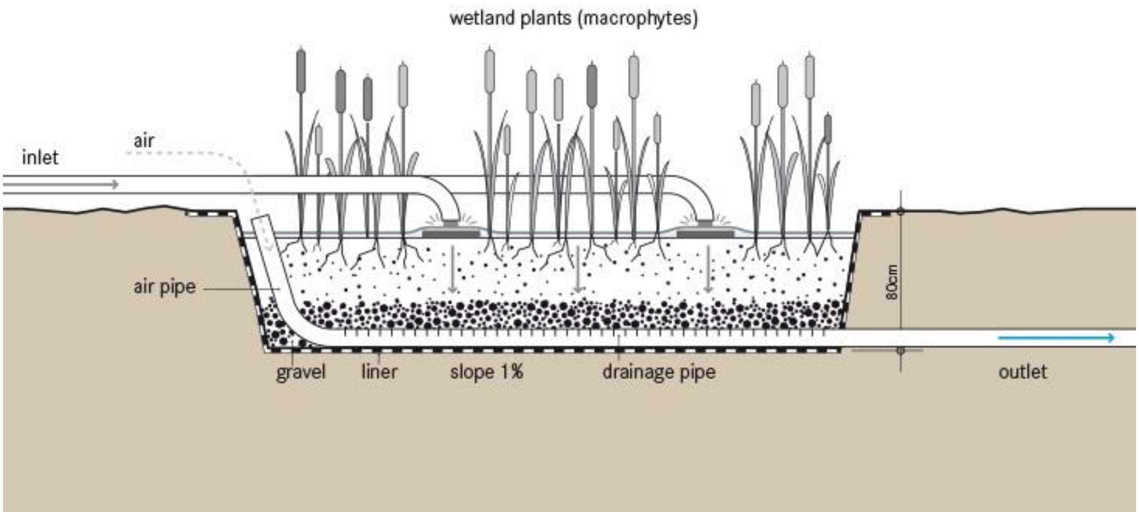


Figure 2.6 Schematic of the vertical subsurface flow constructed wetland (Tilley et al., 2014).

2.4.4 Hybrid Flow Constructed Wetlands

In hybrid flow constructed wetlands, the advantages of various constructed wetlands (surface flow and subsurface flow) can be combined to complement each other (Sayadi et al., 2012; Vymazal, 2013; Ayaz et al., 2016), mainly VSSF and HSSF (Cooper et al., 1999; Liu et al., 2006; Melián et al., 2010; Vymazal and Kröpfelová, 2011; Vymazal, 2013) as shown in Figure 2.7. The idea of this combination is to make the most of the advantages of the one type to offset the disadvantages of the other. For example, the limited oxygen transfer capacity of HSSF constructed wetlands results in this system having a low nitrification process, while VSSF constructed wetlands have better efficiency in this process due to the higher oxygen transfer capacity. Conversely, the denitrification process in HSSF constructed wetlands is better than in VSSF constructed wetlands. By combining HSSF and VSSF constructed wetlands as a hybrid system, good conditions for nitrification and denitrification processes can be produced (Liu et al., 2006; Mena et al., 2008; Vymazal, 2013). Seidel was the first one who tried to combine the various constructed wetlands, parallel VSSF CWs followed by HSSF CWs in series, in Germany in the 1960s, as reported by Vymazal (2005, 2013) and Stefanakis et al. (2014) and that was the foundation for the hybrid constructed wetlands, which was refreshed at the end of the 20th century.

In general, a stage of VSSF units followed by HSSF units in series and a stage of HSSF units followed by VSSF units are the most popular types of hybrid system (Vymazal, 2013). The first combination (Figure 2.7a) includes VSSF units to reduce organic matter and suspended solids, and provide good conditions for the nitrification process. These units are followed by HSSF units, which provide another process to reduce organic matter and suspended solids, and provide good conditions for the denitrification process. This type of hybrid system is widely accepted in treatment of municipal sewage wastewater (Foladori et al., 2012; Vymazal, 2013; Lee et al., 2015). The second combination (Figure 2.7b) includes HSSF units to reduce organic matter and suspended solids, and provide good conditions for the denitrification process followed by VSSF units for the nitrification process, and additional reduction of organic matter and suspended solids. In this type of hybrid, depending on the results of effluent analysis, if there is an increase in concentration of nitrate, which occurs in the wastewater effluent after VSSF because of ammonia nitrogen oxidation, the effluent has to be fed back as an influent in HSSF again or goes to another HSSF unit as shown in Figure 2.7c. These hybrid types are common in treatment of municipal wastewater (Gajewska, 2011; Vymazal, 2013) and can be used in treatment of mixed industrial wastewater (Justin et al., 2009). Vymazal (2005) reported this type of hybrid system, designed by Seidel, consisted of two stages of VSSF units followed by two or three HSSF units in series (Figure 2.7d). The combination of VSSF units followed by HSSF and then FWS as a last stage (Figure 2.7e) was used in treatment of winery wastewater in Italy (Zanieri et al., 2010). Finally, the combination of HSSF units followed by FWS and then HSSF as a last stage (Figure 2.7f) was used in treatment of sewage wastewater in China (Ye and Li, 2009).

The design of hybrid system depends on the type of wastewater, population and the area demand. For instance, Cerezo et al. (2001) used a combination of different constructed wetlands in three stages and four series. FWS units without media and planted with *Phragmites australis*, HSSF CWs with sand as a media, unplanted and planted with *Typha dominguensis*, up-flow VSSF units with gravel as a media, unplanted and planted with *Phragmites australis* and down-flow VSSF units with gravel and stone as a media, unplanted and planted with *Phragmites australis*.

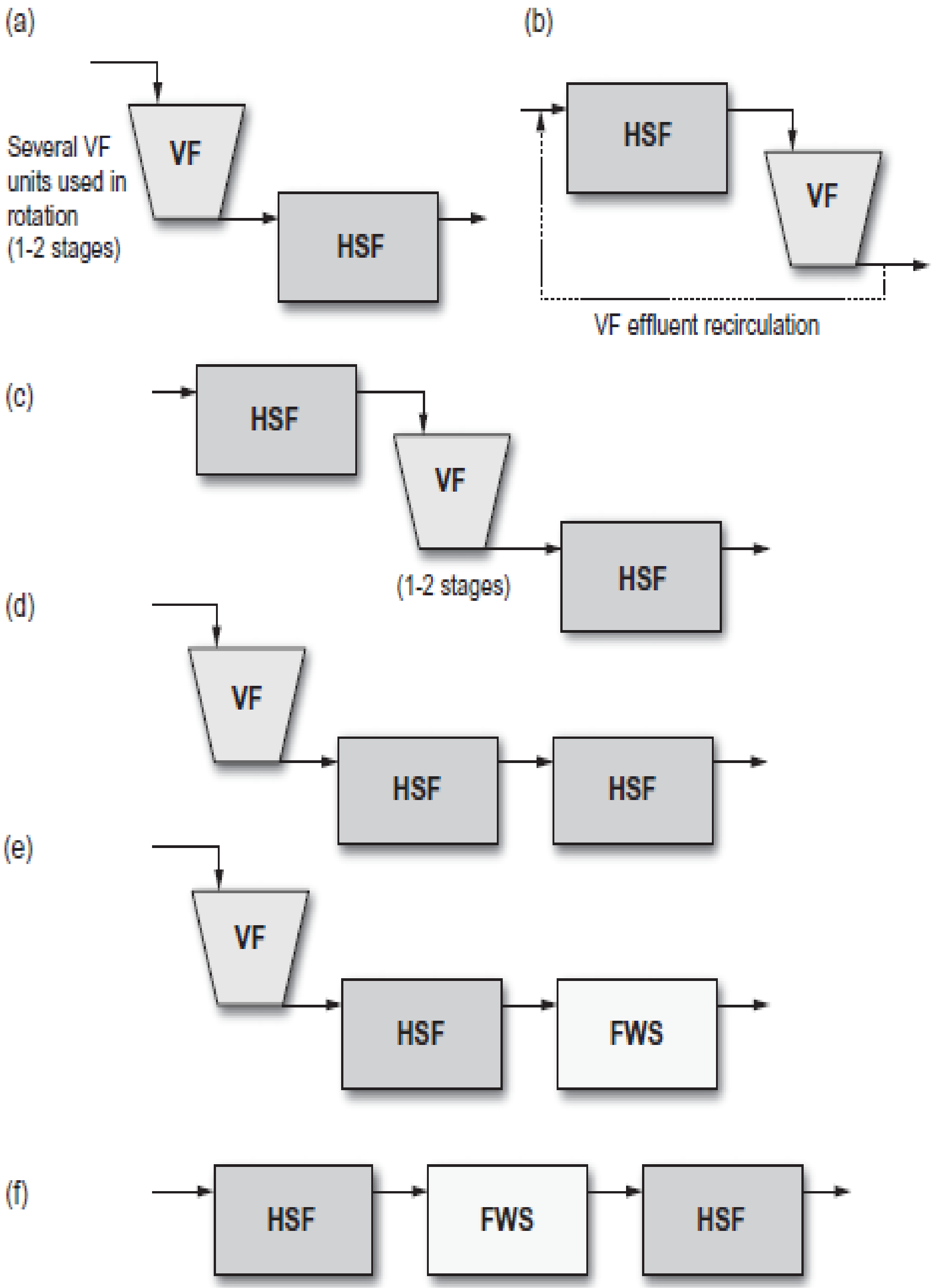


Figure 2.7 Various combinations of different constructed wetland types in hybrid constructed wetlands; (a), VSSF-HSSF, (b); HSSF-VSSF, (c); HSSF-VSSF-HSSF, (d); VSSF-HSSF-HSSF, (e); VSSF-HSSF-FWS, (f); HSSF-FWS-HSSF (Stefanakis et al., 2014).

2.5 Reduction Mechanisms in a Constructed Wetland

Constructed wetlands have an ability to reduce many contaminants, including organic compounds, suspended solids, nutrients, heavy metals (Vymazal et al., 1998b; Sheoran and Sheoran, 2006), dye and colour (Nilratnisakorn et al., 2009; Olejnik and Wojciechowski, 2012).

2.5.1 Total Suspended Solids Reduction Mechanism

Total suspended solids is defined as all particles in wastewater which can be trapped using a filter, they include solid particles which may be organic or inorganic, such as silt, industrial waste and sewage (Stansbury and Branigan, 2013). A high concentration of total suspend solids leads to light being blocked from reaching plants which then affects the rate of photosynthesis (Bilotta and Brazier, 2008), causing a reduction in dissolved oxygen that should be released into the water from the plants. This is not the only physical effect, it is also leads to temperature changes. Furthermore, chemicals alterations, as a result of the higher concentration of total suspended solids, will release contaminants such as heavy metals (Dawson and Macklin, 1998; Bilotta and Brazier, 2008) and nutrients such as phosphorus (Haygarth et al., 2006) into the receiving stream. Also, the higher concentration of TSS may affect biological properties such as reducing the growth of organisms or reducing the population size (Wagener and LaPerriere, 1985; Shaw and Richardson, 2001), as reported by Bilotta and Brazier (2008).

Many researchers have stated that constructed wetlands have the mechanical ability to remove TSS with a high percentage (Green et al., 1997; Manios et al., 2003; Yirong and Puetpaiboon, 2004; Kadlec and Wallace, 2008; Vymazal, 2010a; Sehar et al., 2013). The main parameters affecting the reduction of TSS in vertical-flow constructed wetlands are the hydraulic and microbiological characteristics of the media, and this has been confirmed by many researchers (Kadlec and Wallace, 2008; Mburu et al., 2008; Garcia et al., 2010). However, total suspended solids are reduced primarily by physical settling and filtration (Karathanasis et al., 2003; Mburu et al., 2008). Manios et al. (2003) stated that the ability of a gravel bed to remove total suspended solids is better than any other substrate bed (soil, sand and other compost) due to its physical and chemical structure. The other substrate materials mentioned are compactable, and the applied pressure reduces the large porosity of these substrates, while gravel is less compactible. The presence of plants has a positive effect on reduction of the total suspended solids by increasing retention time (Karathanasis et al., 2003; Kadlec and Wallace, 2008).

Some researchers have stated that the main problem linked to physical processing to reduce total suspended solids is clogging (Manios et al., 2003; Rousseau et al., 2004a; Caselles-Osorio et al., 2007). Xu et al. (2013) studied the effect of the combination of two out of three possible types of loading (hydraulic, organic load and total suspended solids) in different rate (high, medium and low) on clogging in vertical-flow constructed wetlands with a soil media. The results showed that the two loading combinations of high hydraulic and high organic, and high hydraulic and high suspended solids, caused the most clogging. Ye et al. (2008) stated that the main reason for clogging is the accumulation of non-filter materials; their results showed that the clogging occurred when the accumulation concentration of this non-filter materials was more than 18.23 mg/l. This problem can be solved by using a backwashing treatment (Ma et al., 2010), hydrogen peroxide treatment (H_2O_2) (Nivala and Rousseau, 2009) or by using the anaerobic digesters method (De la Varga et al., 2013).

Vertical constructed wetlands are highly efficient in total suspended solids reduction (Tietz et al., 2007; Kadlec and Wallace, 2008), many publications have shown a high reduction percentage value for total suspended solids (Gikas et al., 2007, 93.2%; Gross et al., 2007, 98%; Kadlec and Wallace, 2008, 87%; Paing et al., 2015, 85%).

In the case of textile wastewater treated by constructed wetlands, Bulc and Ojtrsek (2008) used a hybrid system consisting of vertical-flow followed by horizontal-flow constructed wetlands to treat RB5[**], DY211 and VY46 dyes. The results showed that the reduction percentage rate for TSS was 93%. Hussein and Scholz (2017) stated that TSS reduction depends on the type of azo dye. They studied treatment of two azo textile dyes wastewaters (AB113 and BR46) by vertical-flow constructed wetlands. They stated, in the case of BR46, the reduction percentage rate for TSS was 86%, while there was an increase in TSS reduction percentage rate, in the case of AB113 of around 0.07%. The TSS in the influent was 365 mg/l while in the effluent it was 392 m/l.

2.5.2 Organic Matter Reduction Mechanism

In general, organic matter refers to any compounds that contain carbon formed by living organisms. In wastewater effluent, the organic composition is approximately 50% protein, 40% carbohydrate and 10% oil, fat, and other pollutants (Shon et al., 2007). Any increase in organic matter will lead to an increase the decomposers and these decomposers will grow rapidly consuming a great amount of oxygen (Naushad and AL-Othman, 2012). A lack of oxygen causes aquatic life in the receiving stream to be destroyed. The release of organic matter should be reduced to protect the life of downstream users (Ellis, 2004). In constructed wetlands, the reduction of organic matter is achieved through filtration, adsorption, and aerobic, anaerobic and microbial metabolism (Cooper et al., 1996; Vymazal et al., 1998b; Stefanakis et al., 2014). This reduction in organic matter can be assessed by measuring the COD and BOD concentrations (Quayle et al., 2009; Lee and Nikraz, 2014).

The accumulation of organic matter is very important in constructed wetlands. It serves two functions for the micro-organisms, providing energy for micro-organisms to grow and supplying carbon for the long term (Kröpfelová, 2008; Magdoff and van Es, 2010; H Wu et al., 2015). Supplying carbon helps in the denitrification process (nitrogen reduction) and this has been confirmed by many researchers (Van, 1995; Kallner, 2006; Reddy and DeLaune, 2008; Songliu et al., 2009). On the other hand, an increase in the accumulated organic matter plays a significant role in causing clogging (Lianfang et al., 2009; Hua et al., 2010; Fu et al., 2013).

In textile wastewater, chemical oxygen demand is an important index to measure the organic pollution in the wastewater. It is defined as the amount of oxygen equivalent that would be required to oxidize the organic materials in the wastewater (Ding et al., 2005; Chai et al., 2006; Latif and Dickert, 2015). Many researchers have investigated COD concentration in textile wastewater treated by subsurface flow constructed wetlands. Davies et al. (2005), Ojstršek et al. (2007), Ong et al. (2011) and Yalcuk and Dogdu (2014) used vertical-flow constructed wetlands to treat different azo textile dyes; COD reduction percentage rates were 64, 88, 95 and 64%, respectively. Real textile wastewater was treated by horizontal-flow constructed wetlands in Tanzania (Mbuligwe, 2005). The results showed that the COD reduction percentage rate was 72%. Bulc and Ojtrsek (2008) used a hybrid system to treat a mixture of three different dyes. The hybrid system consisted of vertical-flow followed by horizontal-flow constructed wetlands; the COD reduction percentage rate was 84%. Hussein and Scholz (2017) treated two different azo textile dyes (AB113 and BR46) by vertical-flow constructed wetlands. The results showed that COD reduction percentage rates for AB113 and BR46 reached 55% and 89%, respectively.

2.5.3 Nutrients Reduction Mechanism

The presence of nitrogen and phosphorus in wastewater causes eutrophication in natural water and that will lead to lack of oxygen (Sathasivan, 2009; Mazumder, 2013; Yamashita and Yamamoto, 2014). In constructed wetlands, phosphorus occurs as organic and inorganic phosphate (Heal et al., 2005; Vymazal, 2007). Most phosphorus converts to orthophosphate as a result of biological oxidation (Cooper et al., 1996). Vymazal (2007) demonstrated that the reduction mechanisms of phosphorus in constructed wetlands are: fragmentation, desorption, dissolution, leaching, plant and microbial uptake, mineralization, adsorption, precipitation, burial and sedimentation. However, the major reduction mechanisms take place through adsorption, precipitation, and plant and microbial uptake (Richardson and Craft, 1993; Cooper and Findlater, 2013). This means the best reduction mechanism for phosphorus is a combination of physical, chemical and biological treatments (Mazumder, 2013). Orthophosphate promptly amasses in wetland vegetation and media, therefore promoting natural take-up and substance bonding. Kayombo et al. (2004) pointed out that storing and/or reduction of phosphorus is dependent on the type of wetland and its operational mode. Phosphorus can leave the wetland by coming from the media through the water column, if the anaerobic condition is not reversed due to the lack of oxygen demand (Reddy et al., 1999). The filter media of constructed wetlands plays a major role in the reduction of phosphorus (Karczmarczyk, 2004; Seo et al., 2005), and its capacity to reduce the phosphorus depends on the metal content of the wastewater (Brix et al., 2001; Vohla et al., 2011), and the value of pH as well (Cui et al., 2008) through adsorption and precipitation mechanisms. At pH>6, the reactions are a combination of physical adsorption to Fe and Al oxides, and precipitation as calcium phosphates. At pH<6, precipitation as Fe and Al phosphates becomes more important (Priya and Urmila, 2013). These mechanisms might cause clogging in constructed wetlands (Knowles et al., 2011) especially when the wastewater contains a large amount of industry materials in high concentrations (Fleming et al., 1999; Kadlec and Wallace, 2008). Lantzke et al. (1999) conducted a study regarding the percentage reduction of orthophosphate from wastewater by planted vertical-flow constructed wetlands. The results showed the percentage reduction for the plant, gravel and biofilms were 70%, 20% and 10%, respectively, in the long-term.

In general, the main reduction mechanisms for nitrogen are microbial nitrification and denitrification (Tanner et al., 1995; Obarska and Gajewska, 2003), plant uptake (Koottatep and Polprasert, 1997) and accumulation in soil (Lee et al., 2009), as shown in Figure 2.8. Nitrification is the process of oxidizing ammonium or ammonia to nitrite followed by the oxidation of the nitrite to nitrate (Kessel et al., 2015). Denitrification is the process of reducing nitrate to gaseous nitrogen (Schaechter, 2009). Nitrogen enters the wetland as a dissolved organic and/or inorganic form (Sumner, 1999). The relative proportions of the nitrogen composition depend on the source of the wastewater (Vymazal and Kröpfelová, 2009a). Nitrite, nitrate, ammonium and ammonia are the major forms of inorganic nitrogen (Likens, 2010), while amines, urea, amino acids and purine are the forms of organic nitrogen (Bastian and Hammer, 1993; Kadlec and Wallace, 2008). Several publications have reported other processes to reduce nitrogen in constructed wetlands such as ammonification, nitrogen fixation, ammonia volatilization, and anammox and ammonia adsorption (Vymazal, 2007; Mustafa, 2010; Coban et al., 2015). However, the main mechanism to eliminate nitrogen is the combination of nitrification and denitrification (Green, 1996; Lee et al., 2009; Scholz, 2011).

Nitrification and denitrification mechanisms need both aerobic and anaerobic conditions. The nitrification process needs anaerobic conditions to oxidize ammonia and/or ammonium to nitrite as a first step, and aerobic conditions to oxidize nitrite to nitrate as a second step (Kadlec and Knight, 1996; Kyambadde, 2005). Denitrification needs aerobic/anaerobic conditions to reduce nitrogen compounds (nitrate or nitrite) to gaseous nitrogen (Ji et al., 2014; Winkler et al., 2015), and this process needs a carbon source which can be provided either from the decaying plant detritus and/or as COD present in the incoming wastewater (Kallner, 2006; Cakmak and Apaydin, 2010). Hu Jie et al. (2009) studied the effect of the C/N ratio (C, organic carbon; N, inorganic nitrogen) on both the nitrification and denitrification processes. The results showed that increasing the organic carbon leads to a decrease in the nitrification rate, while denitrification was detected after increasing the organic carbon. These results were also confirmed by Ding et al. (2012).

A change in temperature has an effect on the rate of nitrogen reduction. Many publications point out the rate of reduction decreases in the winter period (Kadlec and Reddy, 2001; Kuschk et al., 2003; Mietto et al., 2015). USEPA (2000a) stated that a temperature of >12.9 ˚C is better for both nitrification and denitrification when compared to <12.9 ˚C. Chapanova et al. (2007) pointed out that the reduction rate of ammonia-nitrogen at 5 ˚C is low when compared to that at temperatures between 15-20 ˚C. This means that temperature is an important factor in designing constructed wetlands. Vymazal (2007) stated that the effect of temperature is greater on nitrification than on denitrification. For developing countries (especially when the degree of temperature is over 40 C˚, will be effect on the life of the plants and that depend on them growth cycle and that need to be consult with ecologists.

In the case of textile wastewater treated by constructed wetlands, Bulc and Ojtrsek (2008) found that the reduction percentage rates for TN, $N_{organic}$ and NH_4-N were 52%, 87% and -331% when they treated dyes (RB5, DY211 and VY46) by a hybrid system. This system consisted of vertical-flow followed by horizontal-flow constructed wetlands. Ong et al. (2011) used vertical-flow constructed wetlands to treat dye AO7. The results demonstrated that the reduction percentage rate for NH_4-N was 86%. Yalcuk and Dogdu (2014) studied treatment of azo dye (AY 2G E107) by vertical-flow constructed wetlands. The results showed that the reduction percentage rates for PO_4-P and NH_4-N were 94% and 77%, respectively. Furthermore, Hussein and Scholz (2017) treated textile wastewater of two azo textile dyes (AB113 and BR46) by vertical-flow constructed wetlands. The results stated that the reduction percentage rates of PO_4-P, NH_4-N and NO_3-N for AB113 were 78%, 53% and 73%, respectively and 81%, 70% and 75% in the case of BR46.

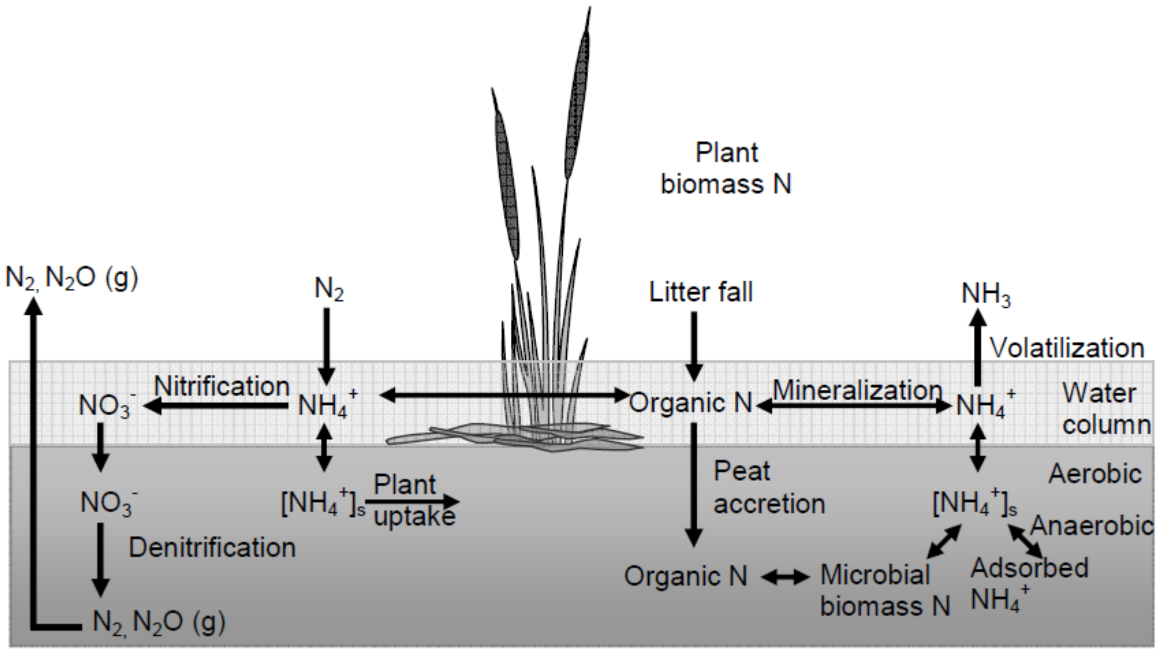


Figure 2.8 Schematic diagram showing the main nitrogen reduction mechanisms (Mustafa, 2010; adapted from Reddy and DeLaune, 2008).

Songliu et al. (2009) investigated the ability of vertical-flow constructed wetlands with different plants to remove nitrate. The constructed wetlands consisted of three parallel wetlands built with concrete. The media was slag at the bottom in a 50 cm layer and 10 cm of local soil above the slag layer. The wetlands included a layer of river gravel with a 30 cm depth and 10-20 mm diameter, as a top layer. The wetlands were planted with three different plants. These constructed wetlands were operated during February 2006 to December 2006 in China. The main conclusion for this study was that the constructed wetland cannot provide enough organic carbon sources to remove the nitrate when it is rich in the effluent and there is a poor organic chemistry contamination in the effluent. The temperature and pH were also investigated in this study and the results showed a strong ability of the constructed wetland to maintain a suitable pH for denitrification, and that the temperature may affect the nitrate reduction in winter.

2.5.4 Heavy Metals Reduction Mechanism

Heavy metals refer to any metallic elements which have a relatively high density compared to water (Fergusson, 1990). Järup (2003) stated that the specific density of heavy metals is more than 5 g/cm^3, for example, cadmium (Cd), mercury (Hg), arsenic (As), thallium (Tl) and lead (Pb) (Martin, 2011). Many publications have stated that most heavy metals ions are known to be carcinogenic or toxic (Singh et al., 2007; Nagajyoti et al., 2010; Fu and Wang, 2011; Lakherwal, 2014), are not biodegradable and are accumulated by living organisms (Gadd, 1990; Barakat, 2011). Serious health problems for humans are caused by heavy metals, such as skin irritation, anaemia, cirrhosis and stomach cramps (Babel and Kurniawan, 2004; Paulino et al., 2006; Oyaro et al., 2007). Therefore, the reduction of heavy metals is very necessary.

In constructed wetlands, there are a number of physical, chemicals and biological processes which reduce heavy metals including settling, sedimentation and adsorption as physical processes; sorption, adsorption, oxidation, precipitation and co-precipitation, metal carbonates and metal sulphides as chemical processes; and microbial and plant uptake as biological processes (Patrick and Verloo, 1998; Cheng et al., 2002; Kosolapov et al., 2004; Sheoran and Sheoran, 2006; Marchand et al., 2010; Sukumaran, 2013; Arivoli et al., 2015). In settling and sedimentation processes, heavy metals associated with particulate matter significantly reduced (Ellis et al., 2003a; Sheoran and Sheoran, 2006) because of dynamic transformation, which occurs in wetland systems whether the water is static or mobile (Johnston, 1993). Heavy metals can be transported from the inflow wastewater to wetland media or microbes (Dunbabin and Bowmer, 1992; Matagi et al., 1998). Sheoran and Sheoran (2006) point out that the sedimentation process is a principle process for the reduction of heavy metals. Sinicrope et al. (1992) founded that a higher reduction rate occurs by plant uptake as a result of the sedimentation process. Walker and Hurl (2002) pointed out that the occurrence of the sedimentation process after another process, like precipitation, leads to the production of larger particles, which act as a trap for heavy metals. Patrick and Verloo (1998) and Lesage (2006) reported that heavy metals can be reduced by physical adsorption (electrostatic attraction). Evangelou (1998) demonstrated that the reduction of heavy metals by chemisorption is better than that by physical adsorption.

Ones of the most important chemical mechanisms of heavy metals reduction is sorption (Sheoran and Sheoran, 2006). This mechanism includes a group of processes: precipitation and adsorption (Marchand et al., 2010). It consists of a transfer of ions from solution phase (inflow wastewater) to solid phase (wetland substance) (Crini and Badot, 2010). The adsorption of heavy metals depends on the type of element, its concentration and media condition (Matagi et al., 1998; Walker and Hurl, 2002; Zeb et al., 2013). For instance, most trace metals such as Pb, Cd, Ni, Zn and Cu exist as cations. The capacity for retention of them depends on the type of wetland media (Sheoran and Sheoran, 2006). Mengzhi et al. (2009) studied the effect of two types of media, coke and gravel, on the reduction rate of three heavy metals (Cu, Pb and Zn). The results showed different absorption efficiencies. Sahu (2014) investigated the effect of different retention times (1-8 days) on the reduction of Cr, Ni, Fe and Hg. the reduction rates increased with increasing retention time. Some authors reported the reduction of heavy metals occurs by the co-precipitation mechanism and cation exchange capacity (Sharma, 2014). For instance, iron can act as a co-precipitation agent for As (Stottmeister et al., 2006). Hedin et al. (1994) and Noller et al. (1994) pointed out that heavy metals co-precipitate with secondary minerals in wetlands. Metals such as Cu, Ni, Zn, and Mn co-precipitated in iron oxides and Co, Fe, Ni and Zn co-precipitated in manganese oxides, as reported by Tumelo Seadira et al. (2014). Hallberg and Johnson (2005) reported that the soluble Mn from AMD can be removed via oxidation and precipitation mechanisms.

Heavy metals reduction by microbial and plant uptake is known as a biological mechanism and it’s the most important pathway to reduce heavy metals (Sheoran and Sheoran, 2006; Rai, 2008; Yeh, 2008; Liu et al., 2015). The reduction of heavy metals in constructed wetlands by plant uptake depends on the type of plant itself, concentration of the heavy metals, pH of sedimentation, temperature, sediment chemical properties and organic matter content (Gambrell, 1994; Siedlecka, 1995; Greaney, 2005; Garcia et al., 2010). For instance, Lim et al. (2001) found there is no significant effect of the plant *Typha angustifolia* on reduction of Cu, Zn, Cd and Pb. Allende et al. (2014) pointed out that the reduction rate of heavy metals was <3% by plant uptake, while >85% by the wetland media. On the other hand, Sinicrope et al. (1992) found a significant effect of the plant *Scirpus lacustris* on the reduction of Cd and Zn. The results showed approximately 13% and 35% as reduction percentages of Zn and Cd, respectively, accumulated in the fine root of the plant. Cooper and Findlater (2013) demonstrated that the higher concentration of heavy metals will affect plant growth. For instance, Wong and Bradshaw (1982) found the average concentrations of 1.9 mg/l for Zn and 1.6 mg/l for Pb would undoubtedly affect plant growth. J Li et al. (2015) indicated that a slightly acidic inflow, with pH= 5.6-6.5, helps the plant to uptake heavy metals, while in the case of an alkaline inflow, pH= 7.5-8.5, the plants hardly uptake any heavy metals. Although the reduction of heavy metals by plant uptake is small, according to some authors, it has an indirect effect on the reduction. The roots of plants provide an environmental culture for the growing micro-organisms (rhizosphere) (Williams, 2002). Also, Dunbabin and Bowmer (1992) found the exudates of roots help to increase bacteria activity, give more space to buffer capacity and keep pH at a neutral level.

2.5.5 Azo Dye Reduction Mechanism

Numerous dyes containing azo compounds are known as azo dyes (Zollinger, 2003; Podczeck and Jones, 2004; Hatice, 2010). Azo compounds are defined as any organic chemical compounds that have the general formula (R-N=N-R’), where (N=N) is a nitrogen atom, R’ and R, are either alkyl (aliphatic) or aryl (aromatic) (Arcos et al., 2013; Castro et al., 2016; Ekambaram, 2016).

Azo dye produces colour in dye pollutant water, and this colour blocks sunlight, which is detrimental to many photo-initiated chemical reactions necessary for aquatic life of water bodies (Pereira and Alves 2012; Yadav et al., 2012). Azo dye decolourization is achieved under aerobic, anaerobic or anoxic conditions (O’neill et al., 2000; Sponza and Işik, 2002; Van der Zee, 2002; Davies et al., 2006). Hatice (2010) stated that there is no correlation between decolourization rate and molecular weight but the rate is dependent on the dye structure and the added organic carbon source. At anaerobic conditions, azo bond (N=N) cleaves and this process releases aromatic amine, which resists any further anaerobic treatment (Brown and Hamburger, 1987; Chung and Stevens, 1993). Aromatic amine can be reduced under aerobic treatment (Weber and Lee Wolfe, 1987; Pinheiro et al., 2004; Ong et al., 2011), while azo dye colour reduction can be achieved under aerobic, anoxic or anaerobic conditions (Pandey et al., 2007). In constructed wetlands, many publications have stated a high colour reduction under anaerobic treatment (Mbuligwe, 2005; Bulc and Ojtrsek, 2008; Ong et al., 2009; Ong et al., 2010).

The biodegradation mechanism works to achieve decolourization of azo dyes under all three conditions (Kodam et al., 2005; Moosvi et al., 2005; Pandey et al., 2007). Under anaerobic conditions, micro-organisms require external carbon as an energy source to degrade azo dye (Carliell et al., 1994; Şen and Demirer, 2003; Haroun and Idris, 2009). Under aerobic conditions, very few micro-organisms (aerobic bacteria, for example: *P. aeruginosa*) are able to grow depending on azo groups as a sole carbon source (Yang et al., 1998; Edwards, 2000; Pandey et al., 2007). These bacteria cleave the nitrogen atom to release the amines as a source of carbon and an energy to help them to grow. Under anoxic conditions, other types of bacteria, *Pseudomonas luteola* as an example, can decolourize azo dye (Zissi and Lyberatos, 1996; Yu et al., 2001; Grekova-Vasileva et al., 2009). These bacteria need a complex organic carbon source to reduce azo dyes (Khehra et al., 2005b). Pandey et al. (2007) stated that the reduction of azo dye is achieved under anaerobic condition only, although many micro-organisms (bacteria) can be grown aerobically under anoxic conditions.

Aromatic amines are released as a result of decolourization of azo dyes under anaerobic conditions. The reduction of azo dyes under aerobic biodegradation by some groups of micro-organisms (bacteria) is more easily achieved (Ekici et al., 2001;Arora, 2015), in the presence of oxygen (Stolz et al., 1992; Jothimani et al., 2003). In spite of this, the reduction of a few simple types of aromatic amines (for example, 5-aminosalicylic acid) has been reported under methanogenic conditions (Kalyuzhnyi et al., 2000; Jothimani et al., 2003; Yemashova et al., 2004). In wetlands, this type of bacteria are known as methanogens and are very common in natural wetlands (Walter and Heimann, 2000; Jerman et al., 2009) as well as in constructed wetlands (Tanner et al., 1997; Inamori et al., 2007; Vymazal and Kröpfelová, 2009b).

As a textile wastewater treatment in the USA, Pervez et al. (1999) studied the colour reduction for two azo textile dyes (AB113 and RB171) by vertical-flow constructed wetland with gravel and sand as media, and planted with *Phragmites australis*. The results showed that the colour reduction rate was 98%. In Portugal, Davies et al. (2005) worked on treatment of an azo textile dye (AO7) by vertical-flow constructed wetland with gravel and sandy clay soil as media, and planted with *Phragmites australis*. The results showed that the colour reduction was 74%. In Tanzania, Mbuligwe (2005) achieved a 77% colour reduction when he treated real textile wastewater by horizontal-flow constructed wetland with gravel and sand as media, and planted with *Typha* and *cocoyam*. Furthermore, in Slovenia, Bulk and Ojtrsek (2008) used a hybrid system to treat three azo textile dyes (RB5, DY211 and VY46). The hybrid system consisted of vertical-flow followed by horizontal-flow constructed wetlands with gravel, sand and tuff as media. The results showed that the colour reduction achieved was 90%.

A survey regarding previous related works studying the reduction of various types of azo dyes is presented in Table 2.2. The survey is categorized according to the dye used, types and design characteristics of wetlands, plant used, reduction performance, duration of experiment and its location and references.

Table 2.2 Some related studies (listed in order of date) on azo textile dye wastewater treatment by constructed wetlands.

Dye used	Type of wetland	Design characteristics	Plants used	Reduction performance	Duration (days)	Country of operation	References
AB113, RB171*	VF	Gravel-sand	*P. australis*	98% colour	70	USA	Pervez et al. (2000)
AO7	VF	Gravel-sandy clay soil	*P. australis*	74% colour, 64% COD and 71% TOC	77	Portugal	Davies et al. (2005)
Various dyes in real wastewater	HF	Gravel-sand	*Typha* and *cocoyam*	77% colour, 72% COD and 59% sulphate	84	Tanzania	Mbuligwe (2005)
AO7	VF	Gravel-sandy clay soil	*P. australis*	99% colour, 93% COD and TOC	48	Portugal	Davies et al. (2006)
RB5**, DY211, VY46	VF-HF	Gravel-sand-tuff	*P. australis*	90% colour, 84% COD, 93% TSS, 52% T-N, 87% $N_{organic}$, -331% NH_4-N, 88% sulphate, 80% anion surfactant and 93% TSS	60	Slovenia	Bulc and Ojstršek (2006)
BB41	VF	Sand	*M. spicatum/C. demersum*	94-96% dye	50	Turkey	Keskinkan and Lugal Göksu (2007)
RR22, VR13, RB5**	VF	Gravel-Sand-Zeolite-Peat	Without plant	70% dye, 60% EC, 88% COD and TOC	90	Slovenia	Ojstršek et al. (2007)
AO7	VF	Gravel	*P. australis*	68% dye, 69% COD, 67% TOC	8	Portugal	Davies et al. (2009)
RR141	VF	Gravel-sand	*Typha*	49% colour, 60% COD, 86% TDS	N/A	Thailand	Nilratnisakorn et al., (2009)
AO7	UF	Glass beads-gravel	*P. australis/ Manchurian wild rice*	98% dye, 86% COD, 96 NH_4-N, 86% NO_3-N, 67% T-N, 26% T-P	42	Japan	Ong et al. (2009)

Table 2.2 continued

AO7	UF	Gravel-glass beads	*P. australis*	98% dye, 90% COD, 67% T-N, 28% T-P, 98% NH_4-N, 100 NO_3-N	365[(a)]	Japan	Ong et al. (2010)
AO7	VF	Gravel-sludge	*P. australis*	94% colour, 95% COD and 86% NH_4-N	27	n/a	Ong et al. (2011)
	FWS-SSF SSF-FWS	Shale	*P. australis*	98% COD, 97%, colour 91% COD, 99% colour	N/A	Thailand	Cumnan and Yimrattanabovorn, (2012)
Various dyes in real textile wastewater	VF	Coconut shavings-Soil with bacteria	*G. pulchella*	70% COD, 74% TOC, 70 BOD	3 and 4	India	Kabra et al. (2013)
Various dyes in real textile wastewater	VF	Coconut shavings-sand - gravel-soil with bacteria	*Portulaca grandifora*	59% COD, 38% BOD, 37% TOC, 41% turbidity, 71% TDS, 60% TSS	3	India	Khandare et al. (2013)
Various dyes in real textile wastewater	VF	Coconut shavings-gravel-sand-soil	*Typha*	79% COD, 77% BOD, 59% TDS, 27% TSS	3	Pakistan	Shehzadi et al. (2014)
AY 2G E107	VF	Gravel-sand-zeolite	*Canna* and *Typha*	95% colour, 64% COD, 94% PO_4-P and 77% NH_4-N	90	Turkey	Yalcuk and Dogdu (2014)

Note: AB, acid blue; RB*, reactive blue; VF; vertical flow; AO, acid orange; COD; chemical oxygen demand; TOC, total organic carbon; HF; horizontal flow; RB**, reactive black; DY, disperse yellow; VY, vat yellow; TSS, total suspended solid; T-N, total nitrogen; N, nitrogen; NH_4-N, ammonium nitrogen; BB; basic blue, RR; reactive red; VR, vat red; EC, electrical conductivity; UF, upper flow; NO_3-N; nitrate nitrogen; TDS; total dissolved solids; FWS; free water surface; SSF; subsurface flow; BOD; biochemical oxygen demand; AY; acid yellow; PO_4-P, Ortho-phosphate-phosphorus; (a) the experimental work was under controlled conditions (indoor); N/A, not applicable.

2.6 Choice of Vertical-Flow Constructed Wetlands over Horizontal-Flow

The use of vertical and horizontal-flow constructed wetlands as a substitutional means of treating wastewater is widely accepted (Haberl et al., 1995; Brix et al., 2001; Bezbaruah and Zhang, 2003; Abou-Elela and Hellal, 2012; Abou-Elela et al., 2013; Fan et al., 2013b; C Li et al., 2015; Sani, 2015) due to the advantages of their use, such as low cost of set-up and operation, ability to tolerate fluctuations in flow, and simplicity of operation and maintenance (Luise et al., 1993; Scholz and Hedmark, 2010). Aslam et al. (2010) concluded that constructed wetlands can be used as an economical option to treat wastewater to help developing countries. Konnerup et al. (2011) stated that vertical-flow and horizontal-flow constructed wetlands have a good ability to improve the quality of the river water, in Vietnam, which is polluted due to aquaculture practices. On the other hand, the vertical-flow constructed wetlands are better than horizontal-flow ones in treatment of wastewater in some water quality variables, according to the results of some research.

2.6.1 Nitrification and Denitrification

Nitrification is the biological oxidation of ammonium-nitrogen (NH_4-N) to nitrite-nitrogen (NO_2-N) and then oxidation of the nitrite-nitrogen (NO_2-N) to nitrate-nitrogen (NO_3-N) by two groups of micro-organisms (Schmidt et al., 2001; Paul, 2006). VFCWs provide excellent conditions for nitrification because their ability to provide oxygen transfer capacity is greater than that of HFCWs (Vymazal, 2002; Vymazal, 2005; Saeed and Sun, 2012; Shi et al., 2012; Paing et al., 2015; Butterworth et al., 2016).

Denitrification is the biological process of production of gaseous nitrogen (N_2) by microbial reduction of nitrite-nitrogen (NO_2-N) (Tiedje, 1982). Although some researchers have been less concerned about nitrate reduction, and denitrification is usually low in VFCWs (Vymazal, 2005; Fuchs, 2009), other researchers have recently demonstrated that VFCWs can denitrify well with some modifications. For example, recycling the treated effluents enhanced the ability of VFCWs to remove the total nitrogen (TN) (Platzer, 1999; Marti et al., 2003; Arias et al., 2005; Gross et al., 2007; Prost-Boucle and Molle, 2012). Lavrova and Koumanova (2014), and Shen et al. (2015) used a solid carbon source (methanol and starch blends, respectively) to achieve a higher denitrification.

2.6.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand is an important index to measure the organic pollution in wastewater. It is defined as the amount of oxygen equivalents required to oxidize the organic materials in the wastewater (Latif and Dickert, 2015). Many researchers have stated that vertical-flow constructed wetland systems perform satisfactorily in the reduction of chemical oxygen demand (COD) (Brix and Arias, 2005; Prochaska et al., 2007; Sharma and Brighu, 2014; Abdelhakeem et al., 2015; Alisawi et al., 2015a).

2.6.3 Heavy Metals

The expression “heavy metals” refers to any metal elements that have a relatively high density compared to water and are toxic or poisonous (Fergusson, 1990), for example Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) and Zink (Zn) (Salomons et al., 2012). Yalcuk and Ugurlu (2009) showed that the ability of vertical-flow constructed wetlands to remove heavy metals found in the effluent of leachate is better than that of horizontal-flow constructed wetlands. Arivoli et al. (2015) used a vertical-flow system to treat heavy metals from the effluent of pulp and paper industry. Their results indicated higher reduction efficiencies for iron, copper, manganese, nickel, zinc and cadmium.

2.6.4 Area Demand

The vertical-flow constructed wetlands system is the state of art design technologies used in wastewater treatment and its use is increasing rapidly (Stefanakis et al., 2014; Scholz, 2015) due to the advantage of its lower area requirement compared to the horizontal-flow constructed wetlands system (Vymazal et al., 1998a; Luederitz et al., 2001; Scholz, 2011 ; Saeed and Sun, 2012; Stefanakis et al., 2014). The required area for VFCW systems varies from 0.4-2 (m^2/PE), while in the case of HFCW systems the required area is from 3-10 (m^2/PE) as shown in Table 2.3 (Cooper and Green, 1995; Vymazal et al., 1998b).

Table 2.3 Examples of area requirements (listed in order of alphabet letters)

Country	Required Area (m^2/PE)		Reference
	VFCWs	HFCWs	
Austria	1.7		(Hoffmann et al., 2011)
Canary Islands	1.5		(Vera et al., 2013)
Czech Republic		4.1-4.5	(Vymazal, 1996)
Czech Republic	1-1.5		(Vymazal and Kröpfelová, 2011)
Germany	1.6,1.7		(Olsson, 2011; Hoffmann et al., 2011)
Greece	1-1.5		(Stefanakis and Tsihrintzis, 2012)
India	1.2	3	(Hoffmann et al., 2011)
Philippines	0.9		(Hoffmann et al., 2011)
Syria	0.4		(Hoffmann et al., 2011)
United Arab Emirates	1.9		(Hoffmann et al., 2011)
United Kingdom		5.6	(Cooper, 2001)
United Kingdom	2		(Cooper, 2005)
United Kingdom	1-2		(Weedon, 2010)

Note: PE, Person Equivalent= 60g BOD/capita/day

2.7 Amine Compounds

2.7.1 Overview

An aromatic amine is an amine in the molecule of which there are one or more aromatic rings directly on the nitrogen atom (Gunawardena, 2015) as shown in Figure 2.9.

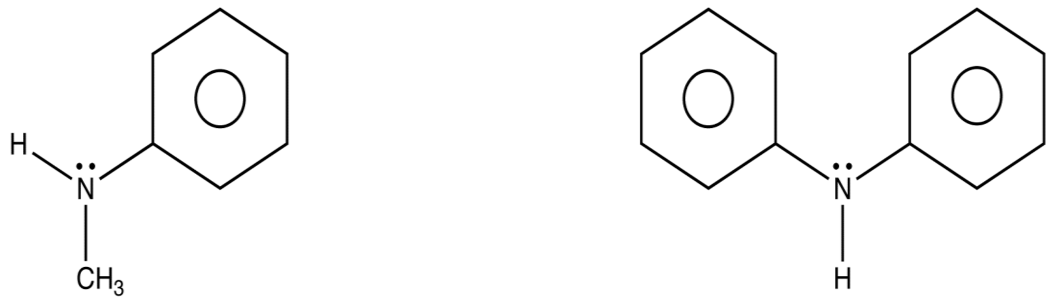


Figure 2.9 Aromatic amine rings

Dyes are the one of major sources of aromatic amines in the environment, (Robinson et al., 2001, Snyderwine et al., 2002 and Pinheiro et al., 2004). In addition, human blood cancer and the other cancers continue to be linked with the present day level of aromatic amines (O'neill et al., 2000; Pielesz et al., 2002; Snyderwine et al., 2002) and they are toxic to aquatic life (Pinheiro et al., 2004; Kirandeep et al., 2015). Each dye has one or more types aromatic amines (Pielesz et al., 2002; Pinheiro et al., 2004). Wetlands can degrade aromatic amines under aerobic conditions (Mbuligwe, 2005; Ong et al., 2010; Ong et al., 2011).

In this study, the two azo textile dyes selected, AB113 and BR46, are toxic to aquatic life (Pinheiro et al., 2004; Mohan et al., 2007; Kirandeep et al., 2015) and carcinogenic (Chung, 2000; Gadaleta et al., 2016). Three types of amines were released as a result of dye AB113 degradation, 3-AminoBenzeneSulfonic Acid (ABSA), 1,4-

DiAminoNaphthalene (DAN) and 5-Amino-8-(phenylamino) Naphthalene-1-Sulfonic Acid (ANSA) (Senthilvelan et al., 2014). The wavelength for each being 288 nm, 255 nm and 225 nm, respectively (Koepernik and Borsdorf, 1983; Paul et al., 1990). In the case of BR46, two aromatic amines were released as a results of its degradation, *N*-benzyi-*N*-mehtylaniline (NBNMA) and *N*-benzyl-*N*-methylbenzene-1,4-diamine (NBNMD), with the wavelength of maximum absorbance for each being 254 nm and 290 nm, respectively (Fihtengolts, 1969; Küçükgüzel et al., 1999).

2.7.2 Classification and Structure of Amines

Amines are classified into three categories (primary, secondary and tertiary) in which one, two or three hydrogen of NH_3 are replaced by aryl or alkyl groups (Bacon and Adams, 1968; Murry and John, 1992; Dobo et al., 2006; Sankar and Nasar 2009).

2.7.2.1 Primary Amines

Primary amines are compounds of amine, which have one group attached to the nitrogen atom as shown in Figure 2.10.

$$\mathbf{CH_3 - \ddot{N}H_2}$$

Figure 2.10 Methylamine (Primary Amines) chemical structure

2.7.2.2 Secondary Amines

Secondary amines are compounds of amine, which have two groups attached to the nitrogen atom as shown in Figure 2.11.

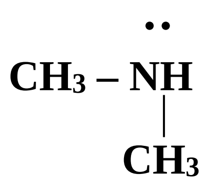


Figure 2.11 Dimethylamine (Secondary Amines) chemical structure

2.7.2.3 Tertiary Amines

Tertiary amines are compounds of amine, which have three groups attached to the nitrogen atom as shown in Figure 2.12.

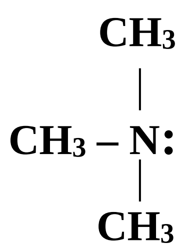


Figure 2.12 Trimethylamine (Tertiary Amines) chemical structure

Amines can be further divided as aliphatic or aromatic: aliphatic, when the nitrogen is bonded to one alkyl group only; and aromatic, when the nitrogen is bonded to one or more aryl groups (Vogt and Gerulis, 2000).

2.8 Textile Wastewater Treatment within Vertical-Flow Constructed Wetlands

The use of constructed wetlands in dye wastewater treatment is still at an experimental stage and is not well established (Nawab et al., 2016). Although many researchers have investigated the performance of CWs to treat textile wastewater (dye reduction, COD reduction, phosphorus and nitrogen compounds), the results rarely cover all seasons and also rarely it is found a researchers work on the mix of dyes and investigate the efficiency of constructed wetlands on the reduction of aromatic amine compounds (Table 2.2). for examples, Pervez et al. (1999) used VFCWs, located in the USA, consisting of nine filters: three replicates were planted-up with *Phragmites australis*, three were replicated with air pumped into the substratum and three replicated without plants used as controls to remove two azo textile dyes (RB*171 and AB113) from a synthetic wastewater simulating textile wastewater over a 70-day time period. The media of the wetlands were filled with layers of gravel of various sizes and sand. The percentage reduction was 98% for both dyes. They observed that the majority of the dye (57%) was removed in the initial period (12 h) by adsorption onto charged surfaces in the substratum. By the addition of peat or other suitable organic substratum, the efficiency was substantially enhanced; however, the period for the experimental work was very short and the researchers did not assess the VFCWs performance in reduction of the COD, phosphorus and nitrogen compounds. Furthermore, the researchers did not investigate the ability of the constructed wetlands system to reduce the amines compounds, which were released due to dye degradation.

Davies et al. (2005) selected AO7 to study the peroxidases (POD) activity of *Phragmites australis* in its degradation in a VFCW. They found a crude plant extract with H_2O_2

(which was produced by the plants) to degrade this kind of dye and its aromatic amines. At the concentration of AO7 (130 mg/l), POD activity was found to be 12.9, 4.3 and 2.1-fold for roots, stems and leaves, respectively. POD activity inhibition occurred immediately at an AO7 concentration of 700 mg/l, but 2 days later, it returned to the previous level. The total period was 312 h in an outside environment. Carias et al. (2008) demonstrated the same fact that the enzymes of *Phragmites australis* are involved in the degradation of AO7. Although the results were important to prove that *Phragmites australis* has a good value to use as a plant with VFCWs, 312 h as a time period is not enough to confirm a final result.

Davies et al. (2006) used aerobic degradation of AO7 (127 mg/l concentration) in a VFCW. The media for this wetland consisted of a 10 cm gravel bottom layer and a 77 cm sandy-clay soil top layer. It was planted with *Phragmites sp.* and the duration of this study was 48 days in an outside environment. In this study, the results showed the percentage of colour reduction reached 99% and COD reduction reached 93%. The time period (48 days) was not long enough to evaluate the effect of plants and micro-organisms on pollutant reduction. In addition, the results depended on the aerobic condition only and did not show the ability of CWs, according to this condition, to remove the phosphorus and nitrogen compounds. Furthermore, the researchers did not investigate the ability of the constructed wetlands system to reduce the amines compounds, which were released due to dye degradation.

Keskinkan and Lugal Göksu (2007) used 20 glass aquaria (4 as control wetlands without plants) as vertical-flow constructed wetlands, located in Turkey, to treat dye (BB41; 11 mg/l influent concentration) with different hydraulic retention times (3, 6, 9 and 18 days). River sand was used as a media and to hold the plant (*P. australis/ Manchurian wild rice*) vertically in selected wetlands. The obtained results indicated that the dye reduction percentage rate in HRTs of 9 and 18 days were higher than those at HRTs of 3 and 6 days. Furthermore, they found that the presence of plants plays a major role in dye reduction. The percentage dye reduction rates for control wetland, wetland planted with *M. spicatum* and wetland planted with *C. demersum* were 96%, 94% and 71%, respectively.

Bulc and Ojtrsek (2008) proved that VFCWs offer an optimal solution to treat textile wastewater and meet the environmental legislation. Their VFCW was packed with sand and gravel, and planted with *Phragmites australis*. Three artificially prepared dye-bath wastewaters were examined (Reactive Black 5, Disperse Yellow 211 and Vat Yellow 46). The results showed that the average treatment efficiency of the VFCW was 90% for colour, 84% for COD, 93% for TSS and -331% for NH_4-N at different hydraulic loads. The time period for this study was five months.

Davies et al. (2009) used two pilot-scale VFCWs, located in Portugal, to treat a 700 mg/l dye AO7 during an eight-day period for the experimental work. One of the pilots was planted with *Phragmites australis* and another one was used as a control, with both using gravel as a media. The obtained results as reduction percentage rates for dye, COD and TOC were 68%, 69% and 67%, respectively. Furthermore, their experimental results indicated that the azo dye (AO7) is a xenobiotic that acts as a chemical stressor agent for the plant which was used in their study.

Ong et al. (2009) used five UFCWs with different plants, aeration and non-aeration to assess the treatment of AO7 and nutrients. According to the study, the reduction efficiencies in organic matter, aromatic amines and NH_4-N were enhanced with supplementary aeration. In the aerated case, the percentage reductions for COD and NH_4-

N were 86% and 96% respectively, while in the case of non-aerated, it was 78-82% for the COD and 41-48% for NH_4-N. In addition, it was found that the percentage reduction for AO7 by the gravel was 0.5% by shaking 100 g gravel with 100 ml AO7, 20 mg/l concentration for 2 days as a contact time. Six weeks was the period of this study and the concentration of AO7 was 50 mg/l.

Ong et al. (2010) used an up-flow constructed wetland (two-parallel laboratory scale) to examine and compare the efficiency treatment of wastewater containing Acid Orange (AO7) at different concentrations and different hydraulic retention times (50 and 100 mg/l and 3 and 6 days, respectively) with the option of supplementary aeration. The constructed wetlands were located indoors under a temperature of 23±3 ˚C during the period from October 2007 to September 2009. The size of the wetlands was 70 cm in height and 18 cm in diameter, the media consisted of 5 mm glass beads at the bottom with a depth of 6 cm and gravel (5.7 mm in diameter) on top of the glass. At 30 cm below the bed surface, there were two areas air inlets as a supplementary aeration in the aerated constructed wetland and the *Phragmites australis* was selected as the plant. The results showed that the organic matters, aromatic amine and NH_4-N were removed with the aerated wetland more effectively than with the non-aerated wetland, while the colour and NO_3-N reduction through the non-aerated were better than through the aerated wetland.

Kabra et al. (2013) used four reactors, located in India, as VFCWs, to treat real textile dyes. Every reactor had two units (A and B); unit A was a plastic container of 40 cm length, 20 cm height and 30 cm width. The solution was pumped from unit B (used as a tank for dye solution to be treated) to unit A, then percolated through the media of unit A and drained into unit B through the porous base. The media consisted of coconut shavings as a bottom layer and soil with bacteria as a top layer, and was planted with *G. pulchella*.

A 10-ml sample was collected from all reactors every 2 h during the 72-h total cycle period. The obtained results from this experimental work were 70% for COD, 74% for TOC and 70% for BOD as reduction percentage rates.

Khandare et al. (2013) treated real textile wastewater containing various dyes by vertical-flow constructed wetlands systems, located in India. These systems consisted of four different reactors planted with *Portulaca grandifora* and the media was fine gravel as a bottom layer of 8 cm depth followed by a 2-cm layer of coconut shavings above which another of 5 cm soil with bacteria was placed. The obtained results from this experimental work were 59% COD, 38% BOD, 37% TOC, 41% turbidity, 71% TDS and 60% TSS as reduction percentage rates.

Yalcuk and Dogdu (2014) used a VFCW to treat Acid Yellow 2G E107 Dye-containing wastewater. The constructed wetland consisted of three filter vertical wetlands, one filled with fine gravel, sand and zeolite as a control and the others planted with Cannaidica and Typha Angustifolia, respectively. The initial dye concentration was 259 mg/l, the loading rate was 0.075kg/m^3/day with a flow rate of 1.2 l/day and the retention time was 3.75 days. The period of operation was three months. The results showed that the average colour reduction percentages were 87% for the control and 98% for the other wetlands. For the NH_4-N, the average percentage reductions were 43%, 61% and 46% for the control, *Canna* and *Typha*, respectively. The average percentage reductions of PO_4-P for the control, *Canna* and *Typha* were 84%, 87% and 88% respectively. For COD, the average percentage reductions were 58%, 61% and 64% respectively. The total time of operation was 90 days including the planning and acclimation periods.

Shehzadi et al. (2014) used endophytic bacteria with VFCWs to enhance the textile effluent degradation and plant growth. The constructed wetlands consisted of four different vertical-flow reactors providing different treatments: reactor with tap water and plant, reactor with textile effluent without plant, reactor with textile effluent and plant and the final one with textile effluent and plant, and endophytic bacteria. The plant used was *T. domingensis*. The results showed that the partnership between the plant and the endophyte could be used to enhance the VFCWs ability to degrade textile wastewater.

Nilratnisakorn et al. (2009) used the plant, *Typha angustifolia Linn*, with a VFCW operating from bottom to top, (HRT= 15 days with the flow rate 0.02 m^3/day), in two pilot scale experiments in a greenhouse. The media consisted of washed gravel (particle size 10-50 mm) as a bottom layer and washed soil (particle size 10-30 mm) as a top layer. The obtained results indicated that the VFCWs achieved good reduction for colour, COD and TDS, at 49%, 60% and 86%, respectively.

2.9 Concluding Remarks

The contribution to performance made by plants in the reduction of COD depends on the type of dye itself (basic or acid; basic dye has two azo atoms while acid dye has three azo atoms), and the structure (some dyes have less or more carbon; carbon is very important for the denitrification process and micro-organisms used it as an energy source) and size of the dye molecule. There was no significant ($p>0.05$) effect of plants on dye BR46 reduction. The influent was 6.9±1.33 mg/l and the reduction percentage rate was 96% and 97% for unplanted and planted wetlands, respectively. There was a significant ($p<0.05$) effect on dye reduction in the case of dye AB113; the influent was 6.6±1.82 mg/l and the reduction percentage rates were 71% and 80% for unplanted and planted wetlands, respectively.

3. CHAPTER THREE: MATERIALS AND METHODS

3.1 Overview

This chapter presents a brief description of the system design, construction and analysis. Section 3.2 describes the experimental set-up, while the sub-sections cover wetland design, media composition, dye details and the ingredient composition of the fertiliser used. Section 3.3 presents operational conditions and other processes. In addition, the sub-sections describe operational conditions for the experimental constructed wetland set-up treating two azo textile dyes only and artificial wastewater containing the two azo textile dyes. The limitations of the experimental research and the concluding remarks are presented in sections 3.4 and 3.5, respectively.

3.2 Experimental Set-up

3.2.1 Site Description

This study was conducted between 1 May 2015 and 31 May 2017. The first month may be viewed as the start-up period. The first period (phase one) between 1 June 2015 and 31 May 2016 was for treatment of azo dyes only, while the second period (phase two) between 1 June 2016 and 31 May 2017 was for treatment of artificial wastewater containing two azo dyes. An experimental constructed wetland rig (Figure 3.1) treating textile wastewater, was constructed and operated within a greenhouse located on the top of the Newton Building at Salford University, England, UK. The experimental rig was designed to assess the system performance by simulating processes occurring within full-scale constructed wetlands. The rig comprised of 21 vertical-flow wetland filters, allowing wastewater to drain vertically, enhancing aerobic biodegradation of organic matter and nitrogen (Fuchs, 2009). The rig was operated in the greenhouse under natural environmental conditions to assess seasonal changes. The experimental wetland filters were located randomly in the experimental rig to minimize random impacts of parameters such as sunlight direction and temperature differences on the wetland performance.

Figure 3.1 Experimental vertical-flow wetland rig located in greenhouse, Newton Building (May 2015)

The impact of operational parameters such as contact time, resting time and hydraulic loading rate on dye reduction were assessed. Contact time is defined as the duration for which the wastewater is in contact with the wetland filter content. In comparison, resting time is the duration when the wetland filter is empty (i.e. no wastewater input).

3.2.2 Wetland Design and Media Composition

Round, black plastic drainage pipes were used to construct the vertical-flow wetlands (Figure 3.1). All 21 wetlands were designed according to the following dimensions: height of 100 cm and inner diameter of 10 cm. One pipe was filled with water, another one was filled to a depth of 90 cm with unwashed gravel and the other wetlands were filled to a depth of 90 cm with gravel. Two different layers of gravel were used (as a filter media) varying in diameter. The diameters of the gravel were: large gravel, as a bottom layer to prevent the clogging of the outlet; and pea gravel, located at the top layer. These types of gravel are commonly available and suitable for constructed wetlands (Crites et al., 2014).

The main characteristics for the vertical-flow constructed wetlands system, used in the experimental work, were as follows: the total number of wetlands was 21 filters with eight replicated filters, the weight of plant (*Phragmites australis*) was 125 g for each selected wetland. These plants were bought from a local source (Botanica Plant Nursery, Chantry Farm, Campsea Ashe, Woodbridge, Suffolk). The total height for every filter was 100 cm with total available volume for the influent of 2.8 l. The media consisted of gravel of two different sizes: gravel with diameter 10-20 mm and height 20 cm as a bottom layer to prevent clogging, and pea gravel with diameter 5-10 mm and height 70 cm as a top player. The outlet valves were located at the centre of the bottom plate of each wetland with 1 cm internal diameter vinyl tubing, and were used for the regulation of flow and sampling. Selected wetlands were planted with *Phragmites australis* and replicated to reduce the variability in experimental results and help to increase both generality and realism of the experimental data findings (Fréchette and Schotter, 2015).

3.2.3 Phragmites Australis

The presence of *Phragmites australis* in VFCWs has a significant impact on promotion of sedimentation of suspended solids and prevention of erosion, by decreasing the rate of water flow through increasing the length per surface area of the hydraulic pathways through the wetland (Lee and Scholz, 2007), and on the reduction of organic matters, aromatic amines and NH_4-N (Ong et al., 2011). The growth cycle of *Phragmites australis* traditionally completes between May and September in Britain (Haslam, 1972). Ferreira et al. (2014) detected normal growth for *Phragmites australis* with absence of toxic signs or depletion of leaf nitrogen content, when they used vertical-flow wetlands to treat an effluent comprising Diazo dye (DR81).

When temperatures begin to decrease in the winter period, plants exhibit yellowing as the part above ground dies and is inactivated. It is common practice, in constructed wetland systems, that the above-ground dried plants are cut at the level of 10-15 cm height only (Stefanakis et al., 2014). The rhizomes were bought in mid-April, planted in the selected wetlands and fertilized regularly. Water levels were dropped to encourage deep rooting and penetration of the gravel matrix by the rhizomes (Brix and Arias, 2005).

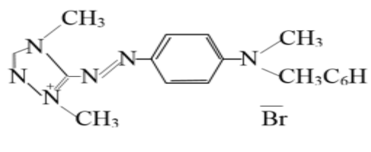
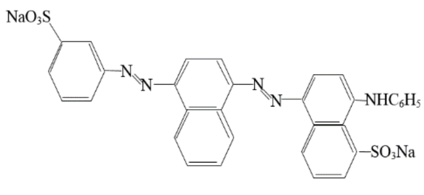
3.2.4 Dye Details

The pollutant features of textile wastewater differ widely among various organic substances, such as dyes, detergents and starches, causing chemical and biological changes which eat up dissolved oxygen from the receiving stream and destroy aquatic life' (Robinson et al., 2002; Deniz and Karaman, 2011; Shirzad-Siboni et al., 2014). Azo dyes AB113 and BR46 were selected as an artificial model because they are commercial dyes and used extensively in the textile industry, (Chung et al., 1992; Riu et al., 1997; Pervez et al., 1999; Olgun and Atar, 2009; Ong et al., 2010; Deniz and Karaman, 2011; Deniz and Saygideger, 2011; Yeddou et al., 2012), which discharges a large quantity of coloured effluents into the water resources and these effluents contain toxic and carcinogenic materials (Chung, 2000; El Qada et al., 2006; Mohan et al., 2007). AB113 is an acid dye with 681.65 g/mol. as a molecular weight and BR46 is a basic dye with 401.3 g/mol. as a molecular weight (Table 3.1). Lade et al. (2012) and Holkar et al. (2014) stated there is a higher degradation rate for dyes with a molecular weight of less than 500 g/mol. and a lower degradation rate for dyes with a molecular weight higher than 500 g/mol. Acid dye is defined as a negatively charged dye at a chemical level, which contains one or more acidic groups such as a sulphonic group (Akbari et al., 2002; Martínez-Huitle and Brillas, 2009). Basic dye is defined as a positively charged stain at a chemical level (Martínez-Huitle and Brillas, 2009; Brillas and Martínez-Huitle, 2015) which therefore reacts well with negatively charged materials (Sun and Yang, 2003).

These dyes were tested at two different concentrations (target concentrations of 7 mg/l and 215 mg/l) for different contact times (approximately 48 h and 94 h) to assess their influence on the performance of vertical-flow constructed wetlands. The details for each dye can be found in Table 3.1. The textile dye BR46 had a wavelength for maximum absorbance (λ_{max}) of 530 nm (Khataee, 2009) and was obtained from Dystar (Am Prime Park, Ruanheim, Germany). The dye AB113 had a λ_{max} of 566 nm (Shirzad-Siboni et al., 2014) and was obtained from Sigma Aldrich (The Old Brickyard, New Road Gillingham, Dorset, United Kingdom) and both of them were used without further purification.

The wavelength for the maximum absorbance of a mixture of both the dyes was determined experimentally by using a Biowave II (WPA) Spectrophotometer. Firstly, the λ_{max} of the mixture of the dyes was determined by scanning the absorption of different mixture dye concentrations between 200 and 800 nm wavelength. The λ_{max} for the mixture dye was found to be 511 nm. The dye stock solutions were prepared by dissolving weighted amounts of each dye (10g) in 1000 ml distilled water and the experimental solutions were obtained by diluting the stock solution to the required concentrations and then mixing.

Table 3.1 Details of azo textile dyes used in the experimental constructed wetlands

Dye	BR46	AB113
Molecular weight (g/mol.)	401.3	681.65
Molecular formula	$C_{18}H_{21}BrN_6$	$C_{32}H_{21}N_5Na_2O_6S_2$
Source	Dystar	Sigma Aldrich
CASRN	12221-69-1	3351-05-1
Purity of dye (%)	70–80	Approximately 50
Chemical structure		

Note: mol., mole; CASRN, chemical abstracts service registry number; BR, basic red; AB, acid blue; C, Carbon; H, hydrogen; N, nitrogen; Na, sodium; O, oxygen; S, sulphur; Br, barium.

3.2.5 Aquarium Plant Nutrient

The fertilizer TNC Complete, which is an aquatic plant nutrient bought from TNC Limited (Spotland Bridge Mill, Mellor Street, Rochdale, United Kingdom), was used in the experimental work as a nutrient for the plant. The corresponding ingredient composition was as follows: phosphorus (0.2%), nitrogen (1.5%), iron (0.08%), manganese (0.018%), potassium (5%), magnesium (0.08%), copper (0.002%), molybdenum (0.001%), boron (0.01%) and zinc (0.01%). TNC Complete also provides ethylene diamine tetra acetic acid (EDTA), which is used as a source for copper, iron, manganese and zinc. The fertilizer was used at a concentration of 1 ml of fertilizer in 10 l of tap water.

3.2.6 Artificial Wastewater Compositions

At the second phase of experimental work in this study, vertical-flow constructed wetlands were operated to treat artificial wastewater containing two azo textile dyes. All the compositions of artificial wastewater (Wießner et al., 2005; Ong et al., 2009) were purchased from Scientific Laboratory Supplies (Wilford Industrial Estate, Wilford, Nottingham, United Kingdom). Details of each composition including its concentration, used in this experimental work and others, are shown in Table 3.2.

Table 3.2 Details of artificial wastewater compositions used in the second phase of experimental work

Material	Chemical structure	Molecular weight (g/mol.)	CAS number	Purity of dye (%)	Concentration (mg/l)
Sodium acetate anhydrous pure	CH_3COONa	82.03	127-09-3	≥99	107.1
Sodium benzoate	C_6H_5COONa	144.11	532-32-1	≥99	204.9
Ammonium nitrate pure	NH_4NO_3	80.04	6484-52-2	≥99	76.1
Sodium chloride pure	NaCl	58.44	7647-14-5	≥99	7.0
Magnesium chloride hexahydrate	$MgCl_2{\cdot}6H_2O$	203.3	7791-18-6	≥99	3.4
Calcium chloride dehydrate	$CaCl_2{\cdot}2H_2O$	147.01	10035-04-8	≥99	4.0
Potassium phosphate dibasic trihydrate	$K_2HPO_4{\cdot}3H_2O$	228.22	16788-57-1	≥99	36.7

Note: mol., mole; CAS, chemical abstracts service; C, Carbon; H, hydrogen; O, oxygen; Na, sodium; N, nitrogen; Mg, Magnesium; Cl, chloride; K, potassium; P, phosphor.

3.3 Environmental Conditions

3.3.1 Operational Conditions

The wetland system was designed to operate in batch flow mode to avoid expenses such as pumping and automatic control costs. Wastewater was poured directly into the wetland filter from the top. The wastewater stayed within the wetland for the duration of the contact time. The treated wastewater was released from the wetland through an outlet pipe located in the centre of the wetland bottom. The duration for which the wetland filter was empty (i.e. no wastewater input) was the resting time. The packing order of the experimental constructed wetland set-up treating dye only and an outline of the application of the simplified statistical wetland filter set-up design to assess the impact of individual key variables are shown in Tables 3.3 and 3.4, respectively. The packing order of the experimental constructed wetland set-up treating artificial wastewater containing two dyes and the impact of its individual key variables are shown in Tables 3.5 and 3.6, respectively.

3.3.1.1 Operational Conditions for Experimental Constructed Wetland Set-Up Treating Azo Textile Dyes Only

Wetland 1 was deliberately left empty (without media and plants). The mean tap water values for dissolved oxygen (DO), pH, electrical conductivity (EC), redox potential, total suspended solids (TSS) and turbidity were 10.1 mg/l, 7.6, 85.1 _ μS/cm , −32 mV, 1 mg/l and 1.3 NTU, respectively. Wetland 2 was filled with unwashed gravel, while Wetland 3 comprised washed gravel and tap water as shown in Table 3.3. Wetlands 4, 8, 9, 10 and 11 were filled with the washed gravel as a media and planted with the plant (*Phragmites australis*), except Wetland 4, which was used as a control, the rhizomes of this plant were washed free of sediments and planted in the selected gravel-filled wetlands. Wetlands 8, 9 and 10, 11 were replicated, respectively. The contact time for Wetlands 8 and 9 was 94 h and the resting time was 2 h, while for Wetlands 10 and 11, the contact time was 48 h and the resting time was 48 h. The replicated wetlands are important to ensure accurate values of their parameters. The purpose of the different contact and resting times is to assess the ability of the wetlands to remove the dye under different design conditions and compare the results with Wetland 4 to assess the importance of the presence of the plant on the dye reduction and the other parameters. The influent was tap water mixed with BR46 (6.9±1.33 mg/l). The same previously explained set-up was used for the Wetlands 5, 6, 7, 12 and 13, but with the dye AB113 at a concentration of about 6.6±1.82 mg/l.

For the high concentration of BR46 (209.3±4.48), Wetlands 14, 15, 18 and 19 were filled with the same media and the plant. Wetlands 14 and 15 were replicated with 48 h as the contact time and 48 h as the resting time, while Wetlands 18 and 19 were replicated with 96 h as the contact time and a resting time of 96 h. Wetlands 16, 17, 20 and 21 are similar to the previous wetlands, just described, but the influent dye is AB113 at a concentration of about 221.5±31.86 mg/l.

Table 3.3 Packing order of the experimental constructed wetland set-up treating two azo textile dyes only. Samples were taken at the end of each contact time period, just before the start of the resting time period between 1 May 2015 and 31 May 2016.

Wetland number	Media	Plants	Dye			Resting time (h)	Contact time (h)
			Type	Mean (mg/l)	SD (mg/l)		
1	Only Water	No	None	N/A	N/A	2	94
2	Unwashed gravel	No	None	N/A	N/A	2	94
3	Washed gravel	No	None	N/A	N/A	2	94
4	Washed gravel	No	BR46	6.9	1.33	2	94
5	Washed gravel	No	AB113	6.6	1.82	2	94
6,7	Washed gravel	Yes	AB113	6.6	1.82	2	94
8,9	Washed gravel	Yes	BR46	6.9	1.33	2	94
10,11	Washed gravel	Yes	BR46	6.9	1.33	48	48
12,13	Washed gravel	Yes	AB113	6.6	1.82	48	48
14,15	Washed gravel	Yes	BR46	209.3	4.48	48	48
16,17	Washed gravel	Yes	AB113	221.5	31.86	48	48
18,19	Washed gravel	Yes	BR46	209.3	4.48	96	96
20,21	Washed gravel	Yes	AB113	221.5	31.86	96	96

Note: SD; standard deviation, N/A; not applicable, BR, basic red; AB, acid blue.

Table 3.4 Application of the simplified statistical wetland filter set-up design (Table 3.3) to assess the impact of individual key variables.

Comparison of two wetland systems with each other		Impact to be assessed
First wetland with number	Second wetland with number	
1	2	Unwashed gravel
2	3	Washed gravel
3	4	BR46
3	5	AB113
4	5	Difference between BR46 and AB113
5	6,7	*Phragmites australis*
6,7	8,9	Difference between BR46 and AB113
8,9	10,11	Decrease in contact time (or increase in resting time)
10,11	12,13	Difference between BR46 and AB113
10,11	14,15	Increased BR46 concentration
12,13	16,17	Increased AB113 concentration
14,15	18,19	Increased contact and resting times
16,17	20,21	Increased contact and resting times
14,15	16,17	Difference between BR46 and AB113
18,19	20,21	Difference between BR46 and AB113

Note: BR46, basic red; AB, acid blue

3.3.1.2 Operational Conditions for Experimental Constructed Wetland Set-Up Treating Artificial Wastewater Containing Two Azo Textile Dyes

All wetlands in this phase have the same media, which is washed gravel (Table 3.5). The influent for Wetlands 1, 5 and 7 was artificial wastewater containing dye BR46 and planted with *Phragmites australis* except for Wetland 1, which was used as a control. The resting and contact times for Wetlands 1 and 5 were 2 h and 94 h, respectively, while for Wetland 7 both resting and contact times were 48 h. The concentration for the influent dye was 6.2 mg/l. Wetlands 2, 3 and 9 followed the same scenario as the previous wetlands but the artificial wastewater contained dye AB113 with an influent concentration 7.5 mg/l. Wetlands 4, 6, 8 and 10 followed the same scenario as the previous wetlands (where Wetlands 8 and 10 follow the same scenario as 7 or 9) but the influent was the artificial wastewater containing the mixture of both of the previous dyes (BR46 and AB113).

Wetlands 11 and 15 were the same as Wetlands 5 and 7 but the dye (BR46) concentration was approximately 206 mg/l with 48 h resting and contact times for Wetland 11 and 96 h resting and contact times for Wetland 15. Wetlands 13 and 17 followed the same scenario as Wetlands 11 and 15 but the influent artificial wastewater contained dye AB113 with an approximate concentration of 207 mg/l. Wetlands 12 and 14 followed the same scenario as the previous Wetlands 11 and 13 but the influent artificial wastewater was the mixture of both of the dyes (BR46 and AB113). The same influent artificial wastewater containing the mixture of the two dyes was used for Wetlands 16 and 18 but with 96 h for both the resting and contact times.

Table 3.5 Packing order of the experimental constructed wetland set-up treating artificial wastewater containing two azo textile dyes. Samples were taken at the end of each contact time period, just before the start of the resting time period between 1 June 2016 and 31 May 2017.

Wetland number[a]	Wetland number[b]	Plants	Dye			Resting time (h)	Contact time (h)
			Type	Mean (mg/l)	SD		
4	1	No	BR46	6.15	0.75	2	94
5	2	No	AB113	7.50	1.66	2	94
6	3	Yes	AB113	7.50	1.66	2	94
7	4	Yes	Mix[c]			2	94
8	5	Yes	BR46	6.15	0.75	2	94
10	6	Yes	Mix			2	94
11	7	Yes	BR46	6.15	0.75	48	48
12	8	Yes	Mix			48	48
13	9	Yes	AB113	7.50	1.66	48	48
14	10	Yes	Mix			48	48
15	11	Yes	BR46	206	9.60	48	48
16	12	Yes	Mix			48	48
17	13	Yes	AB113	207	13.70	48	48
18	14	Yes	Mix			48	48
19	15	Yes	BR46	206	9.60	96	96
20	16	Yes	Mix			96	96
21	17	Yes	AB113	207	13.70	96	96
22	18	Yes	Mix			96	96

Note: [a], wetland used to treat azo textile dye only; [b], wetland used to treat artificial wastewater containing two azo textile dye(s); SD, standard deviation; [c], mixture of BR46 and AB113; BR, basic red; AB, acid blue.

Table 3.6 Application of the simplified statistical wetland filter set-up design (Table 3.5) to assess the impact of individual key variables.

Comparison of two wetland systems with each other		Impact to be assessed
First wetland with number	Second wetland with number	
1	2	Difference between BR46 and AB113
1	5	*Phragmites australis* on BR46
2	3	*Phragmites australis* on AB113
4	6	Mixing dyes (low concentration)
5	7	Decrease in contact time (or increase in resting time on BR46)
3	9	Decrease in contact time (or increase in resting time on AB113)
7	9	Difference between BR46 and AB113
8	10	Mixing dyes (low concentration)
7	11	Increased BR46 concentration
9	13	Increased AB113 concentration
12	14	Mixing dyes (high concentration)
11	15	Increased contact and resting times
13	17	Increased contact and resting times
11	13	Difference between BR46 and AB113
15	17	Difference between BR46 and AB113
16	18	Mixing dyes (high concentration)

Note: BR46, basic red; AB, acid blue.

It is clear that the general rule of a loading period followed by a resting period is used by all researchers/engineers when dealing with this type of constructed wetlands. Applying intervals between the wastewater loadings and allowing the wastewater to drain vertically enhances the aerobic biodegradation of several substances (organic matter, nitrogen, etc.). However, it is also obvious that there is no widely accepted regime concerning the frequency and duration of the loading and resting periods' (Stefanakis et al., 2014).

Two different concentrations for the dyes were studied to find out the effect of concentration of the textile dye effluent on the percentage of dye removed and the other parameters. The low concentration enabled study of the behaviour of the constructed wetland system with different wetlands (filled by media with and without plant), and different contact and resting times. The high concentration enabled assessment of the efficiency of the vertical wetland in treating the effluent of the textile wastewater and assessment of the effect of long-term performance of the constructed wetlands system in treating the textile wastewater (Robinson et al., 2002; Davies et al., 2006; Tee et al., 2015).

3.3.2 Measurement of Water Quality Parameters

Samples were taken regularly at the end of each contact time period, just before the start of the resting time period between 1 May 2015 and 31 May 2017 as shown in Table 3.3 and Table 3.5, and brought to the laboratory (G19) in the Cockcroft Building for testing. The samples were taken at the same time (from 10:00 to 11:00 am) to ensure that the different conditions which occur throughout the day, such as varying pH, would not affect the results. The laboratory tests measured dye concentration, colour, COD, PO_4-P, NO_3-N, NH_4-N, EC, DO, redox, pH, TSS, turbidity and amines, according to the procedures in the standard method (APHA, 1995), unless stated otherwise. Both influent and effluent were analysed. The effluent was obtained from the bottom of each wetland filter. The inflow wastewater was freshly prepared before it was poured into the wetland from the top. These parameters were used to give an indication of the vertical-flow constructed wetland's effectiveness in treating the textile wastewater and water quality.

3.3.2.1 Hydraulic Loading Rate

Hydraulic loading rate (HLR) was calculated according to Equation 3.1 (Tyler, 2001):

$$HLR=\frac{Q}{A} \qquad \text{Equation 3.1}$$

where HLR is hydraulic loading rate (m/d), Q is flow rate (m^3/d), and A is surface area of constructed wetland (m^2).

3.3.2.2 Mass Loading Rate (MLR)

Mass loading rate (MLR) was calculated according to Equation 3.2 (Timmons et al., 2002):

$MLR=C_{i,e}\times HLR$ Equation 3.2

where MLR is mass loading rate ($mg.m^{-2}.d^{-1}$), and $C_{i,e}$ is influent or effluent concentration (mg/l).

3.3.2.3 Percentage Reduction Rate

The percentage reduction rate of any selected variable was calculated according to Equation 3.3 (Kadlec and Wallace, 2008).

$R=\frac{C_{e-}C_i}{C_i}\times 100$ Equation 3.3

where R percentage is reduction rate (%), C_e is effluent value (mg/l, nm, Pt Co.), and C_i is influent value (mg/l, nm, Pt Co.).

3.3.2.4 Analytical Methods and Equipment

The methods and equipment used to measure different parameters during the experimental work are presented below.

3.3.2.4.1 Measurement of Physical Parameters

The physical parameters included dye concentration, colour, TSS, DO, Turbidity, pH, redox potential, EC and temperature. Dye concentration, colour and TSS were measured using a Spectrophotometer Hach Lange DR2800 (www.hach.com). Dye concentration was measured through the selective wavelength at maximum absorbance for each dye. Colour was measured with a unit Pt Co scale. TSS was measured with mg/l as a unit. Samples were filtered using Whatman Grade 1 Qualitative Filter Paper (Standard Grade, Circle, 320 mm), which was bought from Scientific Laboratory Supplies (Wilford Industrial Estate, Wilford, Nottingham, United Kingdom).

The dissolved oxygen (DO) was measured using a hand-held Hach Lange HQ30d Flexi Meter (Pacific Way, Salford, United Kingdom); the unit for the measuring of DO is mg/l. The DO was measured promptly after taking the specimens, to limit, as far as possible, the time for which samples were in contact with air. Turbidity was measured using a Turbicheck Portable Turbidity Meter (Lovibond Water Testing, Tintometer Group, Division Street, Chicago, IL, USA); the unit for the measuring of turbidity is NTU.

The pH and redox potential for all samples were measured by using a hand-held WTW VARIO pH Meter (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany); the unit for the measuring of redox is (mV) while pH is without units, the meter is calibrated with standard buffer solution of pH 4.0, 7.0 and 9.0 every two weeks or whenever required. pH is a value of the amount of free hydrogen ion in water. A value pH of 7 is deemed to be neutral and the range of pH for water quality standards is from 6.5 to 9 (Boyd and Gautier, 2000). For any increase in pH value above the neutral value, alkalinity will increase, while for any decrease below the neutral value, acidity will increase. The measuring of pH value is very important due to its effect on the solubility of many toxic and nutritive chemicals. When the acidity increases, most metals become more toxic and more water-soluble. For example, a slight increase in pH value leads to ammonia becoming more toxic (Thurston et al., 1981). In contrast, the toxicity of sulphides and cyanides increases when the value of pH decreases. Electrical conductivity (EC) was measured using a hand-held Mettler Toledo Education Line Conductivity Meter (Boston Road, Leicester, United Kingdom). The unit for the measurement of EC is micro Siemens per centimetre (μS/cm). Although EC itself is not an aquatic health or human concern, its value gives an indication if there are any other water quality problems. A suddenly increase in EC value indicates that there is a source of dissolved ions in the wetland filter (Kumar and Chopra, 2012). The temperature at the site was recorded each day using a thermometer placed alongside the constructed wetlands.

These hand-held easy to use, robust and waterproof instruments measure, with low costs, the most important parameters for wastewater monitoring. The meters come complete with sensors, and calibration and maintenance solutions for measurement.

3.3.2.4.2 Measurements of Chemical Parameters

The chemical parameters measured included COD, NH_4-N, NO_3-N, PO_4-P and amines. A Spectrophotometer Hach Lange DR2800 (www.hach.com) was applied for the water quality analysis, for variables including chemical oxygen demand (COD), orthophosphate-phosphorus (PO_4-P), nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) with milligrams per litre (mg/l) as a unit. The aromatic amines were measured as an absorbance using a WPA Biowave II UV/Visible Spectrophotometer (Cambourne, Cambridge, United Kingdom), via the wavelength for the selected absorbance for every type of aromatic amines. Also, samples were filtered using Whatman Grade 1 Qualitative Filter Paper (Standard Grade, Circle, 320 mm).

Chemical oxygen demand (COD) does not differentiate between biologically available and inert organic matter and it is a measure of the total amount of oxygen required to oxidize all organic material into water and carbon dioxide. COD was determined with a

Palintest Tube tests system. Palintest Tube tests are integrated with the Palintest heater and photometer system to provide a complete system for COD measurement. COD analysis was performed with three Palintest Tube tests with product codes; LCK 114 (range from 150-1000 mg/l), LCI 400 (range from 0-1000 mg/l) and LCK 049 (range from 50-300 mg/l).

Nutrients such as phosphorus and nitrogen are very important for the growth of plants and algae. Aquatic life in a stream or river depends upon these photo-synthesizers, which exist at surface water in low levels. Any extremes in nutrients concentration lead to a rapid growth for algae and aquatic plants, however, this rapid growth leads to a reduction in dissolved oxygen concentration, depriving invertebrates and fish of available oxygen in the water. Nutrients were determined by automated precision colorimetry methods using a Palintest Tube test with product code LCK 339 for nitrate (range from 0.23-13.5 mg/l), LCK 305 for ammonia (range from 1-12 mg/l) and LCK 049 (range from 1.6-30 mg/l) for ortho-phosphate-phosphorus (Allen et al., 1974). The coloured complexes formed were measured spectrometrically at 540, 655 and 882 nm, respectively. All the tube tests were bought from Hach Lange Ltd. (Pacific Way, Salford, UK).

3.3.2.4.3 Statistical Analysis Methods

After data collection, data were subjected to a normality test before validation and subsequent analysis. Microsoft Excel (www.microsoft.com) was used for general data analysis unless stated otherwise. The IBM SPSS version 23 (IBM Corp., 2013) package was applied to perform the correlation analysis between variables and to assess statistical differences between treatments. In order to investigate statistically significant differences, the Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) was used to investigate data normality. If $p \geq 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed. A one-way analysis of variance (ANOVA) test using Statistical Package for the Social Science software was applied to analyse normal distributed data ($p \geq 0.05$), while the Mann-Whitney test was used to analyse non-normal data ($p < 0.05$) (Stoline, 1981; Kasuya, 2001). These ANOVA and Mann-Whitney tests were computed using IBM SPSS Statistics Version 23 and used to compare means between different treatments, such as the ones highlighted in Tables 3.4 and 3.6. Significant findings have been outlined and discussed. Surprising and/or important insignificant findings have occasionally been highlighted as well.

3.4 Experimental Research Limitations

Highlights and discussions of limitations in this experimental design and methods are presented in this section.

In spite of the reality that the experimental constructed wetlands used are very small in comparison to large-scale constructed wetland systems used in industries, previous studies based on the same design idea as this one proved that the results obtained were applicable in field scale and therefore accepted by the scientific community (Scholz, 2004; Zhao et al., 2004; Sani et al., 2013; Al-Isawi et al., 2015b; Almuktar et al., 2015).

The experimental constructed wetlands in this study, which was carried out in a greenhouse under semi-controlled conditions, may not correspond with other wetlands operated in real field scale because of the variability in environmental factors. However, designers can use the results obtained from this research as a guide in designing and up-scaling field-scale wetlands operated in different climates. In additional, the use and operation of constructed wetland systems in the field require larger land areas and natural energy inputs to achieve to self-maintaining treatment constructed wetlands systems, which will provide a suitable environment for many more types of micro-organisms as a result of the diversity of the micro-environment in a wetland. These facts lead to the conclusion that using small-scale constructed wetlands, as operated in this research, cannot represent the actual requirement of the large land area involved in actual field-scale operation. Moreover, because of the large surface area required for a constructed wetland in the field, many problems are expected to occur. For example, a larger area could be home to many types of animals, affecting the treatment performance and causing health problems for humans, while in these small-scale constructed wetlands, as used in this study, the effects of the presence of animals are not considered.

In the first phase of the experimental work (treating azo textile dyes only), all the planted constructed wetlands were replicated but the control wetlands (unplanted) were not replicated due to the lack of sufficient resources and space to cover the required number of replications needed. Although the obtained results from this experimental work don't mimic the actual set-up for the constructed wetlands with full replication, many publications based on the same idea as this experimental work have been accepted by the scientific community (Al-Isawi et al., 2015a; Sani, 2015; Hussein and Scholz, 2017).

In the second phase of the experimental work (treating artificial wastewater containing two azo textile dyes), one out of each of the replicates used in the first phase, as shown in Table 3.5, was used for the influent of mixed dyes as well as artificial wastewater. This new step depended on the value of significance between each replication.

3.5 Concluding Remarks

The main factor, which has to be considered in case of scaling-up the constructed wetlands is the effluent of the textile industry. If the dye effluent concentration is below 7 mg/l, it will not impact on the performance of VFCW systems. In contrast, a relatively high concentration of 215 mg/l will affect the performance of the system.

4. CHAPTER FOUR: RESULTS AND DISCUSSION FOR TWO AZO TEXTILE DYES REDUCTION

Most of the results and discussion in this chapter have been published in the paper shown below:

Hussein, A. & Scholz, M. 2017. Dye wastewater treatment by vertical-flow constructed wetlands. *Ecological Engineering*, 101, 28-38, doi: 10.1016/j.ecoleng.2017.01.016. (Appendix A).

4.1 Overview

This chapter includes the results and discussion of the dye reduction rates and other physical and chemicals water quality parameters for the period between 1 May 2015 and 31 May 2016. A comparison between wetland filters 1, 2 and 3 is shown in section 4.2, while section 4.3 deals with the test of normality for all variables. The results for plant growth monitoring, redox potential, dissolved oxygen, electrical conductivity, total suspended solids, turbidity, pH, nutrients, dyes and chemical oxygen demand are presented in sections 4.4 to 4.9. Section 4.10 is concerned with the seasonal treatment performance of wetlands in terms of dye reductions. Furthermore, statistical differences between the variables are also presented. Concluding remarks are given in section 4.11.

4.2 Comparison of Unwashed Gravel versus Washed Gravel

Many publications have suggested using a washed gravel in wetlands media. For instance, (Kopec, 2007) stated that using washed gravel is very important to prevent clogging. (Reuter et al., 1992) believed the drop in reduction efficiency of phosphorus has to -38% during the winter period was a result of using unwashed gravel as media. Furthermore, (Rosolen, 2000) stated that sediments in unwashed gravel affect the porosity, which determines hydraulic conductivity. The effluent values of TSS for Wetlands 1, 2 and 3 were 1.0, 1550 and 30 mg/l, respectively. After less than six months, the effluent values became approximately similar, as shown in Figure 4.1, as a result of washing it during the contact time. In the case of turbidity, the effluent values for Wetlands 1, 2 and 3 were 1.88, 870 and 42 NTU, respectively. Within six months, the effluent values were approximately similar, as shown in Figure 4.2, as result of the same previous reason. For the electrical conductivity, the effluent values for Wetlands 1, 2 and 3 were 101, 328 and 151 μS/cm, respectively. After less than six months, the effluent values were approximately similar, as shown in Figure 4.3. For the other variables, pH, redox and DO, there were no significant ($p \geq 0.05$) differences between them.

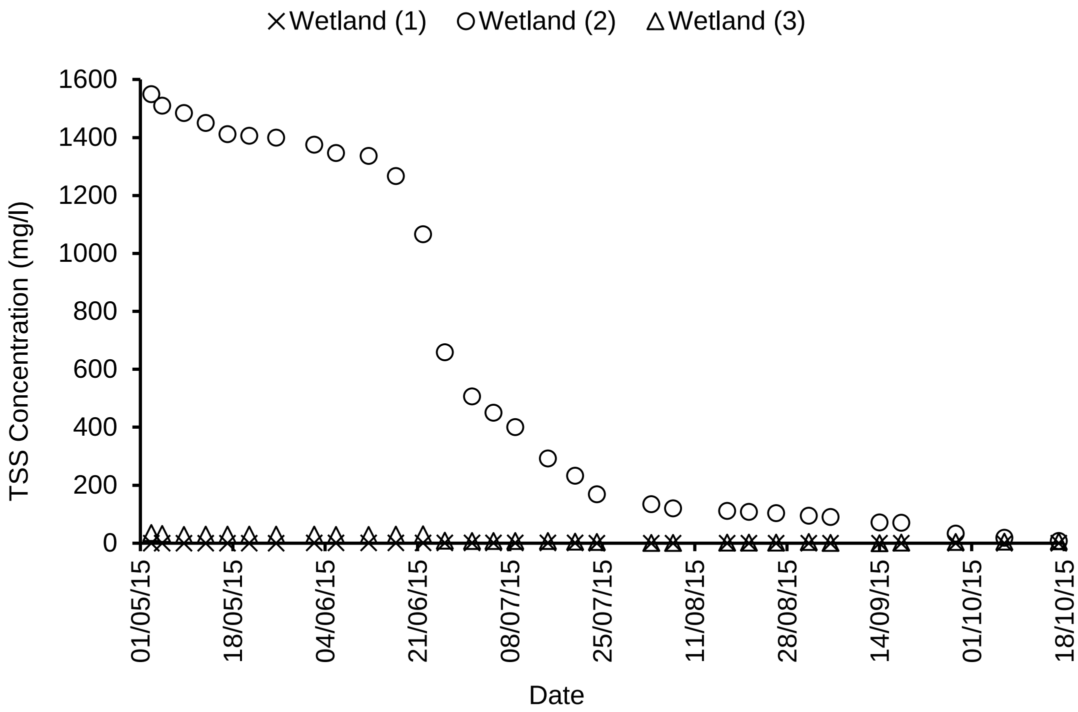


Figure 4.1 Total suspended solids comparison between Wetlands 1, 2 and 3.

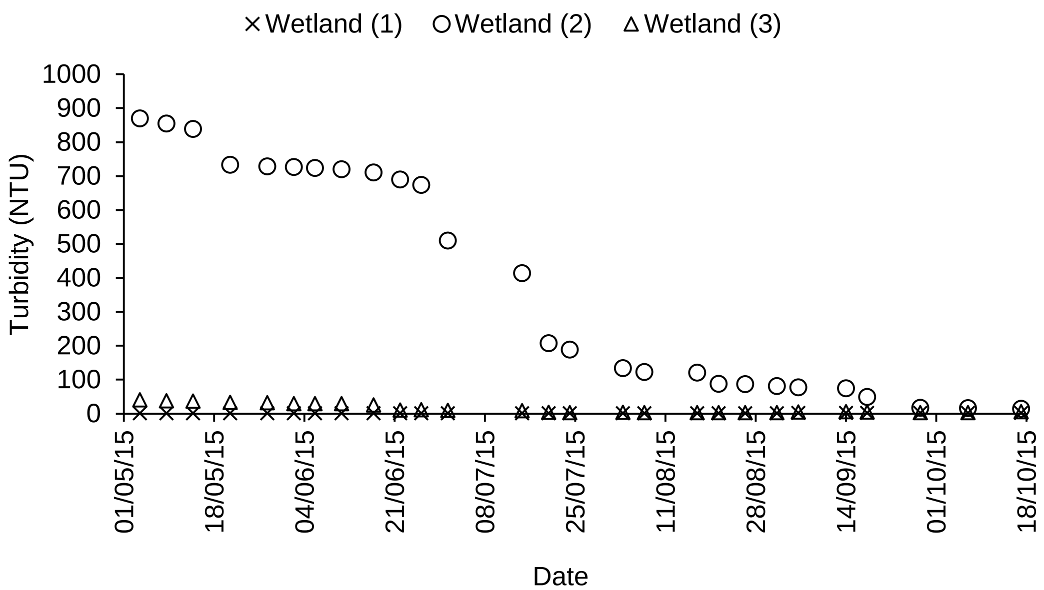


Figure 4.2 Turbidity comparison between Wetlands 1, 2 and 3.

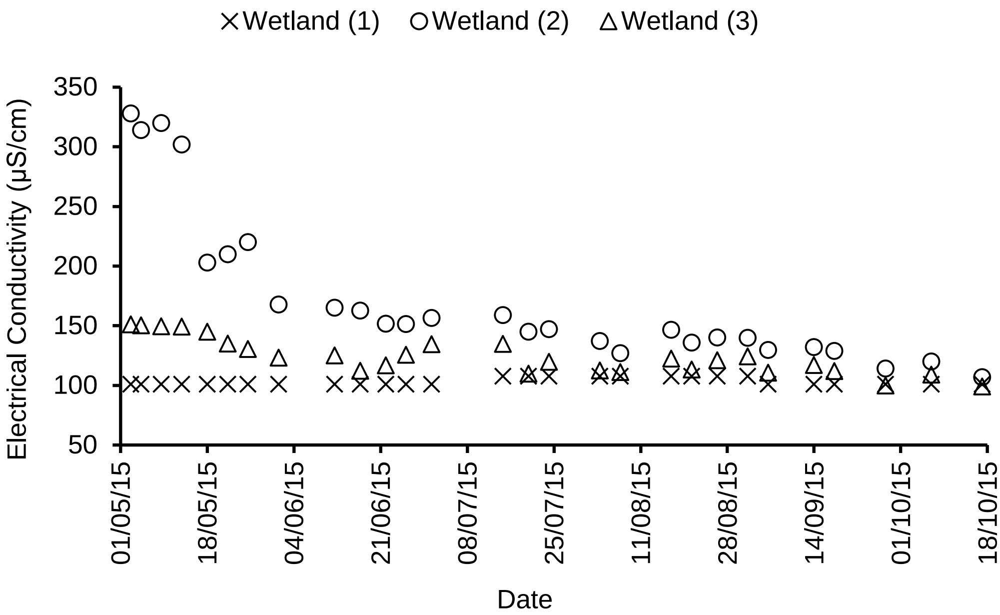


Figure 4.3 Electrical conductivity comparison between Wetlands 1, 2 and 3.

4.3 Test of Normality

Tests of normality results for dimensions of *P. australis* and for effluent water quality characteristics regarding general physical and chemical variables are discussed in Appendix B.1.

4.4 Plant Growth Monitoring

The presence of *Phragmites australis* in vertical-flow constructed wetlands has a significant impact on the reduction of organic matters, aromatic amines and NH_4-N (Ong et al., 2011). The growth cycle of *P. australis* traditionally completes between May and September in Britain (Haslam, 1972). (Ferreira et al., 2014) detected normal growth for *Phragmites* with absence of toxic signs or depletion of leaf nitrogen content, when they used vertical-flow wetlands to treat an effluent comprising Diazo dye (DR81). When temperatures started to decrease in winter (Figure. 4.4), the plants in this experiment began to yellow. The above-ground plant parts died, and were cut and returned to the wetland filters. The authors followed common practice to cut the above-ground plant parts down to between 10 and 15 cm in height according to (Stefanakis et al., 2014).

Based on the experimental results, the most critical observation is that for all considered concentrations and contact times, the plants that were subjected to the dye AB113 revealed more effective growth compared to those that were fed by the dye BR46. Taking into consideration the potential impact of retention time, it has been noted that plants associated with a long contact time showed much better growth than those plants linked to a short one (Table 4.1). These findings support those by (Pagter et al., 2005), who investigated the effect of water stress tolerance of *P. australis* grown in the laboratory by examining effects of different levels of required water. The results showed that a water deficit reduces the leaf biomass per plant and the leaf area.

Regarding the growth of plants, there is no significant ($p \geq 0.05$) difference between experimental wetlands with respect to the length and diameter at the low concentration of the dye AB113, while for the high concentration, there is a significant ($p < 0.05$) difference for the diameter but no significant ($p \geq 0.05$) difference for the length. In the case of plants grown in the presence of the dye BR46, there is a significant ($p < 0.05$) difference for both the length and diameter at the low concentration of the dye, while no significant difference ($p \geq 0.05$) for either parameter was noted at the high concentration of the dye. It follows that no generic interpretation of the data can be made for either dye, indicating further research is needed by ecologists.

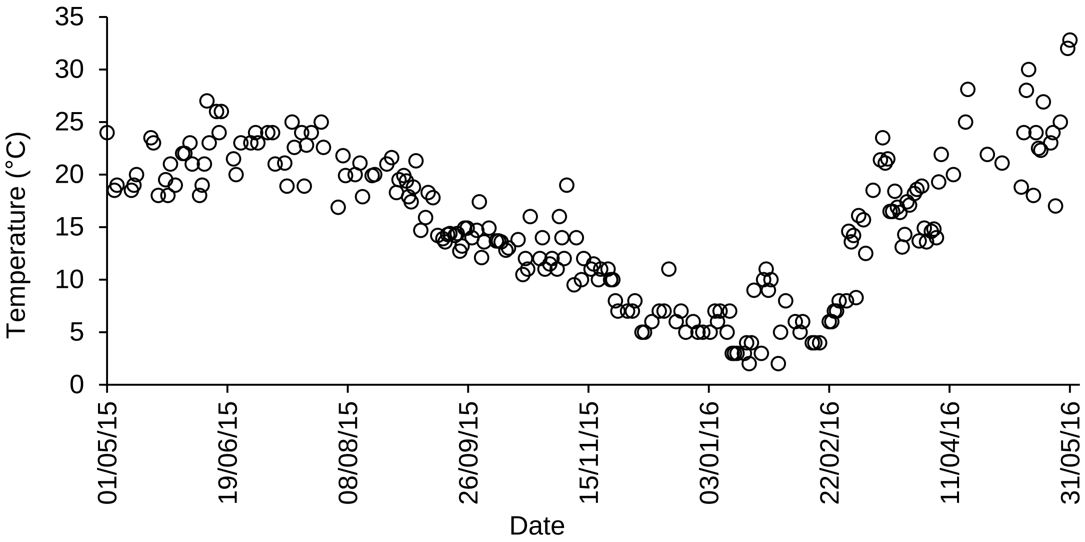


Figure 4.4 Temporal variation of temperature throughout the experimental period (first phase).

Table 4.1 The descriptive statistics of the changes in temperature

Season	Descriptive Statistics			
	Minimum	Maximum	Mean	SD
Summer	13.6	25	22.8	3.25
Autumn	5	19	11.6	3.09
Winter	2	24	8.6	5.59
Spring	13.1	32.8	20.5	5.3

Note: (a)21/06/2015 to 21/9/2015; (b)22/09/2015 to 20/12/2015; (c)21/12/2015 to 9/03/2016; (d)20/03/2016 to 31/05/2016; BR, basic red; AB, acid blue; SD, standard deviation.

The temperature variations cause changes in microbial activity, which in turn creates changes in microbial-mediated water quality improvement (Fan et al., 2016; Xie et al., 2016). The study of Xie et al. (2016) showed that the bacterial numbers and species responsible for ammonification, nitrification and denitrification varied seasonally, with denitrifying bacteria changing the most and nitrifying bacteria changing the least, with also higher numbers in the warm seasons (summer and autumn) and lower numbers in the cold seasons (spring and winter).

Table 4.2 Dimensions of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) planted in the experimental wetlands.

Characteristics			Length (cm)			Diameter (mm)		
Dye	Wetland numbers	Number of stems	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
BR46	8 and 9	40	40	110	86±25.0	2.5	3.8	3.6±0.16
	10 and 11	28	17	44	30±9.0	1.1	1.8	1.5±0.30
	14 and 15	34	14	50	27±13.0	1.0	1.2	1.1±0.07
	18 and 19	23	12	34	23±8.1	0.9	1.5	1.2±0.25
AB113	6 and 7	45	110	141	131±9.5	3.4	3.8	3.6±0.16
	12 and 13	30	23	41	32±4.5	2.1	2.4	2.2±0.10
	16 and 17	36	28	46	35±6.0	2.1	2.3	2.2±0.06
	20 and 21	28	22	52	39±10.7	1.8	2.5	2.1±0.25

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation.

4.5 Redox Potential and Dissolved Oxygen

Redox potentials of less than -100 mV indicate anaerobic environments, while values greater than 100 mV indicate aerobic environments (Suthersan, 2001). DO is an important function in constructed wetlands as it is essential in aerobic respiration for micro-organisms and it regulates the oxidation-redox potential in wastewater (Boyd, 2000). S Wu et al. (2011) and Hou et al. (2016) reported that the main pathways for oxygen transfer in constructed wetlands such as the system in this research (tidal flow) are: wetland macrophytes release via their roots, contact transfer at the interface of biofilm and atmosphere, and dissolved oxygen associated with influent wastewater. Redox potential values for the effluents of both dyes (BR46 and AB113) in the case of low concentration were in the range between -32 and -6 mV as shown in Table 4.2, and the influent and effluent values during the whole period of the experimental work are shown in Figures 4.5 and 4.6, respectively. In the case of high concentration for both dyes (BR46 and AB113), redox potential values for the effluents were in the range between -32 and 4 mV (Table 4.2) and all values for the influents and effluents are shown in Figures 4.7 and 4.8, respectively. Wetlands with low loading rate (18/19 and 20/21 for BR46 and AB113, respectively) have a lower redox potential when compared to Wetlands 14/15 and 16/17 for BR46 and AB113, respectively, which have high loading rate during the period of experimental work. These results can be linked with the dye reductions which are shown in Table 4.4, where dye reduction rate is highest, when the redox potential of the system is at its most negative, findings confirmed by Singh (2014).

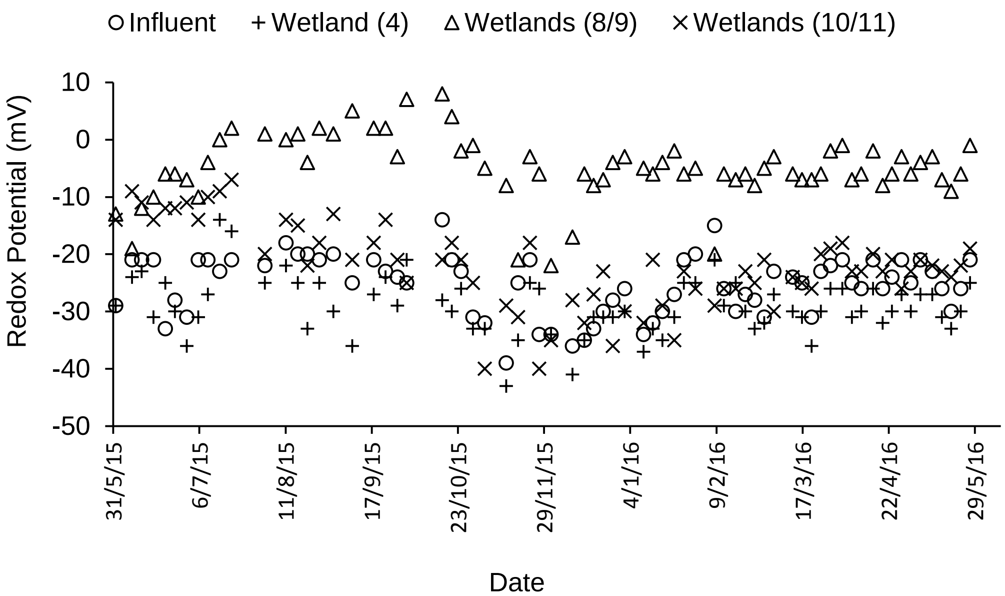


Figure 4.5 Overall variation for redox potential in the influent and effluent for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

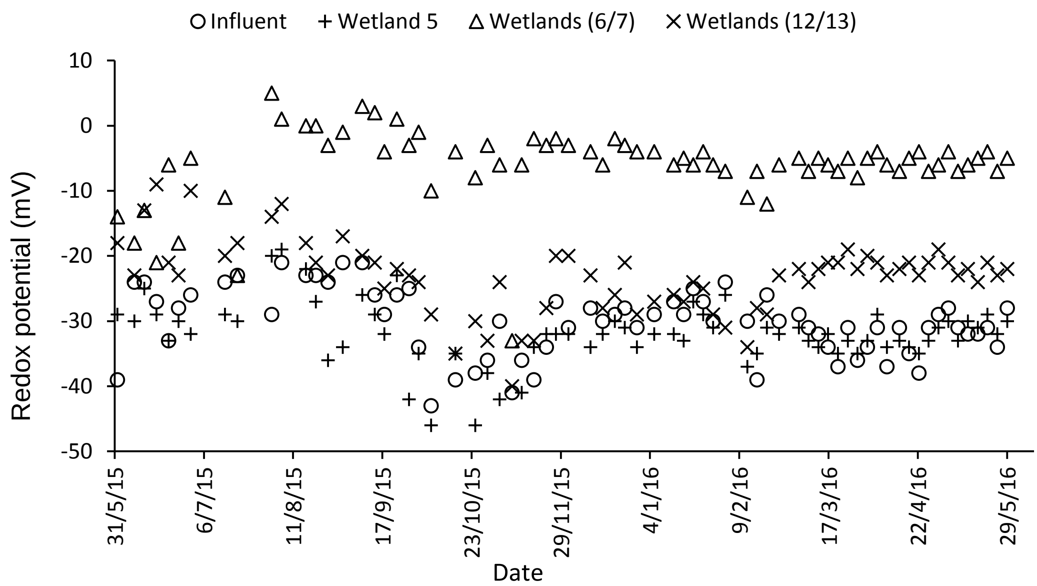


Figure 4.6 Overall variation for redox potential in the influent and effluent for Wetlands 5, 6/7 and 12/13 (low concentration of AB113).

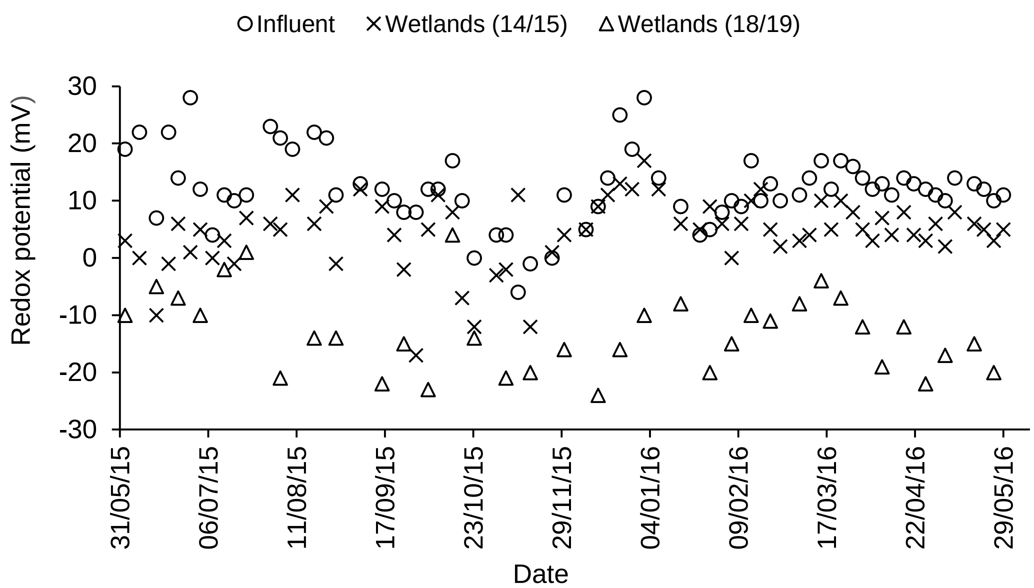


Figure 4.7 Overall variation for redox potential in the influent and effluent for Wetlands 14/15 and 18/19 (high concentration of BR46).

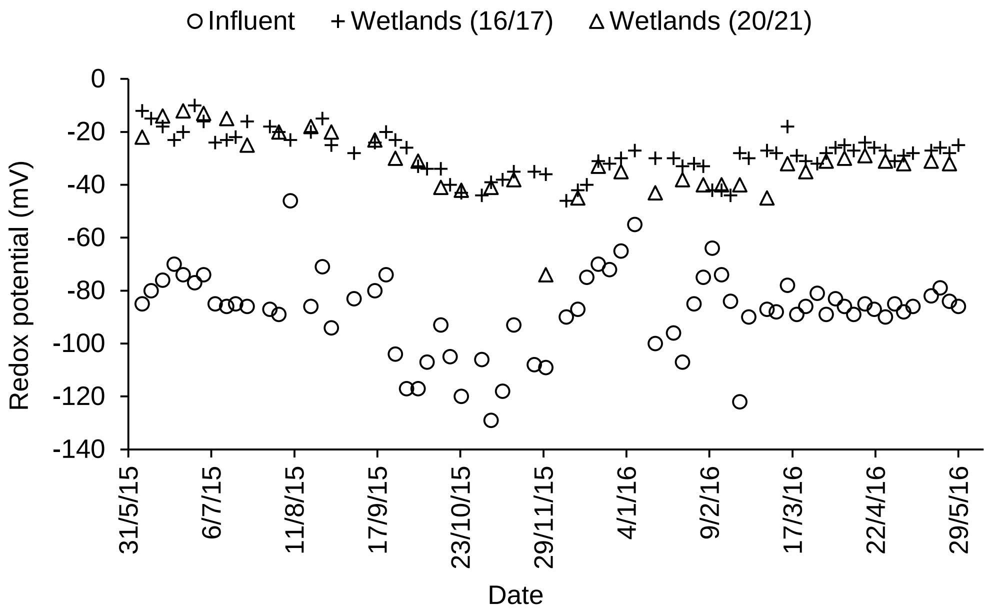


Figure 4.8 Overall variation for redox potential in the influent and effluent for Wetlands 16/17 and 20/21 (high concentration of AB113).

Effluent DO values for BR46 and AB113, in the case of low concentration, were in the range between 5.4 and 8.3 mg/l, as shown in Table 4.2, and were between 6.0 and 7.5 mg/l for the high concentration, as shown in Table 4.2. Furthermore, planted Wetlands 8/9 (Figure 4.9) and 6/7 (Figure 4.10), have the lowest DO values during the whole period of experimental work when compared with unplanted Wetlands 4 and 5 (low resting and high contact times), and planted Wetlands 10/11 and 12/13 (high resting and low contact times), respectively, because of the presence of the plants and the higher period of contact time. In the case of high concentration for both dyes, BR46 and AB113, Wetlands 18/19 and 20/21 (high resting and contact times) have the lower effluent DO values when compared with Wetlands 14/15 and 16/17 (low resting and contact times), as shown in Figure 4.11 and Figure 4.12, respectively. However, there is the possibility that the effluent samples became aerated between taking the samples and the corresponding measurements. Nevertheless, these findings indicate that dye degradation may have taken place in both aerobic and anaerobic environments.

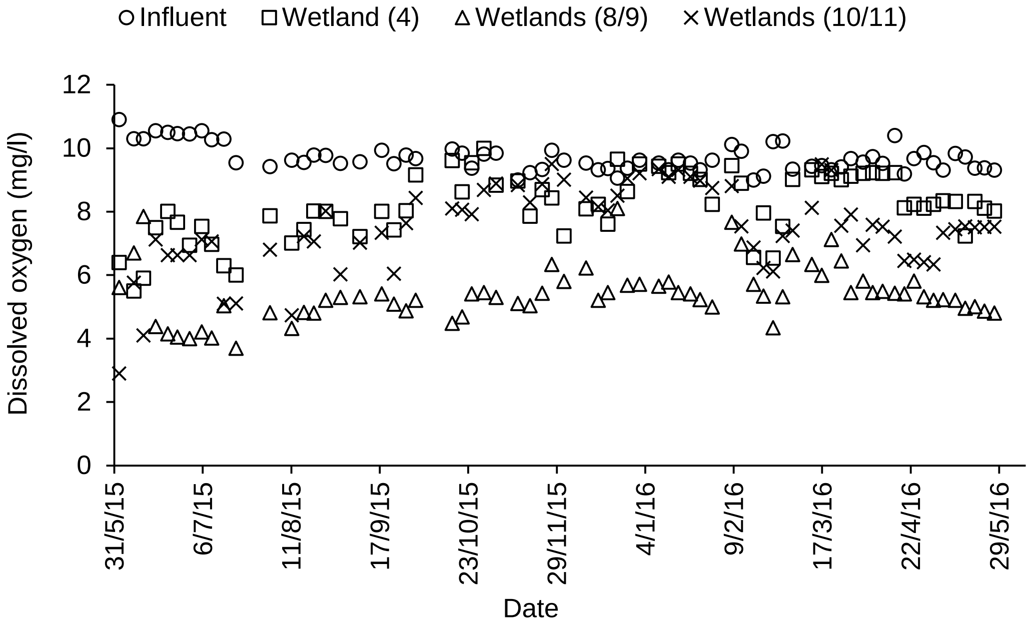


Figure 4.9 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

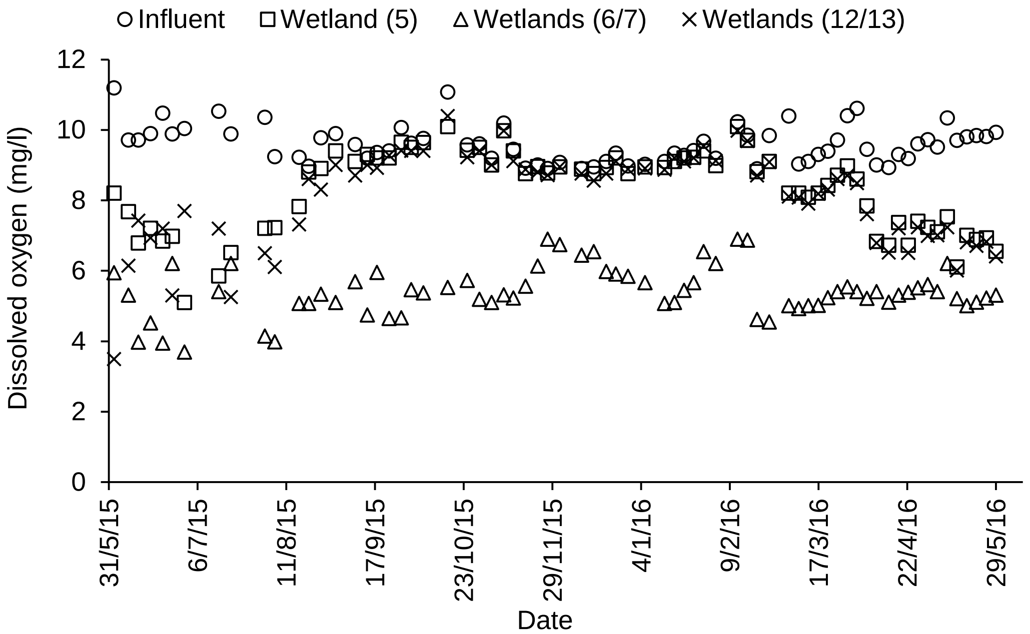


Figure 4.10 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 5, 6/7 and 12/13 (low concentration of AB113).

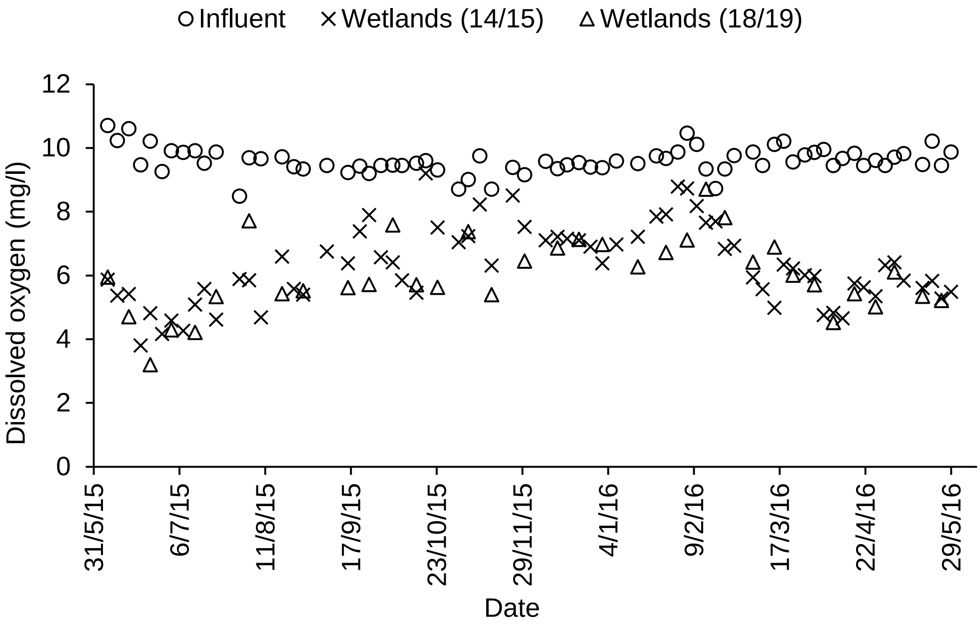


Figure 4.11 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 14/15 and 18/19 (high concentration of BR46).

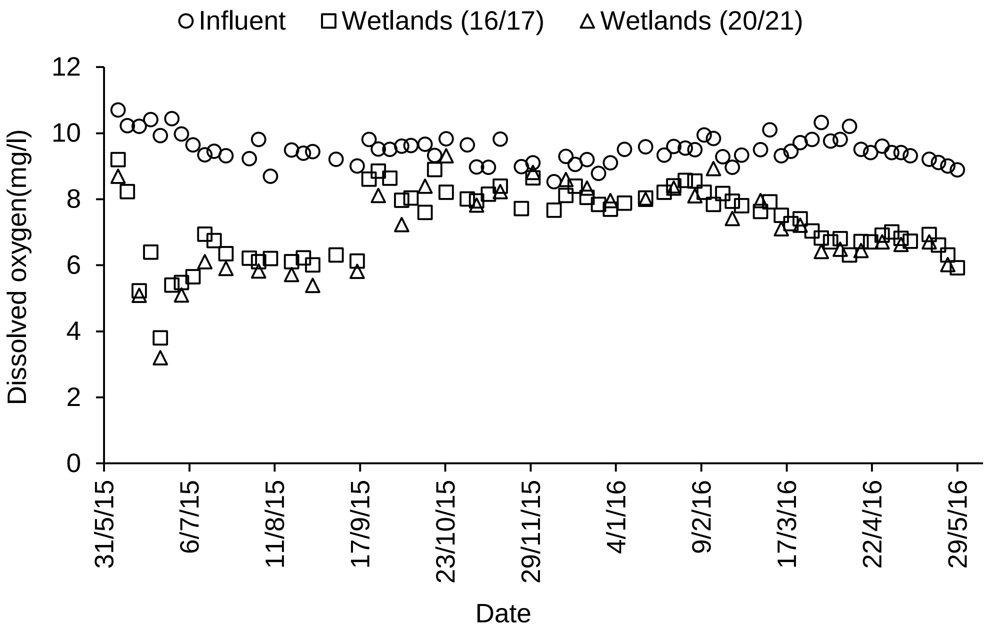


Figure 4.12 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 16/17 and 20/21 (high concentration of AB113).

4.6 Electrical Conductivity, Total Suspended Solids and Turbidity

Electrical conductivity can be used as an indirect measure of the charge (or the ion-carrying species) in the wetland outflow (Islam et al., 2011). EC value can be used as an indicator of other water quality problems. Any sudden increase in EC value indicates that there is a source of dissolved ions in the wetland filter (Kumar and Chopra, 2012). In comparison, all the effluent values for all wetlands in the case of low and high concentrations for both dyes were compliant with the national effluent discharge quality standards set by the Government of Bangladesh, which state that the maximum effluent of EC for inland surface water, public sewer secondary treatment plant and irrigated land is 1200 µS/cm (Ahmed et al., 2002). Furthermore, Sri Lanka central environmental authority (2008) state that the maximum EC discharge on land for irrigation purposes is 2250 µS/cm. For the low concentration of both dyes (BR46 and AB113), as shown in Table 4.2, an inconsiderable increase was noted for planted Wetlands 10/11 and 12/13 (contact time of 48 hours) respectively, when compared to planted Wetlands 8/9 and the unplanted control Wetland 4 as well as planted Wetlands 6/7 and the unplanted control Wetland 5 (contact time of 94 h for each), respectively. For the high concentration, as shown in Table 4.2, in the case of dye AB113, the average value of EC in the effluent of Wetlands 20/21 (high resting and contact times) is the same as for the influent, while for Wetlands 16/17 (low resting and contact times), there is a decrease in the average value, if compared to the influent. In the case of dye BR46, there is a statically insignificant (p<0.05) increase in the average value for the effluent of Wetlands 14/15 (low resting and contact times; 389±99.73 µS/cm), and Wetlands 18/19 (high resting and contact times; 385±83.21 μS/cm), if compared to the influent (357±112.3 μS/cm), as shown in Table 4.2. Yalcuk and Dogdu (2014) have reported similar findings.

The measurement of TSS is an important factor for the design of water treatment facilities (Dzurik, 2003). The Clean Water Act (CWA) lists it as a conventional pollutant (Bell et al. 2011). For the dye BR46 in the case of low concentration, there is an increase in the effluent for unplanted Wetland 4 and planted Wetlands 10/11 (high resting and low contact times), while there is no increase in the average value for planted Wetlands 8/9 (low resting and high contact times), if compared with the influent, as shown in Table 4.2.

In the case of the high concentration scenario, good reduction was noted for Wetlands 14/15 and 18/19. The reduction in Wetlands 18/19 (high resting and contact times) is better than that in Wetlands 14/15 (low resting and contact times), as shown in Table 4.2. For the dye AB113 in the case of low concentration, increased values of TSS in the effluent were noted for the unplanted Wetland 5, and planted Wetlands 6/7, and 12/13. Only a slight increase was noted for Wetlands 6/7 (low resting and high contact times), while a higher increase was recorded for Wetlands 12/13 (high resting and low contact times), as shown in Table 4.2. In comparison during the whole period of study, as shown in Figures 4.13, 4.14 and 4.15 for low concentration of BR46, high concentration of BR46 and low concentration of AB113, respectively, all the effluent values for the wetlands were compliant with the national effluent discharge quality standards set by the Government of Bangladesh, which state the maximum effluent of TSS for inland surface water, public sewer secondary treatment plant and irrigated land are 150 mg/l, 500 mg/l and 200 mg/l, respectively (Ahmed et al., 2002). In the case of the high concentration of AB113, the effluent values for Wetlands 16/17 and

20/21 (Table 4.2) were compliant only with the standard for the public sewer secondary treatment plant, which is 500 mg/l and 600 mg/l as stated by the Government of Bangladesh (Ahmed et al., 2002) and the Government of India (1986), respectively. An increased value was noted for Wetlands 16/17 (low resting and contact times) and 20/21 (high resting and contact times). Increased values for Wetlands 20/21 were noted (392±253.13 mg/l), if compared to Wetlands 16/17 (371.7±177.57 mg/l), as shown in Table 4.2.

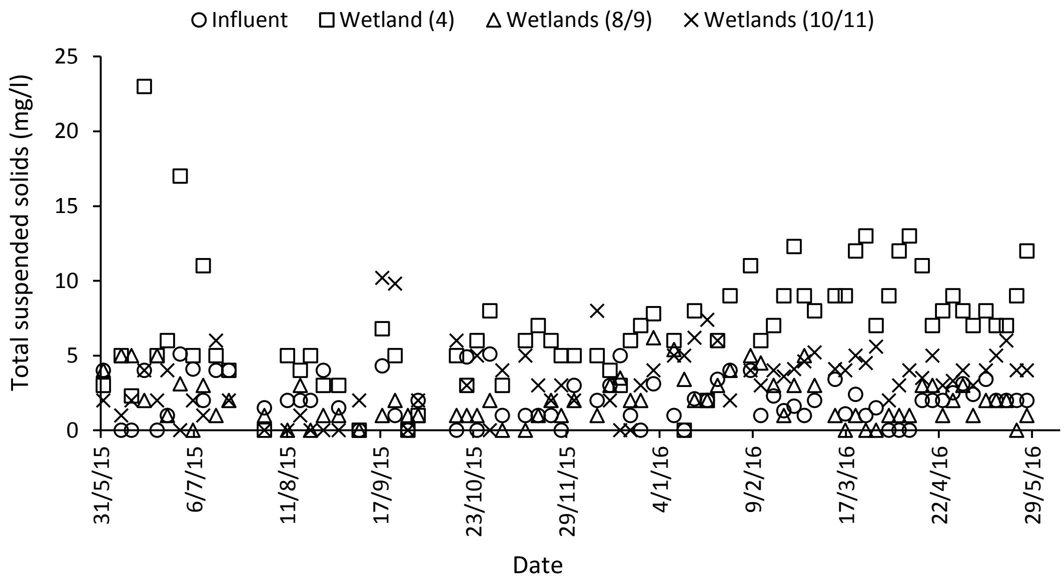


Figure 4.13 Overall variation for total suspended solids in the influent and effluent for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

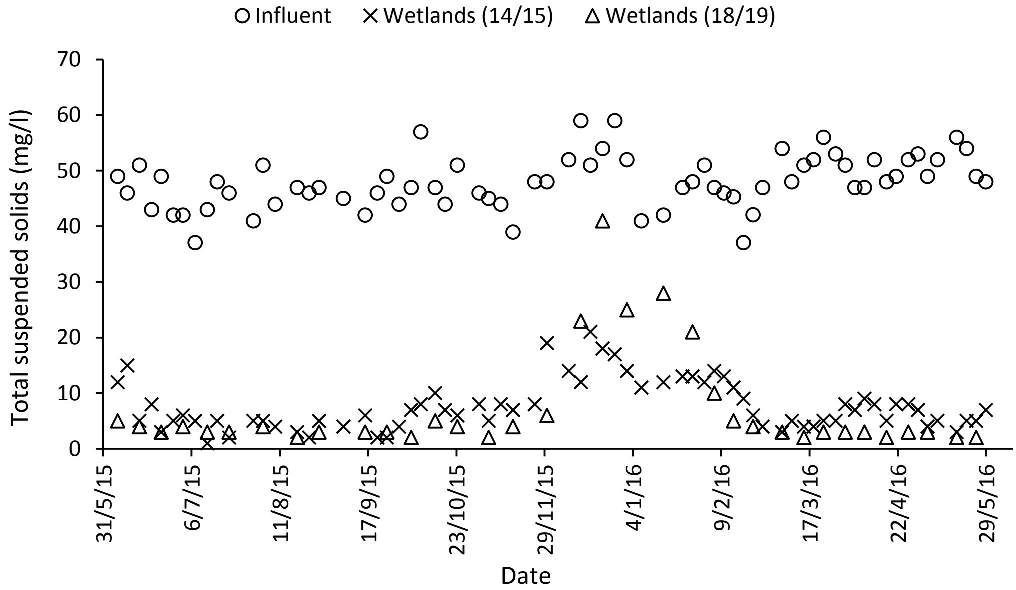


Figure 4.14 Overall variation for total suspended solids in the influent and effluent for Wetlands 14/15 and 18/19 (high concentration of BR46).

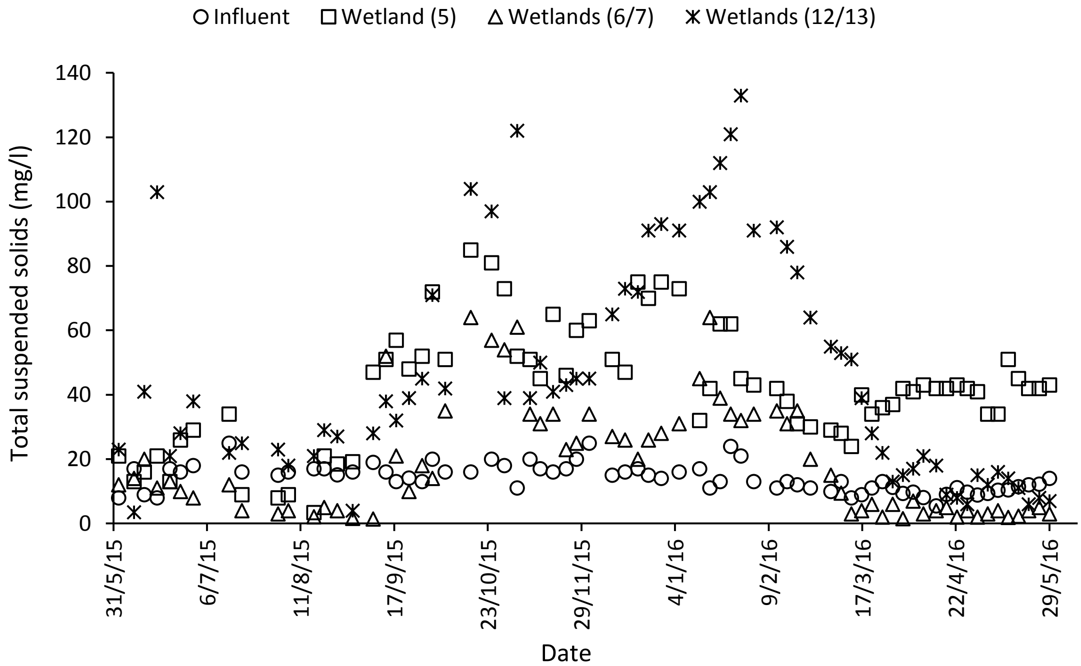


Figure 4.15 Overall variation for total suspended solids in the influent and effluent for Wetlands 5, 6/7 and 12/13 (low concentration of AB113).

Turbidity is often used to interpret the degree of clarity of water. It is a variable often applied as an indicator of the amount of suspended sediments and/or larger micro-organisms in water. A high turbidity of surface water may also indicate elevated concentrations of TSS, reduced algal populations, and potential harm to fish and other aquatic fauna (Postolache et al., 2007). Furthermore, a higher turbidity value can also increase the temperature of surface water as a result of increased absorption of heat from sunlight as well as leading to reduce light penetration, which affects photosynthesis (Håkanson, 2006).

For the dye BR46 ($p<0.05$) in the case of the low concentration scenario, reduction was noted for planted Wetlands 8/9 (low resting and high contact times, 3.0±1.33 mg/l), while there was an increase linked to unplanted Wetland 4 (8.8±7.12 mg/l) and planted Wetlands 10/11 (high resting and low contact times, 3.6±1.89 mg/l), if compared to the influent (3.5±2.89 mg/l), as shown in Table 4.2. The overall variation is shown in Figure 4.16. In the case of high concentrations, relatively good reduction was noted for Wetlands 14/15 (low resting and contact times; 6.6±2.20 mg/l) and 18/19 (high resting and contact times; 6.8±3.90 mg/l), when compared with the influent (16.6±3.10 mg/l), as shown in Table 4.2. The overall variation is shown in Figure 4.17. For the dye AB113 in the case of low concentration ($p<0.05$), increased values in the effluent were noted for unplanted Wetland 5 (39.2±27.40 mg/l), planted Wetlands 6/7 (19.1±16.11 mg/l) and planted Wetlands 12/13 (46.8±32.67 mg/l), if compared to the influent (7.4±5.67 mg/l). A greater increase was recorded for Wetlands 12/13 (high resting and low contact times), if compared to Wetlands 6/7 (low resting and high contact times), as shown in Table 4.2. The overall variation is shown in Figure 4.18. In the case of the high concentration scenario, a reduction was noted for Wetlands 16/17 (low resting and contact times; 208.3±196.67 mg/l; $p<0.05$), while there was an increase in the average value for Wetlands 20/21 (high resting and contact times; 224±202.69 mg/l; $p \geq 0.05$), if compared to the influent (213.4±157.62 mg/l; $p<0.05$), as shown in Table 4.2. The overall variation is shown in Figure 4.19.

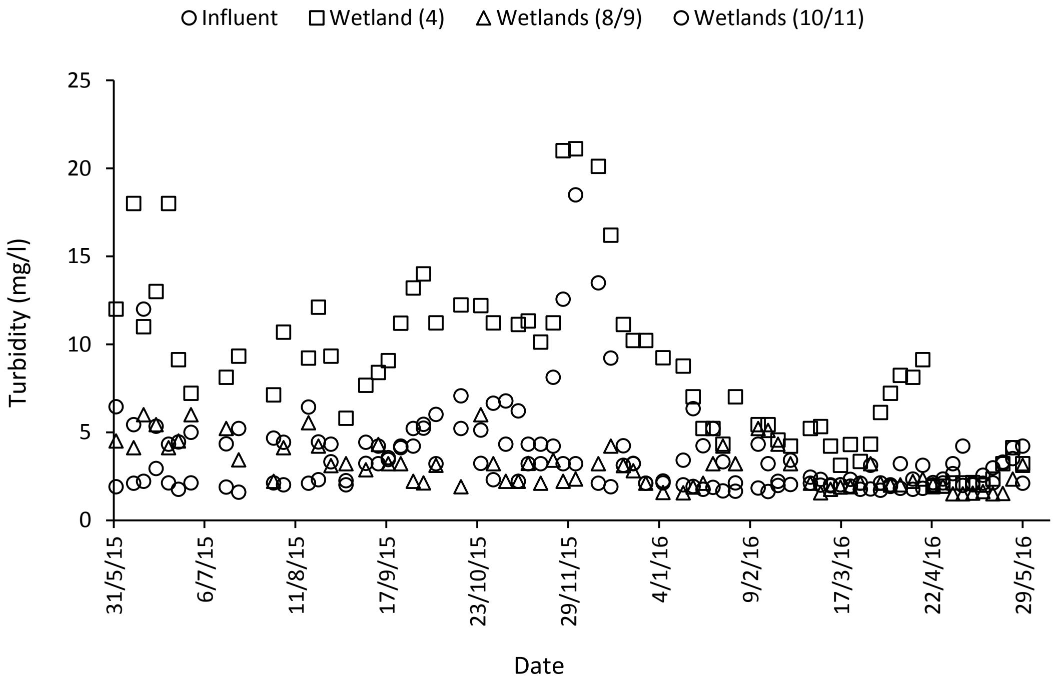


Figure 4.16 Overall variation for turbidity in the influent and effluent for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

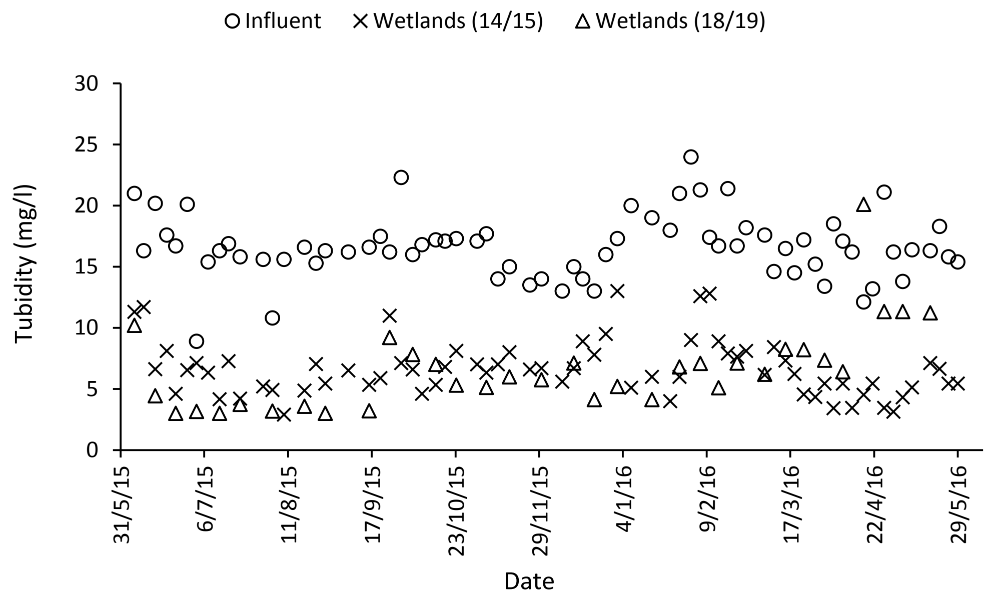


Figure 4.17 Overall variation for turbidity in the influent and effluent for Wetlands 14/15 and 18/19 (high concentration of BR46).

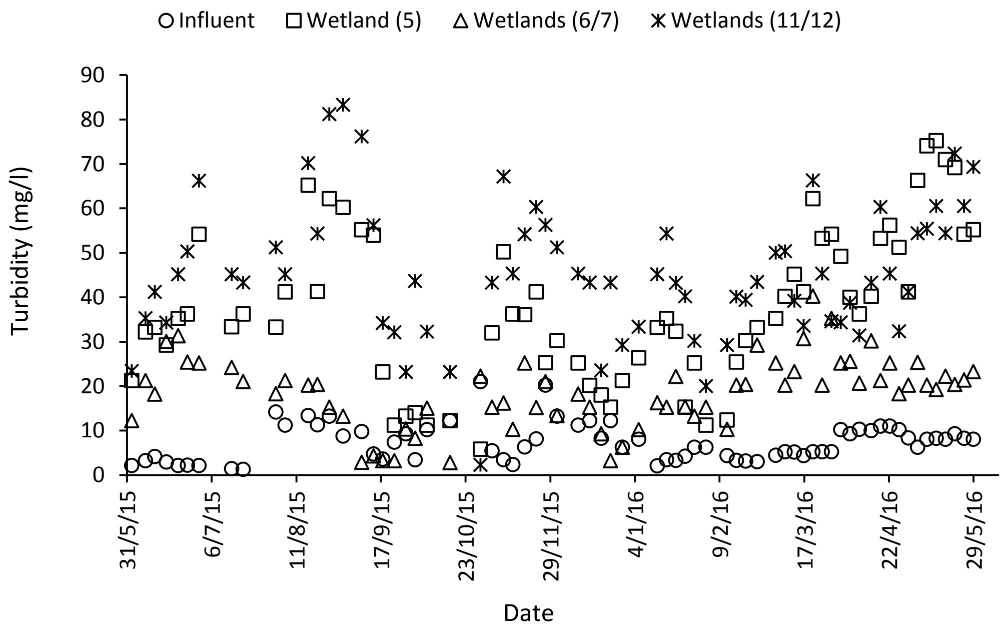


Figure 4.18 Overall variation for turbidity in the influent and effluent for Wetlands 5, 6/7 and 12/13 (low concentration of AB113).

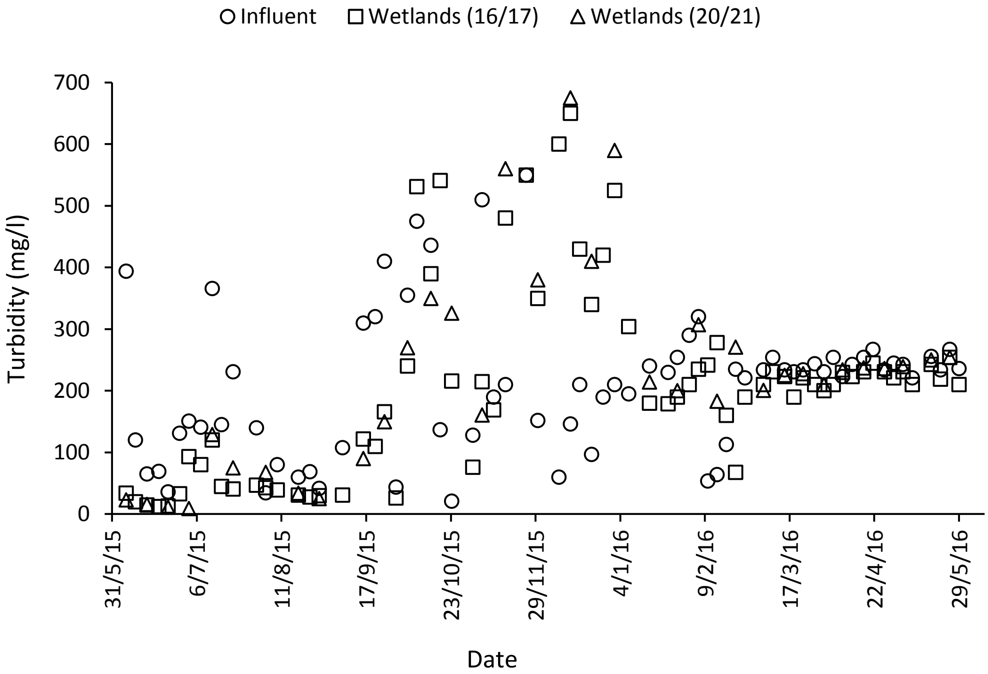


Figure 4.19 Overall variation for turbidity in the influent and effluent for Wetlands 16/17 and 20/21 (high concentration of AB113).

Many researchers confirmed that vertical-flow wetlands have a relatively poor ability to remove TSS and turbidity (Lin et al., 2005, Bulc and Ojtrsek, 2008). Regarding this study, for the low concentration of BR46, a long contact time (94 h) was better than a short (48 h) one in reduction of TSS and turbidity. While for high concentrations, the short contact time was better than the long contact time. In case of low and high concentrations for AB113, the four-day contact time was not enough to reduce TSS and turbidity.

4.7 pH Value

The measuring of pH is very important due to pH conditions having a sensitive impact on the outflow quality including: nutrients, COD and TSS in constructed wetlands. The sensitive impact comes from its effect on the ability of microbial populations to degrade pollutants (Eke and Scholz, 2008; Lavrova and Koumanova, 2013; Paing et al., 2015). In general, all the effluent pH values for all wetlands in the case of low and high concentrations of both dyes (BR46 and AB113) during the whole period of experimental work, as shown in Figure 4.20, Figure 4.21, Figure 4.22 and Figure 4.23, respectively, were compliant with the national effluent discharge quality standards set by all consortia and brand guidelines such as the Government of Bangladesh and STWI, which state the range of the effluent pH value for inland surface water, public sewer secondary treatment plant and irrigated land is between 6 and 9 (Ahmed et al., 2002; STWI, 2012). For the low concentration of the dye BR46, the average influent pH value was 7.2. A slight increase in the effluent pH values of 0.16 and 0.03 was noted for unplanted control Wetland 4 and planted Wetlands 10/11 (low resting and high contact times), respectively, while there was a decrease of 0.31 for planted Wetlands 8/9 (high resting and low contact times), as shown in Table 4.2. For the high concentration, the average influent value was 6.49. Increases of 0.19 and 0.57 were noted for Wetlands 14/15 (low resting and contact times) and Wetlands 18/19 (high resting and contact times), respectively, as shown in Table 4.2. In the case of the dye AB113 for the low concentration scenario, the average influent pH value was 7.32. A slight pH increase of 0.11 was noted for unplanted control Wetland 5, while the pH value decreased for planted Wetlands 6/7 and Wetlands 12/13 by 0.44 and 0.15, respectively, as shown in Table 4.2. For the high concentration, the average influent pH value was 8.39. The pH value decreased for Wetlands 16/17 (low resting and contact times) and 20/21 (high resting and contact times) by 1.10 and 1.06, respectively, as shown in Table 4.2. Regarding the effect of plants on the pH value for both dyes BR46 and AB113, differences of 0.47 and 0.55 were found, as shown in Table 4.2, between the unplanted control Wetlands 4 and 5, and planted Wetlands 8/9 and 6/7 (all these wetlands have the same conditions), respectively. These results suggest that presence of plants has an effect on pH modification in vertical-flow constructed wetland systems used in this study, contrary to the suggestion of Kadlec and Wallace (2000) that the pH modification in this type of constructed wetlands is most likely as a result of interaction between the media and its biofilm, rather than of the plants. Furthermore, there were no changes in pH values in contrast to the findings obtained by Wieder (1989), in which he surveyed 128 constructed wetlands treating acid coal mine wastewater and found the difference between the effluent and the influent to be 0.11 (the influent pH was 2.5). Mitsch and Wise (1998) corroborated this finding, when they found that the difference between the influent and the effluent was 0.52 (the influent pH was 2.83). Furthermore, Kadlec and Wallace (2008) stated that the available pH value range for most degraded bacteria is 4-9.5. Nevertheless, these results indicate the ability of macrophytes such as *P. australis* to modify pH conditions in the rhizosphere, confirming results by (Brix et al., 2002). However, these researches used a different wetland plant (*Typha angustifolia L.*).

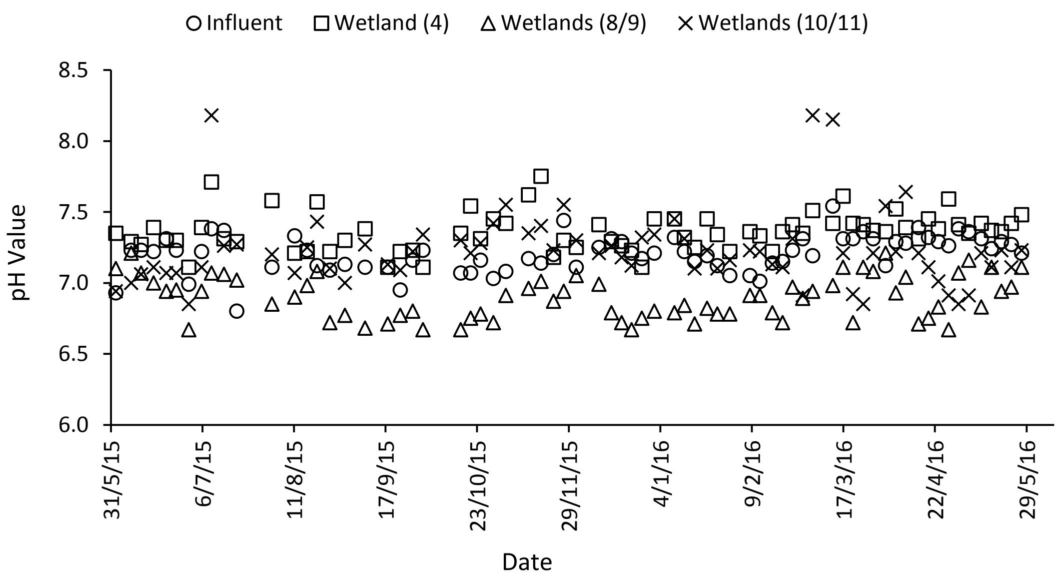


Figure 4.20 Overall variation in pH of the influent and effluent for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

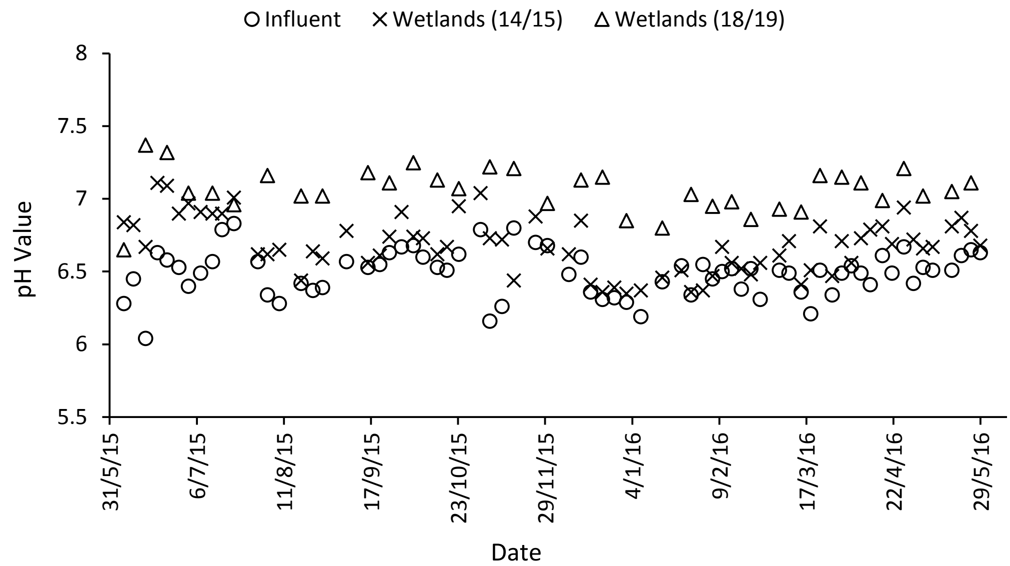


Figure 4.21 Overall variation in pH of the influent and effluent for Wetlands 14/15 and 18/19 (high concentration of BR46).

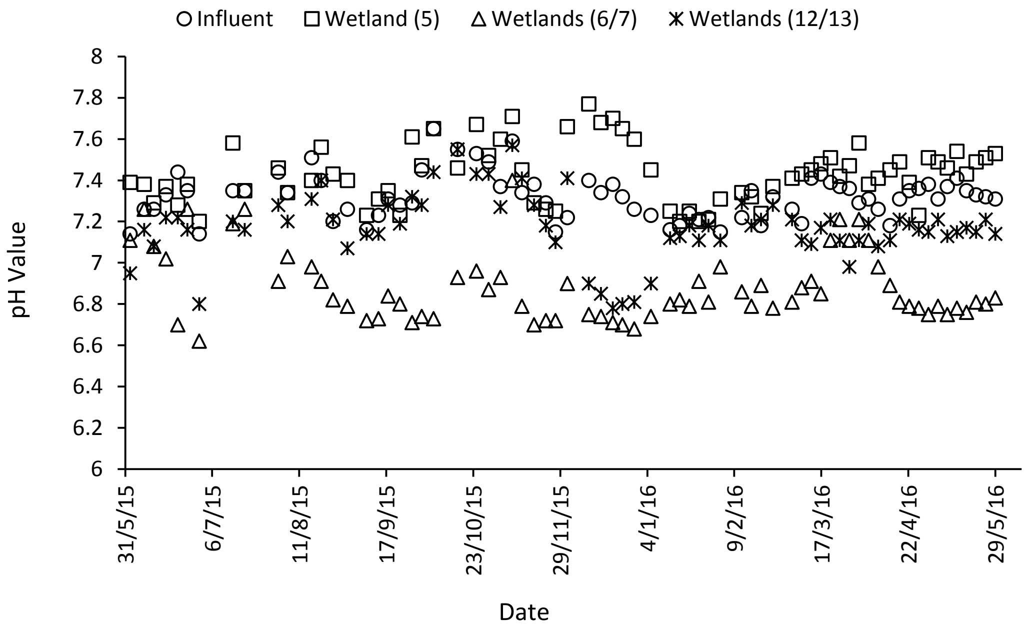


Figure 4.22 Overall variation in pH of the influent and effluent for Wetlands 4, 6/7 and 12/13 (low concentration of AB113).

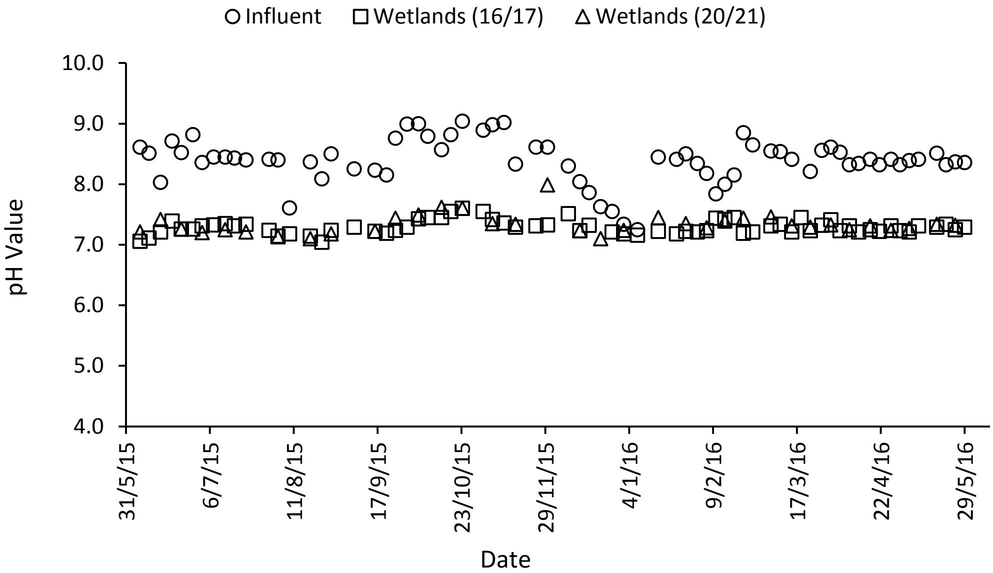


Figure 4.23 Overall variation in pH of the influent and effluent for Wetlands 16/17 and 20/21 (high concentration of AB113).

Table 4.3 Influent and effluent water quality characteristics for general physical and chemical variables.

Characteristics				pH			Redox potential (mV)			Dissolved oxygen (mg/l)		
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
BR46	In	N/A	70	6.80	7.54	7.2±0.147	-39	5	-25±7.7	9.0	10.9	9.7±0.46
	Out	4	70	7.11	7.75	7.36±0.137	-43	-14	-29±6.35	5.5	10.0	8.2±1.12
	Out	8 and 9	70	6.67	7.21	6.89±0.148	-22	8	-5±6.79	3.7	8.1	5.4±0.92
	Out	10 and 11	70	6.85	8.18	7.23±0.212	-40	-7	-22±9.25	2.9	9.5	7.5±1.54
	In	N/A	70	6.04	6.83	6.49±0.179	-6	28	12±8.06	8.7	10.7	9.6±0.41
	Out	14 and 15	70	6.35	7.11	6.68±0.217	-17	17	4±7.53	3.8	9.2	6.3±1.34
	Out	18 and 19	35	6.65	7.37	7.06±0.163	-24	4	-13±7.58	3.2	8.7	6.0±1.29
AB113	In	N/A	70	7.14	7.65	7.32±0.122	-43	-21	-30±5.4	8.9	11.2	9.6±0.55
	Out	5	70	7.20	7.77	7.43±0.165	-51	-19	-32±6.5	5.1	10.1	8.3±1.38
	Out	6 and 7	70	6.62	7.4	6.88±0.184	-33	5	-6±8.2	3.7	6.9	5.4±0.77
	Out	12 and 13	70	6.78	7.57	7.17±0.199	-40	-9	-23±8.2	3.5	10.4	8.1±1.45
	In	N/A	70	7.25	9.04	8.39±0.432	-129	-46	-88±18.2	8.2	10.7	9.5±0.49
	Out	16 and 17	70	7.04	7.6	7.29±0.118	-46	-10	-28±9.6	5.4	9.9	7.5±1.19
	Out	20 and 21	35	7.10	7.99	7.33±0.202	-74	-12	-32±14	3.9	10.0	7.1±1.76

Table 4.2 continued

Characteristics				Total suspended solids (mg/l)			Turbidity (NTU)			Electrical conductivity (μS/cm)		
BR46	In	N/A	70	0	5.1	2.0±1.51	1.6	18.5	3.5±2.89	90	270	159±51.6
	Out	4	70	0	23.0	6.8±5.81	1.9	32	8.8±7.12	70	483	193±79.1
	Out	8 and 9	70	0	6.2	2.0±1.88	1.5	6	3.0±1.33	103	749	257±97.4
	Out	10 and 11	70	0	10.2	3.4±3.33	1.9	12	3.6±1.89	82	419	180±62.4
	In	N/A	70	37.1	59.0	47.9±5.11	8.9	24	16.6±3.1	147	701	357±112.3
	Out	14 and 15	70	1.1	21.3	7.6±4.70	2.9	13	6.6±2.2	253	647	389±99.73
	Out	18 and 19	35	2.3	41.0	6.9±8.41	2.9	20.1	6.8±3.9	251	535	385±83.21
AB113	In	N/A	70	5.5	25.0	14.1±5.40	1.3	21.0	7.4±5.67	70	350	220±104.1
	Out	5	70	3.4	85.0	41.9±23.07	5.8	134.0	39.2±27.40	75	396	242±87.9
	Out	6 and 7	70	1.4	64.0	18.8±15.92	2.8	57.0	19.1±16.11	114	448	294±85.4
	Out	12 and 13	70	3.5	133.0	46.5±33.60	2.3	128.0	46.8±32.67	83	430	235±99.6
	In	N/A	70	119.0	843.0	364.8±195.47	21	660	213.4±157.62	465	976	635±123.2
	Out	16 and 17	70	121.0	706.0	371.7±177.57	12	650	208.3±196.67	456	854	624±99.1
	Out	20 and 21	35	67.0	863.0	392±253.13	9	675	224±202.69	502	844	635±93.3

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

4.8 Nutrient Reduction

The reduction of ortho-phosphate-phosphorous is controlled by chemical and physical adsorption, sedimentation, plant uptake, precipitation and microbial uptake in constructed wetland systems (Brix, 1997; Vymazal, 2007, 2010b; Johari et al., 2016). Moreover, many researchers reported that reduction efficiency of phosphorous compounds is generally poor with constructed wetlands (Choudhary et al., 2011; Lavrova and Koumanova, 2013; Ge et al., 2016). For the low concentrations of BR46 and AB113, the reduction percentages in planted Wetlands 8/9 (89%) as well as planted Wetlands 6/7 (30%), which have low resting and high contact times, were significantly ($p<0.05$) better than those of control unplanted Wetlands 4 (78%) and 5 (27%) as well as planted Wetlands 10/11 (68%) and 12/13 (14%), which have high resting and low contact times, as shown in Table 4.3. For the high concentrations of BR46, the reduction percentages of Wetlands 18/19 (high resting and contact times; 81%) were significantly ($p<0.05$) better than those for Wetlands 14/15 (low resting and contact times; 70%), while for AB113, the reduction percentages of Wetlands 16/17 (low resting and contact times; 78%) were also significantly ($p<0.05$) better than those for Wetlands 20/21 (high resting and contact times; 71%), as shown in Figure 4.24.

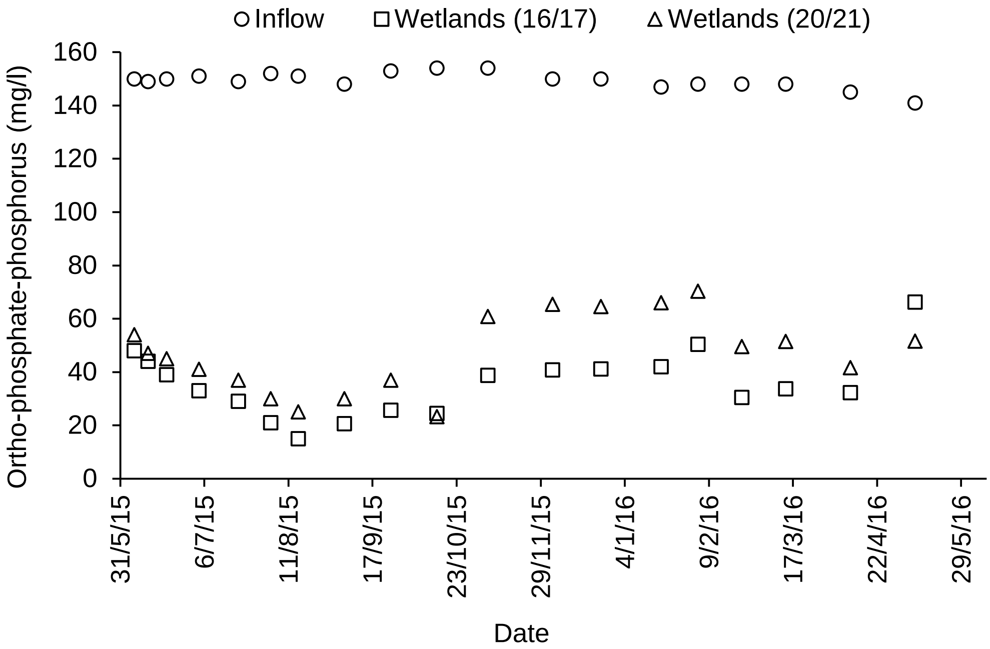


Figure 4.24 Influent and effluent concentrations of ortho-phosphate-phosphorus for Wetlands 16/17 and 20/21 (high concentration of AB113).

A typical standard set by environment agencies for PO_4-P reduction concerning secondary wastewater treatment is 2 mg/l (Royal Commission on Sewage Disposal, 1915). Effluent ortho-phosphate-phosphorus values were compliant with the standard in the case of low concentration of BR46 and the values were relatively high in the case of low concentration of AB113. In the case of high concentration for both dyes, BR46 and AB113, the effluent values of PO4-P were much higher than the standard value.

Nitrification and denitrification are the main reduction mechanisms of nitrogen in constructed wetlands and these mechanisms include two-step processes: firstly, ammonium is oxidized to nitrite followed by oxidation of nitrite to nitrate, these are known as nitrification process. Secondly, the denitrification process includes nitrate being reduced to gaseous nitrogen (Schaechter, 2009; Kessel et al., 2015; Song et al., 2015; Yang et al., 2016). As indicated in Table 4.3, the concentrations of NH_4-N for both dyes are very low due to their chemical structures (Yalcuk and Dogdu, 2014). For the high concentration of dyes, there are no significant (*$p \geq 0.05$*) differences between wetlands. For the low concentration of the dye BR46, the reductions for planted Wetlands 8/9 (low resting and high contact times) were significantly (*$p < 0.05$*) better than those for the corresponding reduction of unplanted Wetland 4, while there were increases in reduction for planted Wetlands 10/11 (high resting and low contact times). For the low concentration of the dye AB113, the reduction percentages with respect to planted Wetlands 12/13 (high resting and low contact times) were better than those for unplanted Wetland 4, and planted Wetlands 6/7 (low resting and high contact times), which was statistically insignificant (*$p \geq 0.05$*). The previous results indicate that aeration availability plays a major function in determining the performance of higher nitrogen reduction. These findings are agreed by many researchers (Vymazal, 2007; H Wu et al., 2011; Fan et al., 2013c).

Regarding NO_3-N reduction for the high concentration of both dyes (BR46 and AB113), the reduction percentages for wetlands with long contact times are better than those for wetlands having short contact times, confirming findings by (Kampf and Claassen, 2005). Regarding the low concentration of BR46, the reduction percentages for planted Wetlands 8/9 (low resting and high contact times) were better than those for unplanted Wetland 4, and planted Wetlands 10/11 (high resting and low contact times). For the low concentrations of AB113, there were significantly (*$p < 0.05$*) better reduction percentages for planted Wetlands 6/7 (low resting and high contact times), but there were increases in the effluents regarding the NO_3-N reduction of planted Wetlands 12/13 (high resting and low contact times) due to the presence of carbon under aerobic conditions (Jie et al., 2009). The reduction percentages for the high concentrations of dyes were better than those for the low concentrations of dyes due to a corresponding increase in carbon percentage (Songliu et al., 2009; Lavrova and Koumanova, 2014; Shen et al., 2015), as shown in Table 4.3. The effluent NO_3-N values for all wetlands in the case of low and high concentration for BR46 and AB113 were compliant with the traditional UK standard, which states that NO_3-N reduction from secondary wastewater is 50 mg/l (Royal Commission on Sewage Disposal, 1915).

Table 4.4 Influent and effluent water quality characteristics for nutrients.

Characteristics				Ammonia-nitrogen (mg/l)				Nitrate-nitrogen (mg/l)				Ortho-phosphate-phosphorus (mg/l)			
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ± SD	Reduction (%)	Min.	Max.	Mean ± SD	Reduction (%)	Min.	Max.	Mean ± SD	Reduction (%)
BR46	In	N/A	20	0.54	0.75	0.62±0.07	N/A	17.9	18.0	18.0±0.03	N/A	5.3	6.3	5.9±0.33	N/A
	Out	4	20	0.45	0.68	0.55±0.09	11	4.5	16.9	9.8±3.98	45	0.9	1.7	1.3±0.27	78
	Out	8 and 9	20	0.21	0.51	0.36±0.11	42	2.5	13.9	7.5±3.69	58	0.19	1.3	0.7±0.4	89
	Out	10 and 11	20	0.49	0.90	0.68±0.12	-10	3.5	16.0	8.9±3.83	50	0.5	2.8	1.9±0.83	68
	In	N/A	20	0.98	1.04	1.02±0.02	N/A	19.3	20.0	19.7±0.18	N/A	55.0	87.0	64.0±8.50	N/A
	Out	14 and 15	20	0.25	0.47	0.35±0.08	66	1.5	16.2	6.2±5.43	69	6.5	47.0	19.3±13.20	70
	Out	18 and 19	20	0.21	0.46	0.31±0.08	70	0.6	12.1	4.9±4.46	75	1.9	43.6	12.1±14.60	81
AB113	In	N/A	20	0.63	0.70	0.66±0.02	N/A	8.4	08.5	8.5±0.03	N/A	6.9	8.3	7.6±0.43	N/A
	Out	5	20	0.37	0.62	0.48±0.08	27	10.5	16.3	12.1±1.38	-42	4.8	6.4	5.6±0.49	27
	Out	6 and 7	20	0.35	0.81	0.52±0.14	21	3.9	6.5	4.9±0.98	42	4.1	6.4	5.3±0.58	30
	Out	12 and 13	20	0.35	0.56	0.47±0.06	29	10.0	15.1	11.6±1.45	-36	5.7	7.7	6.6±0.51	14
	In	N/A	20	2.21	2.77	2.33±0.16	N/A	19.4	20.0	19.8±0.21	N/A	148.0	154.0	151.0±2.10	N/A
	Out	16 and 17	20	0.73	1.54	1.00±0.24	57	0.9	15.2	6.0±5.8	70	15.0	50.4	33.1±11.30	78
	Out	20 and 21	20	0.80	1.97	1.10±0.32	53	0.8	15.2	5.3±5.10	73	23.3	70.3	43.5±15.30	71

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

4.9 Dye and Chemical Oxygen Demand Reductions

The percentage reduction of dye concentrations is shown in Table 4.4. The degradation of azo dyes takes place both in aerobic and anaerobic conditions via various processes involving enzymes and/or chemical reduction (Pandey et al., 2007; Khehra et al., 2005a; Saratale et al., 2011). BR46 is theoretically easier to degrade than AB113 due to its lower molecular weight.

There is no significant (*$p \geq 0.05$*) difference in dye reduction for the low concentration of BR46 between control Wetland 4, planted Wetlands 8/9 (low resting and high contact times) and planted Wetlands 10/11 (high resting and low contact times), as shown in Figure 4.25. It follows that the presence of plants does not affect dye reduction. In comparison, for AB113, the dye reduction in planted Wetlands 6/7 was significantly (*$p<0.05$*) better than that for unplanted control Wetland 5 (Figure 4.26) due to the presence of plants (Keskinkan and Lugal Göksu, 2007).

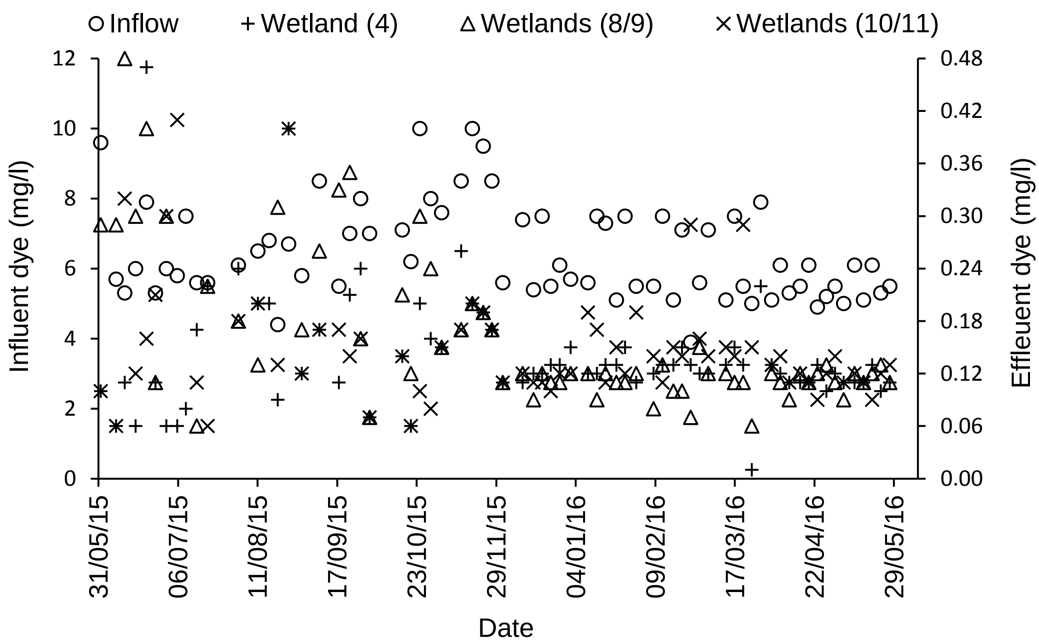


Figure 4.25 Influent and effluent dye concentrations for Wetlands 4, 8/9 and 10/11 (low concentration of BR46).

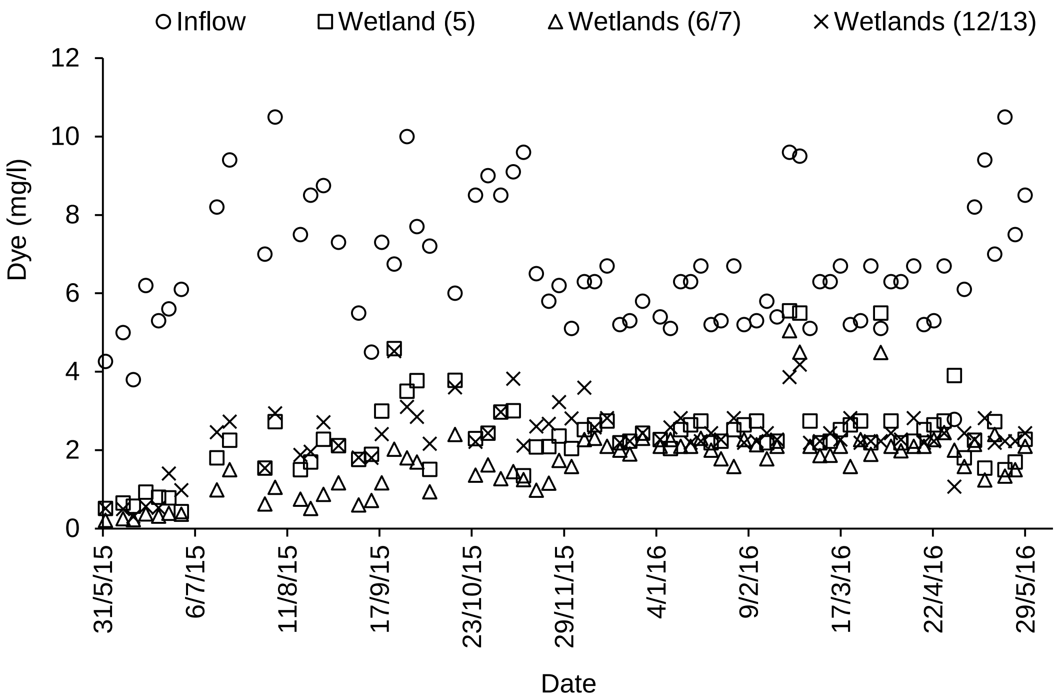


Figure 4.26 Influent and effluent dye concentrations for Wetlands 5, 6/7 and 12/13 (low concentration of AB113).

For high concentrations, the best percentage reductions for BR46 were noted for Wetlands 18/19 (high resting and contact times), which were statistically significantly (*p<0.05*) different, if compared to those of Wetlands 14/15 (low resting and contact times), as shown in Figure 4.27. Wetlands with a low loading rate performed better than those with a high loading rate in terms of a dye reduction percentage rate but the influent mass loading rates for Wetlands 14/15 (high loading rate) and Wetlands 18/19 (low loading rate) were 582.9±12.5 $g.m^{-2}.d^{-1}$ and 291.5±6.3 $g.m^{-2}.d^{-1}$, respectively, as shown in Table 4.5. The final decision about which one is better (low or high loading rate) depends on the design conditions of the constructed wetlands in the field. For the dye AB113, there was no significant (*p≥0.05*) difference in the reduction of dyes between Wetlands 16/17 (low resting and contact times), and Wetlands 20/21 (high resting and contact times), as shown in Table 4.4. The influent mass loading rates for Wetlands 16/17 (high loading rate) and Wetlands 20/21 (low loading rate) were 616.9±88.8 $g.m^{-2}.d^{-1}$ and 308.4±44.4 $g.m^{-2}.d^{-1}$, respectively, as shown in Table 4.5. These results indicate that wetlands with a high loading rate are better than wetlands with a low loading rate.

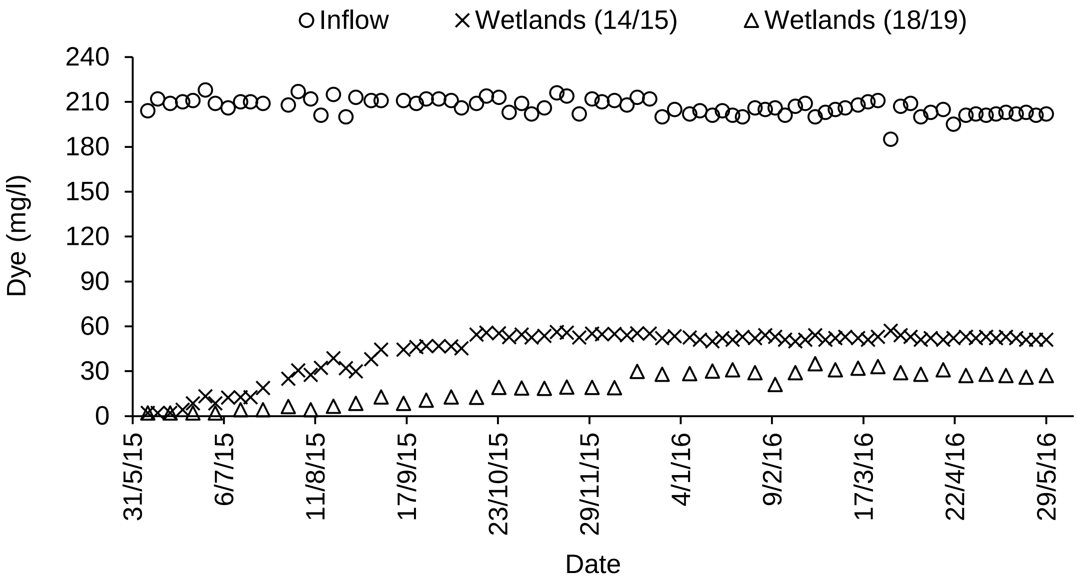


Figure 4.27 Influent and effluent dye concentrations for Wetlands 14/15 and 18/19 (high concentration of BR46).

In textile wastewater, measurement of COD is very important to evaluate organic matter concentration in constructed wetlands. Its reduction mechanisms include anaerobic, filtration, aerobic, adsorption and microbial metabolism (Vymazal, 1998; Song et al., 2006; Stefanakis et al., 2014). For COD reduction concerning low concentrations of dyes (AB113 and BR46), as shown in Table 4.4, planted wetlands with a long contact time had the best COD reduction, if compared to the control and other wetlands having short contact times. Cheng et al., 2011) operated two series of vertical-flow constructed wetlands (one planted and the other unplanted) under different C: N: P ratios. The results they obtained showed that the reduction of COD depended on the presence and development of plants. The reduction rate was higher in the planted wetlands.

Concerning high concentrations of dye BR46, the best reduction percentages for COD were noted for Wetlands 18/19 (high resting and contact times), which were statistically significantly ($p<0.05$) different from those of Wetlands 14/15 (low resting and contact times). Wetlands with a low loading rate performed better than those with a high loading rate as a COD concentration (Table 4.4), but the influent mass loading rate values for Wetlands 14/15 (high loading rate) and Wetlands 18/19 (low loading rate) were 726.9±30.6 $g.m^{-2}.d^{-1}$ and 363.4±15.3 $g.m^{-2}.d^{-1}$, respectively, as shown in Table 4.5. The final decision about which one is better depends on the design conditions of the constructed wetlands in the field. Furthermore, the effluent COD values for both of them, low and high loading rate, were compliant with the national effluent discharge quality standards set by the Government of Bangladesh (most of the textile industries were built in developing countries), which state the maximum COD values for inland surface water, public sewer secondary treatment plant and irrigated land are 200 mg/l, 400 mg/l and 400 mg/l, respectively (Ahmed et al., 2002).

In the case of the dye AB113, there was no significant ($p\geq0.05$) difference in the reduction of COD between Wetlands 16/17 (low resting and contact times) and Wetlands 20/21 (high resting and contact times), as shown in Table 4.4. The influent mass loading rate values for Wetlands 16/17 (high loading rate) and Wetlands 20/21 (low loading rate) were 1626.4±94.7 $g.m^{-2}.d^{-1}$ and 813.2±47.3 $g.m^{-2}.d^{-1}$, respectively, as shown in Table 4.5. These results suggest that wetlands with a high loading rate are better than wetlands with a low loading rate. Furthermore, effluent COD values for both of them, low and high loading rate, were compliant for the purposes of public sewer secondary treatment plant and irrigated land (400 mg/l), and not compliant with the purpose of inland surface water (200 mg/l), according to the national effluent discharge quality standards set by the Government of Bangladesh (Ahmed et al., 2002).

Table 4.5 Dye and chemical oxygen demand (COD) reductions.

Characteristics				Dye concentration (mg/l)			
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ±SD	Reduction (%)
BR46	In	N/A	70	4.4	10.0	6.9±1.33	N/A
	Out	4	70	0.1	0.9	0.3±0.2	96
	Out	8 and 9	70	0.1	0.7	0.2±0.1	97
	Out	10 and 11	70	0.1	0.5	0.2±0.1	97
	In	N/A	70	200.0	218.0	209.3±4.5	N/A
	Out	14 and 15	70	2.0	56	37.7±19	82
	Out	18 and 19	35	2.0	29.8	12.5±9	94
AB113	In	N/A	70	3.5	10.5	6.6±1.8	N/A
	Out	5	70	0.1	4.6	1.9±1.0	71
	Out	6 and 7	70	0.1	4.5	1.3±1.1	80
	Out	12 and 13	70	0.3	4.5	2.1±1	68
	In	N/A	70	155.0	292.0	221.5±31.9	N/A
	Out	16 and 17	70	17.1	191.5	63±48	72
	Out	20 and 21	35	26.91	187.5	59±40	73
Characteristics				COD concentration (mg/l)			
BR46	In	N/A	20	11	32	22±6	N/A
	Out	4	20	2.8	19.5	11±5.4	50
	Out	8 and 9	20	0.3	13.8	7.2±4.3	67
	Out	10 and 11	20	0.7	16.3	9.1±5.1	59
	In	N/A	20	249	280	261±11	N/A
	Out	14 and 15	20	6	100	69±34	74
	Out	18 and 19	20	14	40	28±9	89
AB113	In	N/A	20	36.9	86.4	48.2±12.2	N/A
	Out	5	20	27.6	73	46±14.7	5
	Out	6 and 7	20	14	78	40.6±19.4	16
	Out	12 and 13	20	24	72	44.6±12.9	7
	In	N/A	20	543	610	584±34	N/A
	Out	16 and 17	20	117	454	271±103	54
	Out	20 and 21	20	126	362	262±86	55

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

Table 4.6 Dye and chemical oxygen demand (COD) loading rate.

Characteristics						Dye loading rate ($g.m^{-2}.d^{-1}$)	
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ±SD	Reduction (%)
BR46	In	N/A	70	12.3	27.9	19.2±3.7	N/A
	Out	4	70	0.3	2.5	0.8±0.6	96
	Out	8 and 9	70	0.3	1.9	0.6±0.3	97
	Out	10 and 11	70	0.3	1.4	0.6±0.3	97
	In	14 and 15	70	557	607	582.9±12.5	N/A
	Out	14 and 15	70	5.6	156	105.0±52.9	82
	In	18 and 19	35	278	303	291.5±6.3	N/A
	Out	18 and 19	35	2.8	41.5	17.4±12.5	94
AB113	In	N/A	70	9.7	29.2	18.4±5.0	N/A
	Out	5	70	0.3	12.8	5.3±2.8	71
	Out	6 and 7	70	0.3	12.5	3.6±3.1	80
	Out	12 and 13	70	0.8	12.5	5.8±2.8	68
	In	16 and 17	70	431	813	616.9±88.8	N/A
	Out	16 and 17	70	47.6	533.3	175.5±133.7	72
	In	20 and 21	35	215	406	308.4±44.4	N/A
	Out	20 and 21	35	37.5	261	82.2±55.7	73
Characteristics						COD loading rate ($g.m^{-2}.d^{-1}$)	
BR46	In	N/A	20	30.6	89.1	61.3±16.7	N/A
	Out	4	20	7.8	54.3	30.6±15.0	50
	Out	8 and 9	20	0.8	38.4	20.1±12.0	67
	Out	10 and 11	20	1.9	45.4	25.3±14.2	59
	In	14 and 15	20	694	780	726.9±30.6	N/A
	Out	14 and 15	20	16.7	278.5	192.2±94.7	74
	In	18 and 19	20	347	390	363.4±15.3	N/A
	Out	18 and 19	20	19.5	55.7	39.0±12.5	89
AB113	In	N/A	20	102.8	240.6	134.2±34.0	N/A
	Out	5	20	76.9	203.3	128.1±40.9	5
	Out	6 and 7	20	39.0	217.2	113.1±54.0	16
	Out	12 and 13	20	66.8	200.5	124.2±35.9	7
	In	16 and 17	20	1512	1699	1626.4±94.7	N/A
	Out	16 and 17	20	328	1264	754.7±286.9	54
	In	20 and 21	20	756	849	813.2±47.3	N/A
	Out	20 and 21	20	176	504	364.9±119.8	55

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

4.10 Seasonal Treatment Performance of Wetlands on Dye Reduction

Azo dye decolourization is achieved under aerobic, anaerobic or anoxic conditions (O'neill et al., 2000; Sponza and Işik, 2002; van der Zee, 2002; Davies et al., 2006) through the biodegradation mechanism (Kodam et al., 2005; Moosvi et al., 2005; Pandey et al., 2007). Several publications have stated that micro-organisms are affected by the temperature; they are not active and energetic in cold periods in constructed wetland systems with the best treatment performance occurring during higher temperatures (Song et al., 2006; Imfeld, 2009; Sani et al., 2013). These findings agree with results obtained in this study in the case of low concentration of AB113 and high concentration of BR46 ($p<0.05$), the best reduction percentage rates were in the summer season, as shown in Table 4.6. In the case of high concentration of AB113, the best reduction percentage rates ($p<0.05$) were in the autumn season as a result of well-established microbial populations and plants, as confirmed by many publications (Scholz et al., 2002; Al-Isawi et al., 2015a; Scholz, 2015). In the case of low concentration of BR46 (Wetlands 4, 8/9 and 10/11), as shown in Table 4.6, although the effluent dyes in all seasons were less than 1 mg/l and these effluents do not affect algal growth as stated by Van der Zee (2002), the maximum influent concentration was in the autumn season (7.71 mg/l) and the effluent concentrations for Wetlands 4, 8/9 and 10/11 were 0.33, 0.17 and 0.13 (mg/l), respectively. These results suggest that autumn is better than the other seasons for reduction of BR46 dye.

Table 4.7 Seasonal dyes reduction (mg/l)

Characteristics			Summer[a]		Autumn[b]		Winter[c]		Spring[d]	
Dye	Type of flow	Wetland number(s)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)
BR46	In	N/A	6.26 ±1.07	N/A	7.71±1.38	N/A	6.12±1.11	N/A	5.6±0.72	N/A
	Out	4	0.38±0.12	94	0.33±0.12	96	0.27±0.03	96	0.24±0.08	96
	Out	8 and 9	0.37±0.28	94	0.17±0.08	98	0.11±0.02	98	0.14±0.12	98
	Out	10 and 11	0.26±0.18	96	0.13±0.04	98	0.15±0.04	98	0.16±0.12	97
	In	N/A	210.05±4.6	N/A	212.63±4.16	N/A	211.52±3.45	N/A	207.88±5.65	N/A
	Out	14 and 15	25.4±12.98	88	46.73±3.59	78	46.09±1.35	78	41.41±1.50	80
	Out	18 and 19	5.91±3.28	97	14.95±5.36	93	25.43±3.62	88	21.44±2.24	90
AB113	In	N/A	7.25±1.72	N/A	7.37±1.48	N/A	6.12±1.28	N/A	6.59±1.79	N/A
	Out	5	1.76±0.73	76	2.69±0.85	64	2.69±0.97	56	2.55±0.93	61
	Out	6 and 7	0.79±0.35	89	1.64±0.46	78	2.29±0.85	63	2.08±0.69	68
	Out	12 and 13	1.95±0.69	73	2.95±0.65	60	2.51±0.54	59	2.3±0.38	65
	In	N/A	217.44±36.08	N/A	228.57±33.55	N/A	220.55±15.88	N/A	217.18±10.13	N/A
	Out	16 and 17	97.88±55.54	55	32.71±6.04	86	45.11±8.19	80	51.31±3.32	76
	Out	20 and 21	90.43±48.65	58	37.84±8.76	83	46.51±9.15	79	50.78±4.65	77
Temperature ˚C			22.8		11.6		8.6		20.5	

Note: [a]21/06/2015 to 21/9/2015; [b]22/09/2015 to 20/12/2015; [c]21/12/2015 to 19/03/2016; [d]20/03/2016 to 31/05/2016; BR, basic red; AB, acid blue; N/A, not applicable; SD, standard deviation.

4.11 Concluding Remarks

The presence of *P. australis* did not affect dye removal ($p>0.05$), while it had a minor but insignificant impact on COD removal ($p>0.05$). The use of plants concerning the short contact time scenario for NH4-N and a low concentration of AB113 is linked to a better removal compared to other cases apart from that for all nutrients; particularly PO4-P and NO3-N. In case of low dye concentrations, the presence of plants for the long contact time scenario impacted significantly ($p < 0.05$) positively on the removal efficiencies of PO4-P, NH4-N and NO3-N. The vertical-flow constructed wetlands showed significantly ($p<0.05$) good dye removals for low and high concentrations ofBR46, and low concentrations of AB113 during all seasons under greenhouse conditions. The COD removal was a function of the dye used. This was explained by the complexity of the chemical structure of the dye and seasonal variations in temperature. For high concentrations of BR46, wetlands with a low hydraulic rate were significantly ($p < 0.05$) better that those with a high hydraulic rate for the removal of PO4-P. For AB113, wetlands with a high loading rate removed PO4-P significantly ($p < 0.05$) well. In case of the dye AB113, the four-day contact time was not enough to remove or reduce TSS and turbidity.

5. CHAPTER FIVE: RESULTS AND DISCUSSION FOR ARTIFICIAL WASTEWATER CONTAINING TWO AZO TEXTILE DYES REDUCTIONS

5.1 Overview

This chapter includes the results and discussion of the artificial wastewater containing the two textile dye reduction rates, and the other physical and chemical water quality parameters for the period between 1 June 2016 and 31 May 2017. Section 5.2 deals with the test of normality for all variables. The results concerning the variables plant growth, redox potential, dissolved oxygen, electrical conductivity, total suspended solids, turbidity, pH, dyes, chemical oxygen demand and nutrients are discussed in sections 5.3 to 5.8. Section 5.9 covers the seasonal treatment performance of wetlands on dye reductions. Aromatic amine compound reductions discussed in section 5.10. Furthermore, statistical differences between the variables are also presented in these sections. Concluding remarks are given in section 5.11.

5.2 Test of Normality

Tests of normality results for dimensions of *P. australis* and for effluent water quality characteristics regarding general physical and chemical variables are discussed in Appendix B.2.

5.3 Plant Growth Monitoring

When temperatures started to decrease in winter, the plants in this experiment began to yellow. The above-ground parts died, were cut and returned to the wetland filters. The authors followed common practice to cut the above-ground plant parts down to between 10 and 15 cm height according to Stefanakis et al. (2014).

Based on the experimental results, the most critical observation is that for all considered concentrations and contact times, the plants that were subjected to the dye AB113 revealed less effective growth compared to those that were fed by the dye BR46. Taking into consideration the potential impact of contact time, it has been noted that plants associated with a long contact time showed much better growth than those plants linked to a short one (Table 5.1). These findings support those by Pagter et al. (2005), who investigated the effect of water stress tolerance of *P. australis* grown in the laboratory by examining effects of different levels of required water. The results showed that a water deficit reduces the leaf biomass per plant and the leaf area.

The ANOVA and Mann-Whitney tests were applied for normal and non-normal distributed data, respectively. Regarding the growth of plants, there was a significant ($p<0.05$) difference with respect to the length and diameter at the low and high concentration of the dye AB113 (Wetlands 3, 9, 13 and 17). In the case of plants grown in the presence of the dye BR46, there was a significant ($p<0.05$) difference for both the length and diameter at the low concentration of dye (Wetlands 5 and 7), while no significance ($p\geq0.05$) for either parameter was noted at the high concentration of dye (Wetlands 11 and 15). In the case of the mixed dye, there was no significant ($p\geq0.05$) difference with respect to the length at the low and high concentrations for all wetlands (Wetlands 4, 6, 8, 10, 12, 14, 16 and 18). While with respect to the diameter, there was a significant ($p<0.05$) difference at the high concentration (Wetlands 12, 14, 16 and 18).

Table 5.1 Dimensions of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) planted in the experimental wetlands.

Characteristics			Length (cm)			Diameter (mm)		
Dye	Wetland numbers	Number of stems	Minimum	Maximum	Mean ±SD	Minimum	Maximum	Mean ±SD
BR46	5	68	69	142	108±17.8	1.1	3.1	2.2±0.58
	7	20	60	122	93±16.9	0.8	2.3	1.1±0.39
	11	17	45	90	67±11.2	0.8	1.1	0.9±0.08
	15	14	44	90	60±12.9	0.8	1.4	1.0±0.16
AB113	3	40	79	140	107±18.0	1.0	3.4	2.1±0.63
	9	15	55	98	79±14.8	0.8	1.2	1.0±0.11
	13	20	45	98	70±16.3	0.7	2.9	1.9±0.59
	17	10	29	56	41±9.1	0.7	1.6	1.1±0.26
The mixture of the dyes	4	48	78	142	109±15.9	1.0	3.9	2.4±0.73
	6	55	70	134	109±16.2	1.1	3.7	2.3±0.59
	8	7	85	101	94±5.5	0.9	2.0	1.5±0.39
	10	10	80	110	95±8.2	1.1	2.3	1.8±0.37
	12	10	45	87	64±11.3	0.8	1.6	1.0±0.22
	14	16	46	80	66±9.7	1.3	3.1	2.1±0.48
	16	7	45	61	54±5.8	0.8	1.9	1.3±0.37
	18	9	44	67	58±7.5	1.0	2.9	1.8±0.63

Note: BR, basic red; AB, acid blue; SD, standard deviation.

5.4 Redox Potential and Dissolved Oxygen

Redox potentials of greater than 100 mV indicate aerobic environments, while values less than -100 mV indicate anaerobic environments (Suthersan, 2001). DO is an important function in constructed wetlands since it is essential in aerobic respiration for micro-organisms and it regulates the oxidation-redox potential in wastewater (Boyd, 2000). S Wu et al. (2011) and Hou et al. (2016) reported that the main pathways for oxygen transfer in constructed wetlands such as the system in this research (tidal flow) are: wetland macrophytes release via their roots, contact transfer at the interface of biofilm and atmosphere, and dissolved oxygen associated with influent wastewater. In the case of low concentration, redox potential values (Table 5.2) for the effluent of BR46, AB113 and the mixture of both of them were in the range between -34 mV and -64 mV and the whole values for the influent and effluents are shown in Figures 5.1, 5.2 and 5.3, respectively, for the effluent high concentrations, the value were in the range -56 mV to -95 mV and the whole values for the influent and effluents are shown in Figures 5.4, 5.5 and 5.6, respectively. These findings indicate that dye degradation may have taken place in between aerobic and anaerobic environments.

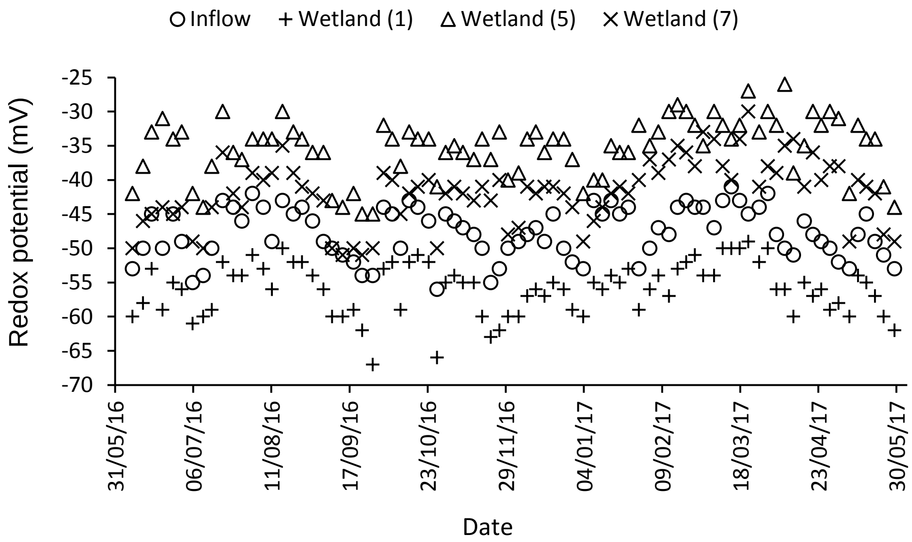


Figure 5.1 Overall variation for redox potential in the influent and effluent for Wetlands 1, 5 and 7 (low concentration of BR46).

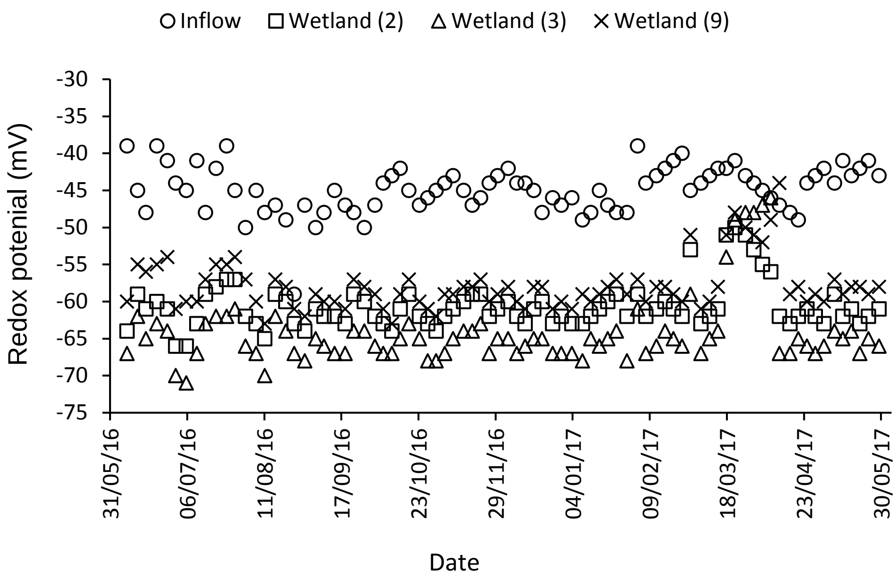


Figure 5.2 Overall variation for redox potential in the influent and effluent for Wetlands 2, 3 and 9 (low concentration of AB113).

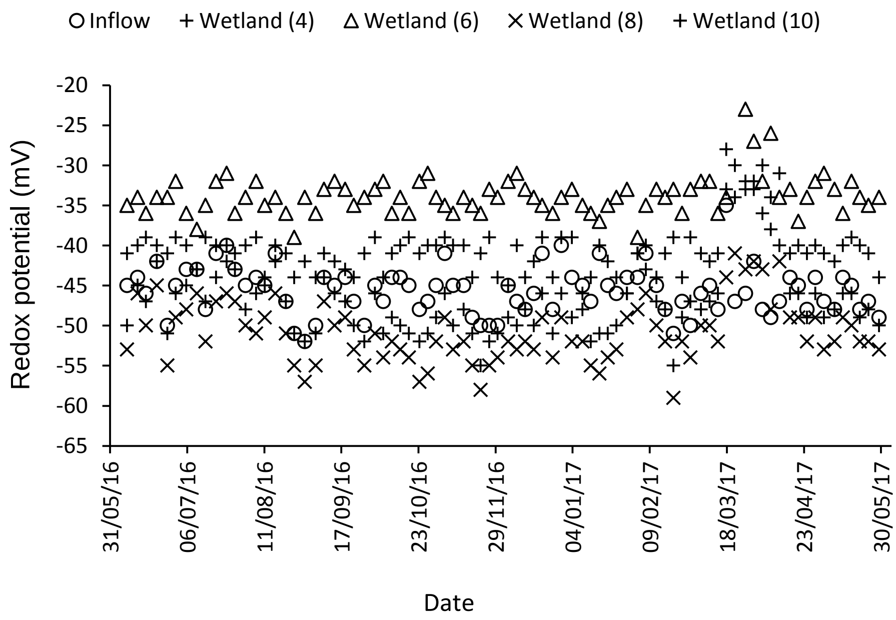


Figure 5.3 Overall variation for redox potential in the influent and effluent for Wetlands 4, 6, 8 and 10 (low concentration of the mixture of dyes BR46 and AB113).

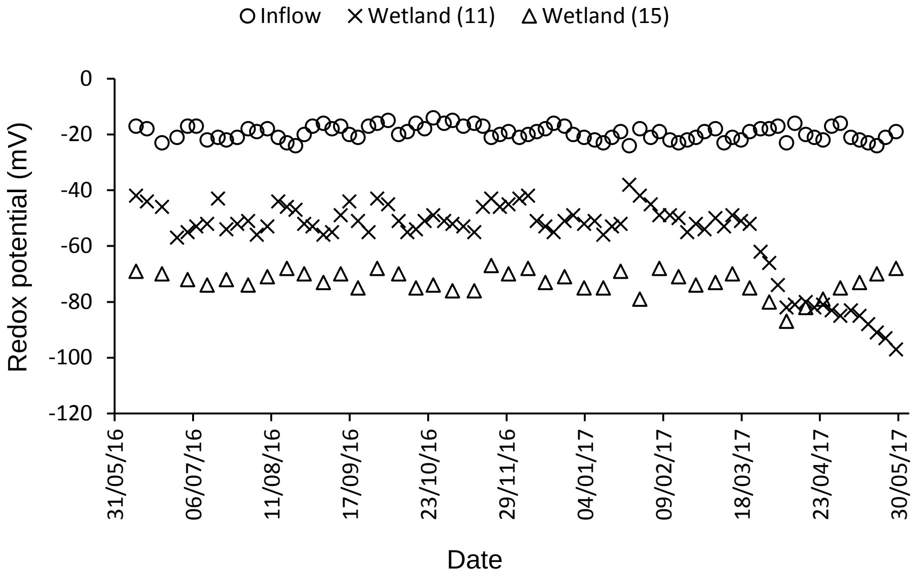


Figure 5.4 Overall variation for redox potential in the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

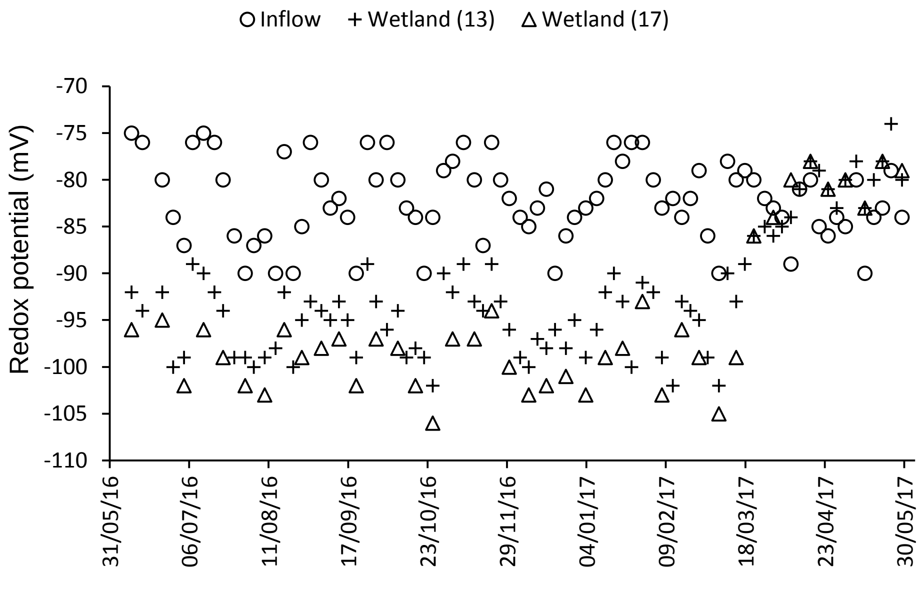


Figure 5.5 Overall variation for redox potential in the influent and effluent for Wetlands 13 and 17 (high concentration of AB113).

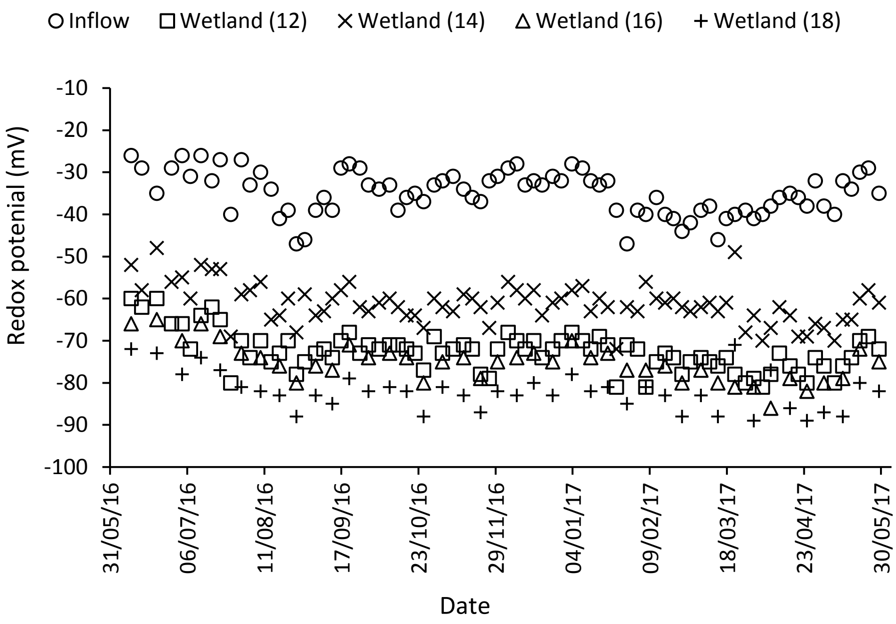


Figure 5.6 Overall variation for redox potential in the influent and effluent for Wetlands 12, 14, 16 and 18 (high concentration of the mixture of dyes BR46 and AB113).

Regarding dissolved oxygen, in case of low concentration, in both of dyes BR46 and AB113, the lowest effluent values (Table 5.2) were in planted Wetlands 5 and 3 (low resting and high contact times; 2.97 mg/l and 3.37 mg/l, respectively) when compared with unplanted Wetlands 1 and 2 (low resting and high contact times), and planted Wetlands 7 and 9 (high resting and low contact times), respectively, during the whole period, as shown in Figures 5.7 and 5.8, respectively, as a result of the higher contact time leading to consumption of more dissolved oxygen by the microbial community. The same was found in the case of high concentration of BR46 and AB113, as shown in Figures 5.9 and 5.10, respectively. In the case of the low concentration of the mixture of the two dyes, the value of DO for Wetlands 4 and 6 (low resting and high contact times) was lower than Wetlands 8 and 10 (high resting and low contact times) as a result of the higher contact time leading to consumption of more dissolved oxygen by the microbial community. The same was found for the DO between Wetlands 12 (low resting and contact times), and 16 (high resting and contact times), in the case of high concentration for the mixture of the two dyes, while the result was opposite to this between Wetlands 14 (low resting and contact times) and 18 (high resting and contact times). Furthermore, in the case of low and high concentration of the two dyes and the mixture of both of them during spring time, wetlands with higher resting time started consuming more dissolved oxygen when compared to wetlands with lower resting time because the increase in aerobic micro-organisms was greater than that of anaerobic macro-organisms.

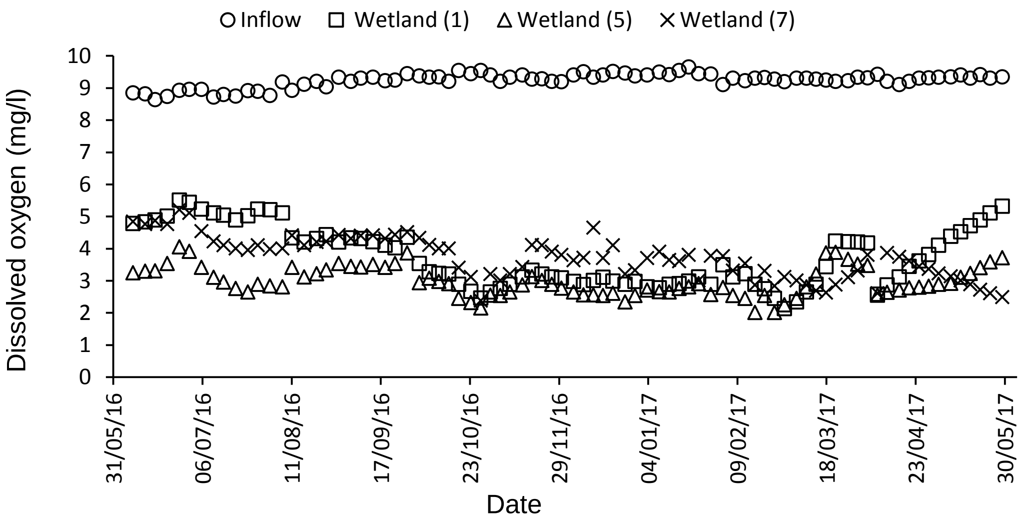


Figure 5.7 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 1, 5 and 7 (low concentration of BR46).

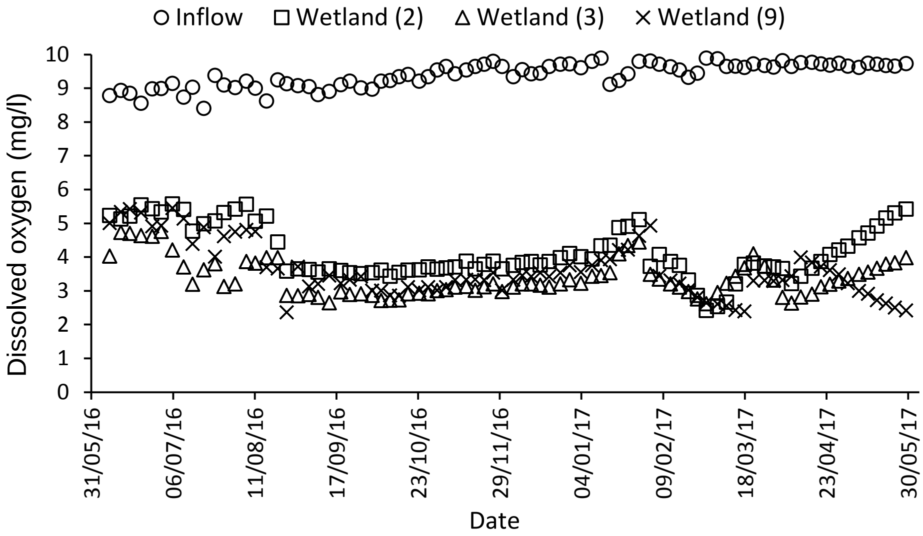


Figure 5.8 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 2, 3 and 9 (low concentration of AB113).

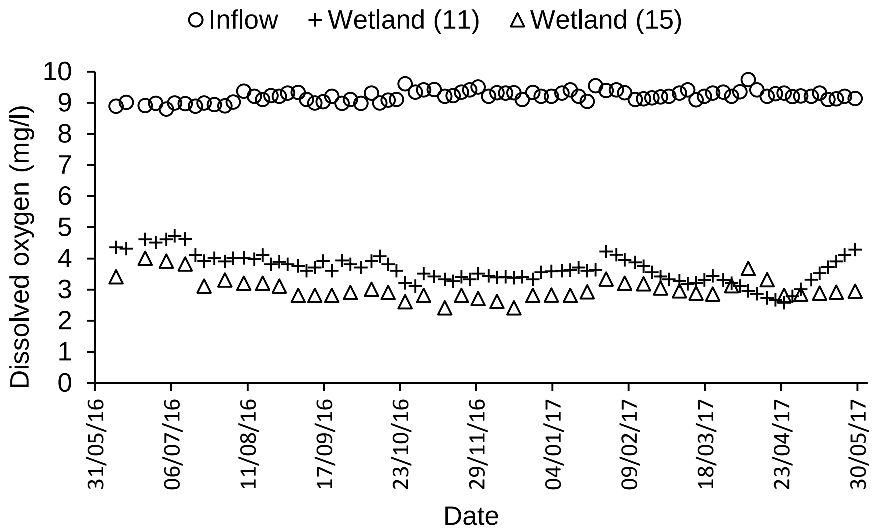


Figure 5.9 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

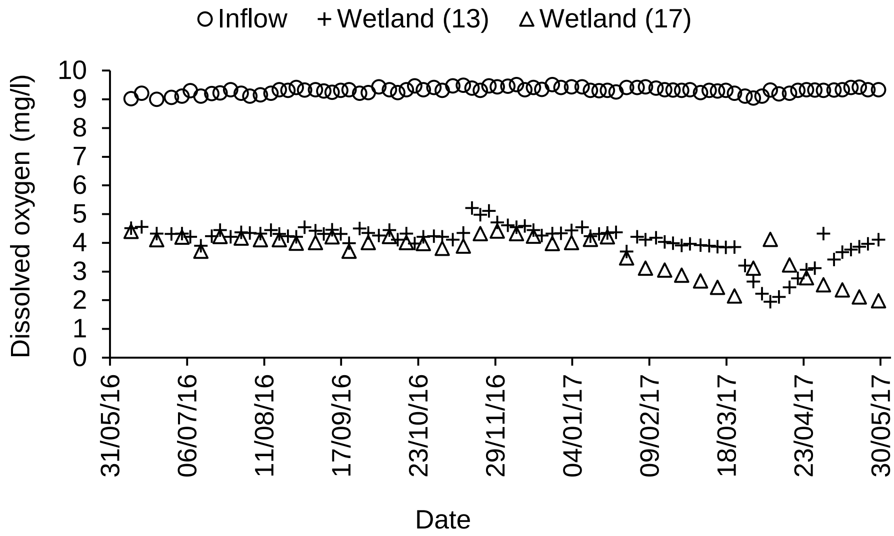


Figure 5.10 Overall variation for dissolved oxygen in the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

5.5. Electrical Conductivity, Total Suspended Solids and Turbidity

Electrical conductivity can be used as an indirect measure of the charge (or the ion-carrying species) in the wetland outflow (Islam et al., 2011). EC value may be used as an indicator of other water quality problems. Any sudden increase in EC value indicates that there is a source of dissolved ions in the wetland filter (Kumar and Chopra, 2012). In comparison, all the effluent values for all wetlands in the case of low and high concentrations for both of the dyes and the mixture of both of them were compliant with the national effluent discharge quality standards set by the Government of Bangladesh, which state that the maximum effluent of EC for inland surface water, public sewer secondary treatment plant and irrigated land is 1200 µS/cm (Ahmed et al., 2002). Furthermore, Sri Lanka central environmental authority (2008) state that the maximum EC discharge on land for irrigation purposes is 2250 µS/cm. For the low concentration of both dyes (BR46 and AB113), as shown in Table 5.2, a higher increase was found in planted Wetlands 5 and 3 (contact time 94 h), when compared to unplanted control Wetlands 1 and 2 (contact time 94 h), respectively, while a decrease in EC effluent values were found in Wetlands 7 and 9 (contact time 48 h), respectively. For the high concentration for both dyes (BR46 and AB113), EC effluent values for all wetlands were less than influent values. Furthermore, Wetlands 15 and 17 (long resting and contact times) had EC values less than Wetlands 11 and 13 (low resting and contact times), respectively. In the case of the mixture of both dyes for both low and high concentrations, all the values of the effluent were less than the influent values, as shown in Table 5.2. Nevertheless, all the previous results indicated no sudden increase in EC values for all wetlands.

The measurement of TSS is important for the design of water treatment facilities (Dzurik, 2003) and the Clean Water Act (CWA) lists it as a conventional pollutant (Bell et al. 2011). In the case of low concentration for dyes BR46 and AB113, there was an increase in effluent for all wetlands when compared to the influent, as shown in Table 5.2. A lower increase and decrease were found in planted Wetlands 7 and 9 (high resting and low contact times) when compared with unplanted Wetlands 1 and 2, and planted Wetlands 5 and 3 (low resting and high contact times), respectively. For the mixture of both dyes, a slight decrease was found in Wetlands 4 and 6 while, a decrease was found in Wetlands 8, and 10. In the case of high concentration for both dyes (BR46 and AB113) and the mixture of the two dyes, a good reduction was recorded in all wetlands, as shown in Table 5.2. Wetlands with high resting and contact times had a lower effluent when compared with wetlands which have low resting and contact times.

In comparing effluents of low and high concentrations of BR46, AB113 and the mixture of both of the dyes during the whole period, as shown in Figures 5.11, 5.12, 5.13, 5.14, 5.15 and 5.16, respectively, it was found that all the effluent results for wetlands were compliant with the national effluent discharge quality standards set by the Government of Bangladesh, which state the maximum effluent values of TSS for inland surface water, public sewer secondary treatment plant and irrigated land are 150 mg/l, 500 mg/l and 200 mg/l, respectively (Ahmed et al., 2002). Furthermore, in the case of low and high concentration of the two dyes and the mixture of both of them during spring time, wetlands with higher resting time started consuming more dissolved oxygen when compared to wetlands with lower resting time because the increase in aerobic micro-organisms was greater than that of anaerobic macro-organisms.

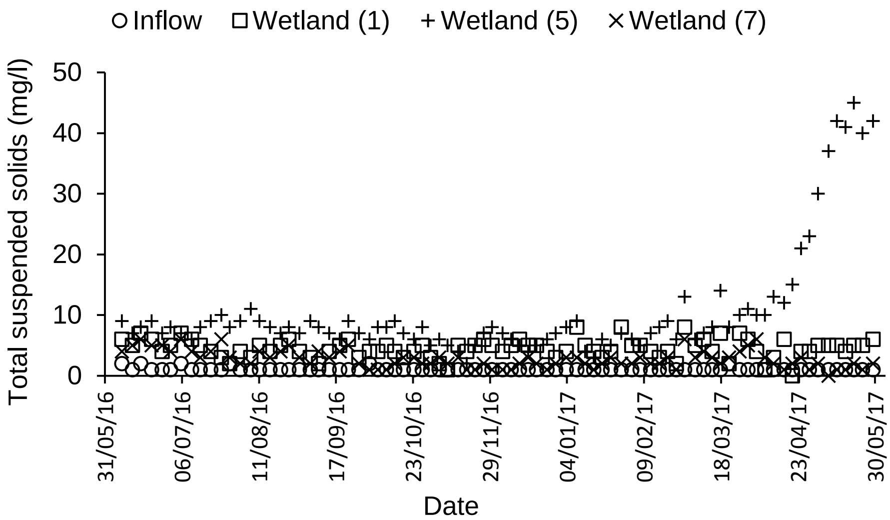


Figure 5.11 Overall variation for total suspended solids in the influent and effluent for Wetlands 1, 5 and 7 (low concentration of BR46).

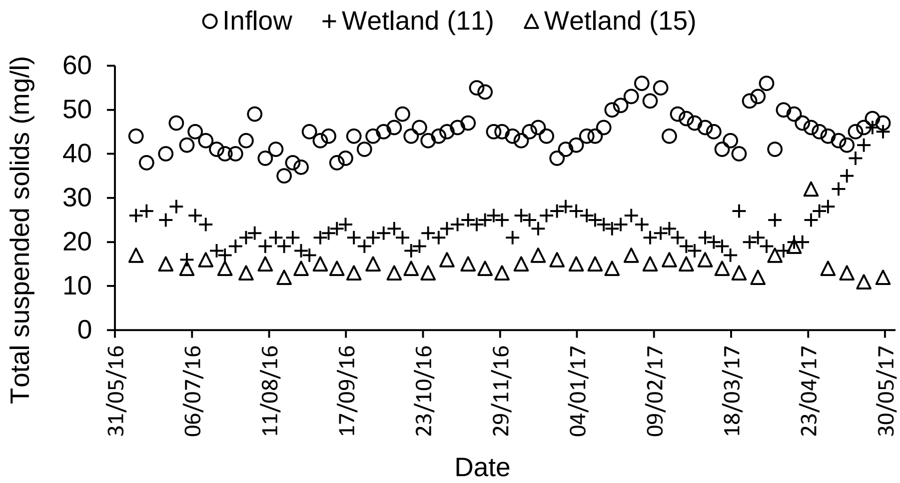


Figure 5.12 Overall variation for total suspended solids in the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

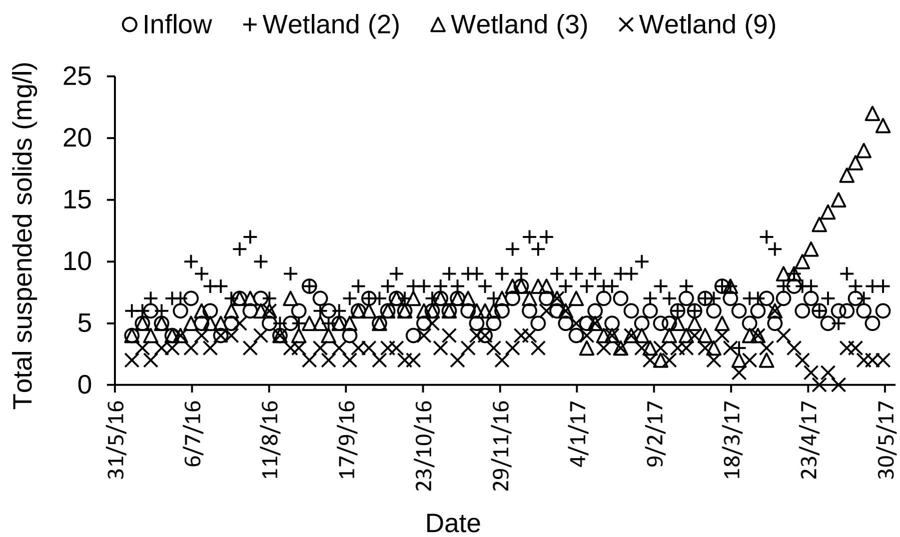


Figure 5.13 Overall variation for total suspended solids in the influent and effluent for Wetlands 2, 3 and 9 (low concentration of AB113).

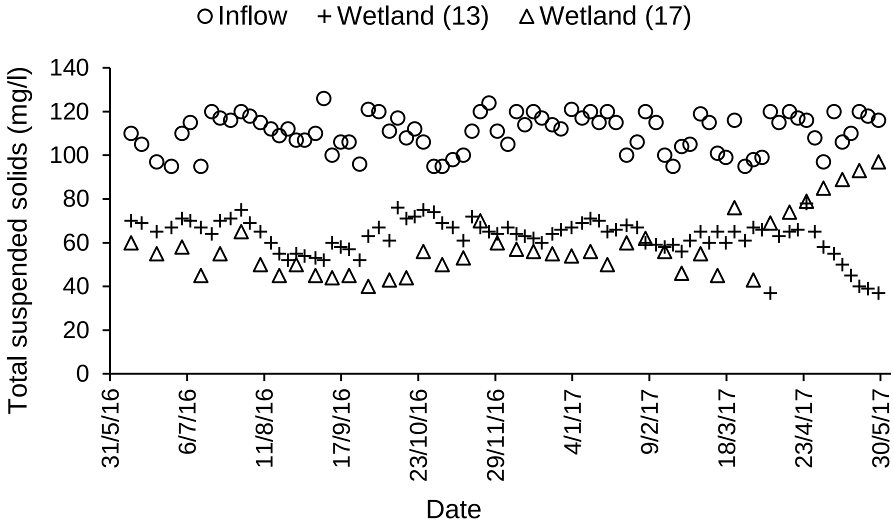


Figure 5.14 Overall variation for total suspended solids in the influent and effluent for Wetlands 13 and 17 (high concentration of AB113).

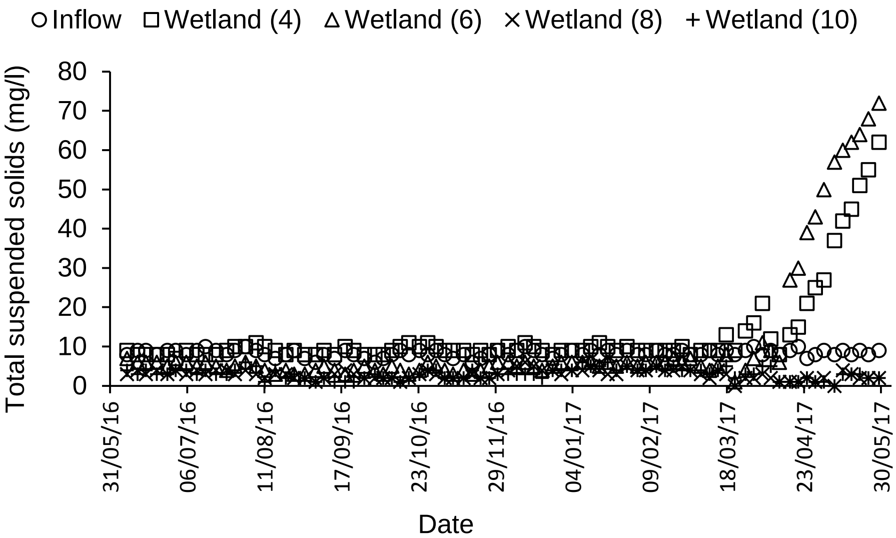


Figure 5.15 Overall variation for total suspended solids in the influent and effluent for Wetlands 4, 6, 8 and 10 (low concentration of the mixture of both of the dyes).

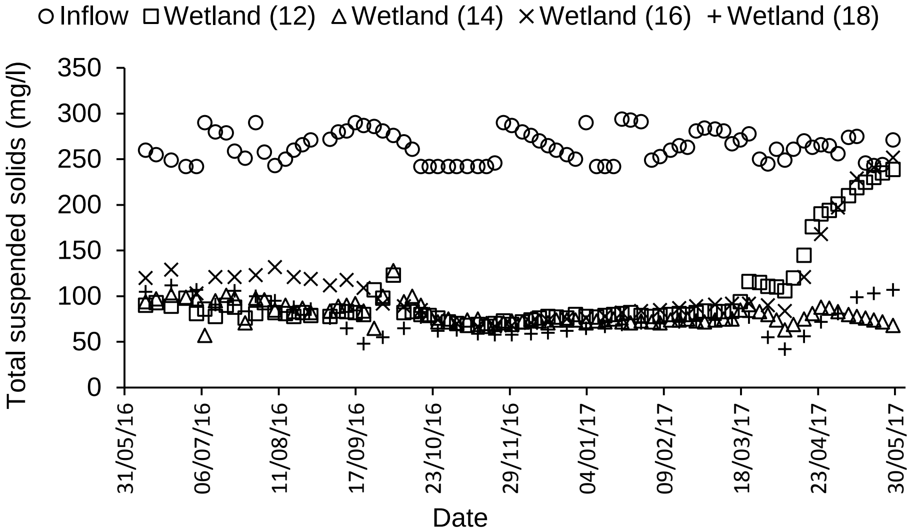


Figure 5.16 Overall variation for total suspended solids in the influent and effluent for Wetlands 12, 14, 16 and 18 (high concentration of the mixture of both of the dyes).

Turbidity is often used to interpret the degree of clarity of water. It is a variable often applied as an indicator of the amount of suspended sediments and/or larger micro-organisms in water. A high turbidity of surface water may also indicate elevated concentrations of TSS, reduced algal populations, and potential harm to fish and other aquatic fauna (Postolache et al., 2007). Furthermore, a higher turbidity value can also increase the temperature of surface water as a result of increased absorption of heat from sunlight, as well as leading to reduced light penetration, which affects photosynthesis (Håkanson, 2006).

For the low concentration of the dye BR46, there was an increase in all effluent wetlands when compared with the influents. Planted Wetland 7 (high resting and low contact times) has a smaller increase when compared with unplanted Wetland 1 and planted Wetland 5 (low resting and high contact times). In the case of dye AB113, a slight increase was recorded in the mean value of planted Wetland 3 (low resting and high contact times) while, a slight decrease was recorded in unplanted Wetland 2 (low resting and high contact times) and planted Wetland 9 (high resting and low contact times). In the case of the mixture of the two dyes, an increase was recorded in Wetlands 4 and 6 (low resting and high contact times), while a decrease was recorded in Wetlands 8 and 10 (high resting and low contact times). For the high concentration for both of the dyes (BR46 and AB113) and the mixture of the two dyes, all the wetlands had a good effluent reduction when compared with the influent. Wetlands 15, 17, 16 and 18 (high resting and contact times) had a greater reduction when compared with Wetlands 11, 13, 12 and 14 (low resting and contact times), respectively.

Lin et al. (2005) and Bulc and Ojtrsek (2008) stated that the ability of vertical-flow constructed wetlands to reduce TSS and turbidity is relatively poor. Regarding this study, for low concentration of AB113, a short contact time (48 h) was better than a long (94 h) one in reduction of TSS and turbidity as well as in the case of the mixture of both of the dyes (BR46 and AB113). While for high concentrations of both dyes and the mixture of the two dyes, the long contact time was better than the short contact time. The percentage TSS reduction rates for BR46, AB113 and the mixture of both dyes were 69%, 47% and 71%, respectively.

5.6 pH Value

The measuring of pH is very important due to pH conditions having a sensitive impact on the outflow quality including: nutrients, COD and TSS in constructed wetlands. The sensitive impact comes from its effect on the ability of microbial populations to degrade pollutants (Eke and Scholz, 2008; Lavrova and Koumanova, 2013; Paing et al., 2015). For the low concentration of the dye BR46, the average influent pH value was 7.47, a slight decrease in the pH effluent value of 0.3 was noted in planted Wetland 5 (low resting and high contact times), while there was a slight increase of 0.25 and 0.1 in unplanted

Wetland 1 (low resting and high contact times) and planted Wetland 7 (high resting and low contact times), respectively. In the case of dye AB113, there was a slight effluent increase of 0.24, 0.22 and 0.16 for the unplanted control Wetland 2, planted Wetland 3 (low resting and high contact times), and planted Wetland 9 (high resting and low contact times), respectively, when compared to the influent value of 7.35, as shown in Table 5.2. In the case of the mixture of both dyes, there was a slight decrease of 0.03, 0.23 and 0.05 for Wetlands 4, 6 (low resting and high contact times) and Wetland 10 (high resting and low contact times), respectively, when compared with the influent value of 7.32, while for Wetland 8 (high resting and low contact times), there was a slight increase of 0.01. For the high concentration for both dyes (BR46 and AB113) and the mixture of the two dyes, a slight increase was found ranging between 0.08 and 0.65 for Wetlands 11, 13, 12, 14 (low resting and contact times), 17, 16 and 18 (high resting and contact times), while an increase of 1.04 was recorded in Wetland 15 (high resting and contact times) when compared with the influent value of 6.94. This increase in effluent pH values is due to the formation of basic aromatic amine metabolites (Chandra, 2015).

Regarding the effect of plants on the pH value for the low concentration of dye AB113, there was a slight difference of 0.02 between unplanted control Wetland 2 and planted Wetland 3 (both of them have the same conditions). This result suggests that the pH modification in vertical-flow constructed wetlands is probably as a result of interactions between the media and its biofilms, rather than due to the plants, this result was confirmed by Kadlec and Wallace (2008). Unlike the result for the dye BR46, there was a difference of 0.55 between unplanted control Wetland 1 and planted Wetland 5 (both of them have the same conditions). The different results regarding the role of plants on pH value are most likely due to each dye having a different chemical structure and molecular weight, as shown in Table 3.1. Furthermore, there were no changes in pH values in contrast to the findings obtained by Wieder (1989), in which he surveyed 128 constructed wetlands treating acid coal mine wastewater and found the difference between the effluent and the influent to be 0.11 (influent pH was 2.5). Mitsch and Wise (1998) corroborated this finding, when they found that the difference between the influent and the effluent was 0.52 (influent pH was 2.82). Furthermore, Kadlec and Wallace (2008) stated that the available pH value range for most degraded bacteria is 4-9.5. Nevertheless, these results indicate the ability of macrophytes such as *P. australis* to modify pH conditions in the rhizosphere, confirming results by Brix et al. (2002). However, these studies used a different wetland plant (*Typha angustifolia L*.). Furthermore, all the effluent pH values for all wetlands in the case of low and high concentrations of both dyes (BR46 and AB113) and the mixture of the two dyes during the whole period, as shown in Figures 5.17, 5.18, 5.19, 5.20, 5.21 and 5.22, respectively, were compliant with the national effluent discharge quality standards set by all consortia and brand guidelines such as the Government of Bangladesh and STWI (Sweden Textile Water Initiative), which state the range of the pH effluent for inland surface water, public sewer secondary treatment plant and irrigated land is between 6 and 9 (Ahmed et al., 2002; STWI, 2012).

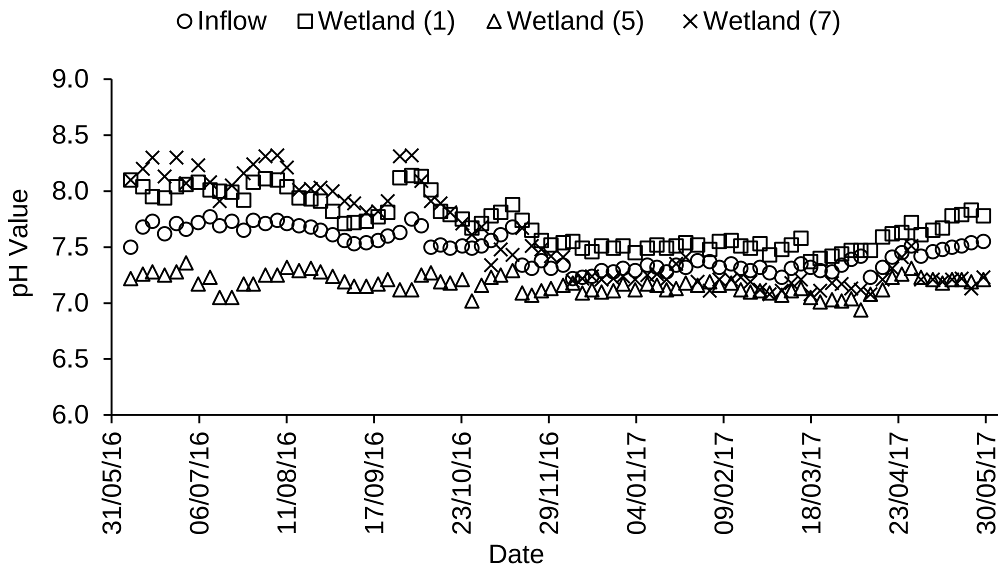


Figure 5.17 Overall variation in pH of the influent and effluent for Wetlands 1, 5 and 7 (low concentration of BR46).

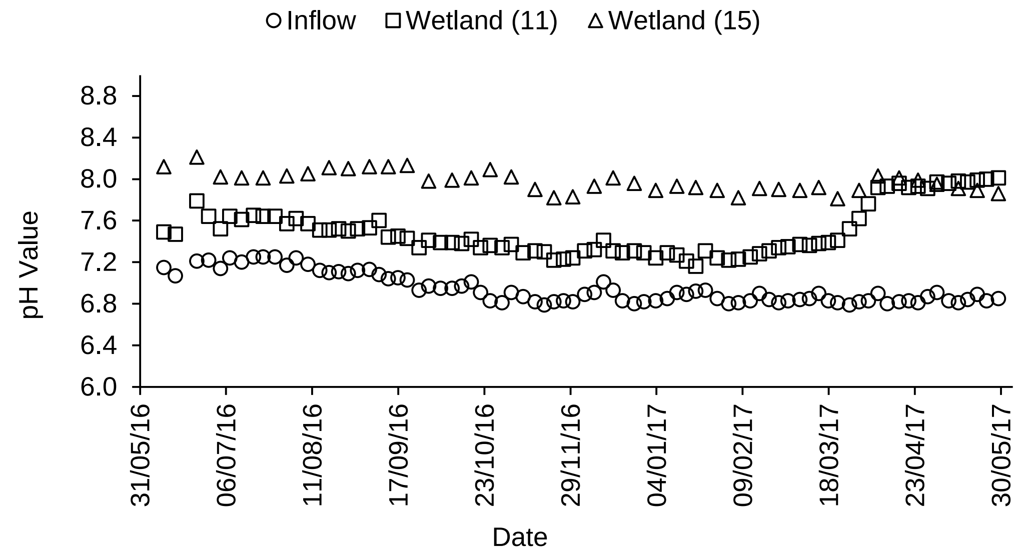


Figure 5.18 Overall variation in pH of the influent and effluent for Wetlands 11and 15 (high concentration of BR46).

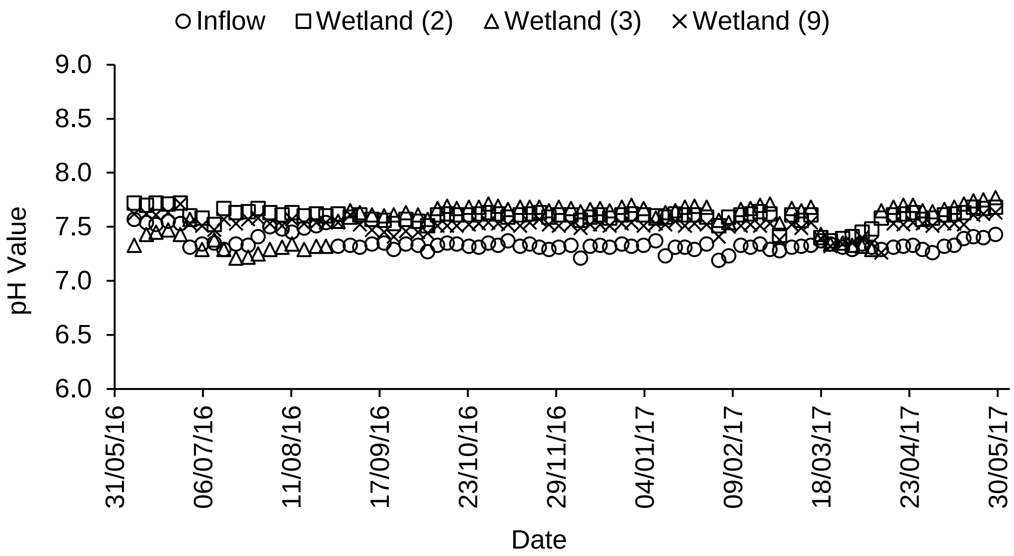


Figure 5.19 Overall variation in pH of the influent and effluent for Wetlands 2, 3 and 9 (low concentration of AB113).

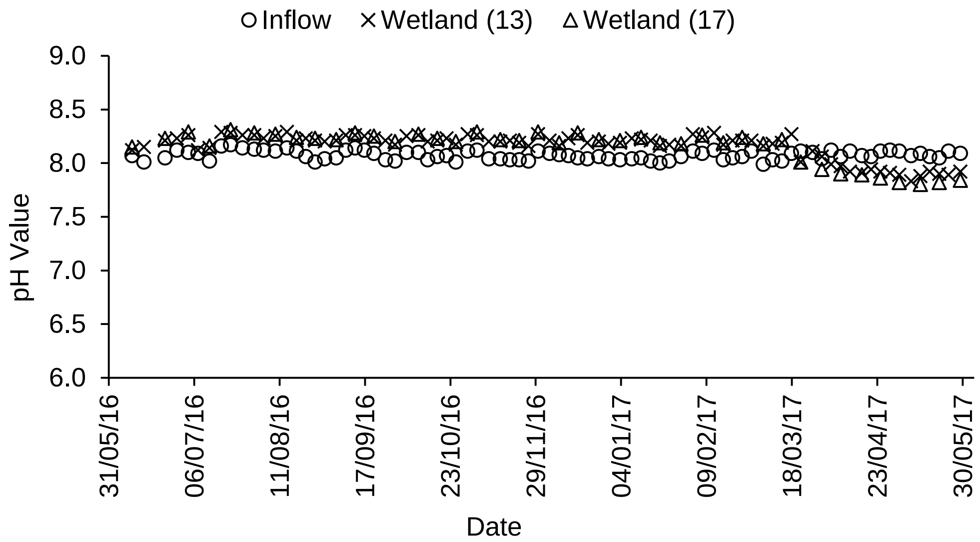


Figure 5.20 Overall variation in pH of the influent and effluent for Wetlands 13 and 17 (high concentration of AB113).

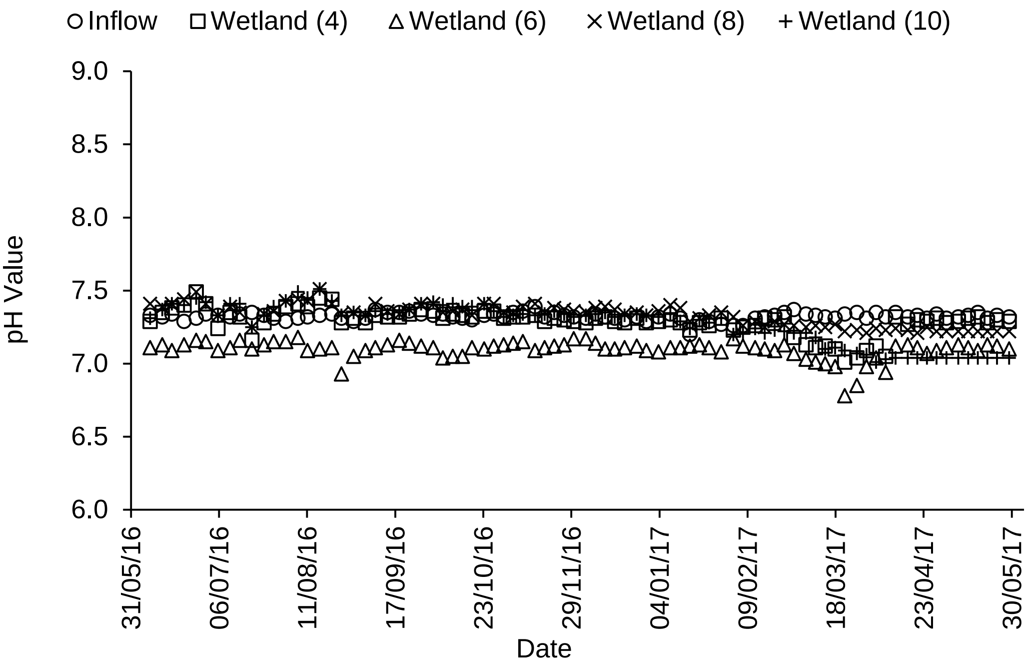


Figure 5.21 Overall variation in pH of the influent and effluent for Wetlands 4, 6, 8 and 10 (low concentration of mixture of both of the dyes).

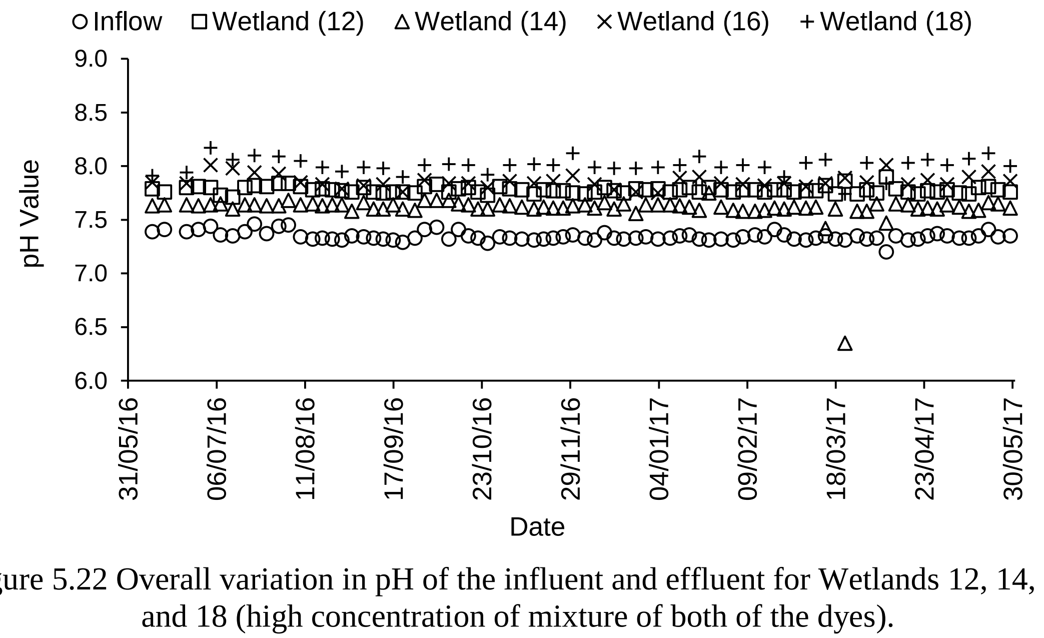


Figure 5.22 Overall variation in pH of the influent and effluent for Wetlands 12, 14, 16 and 18 (high concentration of mixture of both of the dyes).

Table 5.2 Inflow and outflow water quality characteristics for general physical and chemical variables related to different wetlands.

Characteristics				pH			Redox potential (mV)			Dissolved oxygen (mg/l)		
Dye	Type of flow	Wetland number	No. of samples	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
BR46	In	N/A	82	7.22	7.77	7.47±0.17	-56	-41	-47.72±3.74	8.64	9.66	9.24±0.22
	Out	1	82	7.37	8.14	7.72±0.23	-67	-49	-55.98±3.81	2.13	5.51	3.73±0.92
	Out	5	82	6.94	7.36	7.17±0.08	-45	-26	-35.29±4.31	2.01	4.06	2.97±0.46
	Out	7	82	7.08	8.32	7.57±0.42	-51	-30	-41.80±4.74	2.38	5.21	3.71±0.66
	In	N/A	81	6.79	7.25	6.94±0.14	-24	-14	-19.43±2.48	8.8	9.74	9.21±0.18
	Out	11	81	7.16	8.01	7.49±0.24	-97	-38	-56.28±14.03	2.58	4.73	3.65±0.46
	Out	15	41	7.81	8.21	7.98±0.09	-87	-67	-72.90±4.22	2.41	4.01	3.03±0.36
AB113	In	N/A	82	7.19	7.57	7.35±0.08	-59	-39	-44.85±3.21	8.41	9.89	9.40±0.35
	Out	2	82	7.37	7.72	7.59±0.07	-66	-50	-60.71±3.08	2.42	5.57	4.14±0.78
	Out	3	82	7.21	7.77	7.57±0.16	-71	-46	-64.24±4.90	2.62	4.76	3.37±0.54
	Out	9	82	7.26	7.71	7.51±0.07	-63	-44	-57.90±3.42	2.36	5.45	3.59±0.79
	In	N/A	81	7.99	8.17	8.07±0.04	-90	-75	-82.12±4.27	9.00	9.51	9.31±0.11
	Out	13	81	7.83	8.29	8.16±0.12	-102	-74	-92.24±6.78	1.95	5.21	4.07±0.61
	Out	17	41	7.80	8.31	8.15±0.16	-106	-78	-95.27±8.27	1.98	4.41	3.62±0.73
The mixture of the dyes	In	N/A	82	7.2	7.39	7.32±0.03	-52	-35	-45.67±3.01	8.78	9.62	9.36±0.19
	Out	4	82	7.01	7.49	7.29±0.09	-46	-28	-40.62±3.02	1.67	4.47	3.12±0.59
	Out	6	82	6.78	7.18	7.09±0.07	-39	-17	-33.61±3.06	2.21	4.01	2.98±0.44
	Out	8	82	7.21	7.51	7.33±0.07	-59	-41	-50.80±3.81	2.01	5.01	3.85±0.63
	Out	10	82	7.01	7.51	7.27±0.14	-55	-30	-46.66±5.25	1.89	4.84	3.55±0.68
	In	N/A	81	7.2	7.46	7.35±0.04	-47	-26	-34.91±5.16	8.84	9.72	9.24±0.15
	Out	12	81	7.71	7.9	7.78±0.03	-81	-60	-72.64±4.67	2.7	5.05	4.29±0.62
	Out	14	81	6.35	7.75	7.60±0.15	-72	-48	-61.23±4.68	2.19	4.52	2.98±0.63
	Out	16	41	7.75	8.01	7.86±0.06	-86	-65	-75.32±4.54	1.56	5.19	3.52±0.87
	Out	18	41	7.74	8.17	8.00±0.08	-89	-71	-82.07±4.58	1.42	5.50	3.86±1.06

Table 5.2 continued

Characteristics				Total suspended solids (mg/l)			Turbidity (NTU)			Electrical conductivity (μS/cm)		
BR46	In	N/A	82	1	2	1.07±0.26	2.01	6.13	3.91±0.85	545	575	560±9.35
	Out	1	82	0	8	4.48±1.56	3.43	6.61	5.24±0.69	451	558	483±18.04
	Out	5	82	2	45	10.68±9.52	4.11	76.5	11.81±17.02	487	670	644±35.79
	Out	7	82	0	6	2.73±1.45	3	6.53	4.07±0.77	336	421	397±16.62
	In	N/A	81	35	56	45.01±4.51	12.6	15.9	14.62±0.63	685	765	735±13.46
	Out	11	81	16	46	23.68±5.67	7.23	27.1	13.11±3.43	563	684	604±16.09
	Out	15	41	11	32	14.95±3.19	8.04	23.4	12.09±3.94	559	644	581±16.62
AB113	In	N/A	82	4	8	5.87±1.04	5.06	6.71	7.51±0.42	521	575	543±12.85
	Out	2	82	3	12	7.96±1.72	3.91	6.99	5.37±0.87	468	601	559±22.48
	Out	3	82	2	22	6.71±4.04	4.86	10.49	7.03±1.18	497	622	583±21.49
	Out	9	82	0	7	3.16±1.28	2.87	6.42	4.59±0.78	408	511	484±16.42
	In	N/A	81	97	126	110±8.59	42.31	62.34	51.79±3.95	855	892	870±5.63
	Out	13	81	37	78	62.64±8.47	9.95	34.21	24.21±6.61	702	909	765±51.28
	Out	17	41	40	97	58.29±14.40	12.45	25.41	18.62±4.24	672	899	742±60.84
The mixture of the dyes	In	N/A	82	6	10	8.32±0.89	4.23	5.92	5.14±0.34	510	535	522±6.20
	Out	4	82	7	62	12.99±10.90	3.85	64.5	10.67±10.95	478	586	508±14.22
	Out	6	82	1	72	11.40±17.02	4.14	87.4	11.67±17.43	475	568	491±12.96
	Out	8	82	0	6	2.95±1.22	3.54	6.66	4.41±0.55	339	460	399±18.59
	Out	10	82	0	6	3.07±1.38	3.42	6.93	4.65±0.61	335	439	413±24.16
	In	N/A	81	242	294	263±16.54	131	157	144±5.97	760	785	775±4.92
	Out	12	81	67	239	100±44.63	14.2	71.3	43.66±18.77	678	734	710±9.83
	Out	14	81	57	128	80.48±11.56	11.3	55	31.87±11.95	693	760	738±9.44
	Out	16	41	67	252	109±46.20	20.4	68.3	44.59±13.99	670	732	717±11.03
	Out	18	41	42	112	75.27±18.37	14.9	51.2	32.64±10.31	696	785	746±13.33

Note: BR, basic red; AB, acid blue; SD, standard deviation; Min., minimum; Max., maximum; N/A, not applicable.

5.7 Dye, Colour and Chemical Oxygen Demand Reductions

The degradation of azo dyes takes place both in aerobic and anaerobic conditions via various processes involving enzymes and/or chemical reduction (Khehra et al., 2005; Pandey et al., 2007; Saratale et al., 2011). The first contaminant to be recognized in an effluent textile wastewater is the colour, it adsorbs and reflects the sunlight entering the water, thereby interfering with the aquatic species growth and hindering photosynthesis (Pereira and Alves, 2012; Yadav et al., 2012).

For dye and colour reductions concerning low concentration of the dyes (BR46 and AB113), and the mixture of the two dyes, wetlands with a long contact time (Table 5.3) have the best dye and colour reductions (regardless of the planting regime), when compared to wetlands having short contact times. For the high concentration of BR46 and AB113 (Figures 5.23, 5.24, 5.25 and 5.26, respectively), and the mixture of both of them, wetlands which have a low loading rate (high resting and contact times), have the better dye and colour reductions ($p<0.05$) when compared to wetlands with high loading rates (low resting and contact times), as shown in Table 5.3. Although, in terms of dye concentration, wetlands, which have a low loading rate, have the better reduction when compared with wetlands which have a high loading rate, the influents as a mass loading rate for Wetlands 11 and 13 (high loading rate) were 573.71±26.74 and 576.49±38.15 $g.m^{-2}.d^{-1}$, respectively, while for Wetlands 15 and 17 (low loading rate) they were 286.86±13.37 and 288.25±19.08 $g.m^{-2}.d^{-1}$, respectively, as shown in Table 5.4. The final decision about which one is better (low or high loading rate) depends on the design conditions of the constructed wetlands in the field. The effluent colour for the wetlands in the case of low concentrations of BR46, AB113 and the mixture of the two dyes were compliant with the national effluent discharge quality standards set by the Government of India (1986), which state the maximum colour value is 400 (Pt Co.), while in the case of high concentration of BR46, AB113 and the mixture of the two dyes values were not compliant even when compared to maximum value of colour (550 Pt Co.), which is stated by the Government of Taiwan (2003).

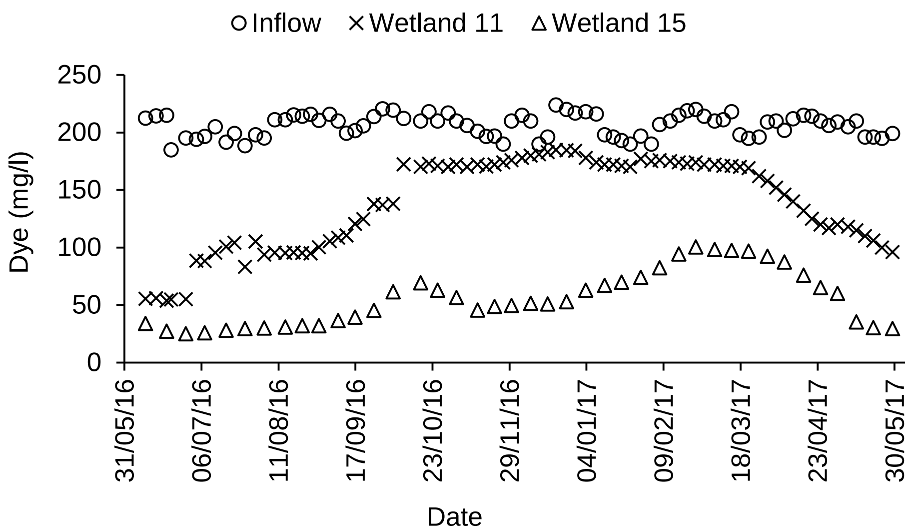


Figure 5.23 Overall variation in dye concentration of the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

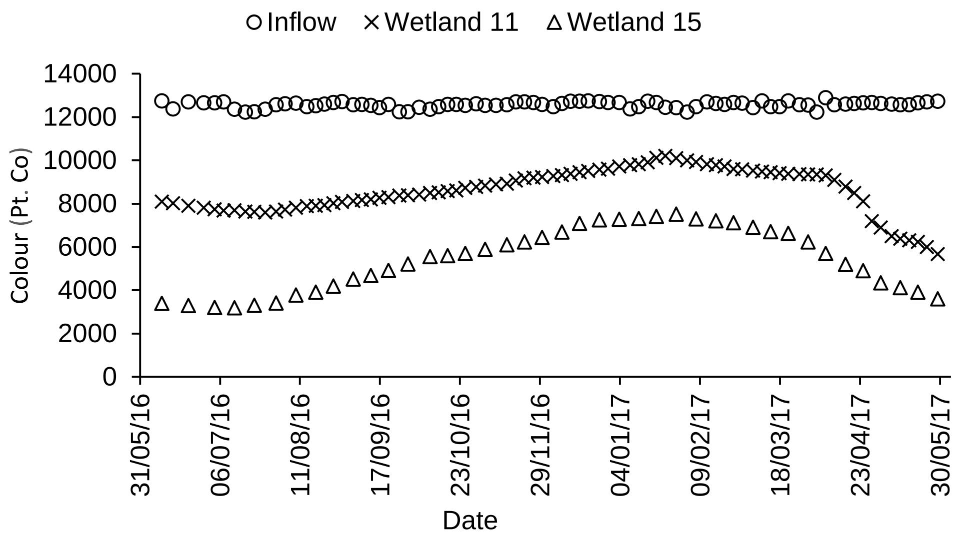


Figure 5.24 Overall variation in dye colour of the influent and effluent for Wetlands 11 and 15 (high concentration of BR46).

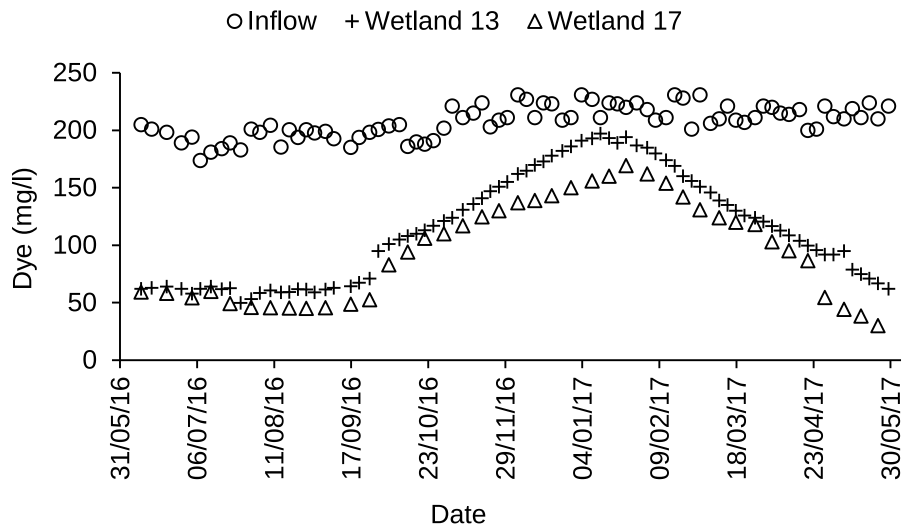


Figure 5.25 Overall variation in dye concentration of the influent and effluent for Wetlands 13 and 17 (high concentration of AB113).

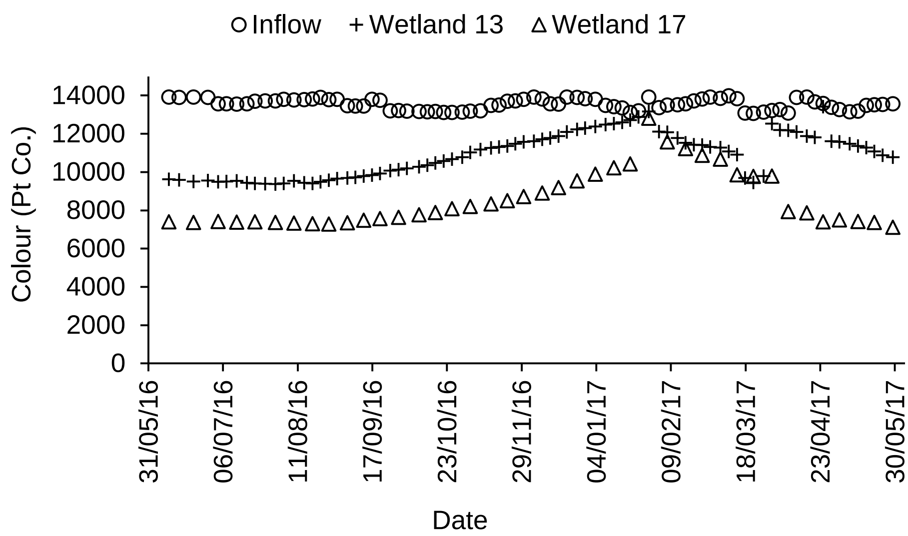


Figure 5.26 Overall variation in dye colour of the influent and effluent for Wetlands 13 and 17 (high concentration of AB113).

In textile wastewater, measurement of COD is very important to evaluate organic matter concentration in constructed wetlands. Its reduction mechanisms include anaerobic, filtration, aerobic, adsorption and microbial metabolism (Vymazal et al., 1998; Song et al., 2006; Stefanakis et al., 2014). The effluent COD values for low concentration of BR46, AB113 and the mixture of both dyes (Table 5.3) were compliant with the national effluent discharge quality standards set by the Government of Bangladesh, which state the maximum COD values for inland surface water, public sewer secondary treatment plant and irrigated land are 200 mg/l, 400 mg/l and 400 mg/l, respectively, while in the case of high concentration of BR46, AB113 and the mixture of both dyes (Table 5.3) values were compliant with public sewer secondary treatment and irrigated land (Ahmed et al., 2002). For COD reduction concerning low concentration of dyes (BR46 and AB113) and the mixture of both of the dyes, the results showed that all wetlands demonstrated good COD reduction, as shown in Table 5.3. Furthermore, wetlands with a long resting time had the best COD reduction, if compared to the control (unplanted wetlands) and/or other wetlands having short resting times. These results indicate that both aerobic and anaerobic environments are the best for COD reduction. Wetlands 7, 9, 8 and 10 have effluent DO values higher than Wetlands 5, 3, 4 and 6. For the high concentration, COD reduction in wetlands which have a low loading rate (high resting and contact times), is better than in the wetlands that have a high loading rate (low resting and contact times) in terms of COD concentration (Table 5.3), but the influent mass loading rate values for Wetlands 11 and 13 (high loading rate) were 1423.14±102.27 and 1668.22±132.73 $g.m^{-2}.d^{-1}$, respectively as shown in Table 5.5 while, for Wetlands 15 and 17 (low loading rate) were 711.57±51.14 and 834.11±66.37 $g.m^{-2}.d^{-1}$. The final decision about which one is better depends on the design conditions of the constructed wetlands in the field. All the previous findings regarding low and high concentration for BR46, AB113 and the mixture of the two dyes indicate that setting aerobic and anaerobic conditions will improve the COD reduction and this has been confirmed by many researchers (Vymazal et al., 1998; Li et al., 2012; Lehl et al., 2016).

Table 5.3 Colour, dye and chemical oxygen demand (COD) reductions for different wetlands.

Characteristics				Colour (Pt Co.)				Dye concentration (mg/l)				Chemical oxygen demand (COD)[a]			
Dye	Type of flow	Wetland number	No. of samples	Min.	Max.	Mean ±SD	Reduction (%)	Min.	Max.	Mean ±SD	Reduction (%)	Min.	Max.	Mean ±SD	Reduction (%)
BR46	In	N/A	82	410	439	422±7.67	N/A	4.3	8.41	6.15±0.75	N/A	200	297	248±20.89	N/A
	Out	1	82	16	187	70.73±35.27	83	0.0	1.18	0.52±0.22	92	49.5	93	76.17±11.79	69
	Out	5	82	26	356	96.96±71.42	77	0.0	0.99	0.57±0.24	91	54	110	73.68±16.44	70
	Out	7	82	9	272	120±80.47	72	0.11	1.87	0.69±0.40	89	20.1	79.5	44.62±13.89	82
	In	N/A	81	12240	12900	12574±142.19	N/A	185	224	206±9.60	N/A	478	576	511±36.72	N/A
	Out	11	81	5670	10210	8604±1053.03	32	53.8	185	139±39.18	33	196	385	311±52.37	39
	Out	15	41	3200	7520	5442±1459.49	57	25.1	100.6	55.75±24.00	73	135	301	225±48.42	56
AB113	In	N/A	82	530	589	555±16.14	N/A	5.32	10.93	7.5±1.66	N/A	234	310	275±18.12	N/A
	Out	2	82	39	386	135±100.27	76	0.48	4.80	1.15±0.87	85	53.9	125	66.47±16.28	76
	Out	3	82	89	400	182±82.52	67	0.61	4.49	1.37±0.84	82	88.8	176	105.75±19.41	62
	Out	9	82	224	490	310±71.22	44	0.57	5.31	1.74±1.08	77	39.7	84.6	53.31±10.30	81
	In	N/A	81	13080	13990	13561±279.67	N/A	174	231	207±13.70	N/A	541	710	599±47.66	N/A
	Out	13	81	7230	13420	10699±1338.87	21	50.0	197	115±46.97	44	199	495	357±75.48	40
	Out	17	41	7290	12820	8707±1432.62	36	30.2	169	95.82±43.72	54	190	345	265±43.31	56
The mixture of both dyes	In	N/A	82	323	450	400±20.82	N/A	0.108[b]	0.182[b]	0.154[b]±0.022	N/A	254	350	292±27.99	N/A
	Out	4	82	145	311	192±37.48	52	0.031	0.156	0.069±0.025	55	85.6	270	132.63±43.60	55
	Out	6	82	80	263	119±33.75	70	0.014	0.157	0.050±0.029	68	58.4	276	116.81±54.38	60
	Out	8	82	114	339	233±34.02	42	0.039	0.136	0.067±0.021	56	36.0	91.2	56.79±15.73	81
	Out	10	82	206	372	273±31.43	32	0.044	0.138	0.073±0.017	53	31.5	120	62.71±24.78	79
	In	N/A	81	16090	16190	16130±29.94	N/A	4.652	5.781	5.339±0.310	N/A	480	594	551±43.48	N/A
	Out	12	81	8620	14520	12199±1593.48	24	3.040	5.333	4.371±0.606	18	145	450	347±76.40	37
	Out	14	81	10790	13720	12020±668.23	25	1.884	5.073	3.587±0.936	33	215	431	340±64.17	38
	Out	16	41	9490	13690	11933±1096.63	26	1.521	4.892	3.429±0.997	36	225	397	310±47.10	44
	Out	18	41	6430	12260	10250±1680.25	36	1.100	4.511	2.819±1.032	47	230	373	294±34.61	46

Note: BR, basic red; AB, acid blue; SD, standard deviation; Min., minimum; Max., maximum; [a] No. of samples is 30; [b] All measurements for the mixture are as a wavelength; N/A, not applicable.

Table 5.4 Dye loading rate.

Characteristics				Dye loading rate ($g.m^{-2}.d^{-1}$)			
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ±SD	Reduction (%)
BR46	In	N/A	82	11.98	23.42	17.13±2.09	N/A
	Out	1	82	0.00	3.29	1.45±0.61	92
	Out	5	82	0.00	2.76	1.59±0.67	91
	Out	7	82	0.31	5.21	1.92±1.11	89
	In	11	81	515.22	623.84	573.71±26.74	N/A
	Out	11	81	149.83	515.23	387.12±109.12	33
	In	15	41	257.61	311.92	286.86±13.37	N/A
	Out	15	41	34.95	140.09	77.63±33.42	73
AB113	In	N/A	82	14.82	30.44	20.89±4.63	N/A
	Out	2	82	1.34	13.39	3.20±2.42	85
	Out	3	82	1.69	12.50	3.82±2.34	82
	Out	9	82	1.59	14.79	4.85±3.01	77
	In	13	81	484.59	643.34	576.49±38.15	N/A
	Out	13	81	139.25	548.65	320.28±130.81	44
	In	17	41	242.29	321.67	288.25±19.08	N/A
	Out	17	41	42.05	235.33	133.43±60.88	54

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

Table 5.5 Chemical oxygen demand (COD) loading rate.

Characteristics				COD loading rate ($g.m-^{2}.d^{-1}$)			
Dye	Type of flow	Wetland number(s)	No. of samples	Min.	Max.	Mean ±SD	Reduction (%)
BR46	In	N/A	30	557.00	827.14	690.68±58.18	N/A
	Out	1	30	137.86	259.01	212.13±32.84	69
	Out	5	30	150.39	306.35	205.19±45.79	70
	Out	7	30	55.98	221.41	124.27±38.68	82
	In	11	30	1331.23	1604.16	1423.14±102.27	N/A
	Out	11	30	545.86	1072.23	866.14±145.85	39
	In	15	30	665.62	802.08	711.57±51.14	N/A
	Out	15	30	187.99	419.14	313.31±67.42	56
AB113	In	N/A	30	651.69	863.35	765.88±50.46	N/A
	Out	2	30	150.11	348.13	185.12±45.34	76
	Out	3	30	247.31	490.16	294.51±54.06	62
	Out	9	30	110.56	235.61	148.47±28.69	81
	In	13	30	1506.69	1977.35	1668.22±132.73	N/A
	Out	13	30	554.22	1378.58	994.25±210.21	40
	In	17	30	753.35	988.68	834.11±66.37	N/A
	Out	17	30	264.58	480.41	369.02±60.31	56

Note: BR, basic red; AB, acid blue; Min., minimum; Max., maximum; SD, standard deviation; N/A, not applicable.

5.8 Nutrient Reduction

The reduction of ortho-phosphate-phosphorous is controlled by chemical and physical adsorption, sedimentation, plant uptake, precipitation and microbial uptake in constructed wetland systems (Brix, 1997; Vymazal, 2007, 2010; Johari et al., 2016). Moreover, many researchers have reported that reduction efficiency of phosphorous compounds is generally poor with constructed wetlands (Choudhary et al., 2011; Lavrova and Koumanova, 2013; Ge et al., 2016).

For the low concentrations, in the case of AB113 and BR46, the reduction percentage in planted Wetlands 3 and 5 (low resting and high contact times) was significantly ($p<0.05$) better than those of control unplanted Wetlands 2 and 1 (low resting and high contact times) and planted Wetlands 7 and 9 (high resting and contact times), as shown in Table 5.6. In the case of the mixture of both dyes (BR46 and AB113), Wetlands 4 and 6 (low resting and high contact times) had better reduction percentages when compared with Wetlands 8 and 10 (high resting and low contact times), respectively, as shown in Table 5.6. For the high concentration for BR46, AB113 and the mixture of the two dyes, Wetlands 15, 17, and 16 and 18 (high resting and contact times) had lower PO_4-P effluent when compared with Wetlands 11, 13, and 12 and 14 (low resting and contact times), respectively, as shown in Table 5.6 The previous results for low and high concentrations, indicate that the reduction efficiency for PO_4-P was relatively good especially with wetlands, regardless of planting regime, which have the longer contact times (the lower in resting times).

Nitrification and denitrification are the main reduction mechanisms of nitrogen in constructed wetlands and these mechanisms include two-step processes: firstly, ammonium is oxidized to nitrite followed by oxidation of nitrite to nitrate, these are known as nitrification process. Secondly, the denitrification process includes nitrate being reduced to gaseous nitrogen (Schaechter, 2009; Kessel et al., 2015; Song et al., 2015; Yang et al., 2016). Regarding NH_4-N reduction percentages for low concentration of BR46 and AB113, as shown in Table 5.4, planted Wetlands 7 and 9 (high resting and low contact times) have better reduction percentages when compared with unplanted control Wetlands 1 and 2 as well as planted Wetlands 5 and 3 (low resting and high contact times), respectively. In the case of the mixture of both dyes, Wetlands 8 and 10 (high resting and low contact times) had better reduction percentages in comparison with Wetlands 4 and 6 (low resting and high contact times), respectively, as shown in Table 5.6. In the case of high concentration for BR46, AB113 and the mixture of both dyes, Wetlands 15, 17, 16 and 18 (high resting and contact times) have better reduction percentages when compared them with Wetlands 11, 13, 12 and 14 (low resting and contact times), respectively. The previous results indicate that aeration availability plays a major function in determining the performance of higher nitrogen reduction. These findings have been confirmed by many researchers (Vymazal, 2007; H Wu et al., 2011; Fan et al., 2013c). The effluent NH_4-N values for all wetlands in case of low and high concentration for BR46, AB113 and the mixture of both dyes were complaint with the traditional UK standard (Royal Commission on Sewage Disposal, 1915), which stated that NH_4-N reduction from the secondary wastewater is 50 mg/l. Furthermore, both of Government of India (1986) and Government of Bangladesh (Ahmed et al., 2002) stated that 50 mg/l is also accepted as an effluent for the surface waters.

Regarding NO_3-N reduction for low concentration for BR46, AB113 and the mixture of both dyes, as shown in Table 5.6, the influent NO_3-N values were in the range (23.53-25.37) mg/l. The reduction percentages for all wetlands were in the range of 83%-100%. For the high concentration for BR46, AB113 and the mixture of both dyes the influent values were approximately 33.45 mg/l and the reduction percentages for all wetlands were in the range of 75%-86%, as shown in Table 5.6. These results for NO_3-N reduction percentages indicate that VFCWs have good ability to reduce the nitrogen in high percentages especially when there is a source of organic carbon, and both of the dyes have carbon in their chemical structure as shown in Table 3.1. These findings have been confirmed by Lavrova and Koumanova (2014) and Shen et al. (2015). Furthermore, Lavrova and Koumanova (2013) demonstrated that VFCWs can effectively reduce NO_3-N with/without plants with a sufficient organic carbon source. The effluent NO_3-N values for all wetlands in case of low and high concentration for BR46, AB113 and the mixture of both dyes were complaint to the traditional UK standard, which stated that NO_3-N reduction from the secondary wastewater is 50 mg/l (Royal Commission on Sewage Disposal, 1915).

Table 5.6 Inflow and outflow water quality characteristics for nutrients related to different wetlands.

Characteristics				Ammonia-nitrogen (mg/l)				Nitrate-nitrogen (mg/l)				Ortho-phosphate-phosphorus (mg/l)			
Dye	Type of flow	Wetland number	No. of samples	Min.	Max.	Mean ± SD	Reduction (%)	Min.	Max.	Mean ± SD	Reduction (%)	Min.	Max.	Mean ± SD	Reduction (%)
BR46	In	N/A	30	16.9	25.7	21.98±2.43	N/A	21.9	30.2	25.07±2.38	N/A	5.41	7.65	6.36±0.68	N/A
	Out	1	30	11	22.1	18.72±2.48	15	0.0	0.49	0.21±0.18	99	2.11	5.91	3.49±0.98	45
	Out	5	30	3.67	19.0	14.41±3.53	34	0.0	0.17	0.02±0.05	100	0.0	3.31	1.76±0.83	72
	Out	7	30	2.55	16.1	6.19±4.78	72	0.0	0.67	0.21±0.20	99	3.44	5.87	4.54±0.69	29
	In	N/A	30	21.3	29.7	26.99±2.41	N/A	28.7	38.1	33.47±1.75	N/A	62.5	68.3	65.78±1.75	N/A
	Out	11	30	12.5	31.4	21.67±4.12	20	4.03	16.6	8.41±3.99	75	33.6	52.4	45.33±6.32	31
	Out	15	30	9.2	26.3	20.29±4.79	25	1.4	13.9	6.23±3.61	81	11.9	35.8	19.09±6.70	71
AB113	In	N/A	30	20.4	24.4	23.36±1.02	N/A	19.9	28.23	23.53±2.19	N/A	7.55	11.8	10.34±1.13	N/A
	Out	2	30	9.86	24.3	19.23±4.16	18	0.0	0.95	0.11±0.23	100	2.25	7.27	4.00±1.62	61
	Out	3	30	13.50	36.2	24.48±7.59	-5	0.03	1.51	0.55±0.43	98	2.01	6.21	3.91±1.07	62
	Out	9	30	9.10	17.4	13.20±2.25	43	0.0	1.1	0.22±0.31	99	5.12	8.34	6.22±0.94	40
	In	N/A	30	26.9	29.0	28.16±0.68	N/A	30.2	37.6	33.05±2.10	N/A	144	158	156±3.27	N/A
	Out	13	30	13.5	27.7	20.89±4.74	26	3.14	7.29	5.59±0.89	83	8.92	95.4	46.15±28.22	70
	Out	17	30	9.65	24.7	18.60±4.98	34	2.85	6.32	4.57±0.99	86	21.2	67.0	42.29±16.47	73
The mixture of both dyes	In	N/A	30	27.2	34.2	30.27±1.81	N/A	22.6	29	25.37±1.72	N/A	6.0	8.7	7.56±0.81	N/A
	Out	4	30	17.42	34.97	26.01±4.75	14	0.0	0.34	0.08±0.09	100	2.45	8.31	4.47±1.48	41
	Out	6	30	7.15	22.24	15.66±4.39	48	0.0	2.1	0.13±0.39	100	0.44	4.22	2.03±1.02	73
	Out	8	30	5.10	17.03	9.78±3.21	68	0.05	13.90	3.71±4.79	85	4.2	8.15	5.85±1.19	23
	Out	10	30	2.30	17.88	10.75±5.39	65	0.0	2.54	0.39±0.59	99	3.9	8.9	6.14±1.54	19
	In	N/A	30	23.2	33.6	30.75±2.81	N/A	31.2	35.1	33.91±1.03	N/A	112.1	123	118±2.72	N/A
	Out	12	30	10.7	24.5	20.72±3.68	33	4.32	9.73	6.45±1.44	81	5.21	86.8	58.37±24.80	51
	Out	14	30	9.8	26.4	19.87±3.85	35	3.71	7.51	5.79±1.04	83	4.71	97.2	56.34±26.13	52
	Out	16	30	7.95	25.2	17.41±5.47	43	2.87	6.11	4.89±0.86	86	19.5	74.3	57.31±15.98	51
	Out	18	30	8.59	27.7	16.84±5.25	45	2.43	8.41	4.71±1.23	86	26.3	69.7	50.48±13.16	57

Note: BR, basic red; AB, acid blue; SD, standard deviation; Min., minimum; Max., maximum; N/A, not applicable.

5.9 Seasonal Comparison of Effluent Dyes Reduction

The overall seasonal comparison of the influent and effluent dyes concentration for all wetlands are shown in Table 5.7. In the case of low concentration of BR46 and AB113, the best reduction percentage rates ($p<0.05$) were in the spring season as a result of well-established microbial populations, favourable operating conditions achieved over time, and plants, as confirmed by many publications (Scholz et al., 2002; Al-Isawi et al., 2015a; Scholz, 2015). In the case of high concentration of BR46, AB113 and the mixture of both dyes, the best reduction percentage rates ($p<0.05$) were in the summer season, as shown in Table 5.7, as a result of the higher temperature, as confirmed by several researchers, who stated that the best treatment performance occurred during the higher temperature (Song et al., 2006; Sani et al., 2013).

Table 5.7 Seasonal reduction (mg/l) of dyes from artificial wastewater containing two azo textile dyes.

Characteristics			Summer[a]		Autumn[b]		Winter[c]		Spring[d]	
Dye	Type of flow	Wetland number(s)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)
BR46	In	N/A	6.26 ±0.99	N/A	6.07±0.49	N/A	6.22±0.76	N/A	6.06±0.66	N/A
	Out	1	0.56±0.13	91	0.67±0.23	89	0.47±0.22	92	0.34±0.08	94
	Out	5	0.58±0.18	91	0.58±0.29	90	0.54±0.31	91	0.58±0.16	90
	Out	7	1.00±0.39	84	0.69±0.16	88	0.66±0.37	89	0.28±0.27	95
	In	N/A	202.80±9.52	N/A	207.47±9.64	N/A	208.64±11.05	N/A	204.65±7.18	N/A
	Out	11	95.9±11.94	53	168.3±14.18	19	174.7±4.63	16	128.58±22.27	37
	Out	15	30.94±4.41	85	54.18±8.07	74	80.01±16.99	62	63.71±26.82	69
AB113	In	N/A	8.96±1.55	N/A	6.66±1.15	N/A	6.51±1.36	N/A	7.78±1.17	N/A
	Out	2	1.98±1.13	78	0.96±0.65	86	0.70±0.11	89	0.80±0.18	90
	Out	3	2.24±1.14	75	1.18±0.76	82	1.03±0.15	84	0.85±0.18	89
	Out	9	3.1±0.94	65	1.56±0.53	77	1.25±0.32	81	0.69±0.02	91
	In	N/A	192.33±8.14	N/A	207.58±13.31	N/A	208.00±9.24	N/A	213.82±7.04	N/A
	Out	13	60.68±3.90	68	129.81±27.93	37	172.16±21.45	17	96.49±20.34	55
	Out	17	49.81±5.65	74	109.38±26.88	47	146.48±16.07	30	71.29±33.29	67

Table 5.7 continued

Characteristics			Summer[a]		Autumn[b]		Winter[c]		Spring[d]	
Dye	Type of flow	Wetland number(s)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)
The mixture of both dyes	In	N/A	0.15±0.02	N/A	0.16±0.02	N/A	0.15±0.02	N/A	0.15±0.03	N/A
	Out	4	0.07±0.00	53	0.06±0.01	63	0.05±0.01	67	0.09±0.05	40
	Out	6	0.05±0.01	67	0.04±0.00	75	0.04±0.00	73	0.08±0.05	47
	Out	8	0.07±0.00	53	0.06±0.01	63	0.06±0.00	60	0.09±0.04	40
	Out	10	0.08±0.00	47	0.07±0.01	56	0.06±0.00	60	0.09±0.03	40
	In	N/A	5.35±0.38	N/A	5.24±0.28	N/A	5.34±0.26	N/A	5.44±0.29	N/A
	Out	12	3.60±0.35	33	4.57±0.21	13	4.89±0.32	8	4.57±0.41	22
	Out	14	2.37±0.44	56	3.98±0.38	24	4.51±0.43	16	3.62±0.59	33
	Out	16	2.09±0.53	61	3.75±0.45	28	4.32±0.54	19	3.75±0.58	31
	Out	18	1.57±0.52	71	3.34±0.44	36	3.96±0.56	26	2.52±0.0.42	54
Temperature ˚C			22.8		11.6		8.6		20.5	

Note: [a]21/06/2016 to 21/9/2016; [b]22/09/2016 to 20/12/2016; [c]21/12/2016 to 19/03/2017; [d]20/03/2017 to 29/05/2017; BR, basic red; AB, acid blue; N/A, not applicable; SD, standard deviation.

5.10 Aromatic Amines Reduction

Azo dyes decolourization is achieved under various conditions including: aerobic, anaerobic or anoxic (O'neill et al., 2000; Sponza and Işik, 2002; van der Zee, 2002; Davies et al., 2006). In anaerobic conditions, azo bond (N=N) cleaves and this process releases aromatic amine, which resists any further anaerobic treatment (Brown and Hamburger, 1987; Chung and Stevens, 1993). Aromatic amine can be reduced under aerobic treatment (Weber and Wolfe, 1987; Pinheiro et al., 2004; Ong et al., 2011). Amine compounds are a toxic material and have an effect on the individual bacteria, which leads to the biomass being insufficient to degrade dyes (Phugare et al., 2011; Holkar et al., 2014). Each dye has one or more types of aromatic amines (Pielesz et al., 2002; Pinheiro et al., 2004). Constructed wetlands can degrade aromatic amines under aerobic conditions (Mbuligwe, 2005; Ong et al., 2010, Ong et al., 2011). In this study, three types of amines were released as a result of dye AB113 degradation: 3-AminoBenzeneSulfonic Acid (ABSA), 1,4-DiAminoNaphthalene (DAN) and 5-Amino-8-(phenyl amino) Naphthalene-1-Sulfonic Acid (ANSA) (Senthilvelan et al., 2014). The wavelength of maximum absorbance for each is 288 nm, 255 nm and 225 nm, respectively (Koepernik and Borsdorf, 1983; Paul et al., 1990). In the case of BR46, two aromatic amines were released as a result of its degradation: *N*-benzyi-*N*-mehtylaniline (NBNMA) and *N*-benzyl-*N*-methylbenzene-1,4-diamine (NBNMD), with the wavelength of maximum absorbance for each being 254 nm and 290 nm, respectively (Fihtengolts, 1969; Küçükgüzel et al., 1999). For the low concentration of AB113, in the case of ABSA (Figure 5.27), Wetland 9 (high resting and low contact times) had a significant ($p<0.05$) reduction efficiency when compared with unplanted Wetland

2 and planted Wetland 3 (low resting and high contact times). For the high concentration of AB113, in the case of ABSA amine (Figure 5.28), Wetland 17 (high resting and contact times) had a significant ($p<0.05$) reduction efficiency when compared with Wetland 13 (low resting and contact times). It follows that reduction of aromatic amine compounds needs aerobic conditions, as stated previously. Amines DAN and ANSA, for low and high concentration, didn't appear in the UV-spectrophotometer equipment. This is because both of them are unstable and therefore they were not detected in solution, as confirmed by Davies et al. (2005) and Davies et al. (2006) who also found that using HPLC analysis did not detect this type of amines. For the low concentration of BR46, in the case of NBNMD amine, Wetland 7 (high resting and low contact times) had a significant ($p<0.05$) reduction efficiency when compared with unplanted Wetland 1 and planted Wetland 5 (low resting and high contact times), as shown in Figure 5.29. NBNMA amine, didn't appear in UV-spectrophotometer equipment regarding unplanted Wetland 1, while for planted Wetlands 5 and 7, it was sometimes detected and sometimes not dependant on the activity of micro-organisms required to degrade this type of amines. For the high concentration BR46, in the case of NBNMD amine, as shown in Figure 5.30, Wetland 15 (high resting and contact times) had a significant ($p<0.05$) reduction efficiency when compared with Wetland 11 (low resting and contact times). Furthermore, during the period between (19/12/16; 6 ˚C) and (3/2/17; 10 ˚C), NBNMD amine was not detected as a result of the decrease in temperature during this period and its effect on the growth and development of microbial communities (Jerman et al., 2009). NBNMA amine,

didn't appear in UV-spectrophotometer equipment for the same reason as stated before.

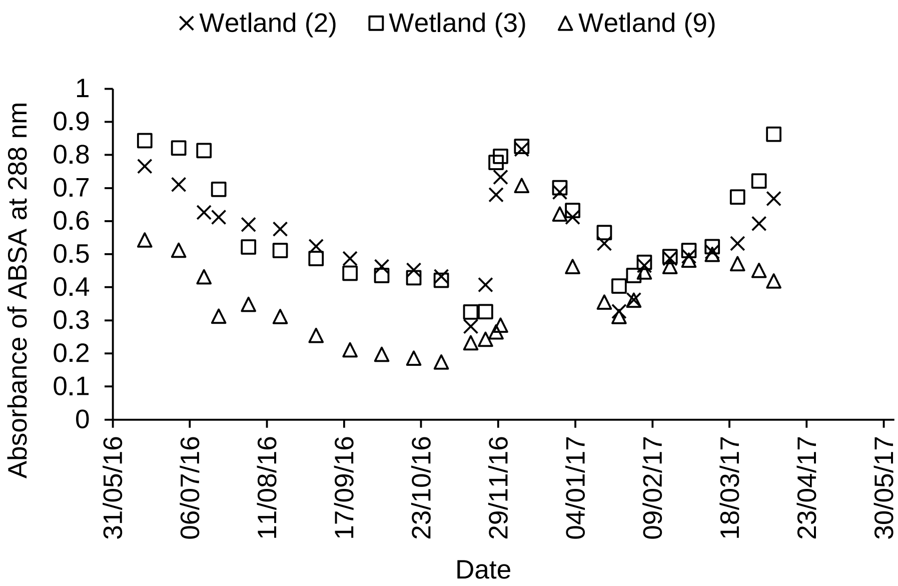


Figure 5.27 Absorbance of ABSA released for Wetlands 2, 3 and 9 (low concentration of AB113)

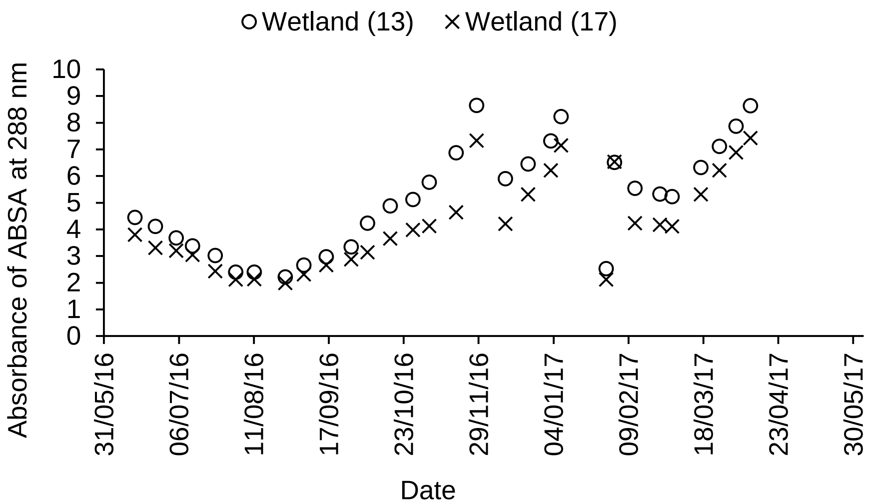


Figure 5.28 Absorbance of ABSA released for Wetlands 13 and 17 (high concentration of AB113)

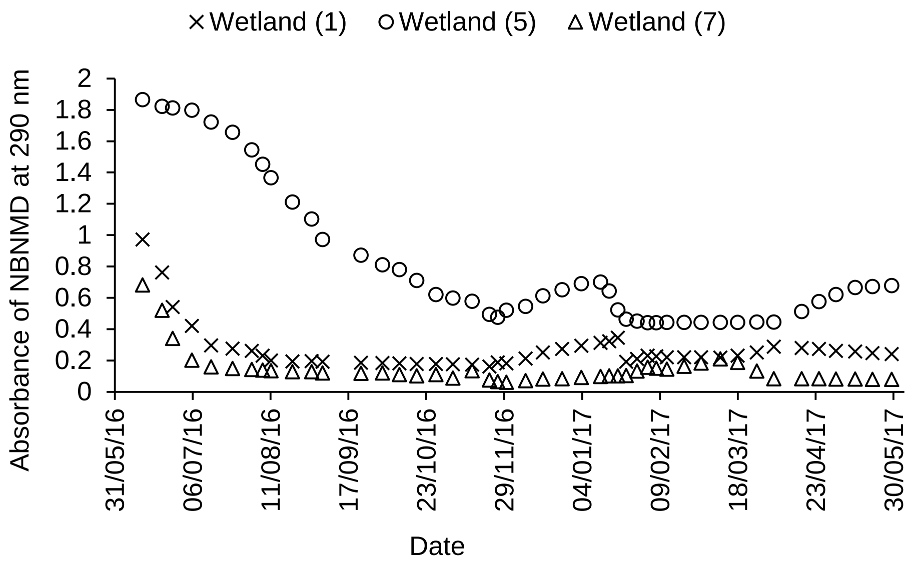


Figure 5.29 Absorbance of NBNMD released for Wetlands 1, 5 and 7 (low concentration of BR46)

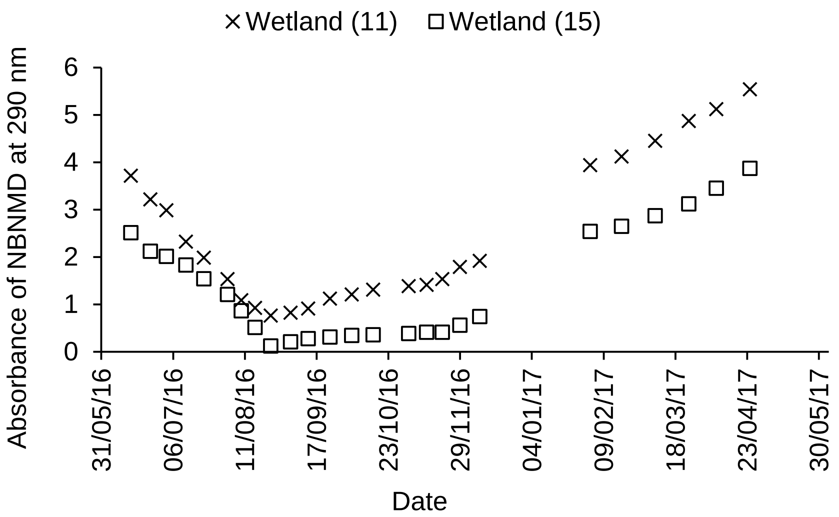


Figure 5.30 Absorbance of NBNMD released for Wetlands 11 and 15 (high concentration of BR46)

5.11 Concluding Remarks

The vertical-flow constructed wetlands showed a significantly ($p<0.05$) good denitrification for low and high concentrations of AB113, BR46 and the mixture of both dyes during all seasons under greenhouse conditions. Furthermore, a good PO4-P reduction was noted, especially with wetlands having long contact times. In addition, good dye reductions were achieved in the case of low and high concentrations of BR46, and low concentrations in the case of AB113 and the mixture of both dyes. The results showed VFCW systems have an ability to cleave the nitrogen atoms and reduce aromatic amine compounds, which are released as a result of nitrogen atom cleavage.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Vertical-flow constructed wetland filters were operated to investigate the performance of treating azo dyes and other water quality variables through different operational variables (low and high concentrations as well as resting and contact times) in two experimental phases (two azo textile dyes only during a first phase and artificial wastewater containing two textile dyes in a second phase). The effect of the presence of plants on dye reduction depends on the dye itself (molecular weight and the chemical structure) and its concentration. Gravel plays a major role to support micro-organisms and nutrients reduction. Furthermore, VFCW systems have the ability to treat different types of dyes, mixtures of them with and without artificial wastewater in case of low and high concentrations. The effects of different operational parameters, resting times, contact times and loading rates on the performance of VFCW systems depends on the group of the selected dyes (acid or basic). Regarding the first experimental phase (two azo textile dyes only), the presence of *P. australis* did not affect dye reductions ($p>0.05$), while it had a minor but insignificant impact on COD reduction ($p>0.05$). The use of plants concerning the short contact time scenario for NH_4-N and a low concentration of AB113 is linked to a better reduction compared to other cases apart from that for all nutrients; particularly PO_4-P and NO_3-N. In the case of low dye concentrations, the presence of plants for the long contact time scenario impacted significantly ($p< 0.05$) positively on the reduction efficiencies of PO_4-P, NH_4-N and NO_3-N. The vertical-flow constructed wetlands showed significantly ($p< 0.05$) good dye reductions for low and high concentrations of BR46, and low concentrations of AB113 during all seasons under greenhouse conditions. The COD reduction was a function of the dye used. This was explained by the complexity of the chemical structure of the dye and seasonal variations in temperature. For high concentrations of BR46, wetlands with a long hydraulic retention time were significantly ($p < 0.05$) better that those with a short hydraulic retention time for the reduction of PO_4-P. For AB113, wetlands with a shorter hydraulic retention time removed PO_4-P significantly ($p<0.05$) well. In the case of the dye AB113, the four-day contact time was not sufficient to remove or reduce TSS and turbidity.

In the second experimental phase (artificial wastewater containing two azo textile dyes), the vertical-flow constructed wetlands showed a significantly ($p<0.05$) good denitrification process for low and high concentrations of AB113, BR46 and the mixture of both dyes during all seasons under greenhouse conditions. Furthermore, a good PO_4-P reduction was noted, especially with wetlands having long contact times. In addition, good dye reductions were achieved in the case of low and high concentrations of BR46, and low concentrations in the case of AB113 and the mixture of both dyes. The results showed VFCW systems have an ability to cleave the nitrogen atoms and reduce aromatic amine compounds, which are released as a result of nitrogen atom cleavage.

6.2 Recommendations

Five main recommendations, which should be considered for further research work, are listed below.

1- The effects of different plant species and an increase of the contact time on particle and dye reduction processes;

2- The effect of pH (low and high) on dye reduction;

3- More investigations of aromatic amine compounds by using HPLC combined with FTIR instruments, especially in the case of the mixture of dyes;

4- Wetland design implications based on the research findings in terms of investment of area and costs; and

5- Working on modelling the results of both controls (without plant) and planted wetlands (dye concentration, nutrients and other pollutants) to help the designer of industrial constructed wetlands in scaling-up tasks.

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APPENDIX A

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Full research paper

Dye wastewater treatment by vertical-flow constructed wetlands

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ABSTRACT

Wetlands have long played an important role as natural purification systems. Textile industry processes are among the most environmentally unsustainable industrial processes, because they produce coloured effluents in large quantities polluting water resources. In this study, two different azo dyes (Acid Blue 113 (AB113) and Basic Red 46 (BR46)) have been fed as part of synthetic wastewater recipes to a laboratory-scale vertical-flow construction wetland set-up comprising wetlands with gravel media as controls and wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) for each dye. Two different concentrations (7 mg/l and 215 mg/l) were used for each dye at two different hydraulic retention times (48 h and 96 h). According to results for the low concentration of BR46, there is no significant ($p > 0.05$) difference between wetlands (unplanted and planted) in terms of dye removal. The use of plants concerning the short contact time scenario for ammonia-nitrogen (NH_4-N) and a low concentration of AB113 is linked to good removal. In case of low dye concentrations, the presence of plants for the long contact time scenario impacted significantly ($p < 0.05$) positive on the removal efficiencies of nutrients. For chemical oxygen demand (COD), the removal percentages were 50%, 59% and 67% for the control and for the wetlands with short and long retention times, respectively. All reductions were statistically significant ($p < 0.05$). For the high concentration of BR46, the removal percentages for this dye and COD were 94% and 82%, and 89% and 74% for the long and short retention times, respectively. For the low concentration of AB113, the percentage corresponding removals for the dye were 71%, 68% and 80%. The COD removals were 4%, 7% and 15% for the control, and the short and long retention times, respectively. Finally, for the high concentration of AB113, the percentage removals for the dye and COD were 71% and 73%, and 50% and 52% for the 48-h and 96-h retention times in this order.

1. Introduction

Azo dyes are important colouring agents in the textile, food and pharmaceutical industries (Tee et al., 2015; Yaseen and Scholz, 2016), and are linked to a relatively high toxicity, mutagenicity and carcinogenicity. Azo dyes and their corresponding breakdown products are difficult to treat in traditional wastewater treatment systems according to Erkurt (2010). Many technological solutions such as coagulation-flocculation and advanced oxidation process (Sivakumar et al., 2013), which have been applied to treat dyes, are not feasible in practice, because of too high costs and complex processes involved. Furthermore, some developing countries such as China and Bangladesh (Chen et al., 2007; Islam et al., 2011), which have a strong textile industry, but unreliable energy sources, may benefit from less-energy demanding methods of wastewater treatment.

Textile wastewater causes considerable environmental pollution. The main challenges are high concentrations of organic matter and particular persistent colorants (dyes) that have to be resistant to the effects of sweat, soap, water, light and oxidants (Olejnik and Wojciechowski, 2012). The azo dyes AB113 and BR46 are, therefore, widely used in the textile industry (Pervez et al., 2000; Olgun and Atar, 2009; Deniz and Karaman, 2011; Yaseen and Scholz, 2016). Typically, textile industry-processing effluents contain dyes in the range between 10 and 200 mg/l (Yassen and Scholz, 2016). Most textile dyes at a rather low concentration of even >1 mg/l can be detected by the human eye (Pandey et al., 2007).

Abbreviations: AB, acid blue; ANOVA, analysis of variance; AO, acid orange; AY, acid yellow; BR, basic red; CASRN, chemical abstracts survey registry number; COD, chemical oxygen demand; DO, dissolved oxygen; DY, disperse yellow; EC, electric conductivity; HF, horizontal-flow; N, nitrogen; NH_4-N, ammonia-nitrogen; n/a, not applicable; NO_2-N, nitrite-nitrogen; NO_3-N, nitrate-nitrogen; PO_4–P, ortho-phosphate-phosphorus; RB, reactive black; SD, standard deviation; SE, standard error; TDS, total dissolved solids; T-N, total nitrogen; TOC, total organic carbon; T-P, total phosphorus; TSS, total suspended solids; VF, vertical-flow; VY, vat yellow; Λ_{max}, maximum absorbance.

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Vertical-flow constructed wetlands are engineered ecosystems designed to remove pollutants from wastewater (Kadlec and Wallace, 2009). These systems mimic the treatment that occurs in natural wetlands by relying on heterotrophic microorganisms, aquatic plants and a combination of naturally occurring processes (Scholz, 2015).

Researchers previously investigated the performance of ponds (Yaseen and Scholz, 2016) and, in particular, constructed wetlands to treat textile wastewater (Table 1). However, results rarely cover all seasons (Vymazal, 2014). Pervez et al. (2000) used aerated vertical-flow wetlands to remove two textile Azo dyes (RB171 and AB113) from synthetic wastewater for a period of 70 days. The percentage removal of 98% was high for both dyes. However, the results did not assess the removal of COD, phosphorus and nitrogen.

Davies et al. (2006) used vertical-flow wetlands for aerobic degradation of Acid Orange 7 (AO7) in a short-term study (Table 1). The results showed colour and COD removals of 99% and 93%, respectively. However, phosphorus and nitrogen removal were not recorded.

Yalcuk and Dogdu (2014) used vertical-flow constructed wetlands to treat Acid Yellow 2G E107 Dye-containing wastewater (Table 1). The constructed wetland consisted of three vertical wetland filters, which were filled with fine gravel, sand and zeolite. One wetland was kept unplanted as a control and the other two were planted with *Canna idica* L. and *Typha angustifolia* L., respectively. The period of operation was only three months. The results showed that the average colour removal percentages were 87% for the control and 98% for the other wetlands. For NH_4-N, the average percentages removals were 43%, 61% and 46% for the control, *C. idica* and *T. angustifolia*, respectively. The average percentages of ortho-phosphate-phosphorus (PO_4-P) for the control, *C. indica* and *T. angustifolia* were 84%, 87% and 88% respectively. Only 90 days was the time of operation.

The presence of *Phragmites australis* in vertical-flow constructed wetlands has a significant impact on the removal of organic matters, aromatic amines and NH_4-N (Ong et al., 2011). The growth cycle of *P. australis* traditionally completes between May and September in Britain (Haslam, 1972). Ferreira et al. (2014) detected normal growth for *Phragmites* with absence of toxic signs or depletion of leaf nitrogen content, when they used vertical-flow wetlands to treat an effluent comprising Diazo dye (DR81).

The aim of this study is to assess the efficiency of vertical-flow constructed wetlands in removing dye, COD, PO_4-P and other nutrients. The corresponding objectives are to assess (a) the role of *P. australis* on dye removal; (b) the influence of two groups of dyes (Acid and Direct) on the performance of constructed wetlands on dyes removal; and (c) the influence of operational parameters such as contact time, resting time and loading rate on dye removal.

2. Materials and methods

2.1. Wetland rig and operation

The study has been conducted between 1 May 2015 and 31 May 2016. The first month may be viewed as the start-up period. An experimental constructed wetland rig (Fig. 1) treating textile wastewater has been operated within a greenhouse located on top of the Newton Building (The University of Salford). The rig has been designed to assess the system performance by simulating processes occurring within full-scale constructed wetlands. The rig comprises twenty-two vertical-flow wetland filters, allowing wastewater to drain vertically, enhancing aerobic biodegradation of organic matter and nitrogen (Fuchs 2009). The experimental wetland filters were located randomly in the experimental rig to minimise random impacts of parameters such as sunlight direction and temperature differences on the wetland performances.

Fig. 1. Experimental vertical-flow constructed wetland rig located within a greenhouse at the beginning of the experiment (Picture taken by Mr. Amjad Hussein on 15 May 2015).

Operational parameters such as contact time, retention time and hydraulic loading rate on dye removal were assessed. Contact time is defined as the duration the wastewater is in contact with the wetland filter content. In comparison, resting time is the duration when the wetland filter is empty (i.e. no wastewater input). The dyes AB113 and BR46 were tested at two different concentrations (target concentrations of 7 mg/l and 215 mg/l) for different retention times (approximately 48 h and 94 h) to assess their influence on the performance of vertical-flow constructed wetlands.

Round and black plastic drainage pipes were used to construct the vertical-flow wetlands (Fig. 1). All twenty-two wetlands were designed according to the following dimensions: height of 100 cm and diameter of 10 cm. One wetland was filled with water, another one was filled to a depth of 90 cm with unwashed gravel and the other wetlands were filled to a depth of 90 cm with washed gravel (Table 2). Two different layers of gravel were used as filter media. Large gravel with a diameter of 10–20 mm was applied as the bottom layer to prevent clogging of the outlet. Pea gravel with a diameter of 5–10 mm was located at the top layer. The outlet valves were located at the centre of the bottom plate of each wetland. The internal diameter of the vinyl outlet tubing was 10 mm.

Selected wetlands were planted with *P. australis* (Table 2). The growth of *P. australis* was monitored. Dead above-ground plant parts were cut down to about13 cm height. The cuttings were recycled by placing them into their corresponding wetland filters.

Table 1
Previous studies (listed in order of date) on textile wastewater treatment by constructed wetlands.

Dye used	Type of wetland	Design characteristics	Plants used	Removal performance	Duration (days)	Country of operation	References
AB113, *RB171	VF	Gravel-sand	*P. australis*	98% colour	70	USA	Pervez et al. (2000)
AO7	VF	Gravel-sandy clay soil	*P. australis*	74% colour, 64% COD and 71% TOC	77	Portugal	Davies et al. (2005)
Various dyes in real wastewater	HF	Gravel-sand	*Typha* and cocoyam	77% colour, 72% COD and 59% sulphate	84	Tanzania	Mbuligwe (2005)
*RB5, DY211, VY46	VF-HV	Gravel-sand-tuff	*P. australis*	90% colour, 84% COD, 93% TSS, 52% T-N, 87% $N_{organic}$, −331% NH_4-N, 88% sulphate, 80% anion surfactant and 93% TSS	60	Slovenia	Bulc and Ojstršek (2008)
AO7	VF	Gravel-sludge	*P. australis*	94% colour, 95% COD and 86% NH_4-N	27	n/a	Ong et al. (2011)
AY 2G E107	VF	Gravel-sand-zeolite	*Canna* and *Typha*	95% colour, 64% COD, 94% PO_4-P and 77% NH_4-N	90	Turkey	Yalcuk and Dogdu (2014)

Note: AB, acid blue; *RB, reactive blue; AO, acid orange; VF, vertical flow; COD, chemical oxygen demand; TOC, total organic carbon; **RB, reactive black; DY, disperse yellow; VY, vat yellow; HV, horizontal flow; TSS, total suspended solid; T-N, total nitrogen; N, nitrogen; NH_4-N, ammonium nitrogen; PO_4-P, Ortho-phosphate-phosphorus; AY, acid yellow.

Table 2
Packing order of the experimental constructed wetland set-up. Samples were taken at the end of each contact time period, just before the start of the resting time period between 1 May 2015 and 31 May 2016.

Wetland number	Media	Plants	Dye			Resting time (h)	Contact time (h)
			Type	Mean (mg/l)	SD (mg/l)		
1	Only Water	No	None	n/a	n/a	2	94
2	Unwashed gravel	No	None	n/a	n/a	2	94
3	Washed gravel	No	None	n/a	n/a	2	94
4	Washed gravel	No	BR46	6.9	1.33	2	94
5	Washed gravel	No	AB113	6.6	1.82	2	94
6,7	Washed gravel	Yes	AB113	6.6	1.82	2	94
8,10	Washed gravel	Yes	BR46	6.9	1.33	2	94
11,12	Washed gravel	Yes	BR46	6.9	1.33	48	48
13,14	Washed gravel	Yes	AB113	6.6	1.82	48	48
15,16	Washed gravel	Yes	BR46	209.3	4.48	48	48
17,18	Washed gravel	Yes	AB113	221.5	31.86	48	48
19,20	Washed gravel	Yes	BR46	209.3	4.48	96	96
21,22	Washed gravel	Yes	AB113	221.5	31.86	96	96

Note: SD, standard deviation; n/a, not applicable; BR, basic red; AB, acid blue.

The dyes AB113 and BR46 were used at two inflow concentrations (low and high) to assess the performance of the vertical-flow wetland systems to treat dye at the presence of fertiliser only. No other contaminants were added to allow for a statistically sound assessment of the impact of dye. The fact that apart from some high dye concentrations all other concentrations in the inflow were relatively low may naturally result in low outflow concentrations as well, indicating good treatment performance, but not necessarily good removal performances.

Details for each dye can be found in Tables 2 and 3. The textile dye BR46 had a wavelength for the maximum absorbance (λ_{max}) of 530 nm (Khatae, 2009), and was obtained from Dystar (Am Prime Park, Raunheim, Germany). The dye AB113 had a λ_{max} of 566 nm (Shirzad-Siboni et al., 2014), and was obtained from Sigma Aldrich (The Old Brickyard, New Road Gillingham, Dorset, United Kingdom). Dye stock solutions were prepared by dissolving 10 g of each dye in 1000 ml of distilled water. The experimental solutions were obtained by diluting stock solution samples to the required concentrations.

The fertiliser TNC Complete, which is an aquatic plant supplement bought from TNC Limited (Spotland Bridge Mill, Mellor Street, Rochdale, United Kingdom), was used in the experimental work as a nutrient and trace element source for the plants. The corresponding ingredient composition was as follows: nitrogen (1.5%), phosphorus (0.2%), potassium (5%), magnesium (0.8%), iron (0.08%), manganese (0.018%), copper (0.002%), zinc (0.01%), boron (0.01%) and molybdenum (0.001%). Ethylene diamine tetra acetic acid (EDTA), which is used as a source for copper, iron, manganese and zinc, is also provided by TNC Complete. For ten litre of freshly prepared influent, one millilitre fertiliser was added.

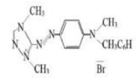
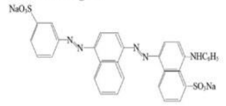
The wetland system has been designed to operate in batch flow mode to avoid expenses such as pumping and automatic control costs. Wastewater was poured directly into the wetland filter from the top. The wastewater stayed within the wetland for the duration of the contact time. The treated wastewater was released from the wetland through an outlet pipe located in the centre of the wetland bottom. The duration the liquid stayed within the wetland is the resting time (see also above).

2.2. Wetland filter set-up

Table 4 outlines the application of the simplified statistical wetland filter set-up design (Table 2) to assess the impact of individual key variables. Wetland 1 was deliberately left empty (without media and plants; Table 2). Only tap water was used as the influent. The mean tap water values for dissolved oxygen (DO), pH, electric conductivity (EC), redox potential, total suspended solids (TSS) and turbidity were 10.1 mg/l, 7.6, 85.1 μS, −32 mV, 1 mg/l and 1.3 NTU, respectively.

Wetland 2 was filled with unwashed gravel, while Wetland 3 comprised washed gravel and tap water. Wetlands 4, 8/10, and 11/12 were filled with the same media as Wetland 3, and planted with *P. australis*, except for Wetland 4, which was used as a control (Table 2). The rhizomes of the plants were washed free of sediment

Table 3
Details of dyes used in the experimental constructed wetlands.

Dye	BR46	AB113
Molecular weight	401.3	681.65
Molecular formula	$C_{18}H_{21}BrN_6$	$C_{32}H_{21}N_5Na_2O_6S_2$
Source	Dystar	Sigma Aldrich
CASRN	12221–69-1	3351–05-1
Purity of dye (%)	70–80	Approximately 50
Nutrient content for 200 mg of dye (mg/l)	50.0 ortho-phosphate-phosphorus, 7.5 ammonia-nitrogen and 0.93 nitrate-nitrogen	137.0 ortho-phosphate-phosphorus, 7.7 ammonia-nitrogen and 1.8 nitrate-nitrogen
Chemical structure		

Note: CASRN, chemical abstracts survey registry number; BR, basic red; AB, acidic blue.

Table 4
Application of the simplified statistical wetland filter set-up design (Table 2) to assess the impact of individual key variables.

Comparison of two wetland systems with each other		Impact to be assessed
First wetland with number	Second wetland with number	
1	2	Unwashed gravel
2	3	Washed gravel
3	4	BR46
3	5	AB113
4	5	Difference between BR46 and AB113
5	6,7	*Phragmites australis*
6,7	8,10	Difference between BR46 and AB113
8,10	11,12	Decrease in contact time(or increase in resting time)
11,12	13,14	Difference between BR46 and AB113
11,12	15,16	Increased BR46 concentration
13,14	17,18	Increased AB113 concentration
15,16	19,20	Increased contact and resting times
17,18	21,22	Increased contact and resting times
15,16	17,18	Difference between BR46 and AB113
19,20	21,22	Difference between BR46 and AB113

and planted in selected gravel-filled wetlands. The influent was tap water mixed with BR46 (6.9±1.33 mg/l).

The contact time for Wetlands 8 and 10 was 94 h, and the resting time was 2 h, while for Wetlands 11 and 12, the contact time was 48 h and the resting time was 48 h (Table 2). The purpose for different contact and resting times was to assess the performance of wetlands in removing dyes under different design conditions, and compare the results with those for Wetland 4 to assess the importance of the presence of plants on dye removal and other parameters. The same previously explained set-up was used for Wetlands 5, 6/7, and 13/14, but with the dye AB113 at a concentration of about 6.6±1.82 mg/l.

The Wetlands 15/16, and 19/20 contained the same media, plants and dye (BR46) as Wetlands 8/10, and 11/12, but the dye concentration was approximately 209 mg/l. The Wetlands 15 and 16 are replicates with 48-h contact time and 48-h resting time, while the Wetlands 19 and 20 are replicates with 96-h contact time and a resting time of 96 h. Wetlands 17, 18, 21 and 22 are similar to Wetlands 15, 16, 19 and 20, but the influent dye was AB113 at a concentration of about 216 mg/l (Table 2).

2.3. Experimental and statistical analysis

The water quality analysis was performed according to APHA (1995), unless stated otherwise, to assess the annual and seasonal treatment performance. Both influent and effluent were analysed. The effluent was obtained from the bottom of each wetland filter. The inflow wastewater was freshly prepared before it was poured into the wetland from the top.

The spectrophotometer DR 2800 Hach Lange (www.hach.com) was applied for the water quality analysis for variables including dyes, COD, NH_4-N, NO_3-N, PO_4-P and TSS. Turbidity was determined with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, Division Street, Chicago, IL, USA). The pH and redox potential for all samples was measured using a VARIO pH meter (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). This meter was calibrated with standard buffer solutions of pH 4, 7 and 9 each two weeks or whenever required. The EC and total dissolved solids (TDS) were measured using a Mettler Toledo Education Line Conductivity Meter (Boston Road, Leicester, UK). The DO was measured using a Hach Lange HQ30d Flexi Meter (Pacific Way, Salford, UK). Water samples were taken regularly as shown in Table 2, and they were brought to the university-based laboratory for testing.

Water samples were always taken at the same time (10:00 to 11:00 am) to ensure that the various environmental boundary conditions, which are variable throughout the day, such as diurnal variations of pH and DO, would not impact on the results. Laboratory tests were performed to measure dyes, COD, PO_4-P, NO_3-N, NH_4-N, EC, DO, redox potential, pH, TSS, TDS and turbidity. The temperature at the site is recorded each week using a thermometer placed alongside the constructed wetlands.

In order to investigate statistically significant differences, the Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) was used to assess the normality of data. A one-way analysis of variance (ANOVA) test using Statistical Package for the Social Science software was applied to analyse normal distributed data, while the Mann-Whitney test was used to analyse non-normal data

(Stoline, 1981; Kasuya, 2001). These ANNOVA and Mann-Whitney tests were used to compare means between different treatments such as the ones highlighted in Table 4. Significant findings have been outlined and discussed. Somehow surprising and/or important insignificant findings have occasionally been highlighted as well.

3. Results and discussion

3.1. Test of normality for plant and water quality variables

Tests of normality results for dimensions of *P. australis* and for effluent water quality characteristics regarding general physical and chemical variables are discussed in the supplementary material S1

3.2. Plant growth assessment

When temperatures started to decrease in winter (see Supplementary material S2), the plants in this experiment began to yellow. The above-ground parts died, were cut and returned to the wetland filters. The authors followed common practice to cut the above-ground plant parts down to between 10 and 15 cm height according to Stefanakis et al. (2014).

Based on the experimental results, the most critical observation is that for all considered concentrations and contact times, the plants that were subjected to the dye AB113 revealed more effective growth compared to those that were fed by the dye BR46. Taking into consideration the potential impact of retention time, it has been noted that plants associated with a long contact time showed much better growth than those plants linked to a short one (Table 5). These findings support those by Pagter et al. (2005), who investigated the effect of water stress tolerance of *P. australis* grown in the laboratory by examining effects of different levels of required water. The results showed that a water deficit reduces the leaf biomass per plant and the leaf area.

The ANOVA and Mann-Whitney tests were applied for normal and non-normal distributed data, respectively (see above). Regarding the growth of plants, there is no significant ($p > 0.05$) difference with respect to the length and diameter at the low concentration of the dye AB113, while for the high concentration, there is a significant ($p > 0.05$) difference for the diameter but no significant ($p > 0.05$) difference for the length. In case of plants grown in the presence of the dye BR46, there is a significant ($p < 0.05$) difference for both the length and diameter at the low concentration of dye, while no significance ($p > 0.05$) for both parameters was noted at the high concentration of dye. Readers may wish to consult Table S1 and the corresponding discussion in the supplementary material S1 for more details. However, no generic interpretation of the data can be made for both dyes, indicating further research needs by ecologists.

3.3. Dissolved oxygen and redox potential

Redox potentials of less than −100 mV indicate anaerobic environments, while values greater than 100 mV indicate aerobic environments (Suthersan, 2001). Redox potential and DO values for the effluents of both dyes were in the range between −32 and −6 mV and between 5.4 and 8.2 mg/l, respectively, for low dye concentrations, and between −32 and 4 mV and between 6.0 and 7.5 mg/l, respectively, for high dye concentrations (Tables 2 and 5). However, there is the possibility that the effluent samples got aerated between taking the samples and corresponding measurements. Nevertheless, these findings indicate that dye degradation may have taken place in both aerobic and anaerobic environments.

3.4. Electrical conductivity, total suspended solids and turbidity

Electrical conductivity can be used as an indirect measure of the charge (or the ion-carrying species) in the wetland outflow (Islam et al., 2011). For the low concentration of both dyes (BR46 and AB113), as shown in Table 6, an increase was noted for Wetlands 11/12 and Wetlands 13/14 (contact times of 48 h for each), respectively, when compared to Wetlands 8/10 and 4 as well as Wetlands 6/7 and 5 (contact times of 94 h for each), respectively. For the high concentration in case of dye AB113, the average value of EC in the effluent of Wetlands 21/22 (high resting and contact times) is the same as for the influent, while for Wetlands 17/18 (low resting and contact times), there is a decrease in the average value, if compared to the influent. In case of dye BR46, there is a statistically insignificant ($p > 0.05$) increase in the average value for the effluent of Wetlands 15/16 (low resting and contact times; 389 ± 99.73 μS/cm), and Wetlands 19/20 (385 ± 83.21 μS/cm), if compared to the influent (357 ± 112.3 μS/cm).

Yalcuk and Dogdu (2014) have reported similar findings. The measurement of TSS is important for the design of water treatment facilities (Dzurik, 2003). For the dye BR46 in case of low concentration, there is an increase in the effluent for Wetlands 4, 11 and 12 (Tables 2 and 6), while there is no increase in the average value for Wetlands 8 and 10 (Tables 2 and 6), if compared with the influent.

For the high concentration scenario, the removal in Wetlands 19 and 20 (high resting and contact times) is better than that in Wetlands 15 and 16 (low resting and contact times). For the dye AB113 in case of low concentration, increased values of TSS in the effluent were noted for the Wetlands 5, 6/7, and 13/14 (Tables 2 and 6). Only a slight increase was noted for Wetlands 6/7 (low resting and high contact times), while a higher increase was recorded for Wetlands 13/14 (high resting and low contact times). In case of the high concentration scenario, a statistically insignificant ($p > 0.05$) value was noted for the Wetlands 17/18 (low resting and contact times) and 21/22 (high resting and contact times). Increased values for Wetlands 21/22 were noted (392 ± 253.13 mg/l), if compared to the Wetlands 17/18 (371.7 ± 177.57 mg/l).

Turbidity is often used to interpret the degree of clarity of water. It is a variable often applied as an indicator of the amount of suspended sediments and/or larger micro-organisms in water. A high turbidity of surface water may also indicate elevated concentrations of TSS, reduced algal populations, and potential harm to fish and other aquatic fauna (Postolache et al., 2007).

For the dye BR46 ($p < 0.05$) in case of the low concentration scenario, removal was noted for the planted Wetlands 8/10 (3.0 ± 1.33 mg/l), while there was an increase linked to the unplanted Wetlands 4 (8.8 ± 7.12 mg/l; $p > 0.05$) and planted Wetlands 11/12 (3.6 ± 1.89 mg/l) (see also Tables 2 and 6), if compared to the influent (3.5 ± 2.89 mg/l). In case of high concentrations, good removal was noted for the Wetlands 15/16 (low resting and contact times; 6.6 ± 2.20 mg/l; $p > 0.05$) and the Wetlands 19/20 (high resting and contact times; 6.8 ± 3.90 mg/l; $p > 0.05$), when compared with the influent (16.6 ± 3.10 mg/l). For the dye AB113 in case of low concentration ($p < 0.05$), increased values in the effluent were noted for the unplanted Wetland 5 (39.2 ± 27.40 mg/l), the planted Wetlands 6/7 (19.1 ± 16.11 mg/l) and the planted Wetland 13/14 (46.8 ± 32.67 mg/l) (see also Tables 2 and 6), if compared to the influent (7.4 ± 5.67 mg/l). A greater increase was recorded for Wetlands 13/14 (high resting and low contact times), if compared to Wetlands 6/7 (low resting and high contact times). In case of the high concentration scenario, a removal was noted for Wetlands 17/18 (low resting and contact times; 208.3 ± 196.67 mg/l; $p < 0.05$), while there was an increase in the average value for Wetlands 21/22 (high resting and contact times; 224 ± 202.69 mg/l; $p > 0.05$), if compared to the influent (213.4 ± 157.62 mg/l; $p < 0.05$).

Table 5
Dimensions of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) planted in the experimental wetlands (Tables 2 and 4).

Characteristics			Length (cm)			Diameter (mm)		
Dye	Wetland number	Number of steams	Mini-mum	Maxi-mum	Mean ±SD	Mini-mum	Maxi-mum	Mean ±SD
BR46	8 and 10	40	40	110	86 ± 25.0	2.5	3.8	3.6 ± 0.16
	11 and 12	28	17	44	30 ± 9.0	1.1	1.8	1.5 ± 0.30
	15 and 16	34	14	50	27 ± 13.0	1.0	1.2	1.1 ± 0.07
	19 and 20	23	12	34	23 ± 8.1	0.9	1.5	1.2 ± 0.25
AB113	6 and 7	45	110	141	131 ± 9.5	3.4	3.8	3.6 ± 0.16
	13 and 14	30	23	41	32 ± 4.5	2.1	2.4	2.2 ± 0.10
	17 and 18	36	28	46	35 ± 6.0	2.1	2.3	2.2 ± 0.06
	21 and 22	28	22	52	39 ± 10.7	1.8	2.5	2.1 ± 0.25

Note: BR, basic red; AB, acidic blue; SD, standard deviation.

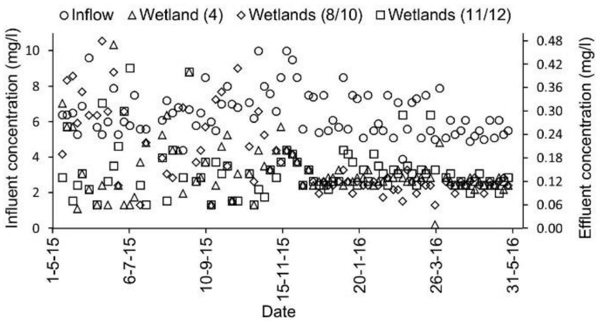


Fig. 2. Inflow and outlow concentrations of Basic Red 46 for Wetlands 4, 8/10 and 11/12 (see also Tables 2 and 4).

Many researchers confirmed that vertical-flow wetlands have a relatively poor ability to remove TSS and turbidity (Lin et al., 2005; Bulc and Ojstršek, 2008). Regarding this study, for the low concentration of BR46, a long contact time (94 h) was better than a short (48 h) one in removal of TSS and turbidity. While for high concentrations, the short contact time was better than the long contact time. In case of low and high concentrations for AB113, the four-day contact time was not enough to reduce TSS and turbidity.

3.5. pH value

For the low concentration of the dye BR46, the average pH inflow value was 7.2. A slight increase in the effluent pH values of 0.16 and 0.03 were noted for the unplanted Wetland 4 and the planted Wetlands 11/12 (see also Tables 2 and 6), respectively, while there was a decrease of 0.31 for Wetlands 8/10 (replicates), as shown in Table 6. For the high concentration, the average influent value was 6.49. Increases of 0.19 and 0.57 were noted for Wetlands 15/16 (low contact and resting times) and Wetlands 19/20 (high contact and resting times), respectively. In case of the dye AB113 for the low concentration scenario, the average influent pH value was 7.32. A slight pH increase of 0.11 was noted for the unplanted Wetland 5, while the pH value decreased for the planted Wetlands 6/7 and the planted Wetlands 13/14 (see also Tables 2 and 6) by 0.44 and 0.15, respectively. For the high concentration, the average influent pH value was 8.39. The pH value decreased for the Wetlands 17/18 (low resting and contact times) and the Wetlands 21/22 (high resting and contact times) by 1.10 and 1.06, respectively, as shown in Table 6.

These results indicate the ability of macrophytes such as *P. australis* to modify pH conditions in the rhizosphere, confirming results by Brix et al. (2002). However, these researches used a different wetland plant (*Typha angustifolia* L.).

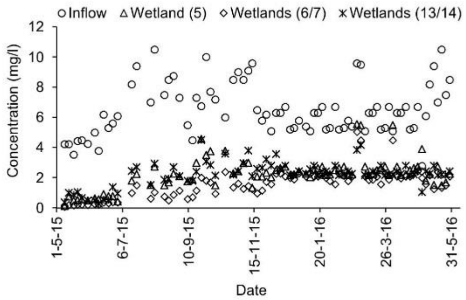


Fig. 3. Inflow and outlow concentrations of Acid Blue 113 for Wetlands 5, 6/7 and 13/14 (see also Tables 2 and 4).

3.6. Dye removal and chemical oxygen demand removal

The percentage removal of dyes is shown in Table 7. The degradation of azo dyes takes place both in aerobic and anaerobic conditions via various processes involving enzymes and/or chemical reduction (Pandey et al., 2007). BR46 is theoretically easier to degrade than AB113 due to a lower molecular weight (Table 3).

There is no significant ($p > 0.05$) difference in dye removal for the low concentration of BR46 between the unplanted control Wetland 4, the planted Wetlands 8/10 and the planted Wetlands 11/12 (Tables 2 and 6, and Fig. 2). It follows that the presence of plants does not affect dye removal. In comparison, for AB113, the dye removal in the planted Wetlands 6 and 7 was significantly ($p < 0.05$) better

Table 6
Inflow and outflow water quality characteristics for general physical and chemical variables related to different wetlands (Tables 2 and 4).

Characteristics				pH			Redox potential (mV)			Dissolved oxygen (mg/l)		
Dye	Type of flow	Wetland number	No. of samples	Mini-mum	Maxi-mum	Mean ±SD	Mini-mum	Maxi-mum	Mean ±SD	Mini-mum	Maxi-mum	Mean ±SD
BR46	In	n/a	70	6.80	7.54	7.2 ± 0.147	−39	5	−25 ± 7.7	9.0	10.9	9.7 ± 0.46
	Out	4	70	7.11	7.75	7.36 ± 0.137	−43	−14	−29 ± 6.35	5.5	10.0	8.2 ± 1.12
	Out	8 and 10	70	6.67	7.21	6.89 ± 0.148	−22	8	−5 ± 6.79	3.7	8.1	5.4 ± 0.92
	Out	11 and 12	70	6.85	8.18	7.23 ± 0.212	−40	−7	−22 ± 9.25	2.9	9.5	7.5 ± 1.54
	In	n/a	70	6.04	6.83	6.49 ± 0.179	−6	28	12 ± 8.06	8.7	10.7	9.6 ± 0.41
	Out	15 and 16	70	6.35	7.11	6.68 ± 0.217	−17	17	4 ± 7.53	3.8	9.2	6.3 ± 1.34
	Out	19 and 20	35	6.65	7.37	7.06 ± 0.163	−24	4	−13 ± 7.58	3.2	8.7	6.0 ± 1.29
AB113	In	n/a	70	7.14	7.65	7.32 ± 0.122	−43	−21	−30 ± 5.4	8.9	11.2	9.6 ± 0.55
	Out	5	70	7.20	7.77	7.43 ± 0.165	−51	−19	−32 ± 6.5	5.1	10.1	8.3 ± 1.38
	Out	6 and 7	70	6.62	7.4	6.88 ± 0.184	−33	17	−6 ± 8.2	3.7	6.9	5.4 ± 0.77
	Out	13 and 14	70	6.78	7.57	7.17 ± 0.199	−40	5	−23 ± 8.2	3.5	10.4	8.1 ± 1.45
	In	n/a	70	7.25	9.04	8.39 ± 0.432	−129	−46	−88 ± 18.2	8.2	10.7	9.5 ± 0.49
	Out	17 and 18	70	7.04	7.6	7.29 ± 0.118	−46	−10	−28 ± 9.6	5.4	9.9	7.5 ± 1.19
	Out	21 and 22	35	7.10	7.99	7.33 ± 0.202	−74	−12	−32 ± 14	3.9	10.0	7.1 ± 1.76
Characteristics				Total suspended solids (mg/l)			Turbidity (NTU)			Electric conductivity (μS/cm)		
BR46	In	n/a	70	0	5.1	2.0 ± 1.51	1.6	18.5	3.5 ± 2.89	90	270	159 ± 51.6
	Out	4	70	0	23.0	6.8 ± 5.81	1.9	32	8.8 ± 7.12	70	483	193 ± 79.1
	Out	8 and 10	70	0	6.2	2.0 ± 1.88	1.5	6	3.0 ± 1.33	103	749	257 ± 97.4
	Out	11 and 12	70	0	10.2	3.4 ± 3.33	1.9	12	3.6 ± 1.89	82	419	180 ± 62.4
	In	n/a	70	37.1	59.0	47.9 ± 5.11	8.9	24	16.6 ± 3.1	147	701	357 ± 112.3
	Out	15 and 16	70	1.1	21.3	7.6 ± 4.70	2.9	13	6.6 ± 2.2	253	647	389 ± 99.73
	Out	19 and 20	35	2.3	41.0	6.9 ± 8.41	2.9	20.1	6.8 ± 3.9	251	535	385 ± 83.21
AB113	In	n/a	70	5.5	25.0	14.1 ± 5.40	1.3	21.0	7.4 ± 5.67	70	350	220 ± 104.1
	Out	5	70	3.4	85.0	41.9 ± 23.07	5.8	134.0	39.2 ± 27.40	75	396	242 ± 87.9
	Out	6 and 7	70	1.4	64.0	18.8 ± 15.92	2.8	57.0	19.1 ± 16.11	114	448	294 ± 85.4
	Out	13 and 14	70	3.5	133.0	46.5 ± 33.60	2.3	128.0	46.8 ± 32.67	83	430	235 ± 99.6
	In	n/a	70	119.0	843.0	364.8 ± 195.47	21	660	213.4 ± 157.62	465	976	635 ± 123.2
	Out	17 and 18	70	121.0	706.0	371.7 ± 177.57	12	650	208.3 ± 196.67	456	854	624 ± 99.1
	Out	21 and 22	35	67.0	863.0	392 ± 253.13	9	675	224 ± 202.69	502	844	635 ± 93.3

Note: BR, basic red; AB, acidic blue; SD, standard deviation; n/a, not applicable.

Table 7
Dye and chemical oxygen demand (COD) removal for different wetlands (Tables 2 and 4).

Characteristics				Dye concentration (mg/l)			Dye loading rate (mg/l/d)				
Dye	Type of flow	Wetland number	No. of samples	Mini-mum	Maxi-mum	Mean ±SD	Removal (%)	Mini-mum	Maxi-mum	Mean ±SD	Removal (%)
BR46	In	n/a	70	4.4	10.0	6.9 ± 1.33	n/a	0.42	0.94	0.65 ± 0.12	n/a
	Out	4	70	0.1	0.9	0.3 ± 0.2	96	0.006	0.08	0.03 ± 0.02	95
	Out	8 and 10	70	0.1	0.7	0.2 ± 0.1	97	0.006	0.07	0.02 ± 0.01	97
	Out	11 and 12	70	0.1	0.5	0.2 ± 0.1	97	0.005	0.02	0.02 ± 0.01	97
	In	n/a	70	200.0	218.0	209.3 ± 4.48	n/a	19.4	21.1	20.3 ± 0.39	n/a
	Out	15 and 16	70	2.0	56	37.7 ± 19	82	0.19	5.4	3.7 ± 1.8	82
	Out	19 and 20	35	2.0	29.8	12.5 ± 9	94	0.12	1.74	0.7 ± 0.5	89
AB113	In	n/a	70	3.5	10.5	6.6 ± 1.82	n/a	0.27	0.82	0.51 ± 0.11	n/a
	Out	5	70	0.1	4.6	1.9 ± 1.0	71	0.007	0.33	0.14 ± 0.07	73
	Out	6 and 7	70	0.1	4.5	1.3 ± 1.1	80	0.006	0.32	0.09 ± 0.08	82
	Out	13 and 14	70	0.3	4.5	2.1 ± 1	68	0.216	0.32	0.15 ± 0.07	71
	In	n/a	70	155.0	292.0	221.5 ± 31.86	n/a	12.77	22.6	17.23 ± 2.5	n/a
	Out	17 and 18	70	17.1	191.5	63 ± 48	71	1.23	13.8	4.54 ± 3.45	74
	Out	21 and 22	35	26.91	187.5	59 ± 40	73	0.97	6.77	2.12 ± 1.44	75
Characteristics				COD concentration (mg/l)			COD loading rate (mg/l/d)				
BR46	In	n/a	20	11	32	22 ± 6	n/a	0.88	2.56	1.76 ± 0.48	n/a
	Out	4	20	2.8	19.5	11 ± 5.4	50	0.21	1.44	0.81 ± 0.4	54
	Out	8 and 10	20	0.3	13.8	7.2 ± 4.3	67	0.02	1.02	0.53 ± 0.32	70
	Out	11 and 12	20	0.7	16.3	9.1 ± 5.1	59	0.05	1.2	0.67 ± 0.38	62
	In	n/a	20	249	280	261 ± 11	n/a	19.9	22.4	20.88 ± 0.8	n/a
	Out	15 and 16	20	6	100	69 ± 34	74	0.44	7.4	5.1 ± 2.52	76
	Out	19 and 20	20	14	40	28 ± 9	89	0.52	1.48	1.04 ± 0.33	95
AB113	In	n/a	20	36.9	86.4	48.2 ± 12.2	n/a	2.95	6.91	3.84 ± 1.04	n/a
	Out	5	20	27.6	73	46 ± 14.7	5	2.04	5.4	3.4 ± 1.11	11
	Out	6 and 7	20	14	78	40.6 ± 19.4	16	1.04	5.77	3.03 ± 1.55	21
	Out	13 and 14	20	24	72	44.6 ± 12.9	7	1.78	5.32	3.18 ± 0.96	17
	In	n/a	20	543	610	584 ± 34	n/a	43.44	48.8	47.84 ± 1.3	n/a
	Out	17 and 18	20	117	454	271 ± 103	54	8.66	33.6	22.34 ± 7.7	53
	Out	21 and 22	20	126	362	262 ± 86	55	9.32	26.78	21.46 ± 6.3	55

Note: BR, basic red; AB, acidic blue; SD, standard deviation; n/a, not applicable.

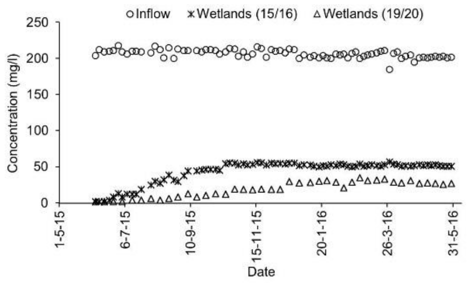


Fig. 4. Inflow and outlow concentrations of Basic Red 46 for Wetlands 15/16 and 19/20 (see also Tables 2 and 4).

than the one for the unplanted control Wetland 5 (Fig. 3) due to the presence of plants (Keskinkan and Lugal Göksu, 2007).

For high concentrations, the best percentage removals for BR46 were noted for Wetlands 19/20 (high resting and contact times), which were statistically significantly ($p < 0.05$) different, if compared to those of Wetlands 15/16 (low resting and contact times). Wetlands with a low loading rate performed better than those wetlands with a high loading rate (Fig. 4). For the dye AB113, there was no significant ($p > 0.05$) difference in the removal of dyes between Wetlands 17 and 18 (low resting and contact times), and Wetlands 21 and 22 (high resting and contact times) as shown in Table 7.

For COD removal concerning low concentrations of dyes (AB113 and BR46), wetlands with a long retention time had the best COD removal (regardless of planting regime), if compared to the control and other wetlands having short retention times. Cheng et al. (2011) operated two series of vertical-flow constructed wetlands (one planted and other one unplanted) under different C: N: P ratios. Their results obtained showed that the removal of COD depended on the presence and development of plants. The removal rate was higher in the planted wetlands (Cheng et al., 2011).

Concerning high concentrations of dye BR46, the best removal percentages for COD were noted for Wetlands 19/20 (high resting and contact times), which were statistically significantly ($p < 0.05$) different from Wetlands 15/16 (low resting and contact times). Wetlands with a low loading rate performed better than those wetlands with a high loading rate (Table 7). In case for the dye AB113, there was no significant ($p > 0.05$) difference in the removal of COD between Wetlands 17/18 (low resting and contact times) and Wetlands 21/22 (high resting and contact times) as shown in Table 7.

3.7. Nutrient removal

The removal of phosphorous is controlled by chemical and physical adsorption, sedimentation, plant uptake and precipitation in constructed wetland systems (Brix, 1997). For the low concentrations of BR46 and AB113, the removal percentages in Wetlands 8/10 (89%) as well as Wetlands 6/7 (30%) were significantly ($p < 0.05$) better than those of the control Wetlands 4 (78%) and 5 (27%) as well as Wetlands 11/12 (68%) and 13/14 (14%), which have short and long retention times in that order (Table 8). For the high concentrations of BR46, the removal percentages of Wetlands 19/20 (high resting and contact times; 81%) were significantly ($p < 0.05$) better than those for Wetlands 15/16 (low resting and contact times; 70%), while for AB113, the removal percentages of Wetlands 17/18 (low resting and contact times; 78%) ($p < 0.05$) were also significantly better than those for Wetlands 21/22 (high resting and contact times; 71%) as shown in Fig. 5.

Table 8
Inflow and outflow water quality characteristics for nutrients related to different wetlands (Tables 2 and 4).

Characteristics				Ammonia-nitrogen (mg/l)				Nitrate-nitrogen (mg/l)				Ortho-phosphate-phosphorus (mg/l)			
Dye	Type of flow	Wetland number	No. of samples	Mini-mum	Maxi-mum	Mean ± SD	Removal (%)	Mini-mum	Maxi-mum	Mean ± SD	Removal (%)	Mini-mum	Maxi-mum	Mean ± SD	Removal (%)
BR46	In	n/a	20	0.54	0.75	0.62 ± 0.065	n/a	17.9	18.0	18.0 ± 0.03	n/a	5.3	6.3	5.9 ± 0.33	n/a
	Out	4	20	0.45	0.68	0.55 ± 0.086	11	4.5	16.9	9.8 ± 3.98	45	0.9	1.7	1.3 ± 0.27	78
	Out	8 and 10	20	0.21	0.51	0.36 ± 0.111	42	2.5	13.9	7.5 ± 3.69	58	0.19	1.3	0.7 ± 0.4	89
	Out	11 and 12	20	0.49	0.90	0.68 ± 0.118	−10	3.5	16.0	8.9 ± 3.83	50	0.5	2.8	1.9 ± 0.83	68
	In	n/a	20	0.98	1.04	1.02 ± 0.016	n/a	19.3	20.0	19.7 ± 0.18	n/a	55.0	87.0	64.0 ± 8.50	n/a
	Out	15 and 16	20	0.25	0.47	0.35 ± 0.075	66	1.5	16.2	6.2 ± 5.43	69	6.5	47.0	19.3 ± 13.20	70
	Out	19 and 20	20	0.21	0.46	0.31 ± 0.078	70	0.6	12.1	4.9 ± 4.46	75	1.9	43.6	12.1 ± 14.60	81
AB113	In	n/a	20	0.63	0.70	0.66 ± 0.022	n/a	8.4	08.5	8.5 ± 0.03	n/a	6.9	8.3	7.6 ± 0.43	n/a
	Out	5	20	0.37	0.62	0.48 ± 0.079	27	10.5	16.3	12.1 ± 1.38	−42	4.8	6.4	5.6 ± 0.49	27
	Out	6 and 7	20	0.35	0.81	0.52 ± 0.143	21	3.9	6.5	4.9 ± 0.98	42	4.1	6.4	5.3 ± 0.58	30
	Out	13 and 14	20	0.35	0.56	0.47 ± 0.062	29	10.0	15.1	11.6 ± 1.45	−36	5.7	7.7	6.6 ± 0.51	14
	In	n/a	20	2.21	2.77	2.33 ± 0.159	n/a	19.4	20.0	19.8 ± 0.21	n/a	148.0	154.0	151.0 ± 2.10	n/a
	Out	17 and 18	20	0.73	1.54	1.00 ± 0.236	57	0.9	15.2	6.0 ± 5.8	70	15.0	50.4	33.1 ± 11.30	78
	Out	21 and 22	20	0.80	1.97	1.10 ± 0.315	53	0.8	15.2	5.3 ± 5.10	73	23.3	70.3	43.5 ± 15.30	71

Note: BR, basic red; AB, acid blue; SD, standard deviation; n/a, not applicable.

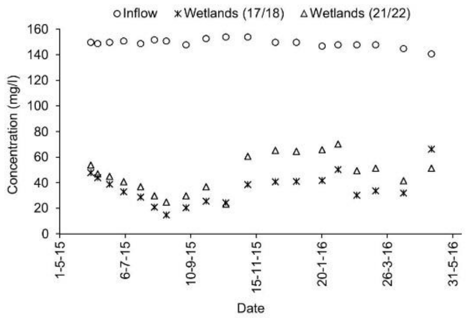


Fig. 5. Inflow and outlow concentrations of ortho-phosphate-phosphorus for Wetlands 17/18 and 21/22 (see also Tables 2 and 4).

As indicated in Table 8, the concentrations of NH_4-N for both dyes are very low due to their chemical structures (Yalcuk and Dogdu, 2014). For the high concentration of dyes, there are no significant ($p > 0.05$) differences between wetlands. For the low concentration of the dye BR46, the removals for the planted Wetlands 8 and 10 were significantly ($p < 0.05$) better than those for the corresponding removal of the unplanted Wetland 4, while there are increases in removal for planted Wetlands 11 and 12 (Table 8). For the low concentration of the dye AB113, the removal percentages with respect to planted Wetlands 13 and 14 were better than those for the unplanted Wetland 4 and the planted Wetlands 6 and 7 (Table 8), which was statistically insignificant ($p > 0.05$).

The removal of NO_3-N is a relatively time-consuming anaerobic process, and naturally takes place mostly in the sediment (Mustafa et al., 2009). For the high concentration of both dyes (BR46 and AB113), the removal percentages for wetlands with a long retention times are better than those for wetlands having short retention times, confirming findings by Van Loosdrecht and Clement (2005). Regarding the low concentration of BR46, the removal percentages for the planted Wetlands 8 and 10 were better than those for the unplanted Wetland 4 and the planted Wetlands 11 and 12 (Table 8). For the low concentrations of AB113, there were significantly ($p < 0.05$) better removal percentages for the Wetlands 6 and 7 (low resting and high contact times), but there were increases in the effluents regarding the NO_3-N removal of Wetlands 13 and 14 (high resting and low contact times) due to the presence of carbon under aerobic conditions (Hu et al., 2009). The removal percentages for the high concentrations of dyes were better than the ones for the low concentration of dyes due to a corresponding increase in carbon percentage.

4. Conclusions and recommendations for further research

The presence of *P. australis* did not affect dye removal ($p > 0.05$), while it had a minor but insignificant impact on COD removal ($p > 0.05$). The use of plants concerning the short contact time scenario for NH_4-N and a low concentration of AB113 is linked to a better removal compared to other cases apart from that for all nutrients; particularly PO_4-P and NO_3-N. In case of low dye concentrations, the presence of plants for the long contact time scenario impacted significantly ($p < 0.05$) positive on the removal efficiencies of PO_4-P, NH_4-N and NO_3-N.

The vertical-flow constructed wetlands showed significantly ($p < 0.05$) good dye removals for low and high concentrations of BR46, and low concentrations of AB113 during all seasons under greenhouse conditions. The COD removal was a function of the dye used. This was explained by the complexity of the chemical structure of the dye and seasonal variations in temperature.

For high concentrations of BR46, wetlands with a long hydraulic retention time were significantly ($p < 0.05$) better that those with a short hydraulic retention time for the removal of PO_4-P. For AB113, wetlands with a shorter hydraulic retention time removed PO_4-P significantly ($p < 0.05$) well. In case of the dye AB113, the four-day contact time was not enough to remove or reduce TSS and turbidity.

The authors recommend to assess (a) the effects of different plant species and an increase of the contact time on particle and dye removal processes; (b) the effect of pH (low and high) on dye removal; (c) the treatment of synthetic textile wastewater contaminated with different dyes, matching the typical characteristics of real effluent from a textile factory; (d) the impact of different dyes mixed with other contaminants and plant growth parameters such as stem diameter; and (e) wetland design implications based on the research findings in terms of investment of area and costs.

Competing interest

Authors have no competing interests.

Supplementary material

Note that on-line supplementary material is associated with this article. Supplementary material S1 comprises a discussion on tests of normality supported by two tables. Supplementary Material S2 shows the temporal variation of temperature throughout the experimental time period.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2017.01.016.

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APPENDIX B

Test of normality for plant and water quality variables

B.1 Azo textile Dyes

The corresponding results for the plant dimensions are shown in Table B.1. The lengths were approximately normally distributed for Wetlands, 12 and 13, 16 and 17, and 18 and 19, while the opposite was the case for Wetlands 6 and 7, 8 and 9, 10 and 11, 14 and 15, and 20 and 21. The diameters were approximately non-normally distributed for all wetlands as shown in Table B.1 and Figures B1 and B2 as s sample

Table B.1 Test of normality for dimensions of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) related to Table 4.1

Characteristic			Test of normality (if $p \geq 0.05$, data are normally distributed; if $p<0.05$, data are not normally distributed)	
Dye	Wetland numbers	Number of stems	Length (mm)	Diameter (mm)
BR46	8 and 9	40	0.001	0.000
	10 and 11	28	0.024	0.011
	14 and 15	34	0.000	0.006
	18 and 19	23	0.158	0.007
AB113	6 and 7	45	0.000	0.004
	12 and 13	30	0.291	0.002
	16 and 17	36	0.518	0.038
	20 and 21	28	0.017	0.049

Note: BR, basic red; AB, acid blue; Sig., *p* value depending on the Shapiro-Wilk test.

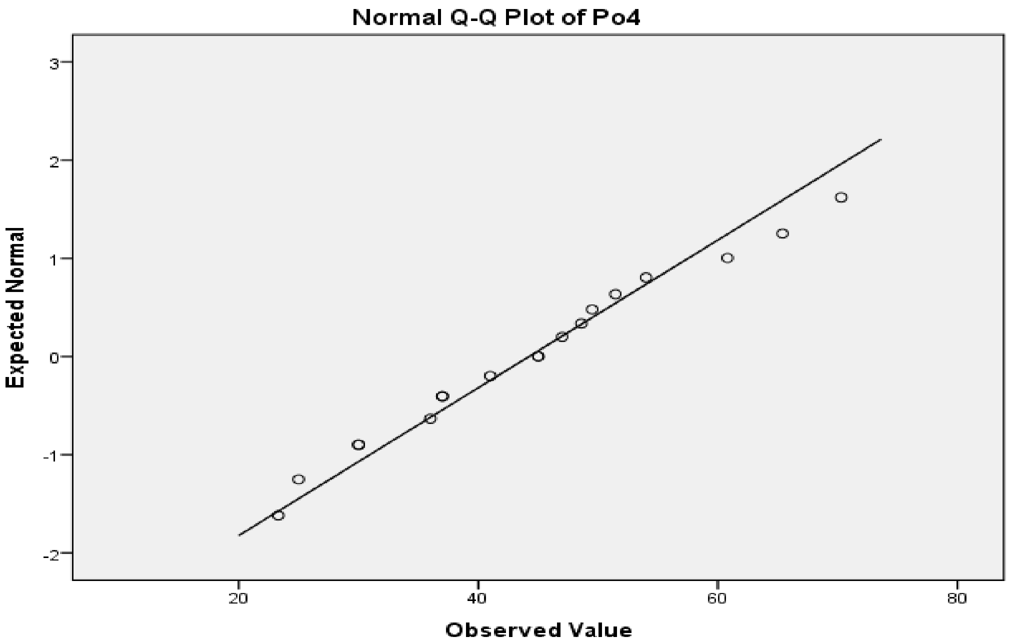

Figure B1 Normal Q-Q plot for ortho-phosphate-phosphorus for Wetlands 20/21

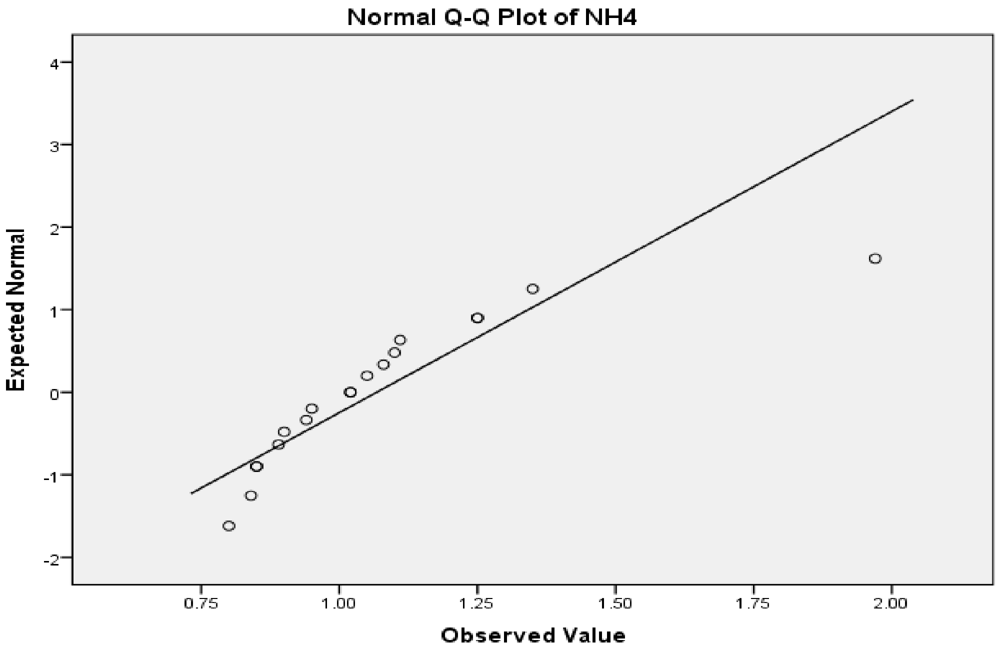

Figure B2. Normal Q-Q plot for ammonia-nitrogen for Wetlands 20/21

Moreover, the corresponding results for AB113 are shown in Table B.2. Wetland 5 was approximately normally distributed for COD and NH_4-N, while the opposite was the case for dye concentration, pH, redox potential, EC, TSS, turbidity, DO, PO_4-P and NO_3-N. For Wetlands 6 and 7, the results were approximately normally distributed for DO, COD, PO_4-P and NH_4-N, while findings were not normally distributed for dye concentration, pH, redox potential, EC, TSS, turbidity and NO_3-N. For Wetlands 12 and 13, the obtained results were approximately normally distributed for dye concentration, COD and NH_4-N, while not normally distributed for pH, redox potential, EC, DO, TSS, turbidity, PO_4-P and NO_3-N. For the high concentration, the obtained results for Wetlands 16 and 17 showed approximately normally distributed data for redox potential, COD and PO_4-P, while they were not normally distributed for dye concentration, pH, DO, EC, TSS, turbidity, NH_4-N and NO_3-N. For Wetlands 20 and 21, the obtained results showed data that were normally distributed for redox potential, EC, DO and PO_4-P, while they were not normally distributed for the dye, pH, TSS, turbidity, COD, NH_4-N and NO_3-N.

For the dye BR46, in the case of low concentration, the obtained results for Wetland 4 (Table B.2) showed normally distributed data for pH, redox potential, COD, NH_4-N, NO_3-N and PO_4-P, while not normally distributed findings were recorded for dye concentration, DO, TSS, turbidity and EC. For Wetlands 8 and 9, the obtained results showed normally distributed data for COD, NH_4-N, NO_3-N and PO_4-P, while not normally distributed ones were noted for dye concentration, pH, redox potential, DO, TSS, turbidity and EC. For Wetlands 10 and 11, the results showed normal distributions for redox potential, COD, NH_4-N and NO_3-N, while not normal distributed data for dye concentration, pH, DO, TSS, turbidity, EC and PO_4-P. In the case of high concentration, for Wetlands 14 and 15, the obtained results showed normal distributions for pH and DO, while not normal distributed output for dye concentration, redox potential, TSS, turbidity, EC, COD, PO_4-P, NO_3-N and NH_4-N. For Wetlands 18 and 19, the obtained results showed normal distributions for pH, redox potential, DO, EC and COD, while not normally distributed data were noted for dye concentration, TSS, turbidity, PO_4-P, NH_4-N and NO_3-N as shown in Table B.2.

Table B.2 Test of normality for effluent water quality characteristics regarding general physical and chemical variables related to Tables 4.2, 4.3 and 4.4

Characteristic			Test of normality (if $p \geq 0.05$, data are normally distributed; if $p<0.05$, data are not normally distributed)			
Dye	Wetland number(s)	No. of samples	Dye concentration (mg/l)	pH (–)	Redox potential (mV)	Dissolved oxygen (mg/l)
BR46	4	70	0.000	0.009	0.532	0.029
	8 and 9	70	0.000	0.019	0.006	0.020
	10 and 11	70	0.002	0.000	0.110	0.000
	14 and 15	70	0.000	0.071	0.034	0.287
	18 and 19	35	0.029	0.930	0.381	0.955
AB113	5	70	0.000	0.005	0.031	0.000
	6 and 7	70	0.000	0.000	0.000	0.277
	12 and 13	70	0.068	0.029	0.019	0.000
	16 and 17	70	0.000	0.050	0.302	0.017
	20 and 21	35	0.000	0.004	0.051	0.100
			Total suspended solids (mg/l)	Turbidity (NTU)	Electrical conductivity (μS/cm)	
BR46	4	70	0.000	0.000	0.021	
	8 and 9	70	0.000	0.000	0.000	
	10 and 11	70	0.000	0.000	0.006	
	14 and 15	70	0.000	0.000	0.027	
	18 and 19	35	0.000	0.000	0.238	
AB113	5	70	0.047	0.000	0.033	
	6 and 7	70	0.000	0.000	0.001	
	12 and 13	70	0.000	0.003	0.011	
	16 and 17	70	0.006	0.000	0.041	
	20 and 21	35	0.029	0.021	0.113	

Table B.2 continued

Dye	Wetland number(s)	No. of samples	Chemical oxygen demand (mg/l)	Ortho-phosphate-phosphorus (mg/l)	Ammonia-nitrogen (mg/l)	Nitrate-nitrogen (mg/l)
BR46	4	20	0.327	0.196	0.122	0.107
	8 and 9	20	0.578	0.416	0.075	0.275
	10 and 11	20	0.850	0.017	0.243	0.096
	14 and 15	20	0.029	0.017	0.012	0.015
	18 and 19	20	0.420	0.000	0.010	0.031
AB113	5	20	0.261	0.000	0.352	0.001
	6 and 7	20	0.197	0.069	0.360	0.036
	12 and 13	20	0.466	0.000	0.449	0.011
	16 and 17	20	0.283	0.862	0.012	0.018
	20 and 21	20	0.015	0.857	0.001	0.006

Note: BR, basic red; AB, acid blue; Sig., *p* value depending on the Shapiro-Wilk test.

B.2 Artificial wastewater containing two azo textile dyes

The corresponding results for the plants' dimension are shown in Table B.3. The lengths were approximately normally distributed for Wetlands 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 while the opposite was the case for Wetlands 5 and 6. The diameters were approximately normally distributed for Wetlands 3, 4, 6, 8, 9, 10, 11, 13, 14, 16, 17 and 18 while the opposite was the case for Wetlands 7, 12 and 15.

Regarding water quality variables in case of AB113, as shown in Table B.4, Wetlands 2, 3 and 9 were approximately no normally distribution for all variables (dye concentration, pH, redox, EC, TSS, turbidity, DO, colour, COD, PO_4-P, NH_3-N and NO_3-N). Wetland 13 was approximately normally distribution for redox, NH_4-N and COD, while no normally distribution for dye concentration, pH, EC, TSS, turbidity, DO, PO_4-P, NO_3-N and colour. Wetland 17 was normally distribution for NO_3-N, while the opposite for dye concentration, pH, redox, EC, DO, TSS, turbidity, PO_4-P, NH_4-N, COD and colour.

In case of BR46, Wetland 1 was approximately normally distribution for COD, while the opposite for dye concentration, pH, redox, EC, TSS, turbidity, DO, colour, NH_4-H, NO_3-N and PO_4-P. Wetland 5 was approximately normally distribution for pH and DO, while the opposite for dye concentration, redox, EC, TSS, turbidity, colour, NH_4-N, NO_3-N, PO_4-P and COD. Wetland 7 was approximately normally distribution for DO, PO_4-P and COD, while the opposite for dye concentration, pH, redox, EC, TSS, turbidity, colour, NH_4-N and NO_3-N. Wetland 11 was approximately normally distribution for DO, while the opposite for dye concentration, pH, redox, EC, TSS, turbidity, colour, NH_4-N, NO_3-N, PO_4-P and COD. Wetland 15 was approximately normally distribution for PO_4-P and COD, while the opposite for dye concentration, pH, redox, TSS, turbidity, DO, colour, EC, NH_4-N and NO_3-N.

In case of the mixture between both of previous dyes, Wetlands 4 and 12 were approximately no normally distribution for all variables (dye concentration, pH, redox, DO, TSS, turbidity, EC, colour, NH_4-N, NO_3-N, PO_4-P and COD. Wetlands 6 was approximately normally distribution for PO_4-P, while the opposite for the dye concentration, pH, redox, TSS, turbidity, EC, colour, DO, NH_4-N, NO_3-N and COD. Wetland 8 was approximately distribution for redox, DO and PO_4-P, while the opposite for dye concentration, pH, EC, TSS, turbidity, colour, COD, NH_4-N and NO_3-N. Wetland 10 was approximately normally distribution for dye concentration, while the opposite for pH, redox, EC, TSS, turbidity, DO, colour, COD, NH_4-N, NO_3-N and PO_4-P. Wetland 14 was approximately normally distribution for redox, while the opposite for dye concentration, pH, EC, TSS, DO, turbidity, colour, COD, NH_4-N, NO_3-N and PO_4-P. Wetland 16 was approximately normally distribution for COD, NH_4-N, NO_3-N and PO_4-P, while the opposite for dye concentration, pH, redox, TSS, EC, DO, turbidity and colour. Wetland 18 was approximately normally distribution for dye concentration, turbidity, DO, COD, PO_4-P, NH_4-N and NO_3-N, while the opposite for pH, redox, EC, TSS and colour.

Table B.3 Test of normality for dimensions of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) related to Table 5.1

Characteristic			Test of normality (if $p \geq 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed)	
Dye	Wetland number(s)	Number of stems	Length (mm)	Diameter (mm)
BR46	5	68	0.042	0.015
	7	20	0.513	0.000
	11	17	0.720	0.062
	15	14	0.131	0.025
AB113	3	40	0.060	0.402
	9	15	0.082	0.547
	13	20	0.113	0.937
	17	10	0.335	0.824
The mixture of the dyes	4	48	0.287	0.364
	6	55	0.004	0.545
	8	7	0.729	0.970
	10	10	0.592	0.822
	12	10	0.655	0.004
	14	16	0.548	0.972
	16	7	0.685	0.996
	18	9	0.122	0.790

Note: BR, basic red; AB, acid blue.

Table B.4 Test of normality for effluent water quality characteristics regarding general physical and chemical variables related to Tables 5.2, 5.3 and 5.6

Characteristic			Test of normality (if $p \geq 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed)			
Dye	Wetland number(s)	No. of samples	Dye concentration (mg/l)	pH (–)	Redox potential (mV)	Dissolved oxygen (mg/l)
BR46	1	82	0.003	0.000	0.038	0.000
	5	82	0.002	0.916	0.004	0.050
	7	82	0.000	0.000	0.033	0.564
	11	81	0.000	0.000	0.000	0.445
	15	41	0.010	0.000	0.000	0.014
AB113	2	82	0.000	0.000	0.000	0.000
	3	82	0.000	0.000	0.000	0.000
	9	82	0.000	0.000	0.000	0.000
	13	81	0.000	0.000	0.110	0.000
	17	41	0.001	0.000	0.002	0.000
The mixture of both of dyes	4	82	0.015	0.000	0.000	0.000
	6	82	0.021	0.000	0.000	0.000
	8	82	0.000	0.001	0.053	0.585
	10	82	0.059	0.000	0.000	0.024
	12	81	0.000	0.000	0.000	0.000
	14	81	0.000	0.000	0.194	0.000
	16	41	0.000	0.000	0.014	0.000
	18	41	0.051	0.021	0.033	0.411

Table B.4 continued

Dye	Wetland number(s)	No. of samples	Total suspended solids (mg/l)	Turbidity (NTU)	Electric conductivity (μS/cm)	Colour (Pt Co.)
BR46	1	82	0.006	0.043	0.000	0.000
	5	82	0.002	0.000	0.000	0.000
	7	82	0.000	0.000	0.000	0.000
	11	81	0.009	0.042	0.000	0.001
	15	41	0.000	0.002	0.000	0.005
AB113	2	82	0.001	0.000	0.000	0.000
	3	82	0.006	0.000	0.000	0.014
	9	82	0.000	0.002	0.000	0.005
	13	81	0.012	0.004	0.000	0.000
	17	41	0.004	0.003	0.000	0.000
The mixture of both of dyes	4	82	0.000	0.000	0.000	0.000
	6	82	0.000	0.000	0.000	0.000
	8	82	0.000	0.000	0.000	0.000
	10	82	0.000	0.000	0.000	0.000
	12	81	0.000	0.000	0.000	0.000
	14	81	0.000	0.000	0.000	0.021
	16	41	0.000	0.000	0.002	0.015
	18	41	0.026	0.062	0.000	0.000

Table B.4 continued

Dye	Wetland number(s)	No. of samples	Chemical oxygen demand (mg/l)	Ortho-phosphate-phosphorus (mg/l)	Ammonia-nitrogen (mg/l)	Nitrate-nitrogen (mg/l)
BR46	1	30	0.357	0.018	0.026	0.006
	5	30	0.000	0.015	0.000	0.000
	7	30	0.210	0.324	0.000	0.000
	11	30	0.001	0.000	0.038	0.009
	15	30	0.318	0.151	0.012	0.004
AB113	2	30	0.000	0.000	0.021	0.000
	3	30	0.017	0.009	0.002	0.000
	9	30	0.035	0.000	0.006	0.000
	13	30	0.112	0.003	0.073	0.002
	17	30	0.000	0.001	0.000	0.587
The mixture of both of dyes	4	30	0.000	0.000	0.037	0.000
	6	30	0.000	0.079	0.000	0.000
	8	30	0.000	0.055	0.000	0.000
	10	30	0.000	0.000	0.008	0.000
	12	30	0.000	0.000	0.010	0.000
	14	30	0.000	0.000	0.016	0.000
	16	30	0.175	0.612	0.075	0.109
	18	30	0.990	0.149	0.092	0.006

Note: BR; basic red, AB; acid blue