TREATMENT OF SYNTHETIC WASTEWATER CONTAINING TEXTILE DYES WITH EXPERIMENTAL CONSTRUCTED WETLANDS

By

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Civil Engineering

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2018

DEDICATION

This thesis is dedicated to

My Parents

for their endless love and support

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ACKNOWLEDGMENTS

Firstly, I would like to give praise and thanks to the great Allah, for giving me this wonderful opportunity and for giving me the strength and courage to complete my study after years of hard work.

My sincere thanks to my supervisor Prof. Miklas Scholz for his supervision, valuable academic guidance, advice, continuous encouragement, help and support throughout the PhD to make sure that my research was heading in the right direction. Also, I would like to thank my co-supervisor Dr. John Hadfield for his help and support.

My special thanks to all my colleagues and friends for their support, and also, I would like to thank all the staff in the Library and the School of Computing, Science, and Engineering, as well as Catriona Nardone and Maggie Hardman for their great support. My deep gratitude goes to the staff of the Environment and Life Sciences school, especially Dr. Matthew, Kath and Sally for their technical support. I would like also to express my appreciation to the Pattern Recognition and Image Analysis research lab at the University of Salford, especially Dr. Apostolos Antonacopoulos and Christian Clausner regarding the Aletheia Lemna Edition software development.

My deepest heartfelt gratitude goes to my dear father , mother, sisters and brothers for their love, ongoing support, encouragement and prayer. Also, I would like to express my deepest heartfelt thanks to my dear husband, for his love, consideration, and prayers, and to my daughters for their love, understanding, and support.

Finally, special thanks go to the Ministry of Higher Education and Scientific Research in Iraq, for funding this research and deep thanks goes to the Iraqi Cultural Attaché, as well as the Civil Engineering Department, Collage of Engineering, University of Basra in Iraq for their support during the period of my study.

LIST OF PUBLICATIONS

The approaches and findings of this thesis have been published and submitted as listed below:

Journal papers

1. **Yaseen, D.A.,** & Scholz, M. (2016). Shallow pond systems planted with *Lemna minor* treating azo dyes. *Ecological Engineering, 94,* 295–305.
2. **Yaseen, D. A.,** & Scholz, M. (2017). Comparison of experimental ponds for the treatment of dye wastewater under controlled and semi-natural conditions. Environmental *Science and Pollution Research, 24*(19), 16031–16040. doi: 10.1007/s11356-017-9245-5.
3. **Yaseen, D. A.,** & Scholz, M. (2017b). Textile dye removal using experimental wetland ponds planted with common duckweed under semi-natural conditions. *Environment Protection Engineering, 43*(3), 39–60. doi: 10.5277/epe170303.
4. **Yaseen, D. A.,** & Scholz, M. (2018). Treatment of synthetic textile wastewater containing dye mixtures with microcosms. *Environmental Science and Pollution Research,* 25, 1980–1997. doi: 10.1007/s11356-017-0633-7.
5. **Yaseen D. A.,** & Scholz M. Textile Dye Wastewater Characteristics, and constituents of synthetic effluents: a critical review. *International Journal of Environmental Science and Technology*. (Submitted).
6. **Yaseen D. A.,** & Scholz M. Impact of pH on the treatment of artificial textile wastewater containing azo dyes using pond systems. *International Journal of Environmental Science and Technology*.(Submitted).

Conference papers and abstracts conferences

1. **Yaseen, D. A.,** Scholz, M., Christian, C., & Antonacopoulos, A. (2017). Assessing the impact of dyes accumulation on the growth of *Lemna minor* L. using image processing technique. *Proceeding of CSE 2017 Annual PGR Symposium (CSE-PGSym17) 17th March 2017.* The University of Salford, Salford. **http://usir.salford.ac.uk/42484/.** (short paper).

2. **Yaseen, D. A.,** & Scholz, M. (2015). Dye removal in experimental ponds treating textile wastewater with *Lemna minor*. (Abstract). *The 5th World Sustainability Forum (WSF 2015) (7-9 September, 2015),* Basel, Switzerland. Web: **www.sciforum.net/conference/wsf-5.**
3. **Yaseen, D. A.,** & Scholz, M. (2017). Treatment of artificial wastewater contaminated with azo dyes by simulated shallow ponds systems. (Abstract). *The Ninth Manchester Metropolitan University Postgraduate Research Conference 22nd February 2017.* Manchester Metropolitan University, Greater Manchester, United Kingdom.

   http://www2.mmu.ac.uk/graduate-school/events-conferences/postgraduate-research-conference-2017/
4. **Yaseen, D. A.,** & Scholz, M. (2017). Impact of different environmental conditions on the treatment of azo dyes Acid Blue 113 and Basic Red 46 using wetland ponds. (Abstract). *Postgraduate Annual Research Conference (SPARC) 27th-29th June 2017.* The University of Salford, Salford.

   http://www.pg.salford.ac.uk/sparc_conference.
5. **Yaseen, D. A.,** & Scholz, M. (2017). Potential of algal-based ponds in treatment of textile dyes containing wastewater. (Poster presentation). *Postgraduate Annual Research Conference (SPARC), 27th-29th June 2017.* The University of Salford, Salford.

   http://www.pg.salford.ac.uk/sparc_conference
6. **Yaseen, D. A.,** & Scholz, M. (2018). Artificial textile wastewater treatment using Duckweed-based systems. (Poster presentation). *Postgraduate Annual Research Conference (SPARC), 5th July 2018.* The University of Salford, Salford. (Submitted).

   http://www.pg.salford.ac.uk/sparc_conference

LIST OF ABBREVIATIONS

AB113	Acid blue 113
BC	Before Christ
Abs	Absorbance
ADMI	American Dye Manufactures Institute unit
AO7	Acid orange 7
AOX	Adsorbable organic halogens
APHA	American Public Health Association standard
AY 2G	Acid yellow 2G
BB41	Basic blue 41
BDL	Below detected limits
BOD	Biochemical oxygen demand
BR46	Basic red 46
C	Carbon
C. demersum	*Ceratophyllum demersum*
C1	Concentration of last grab sample in the end of week n-1
C2	Concentration of the dose added in the beginning of week n
C3	Concentration of grab sample at the end of week n
CA	Coverage area
CAS	Chemical abstracts survey registry
Cl-	Chlorine
COD	Chemical oxygen demand
CWs	Constructed wetlands
DF	Down flow
DFCWs	Down flow constructed wetlands
DO	Dissolved oxygen
DO46	Direct orange 46
DS	Dissolved solids
DSTWW	Diluted synthetic textile wastewater
DTW	Dechlorinated tap water.
DW	Dry weight
EC	Electrical conductivity
E. cordifolius	*Echinodorus cordifolius L.*
EC50	Half maximal effective concentration
EWA	European Water Association
FAO	Food and Agricultural Organisation
FW	Fresh weight
FWS	Free water surface

GCMS	Gas chromatography mass spectrometry
H	Hydrogen
HF	Horizontal subsurface flow
HPLC	High-performance liquid chromatography
HRT	Hydraulic retention time
IBM SPSS	International Business Machine Statistical Package for Social Sciences
ICP – OES	Inductively Coupled Plasma – Optical Emission Spectrometer
IF	Inflow
ITRC	Interstate Technology and Regulatory Council Wetlands Team
K-W	Kruskal-Wallis test
L. minor	*Lemna minor* L.
M. spicatum	*Myriophyllum spicatum*
Mixture 1 (M1)	8 mg/l of basic red 46 + 2 mg/l of reactive blue 198
Mixture 2 (M2)	2 mg/l of basic red 46 + 8 mg/l of reactive blue 198
Mixture 3 (M3)	5 mg/l of basic red 46 + 5 mg/l of reactive blue 198
MLSS	Mixed liquor suspended solids
mV	Redox potential
M-W	Mann- Whitney U test
N	Nitrogen
N/A	Not applicable
N/V	Not available
Na	Sodium;
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_4	Ammonium
NH_4–N	Ammonium-nitrogen
NO_2	Nitrite
NO_2–N	Nitrite nitrogen
NO_3–N	Nitrate-nitrogen
NTU	Nephelometric turbidity unit
O	Oxygen
ORP	Oxidation redox potential
P	Phosphorus
P. australis	*Phragmites australis*
P1	*Lemna minor* L. and algae ponds
P2	Algae ponds
P3	*Lemna minor* L. ponds receiving inflow at normal pH
P4	Control ponds receiving inflow at normal pH

P5	*Lemna minor* L. ponds receiving inflow at pH of 9
P6	Control ponds receiving inflow at pH of 6
P7	*Lemna minor* L. ponds receiving inflow at pH of 6
P8	Control at pH of 6
PO_4–P	Ortho-phosphate-phosphorus
Pt Co	Platinum-Cobalt
R	Removal efficiency
RB171	Reactive blue 17
RB198	Reactive blue 198
RB5	Reactive black 5
RFN	Relative frond number
RGR	Relative growth rate
RR120	Reactive red 120
RR141	Reactive red 141
RR2	Reactive red 2
RR22	Reactive red 22
RZM	Root Zone Method
S	Sulphur
SA	Surface area of pond
SO_4	Sulphate
SPSs	Shallow pond systems
SRDW	Synthetic reactive dye wastewater
SS	Suspended solids
SSF	Subsurface flow
SSFCWs	Subsurface flow constructed wetlands
STWW	Synthetic textile wastewater
TA	Total alkalinity
TBD	Turbidity
TDS	Total dissolved solids
Temp	Temperature
TH	Total hardness
TKN	Total kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
UF	Up flow
UFCWs	Up flow constructed wetlands
UK	United Kingdom

UN-HABITAT	United Nations Human Settlements Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet.
V1	Volume of water remained in the ponds in the end of week n-1
V2	Volume of the dose added in the beginning of week n
V3	Volume of water remaining in the pond at the end of week n
VF	Vertical subsurface flow
VFCWs	Vertical flow constructed wetlands
VHF	Vertical and horizontal flow
VR13	Vat red 13
λ_{max}	Wavelength at maximum absorption

ABSTRACT

A global increase in industrialisation has resulted in the rapid growth of textile industries in developing countries, leading to a high rise in the overall discharge of a broad range of pollutants. Amongst these pollutants is dye wastewater, which frequently has mutagenic and carcinogenic effects on humans and animals, depending on the receiving watercourses as a source for drinking water. In contrast to traditional high-rate wastewater treatment units, passive biological treatment technologies, such as constructed wetlands are a sustainable and cost-effective alternative technology to treat large quantities of contaminated water, especially in places where land costs are low.

Four small scale experiments were conducted between July 2014 and June 2017 using plastic containers simulating constructed wetland planted with Common Duckweed (*Lemna minor*) for assessing the system performance, as a polishing stage, for the treatment of synthetic wastewater containing dyes: acid blue 113 (AB113), reactive blue 198 (RB198), basic red 46 (BR46) and direct orange 46 (DO46), with the main focus on removal of the dyes.

The novelty of this research was to cover five prominent gaps in the literature, related to the treatment of dye effluents using free-floating plant-based constructed wetland systems under hydroponic conditions. These gaps have not been previously investigated with this system, which include: treatment of four dyes, which have not been treated before using this economic system; evaluate the system performance and the removal mechanism, in detail, for long-term operation as a polishing stage; assess the performance of identical systems for treating the same dye wastewaters under both semi-natural and controlled conditions; studying the effect of pH adjustment during the operation period; and treatment of mixed dyes (real cases) of textile effluents.

The overall findings showed that the systems removed BR46 more efficiently than the other dyes studied, and ponds containing *L. minor* significantly ($p < 0.05$) outperformed algae-dominated and control ponds. The potential of *L. minor* ponds for the treatment of BR46 was significantly ($p < 0.05$) better under controlled conditions than those under semi-natural conditions. The impact of pH was negligible concerning the treatment of BR46. In addition, the potential for BR46 degradation when it forms only part of a dye mixture is lower, compared to its corresponding removal as an individual dye. Furthermore, only ponds

containing *L. minor* completely eliminated BR46 by removing the aromatic amines after dye decolourisation. Regarding the main water quality parameters, the findings showed that the removal efficiencies of chemical oxygen demand were not significantly different in all design variables. The reduction of nutrients was considerably higher in planted ponds than in controls. Based on *L. minor* monitoring, all dyes reduced the growth of *L. minor*, and the synthetic textile wastewater negatively affected the plants' growth compared with wastewater containing fertiliser.

The overall outcomes of this research provide a better understanding of the long-term performance of shallow ponds technology using different al conditions and design variables for the treatment of dye wastewater. Consequently, this will be a tremendous value for wetland ponds engineers to foster the practical development of this system as a low-cost alternative for helping developing countries.

Chapter 1
Introduction

1.1 Overview

This chapter briefly demonstrates the adverse impact of textile factories focusing on their contaminated effluents, and the valuable approach of such wastewater treatment for environmental protection and subsequently to reduce the related water shortage problem. The chapter is partitioned into five sections. Section 1.1 presents an overview to the chapter. Section 1.2 defines the main textile industry problems and wetland features as a promising treatment technology, which is also the main motivation of the research. The gaps in knowledge, study problem and contribution are presented in Section 1.3. The aim and objectives are introduced in Section 1.4, and finally, Section 1.5 presents the thesis outline.

1.2 Research background and motivations

Population growth, economic improvement and rapid industrialisation have resulted in a higher demand for textile materials, which have consequently increased the number of textile industries and their effluents. This is one of the major causes of global environmental pollution challenges, particularly in the developing world (Dos Santos et al., 2007; Khataee et al., 2012; Gupta et al., 2015; Yaseen & Scholz, 2016). It is common for the textile industry to release huge amounts of wastewater, as well as using a large quantity of potable water (Babu et al., 2007; Hai et al., 2007; Ohioma et al., 2009; Carmen & Daniela, 2012; Zhang et al., 2012; Kamat, 2014; Sivakumar, 2014). It is estimated that between 200 litres (Ntuli et al., 2009; Kant, 2012; Ghaly et al., 2014; Suresh, 2014; Holkar et al., 2016) and 500 litres (Kalliala & Talvenmaa, 2000; Karcher et al., 2002) of fresh water is used to produce 1 kg of textile material. It is utilised during the processes of applying the chemical onto the fabric materials and washing the final products (Ntuli et al., 2009; Ananthashankar, 2012; Ghaly et al., 2014). This huge quantity of water consumed is unacceptable, especially in countries that are threatened by or are suffering from water scarcity problems (Dos Santos et al., 2007; Reham, 2011). This extensive use of natural water resources via fabric industries leads to disturbance of the environmental equilibrium, and consequently contributes to increasing the risk of water shortage. It is reported that industrial activities in time will lead to the existing potable water resources becoming even more scarce (Research priorities for earth science and public health, 2007; Reddy & Lee, 2012; Tahir et al., 2016). The Food and Agricultural Organisation (FAO) (2012) stated that even the regions that are characterised by plentiful resources of freshwater could also become under risk of water scarcity and drought. The issue of water scarcity has worsened and is set to worsen even further, according to the FAO (2016); the estimations report that by 2025 two-thirds of the world will live in regions under the risk of water scarcity, and around 1.8 billion people will be suffering from total water shortage problems in their areas (Chartres & Varma, 2010; Al-Isawi, 2016), which consequently makes the utilisation of natural water by textile mills even more unacceptable worldwide. In addition to using vast quantities of water, textile industries discharge approximately 1000–3000 m^3 of wastewater daily, whilst processing 12–20 tonnes of fabric (Pegga & Brown, 1986; Kdasi et al., 2004; Ananthashankar, 2012; Ghaly et al., 2014). This wastewater contains a mixture of dyes, auxiliaries, and heavy metals (e.g., zinc, copper, chromium, and lead), which are added during the textile production process and this causes serious environmental concerns (Cheng et al., 2002; Merzouk et al., 2009; Ananthashankar, 2012; Carmen & Daniela, 2012; Paul et al., 2012; Sekomo, 2012; Gupta et al., 2015; Holkar et al., 2016). However, the main problematic pollutants from textile mills in aquatic life are dyes (Dos Santos et al., 2007; Morali, 2010; Reema et al., 2011; Yaseen et al., 2017; Yaseen & Scholz, 2018).

Dyes are generally classified according to their origin into natural and synthetic; synthetic dyes are widely used as colouring agents and textile mills utilise around 60% of the dyes produced globally (Morali, 2010). In commercial terms, azo dyes are seen as the largest group of synthetic dyes (Stolz, 2001; Kim et al., 2004; Pandey et al., 2007; Joshi et al., 2008; Lim et al., 2011) and it is estimated that between 60% and 70% of the dyes applied in the textile industry are azo compounds (Van Der Zee et al., 2003; Davies et al., 2006; Puvaneswari et al., 2006; Cumnan & Yimrattanabovorn, 2012; Solis et al., 2012; Al-Amrani et al., 2014). This group of dyes is defined by the presence of one or more double bonds between nitrogen atoms (Zollinger, 1991; Wallace, 2001; Pandey et al., 2007; Yaseen & Scholz, 2016). In textile dye-baths, most azo dyes cannot bind with fabrics completely, resulting in some of the dyes being lost and therefore released into the environment (Manu & Chaudhari, 2003; Pearce et al., 2003; Van Der Zee & Villaverde, 2005; Ratna & Padhi, 2012). The degree of loss varies between 2% and 50% depending on the type of dye (Ganesh et al., 1994; O'Neill et al., 1999; Pandey et al., 2007; Bedekar et al., 2015). Typically, textile industry processing effluents contain dyes in the range between 10 and 200 mg/l which means they are highly coloured (O'Neill et al., 1999; Saleh, 2005; Pandey et al., 2007; Arslan-Alaton et al., 2008; Shertate & Thorat, 2014; Yaseen & Scholz, 2016). The dye wastewater effluents are also high in pH, suspended solids (SS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), salts, total dissolved solids (TDS) and metals (Sekomo et al., 2012; Verma et al., 2012; Kabra et al., 2013; Chandanshive et al., 2016). Consequently, the dissemination of these dye effluents into the environment, in addition to their unacceptable appearance (Banat et al., 1996; Nguyen & Juang, 2013), can cause many environmental problems, such as inhibiting the aquatic life in receiving watercourses, contaminating the water surface vicinity and contaminating the groundwater and the soil layer properties (Carmen & Daniela, 2012; Sivakumar, 2014; Tahir et al., 2016; Yaseen & Scholz, 2016). Furthermore, some textile dyes and their intermediate products, such as aromatic amine, which occurs after dye decolourisation when the azo bond breaks down, are deleterious due to their toxicity, mutagenicity and carcinogenicity to all life forms (Forgacs et al., 2004; Moussavi & Mahmoudi, 2009; Ong et al., 2010; Kabra et al., 2011; Khataee et al., 2012; Balarak et al., 2015; Yaseen & Scholz, 2017a). Accordingly, these dyes should be decolourised and degraded prior to their discharge, not only to solve the aesthetic problem, but also to detoxify and disinfect the contaminated site (Hai et al., 2007; Joshi et al., 2008; Tahir et al., 2016).

In developing countries, where water scarcity may already exist, the problems are exacerbated, since the extreme usage of freshwater resources (Sekomo, 2012) is combined with the "*direct*" discharge of untreated or poorly treated effluents to the natural water resources (Sekomo, 2012; Rane et al., 2014; Chandanshive et al., 2016). These effluents are loaded with pollutants at concentrations exceeding standards limits and their reuse for agriculture (irrigation) and industrial purposes is unacceptable. Hence, it has become necessary to solve the problems associated with textile industries by treating their effluents using adequate economic and effective strategies for environmental protection and/or for reuse purposes (Mugdha & Usha, 2012; Tahir et al., 2016). Various efficient methods have been used for dye wastewater purification, including physical, chemical and biological processes (Kumar & Bhat, 2012; Holkar et al., 2016). The known chemical and physical treatment methods are advanced oxidation, ozonation, ion exchange, coagulation and flocculation, membrane filtration and adsorption by activated carbon or other cheap adsorbent materials, such as fly ash or peat (Golob et al., 2005; Zonoozi et al., 2008; Vijayaraghavan et al., 2009; Aouni et al., 2012; Syafalni, 2012; El Haddad et al., 2014; Sheshdeh et al., 2014). However, each method has some drawbacks, such as expense and the generation of large quantities of sludge, which is then difficult to dispose of and may cause secondary pollution problems. Other drawbacks include the inefficient removal of colour or dye intermediates, low COD reduction, involving complex processes, regeneration difficulties of adsorbent materials, and dealing with a small volume of effluents (Robinson et al., 2001; Forgacs et al., 2004; Gupta et al., 2011; Saratale et al., 2011; Babuponnusami & Muthukumar, 2014; Gosavi & Sharma, 2014; Adegoke et al., 2015; Asghar et al., 2015; Gupta et al., 2016). In addition, it is difficult to apply most of these methods under field conditions (Ji et al., 2007; Sani, 2015). Furthermore, conventional aerobic treatment methods in municipal sewage were found to be unsuitable to operate, due to the toxicity effect of some textile dyes on the living micro-organisms in the treatment plants (Wilimott et al., 1998; Otero et al., 2003; Reema et al., 2011). Consequently, it remains imperative to search for natural, low cost and simple techniques to treat contaminated water, especially dye effluents.

Biological treatment alternatives using constructed wetlands (CWs) are sustainable and cost-effective compared with conventional methods of remediation, especially when land value is low (Vymazal, 2007; Kadlec & Wallac, 2009; Kayranli et al., 2010; Wu et al., 2014; Scholz, 2015; Yaseen & Scholz, 2016). This technology is well-accepted as being environmentally friendly, cheap, simple-to-use, and effective to treat diverse domestic and municipal sewage, storm water, agricultural runoff, and industrial wastewaters worldwide (Scholz & Xu, 2002; Scholz & Lee, 2005; Scholz, 2006; Singh & Singh, 2006; Ozengin & Elmac, 2007; Patel & Kanungo, 2010; Sekomo et al., 2012; Sani et al., 2013; Vymazal, 2014; Scholz, 2015). CWs are man-made wetland or marsh created as a new or redesigned habitat for plants, organisms and other wildlife to emulate the optimal biological, chemical and physical conditions that occur in natural wetlands, which act as a biofilter, (Kadlec & Knight, 1996; Cooper et al., 1997; Vymazal et al., 1998; Scholz, 2010, 2015; Al-Isawi et al., 2017) to purify different types of contaminated water at different concentrations and under various climate conditions (Kadlec & Wallace, 2009). Literature indicates promising results in the treatment of textile dye effluents using artificial wetland systems planted with emergent plants, such as *Phragmites australis* (Cav.) Trin. ex Steud. (Davies et al., 2005; Mbuligwe, 2005; Bulc & Ojstrsek, 2008; Ong et al., 2009a, 2010, 2011; Cumnan & Yimrattanabovorn, 2012; Hussein & Scholz, 2017) and *Echinodorus cordifolius* L. (Noonpui & Thiravetyan, 2011), submerged plants such as *Myriophyllum spicatum* and *Ceratophyllum demersum* (Keskinkan & Lugal Goksu, 2007), and free-floating plants such as *Lemna minor* L. (Sivakumar, 2014) and *Eichhornia crassipes* (Mart.) Solms (Muthunarayanan et al., 2011). The high removal efficiency in CWs for dyes, metals, nutrients, COD and other contaminants was found to be due to the complex interactions between plants, water, soil and micro-organisms (Yaseen & Scholz, 2016).

Recently, among all types of CWs, there has been increased attention towards using free-floating macrophytes-based CWs systems, which refer to shallow ponds vegetated with free-floating plants, to treat dye wastewater, as the cheapest method in addition to its effectiveness and sustainability (Dalu & Ndamba, 2003; Tsalkatidou et al., 2009; Babu, 2011; United States Environmental Protection Agency (USEPA), 2011; Mburu et al., 2013; Yaseen & Scholz, 2016). CWs with free-floating macrophytes consist of one or more shallow earthen, concrete, plastic, or steel basins (pits) constructed in or on ground level as shallow ponds, in which free-floating plants float on the water surface (Kadlec et al., 2000; Adeola, 2007; Vymazal, 2008), and could exist in combination with other treatment systems for primary, secondary or polishing units (Faleschini et al., 2012). The presence of algae bloom on the surface of ponds instead of aquatic plants refers to the difference between stabilisation ponds and CWs with free-floating plants (Kadlec et al., 2000; Vymazal, 2008), although some authors indicate that the term ‘wetlands’ refers to all marshlands and ponds (Mitsch & Gosselink, 1993, 2000; Ramsar, 2010). The main feature of using constructed wetlands technology with free-floating plants (shallow ponds) as a method for management and purification of wastewater, is that it enhances the eco-friendly values as well as being inexpensive to establish (Scholz, 2011; Sani, 2015). In developing countries, where much lower capital cost of treatment system is preferable, this system, compared with other wetland systems, is beneficial because free-floating plants normally do not require substrates to support their roots, that grow hydroponically (Chen et al., 2016), which saves the extra charge involved for soil and/or gravel (Vymazal, 2010). Regarding the operation and maintenance, such as pumping energy, screen cleaning, plant harvesting, and equipment repairs, the costs are very low for all wetlands (Vymazal, 2010). Several investigations confirmed the effectiveness of different algae species and free-floating plants-based systems for the treatment of effluents from the textile industry (Lim et al., 2010; Sekomo, 2012; Sivakumar et al., 2013; Uysal et al., 2014) and other effluents (Al-Nozaily et al., 2000;

Zimmo et al., 2005; Ozengin & Elmaci, 2007; Tsalkatidou et al., 2009; Patel & Kanugo, 2010; Babu, 2011; Mburu et al., 2013). Generally, the treatment of wastewater using these systems is based on the ability of living organisms, such as algae and/or bacteria to clean up pollutants, and the ability of plants to assimilate and accumulate soil, wastewater, water and air contaminants (Sivakumar, 2014; Yaseen & Scholz, 2017a). However, the performance abilities of the systems vary depending on the design variables and operational conditions, such as system arrangement, contact time, environmental conditions, plants and/or algae species, types of substrate (if applicable), types of wastewater, and pollutant concentrations (Scholz, 2011; Sani, 2015). Another variable is the growth rates of plants utilised in the system (Picard et al., 2005; Sani et al., 2013; Sani, 2015). Therefore, these parameters must be considered carefully for achieving optimal results.

As a result, this research is motivated by all these challenges linked with textile factories in the low-income developing countries, including the environmental pollution by dye effluents and the consequent water scarcity problems, which necessitate a solution by treating the textile effluents using an adequate economic and effective strategy that helps to protect the eco-system and enable subsequent recycling of the treated effluents for irrigation purposes or reuse within the textile factory processes (Mugdha & Usha, 2012; Chandran, 2016). Additionally, although previous studies highlighted the importance of using free-floating plants-based treatment systems for the remediation of textile dye wastewater as an effective, green, sustainable and cheap method, the application of these systems during the polishing stage is still limited in this field and there are only short-term (few weeks) studies treating wastewater contaminated with textile dyes in Turkey and India without full assessment of the system performance (Muthunarayanan et al., 2011; Sivakumar et al., 2013; Sivakumar, 2014; Uysal et al., 2014). This lack of clear data was the main motivation for this study, in order to address the ecological problems caused by textile industries. The polishing stage is the last purification stage for pollutants in a multi-stage treatment system, which deals with wastewater characterised by relatively low concentrations of contaminants (Reed et al., 1995). The importance of this stage has increased as secondary treatment systems of a biological, chemical or physical nature are unable to remove dye contaminations completely, and therefore, a further step is required (Bejarano, 2005).

1.3 Problem statement, knowledge gaps, and contribution

This research is driven by the need to address problems associated with the effluents from the textile industry, including the dyes used in the production process. A solution using constructed wetlands with free-floating plants that simulate shallow ponds is suggested in this research as a cheap, effective and green technology.

There have been some investigations of free-floating plants-based treatment systems to treat textile effluents in recent years (Sekomo et al., 2012; Sivakumar et al., 2013; Chandanshive et al., 2016). However, the literature in this area is still limited, and there are only few previous studies that focus particularly on removing textile dyes from contaminated wastewater using free-floating plants-based systems (Muthunarayanan et al., 2011; Sivakumar, 2014; Uysal et al., 2014). In addition, the available publications have not considered the mechanism of dye removal in detail, and there is a lack of information concerning the main water quality parameters and the system performance from an engineering point of view.

Several reports have been published for wastewater treatment by constructed wetland systems planted with emergent and submerged macrophytes as a post (polishing) treatment stage (Keskinkan & Lugal Goksu, 2007; Ayaz, 2008; Sekomo et al., 2012), and despite the importance of this stage, the literature review showed limited attention towards operating wetland systems with free-floating plants for dye wastewater treatment during the polishing stage.

The previous few investigations on free-floating plants-based treatment systems provide promising results for treating wastewater containing dyes (Muthunarayanan et al., 2011; Sivakumar, 2014; Uysal et al., 2014; Chandanshive et al., 2016). However, there remain prominent gaps in all these studies concerning the system performance for dye effluents treatment in long-term operation, as well as the treatment of mixed dyes (real case of textile effluents), which have not been investigated using constructed wetlands planted with free-floating plants. In addition, many approaches showed a noticeable impact for the pH factor on the dye molecule removal (Deniz & Karaman, 2011; Reema et al., 2011; Khataee et al., 2012; Movafeghi et al., 2013; Shirzad-Siboni et al., 2014; Balarak et al., 2015, 2016a). Surprisingly, no articles have focused on studying the effect of pH adjustment on the full assessment of long-term performance of free-floating plants-based treatment systems in terms of the removal of dyes and other contaminants. Furthermore, researchers concentrated on examining small-scale experimental wetlands for dye effluent treatment under either controlled (indoor) conditions (Ong et al., 2010; Noonpui & Paitip, 2011; Sivakumar, 2014) or semi-natural (outdoor) conditions (Mbuligwe, 2005; Davies et al., 2009; Muthunarayanan et al., 2011). However, the performance of identical systems for treating the same dye wastewaters under both semi-natural and controlled conditions has not been previously published. With regard to the dyes tested in this research, there is no previous literature about using them with free-floating plants-based constructed wetland systems, and this will be the first study to examine the feasibility of removing these dyes using this system.

Therefore, it is necessary to fill the gaps in knowledge in this field by operating small-scale free-floating plants-based constructed wetlands simulated shallow ponds for the treatment of textile azo dyes containing wastewater, and evaluating the system performance in terms of different design variables and operational conditions in long-term experiments during the polishing stage.

This work would offer valuable data to allow for a sustainable solution using a promising strategy to mitigate water pollution problems, particularly for developing countries which suffer from the impact of dye effluents, especially when financial resources are limited and land costs are relatively cheap. Also, the research provides a practical contribution to designers and operators of wetland ponds, in terms of the information about the system performance for treating textile dyes and related water. Furthermore, the findings are expected to support the possible application of large-scale treatment plants.

1.4 Aim and objectives

The overall aim of the research study is to assess the potential of constructed wetland systems vegetated with free-floating plants (*Lemna minor* L.), simulating shallow pond systems (SPSs), for the treatment of synthetic wastewater containing textile azo dyes, as a polishing stage, in a long-term study.

To achieve the study aim, the specific objectives are as follows:

1. To examine the influence of different design variables on the SPS performance for treating synthetic wastewater contaminated with 5 mg/l of four azo dyes, under different environmental conditions, and to evaluate the inflow and outflow water quality parameters including dyes and COD.

2. To compare the SPS efficiency using *L. minor* under controlled and uncontrolled (semi-natural) conditions in Salford, for remediation of synthetic wastewater contaminated with 5 mg/l of four azo dyes and to evaluate the main water quality parameters including dye removal.
3. To assess the impact of pH variations on the SPS performance (with/without *L. minor*) under controlled conditions for the treatment of 10 mg/l of azo dyes containing synthetic textile wastewater with and without dilution, and to evaluate dye removal and other water quality parameters (COD, nutrients, etc.).
4. To investigate the SPS performance with/without *L. minor* under controlled conditions for the handling of diluted synthetic textile wastewater comprising three mixtures of RB198 and BR46 in different percentages at a total concentration of 10 mg/l, and to evaluate the dye removal and other water quality parameters (COD, nutrients, etc.).
5. To assess the impact of operating conditions, synthetic wastewater, pH variation and dye accumulation on *L. minor* growth rate, by monitoring and comparing the growth parameters with ponds without dyes and synthetic wastewater.

Figure 1.1 demonstrates the methodology of the research and how objectives are linked to achieve the aim.

1.5 Thesis structure

This thesis is organised into the following seven chapters:

Chapter One: Introduction

This chapter includes the background information on environmental problems caused by the effluents from the textile industry, and previous studies related to this research. It is also presents the research motivation, gaps in knowledge, problem statement, contribution, aim and objectives.

Chapter Two: Critical Literature Review

This chapter provides a background to the textile industry, and includes an overview of textile wastewater characteristics, variation and simulation, textile dye classification and environmental challenges linked with textile dye effluents. A critical review of previous research work is also given for treatment of textile dyes containing wastewater using CWs and algae ponds. Furthermore, a significant part of the chapter is focused on the literature related to the role of different contaminant treatment mechanisms.

Chapter Three: Materials and Methods

This chapter describes the research methodology including materials, experimental set-up and system operation, under controlled and uncontrolled (semi-natural) environmental conditions. Furthermore, the sampling procedure and subsequent data analysis are also stated.

Chapter Four: Impact of Design Variables and Environmental Conditions on System Efficiency

This chapter presents the overall treatment results and related discussion of pond performance using different design variables for treating synthetic wastewater containing 5 mg/l of dyes under laboratory and semi-natural conditions, as well as the comparison between them.

Chapter Five: Impact of pH Adjustment on System Performance

This chapter presents the overall treatment results and discussion related to the short-term and long-term impact of pH variation on system performance in terms of dye removal at concentration of 10 mg/l, and for improvement of other water quality parameters.

Chapter Six: Assessment of System Performance for Treating Dye Mixtures

This chapter presents the overall results and discussion of the system performance for the treatment of diluted synthetic textile wastewater containing three mixtures of two dyes at concentration of 10 mg/l.

Chapter Seven:Conclusions and Recommendations

This chapter concludes the main results of each experiment. In addition, suggestions for further related work are also provided.

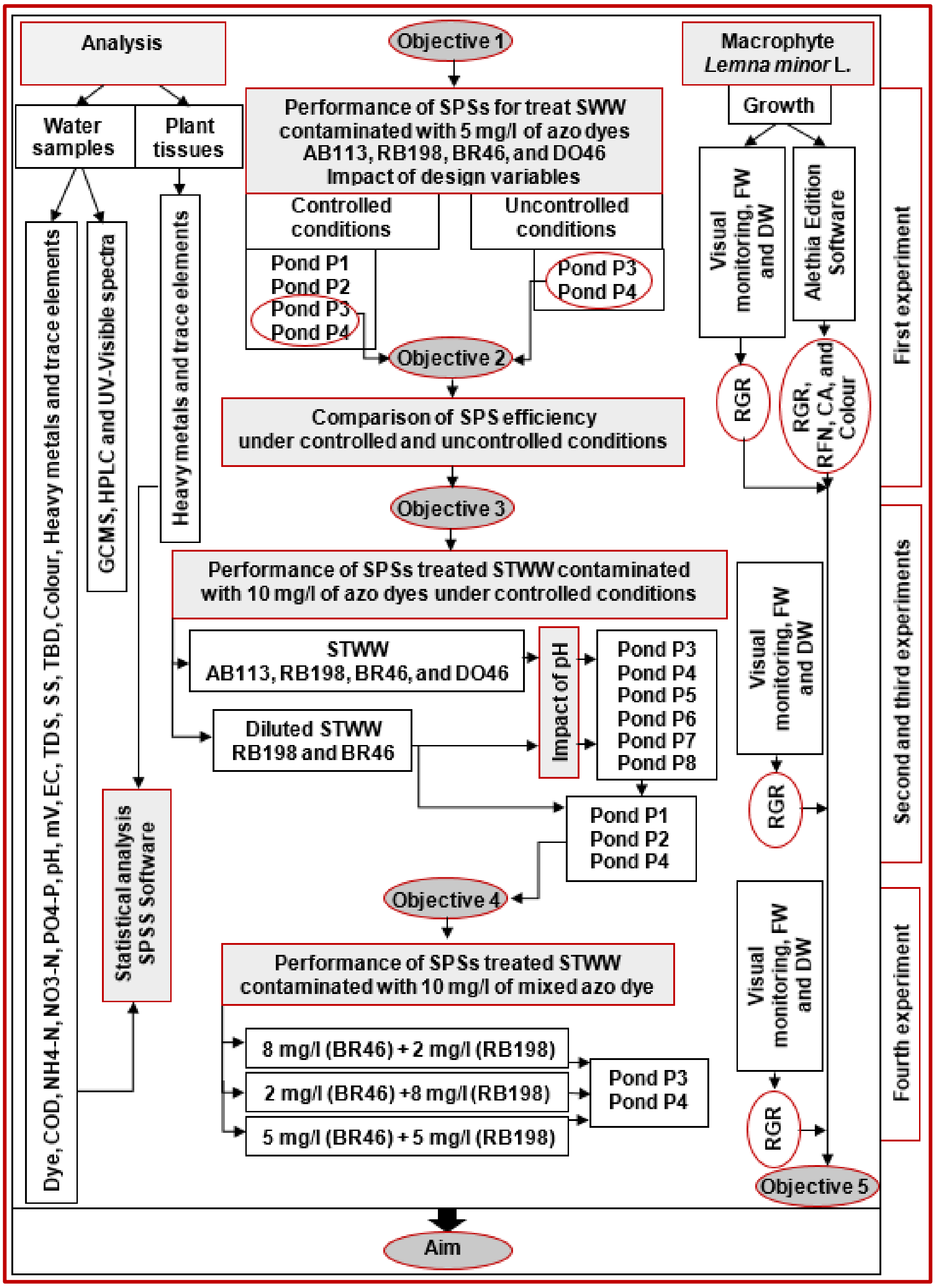


Figure 1.1 Research aim and related objectives diagram. Note: SPSs, shallow pond systems; COD, chemical oxygen demand; PO_4–P, ortho-phosphate-phosphorus; NH_4–N, ammonium-nitrogen; NO_3–N, nitrate-nitrogen; SS, suspended solids; TDS, total dissolved solids; TBD, turbidity; EC, electrical conductivity; mV, redox potential; DO, dissolved oxygen; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; FW, fresh weight; DW, dry weight; RGR, relative growth rate; RFN, relative frond number; CA, coverage area, P1, *Lemna minor* L. and algae; P2, algae; P3, *Lemna minor* L.; P4, control; P5, *Lemna minor* L. at pH of 9; P6, control at pH of 9; P7, *Lemna minor* L. at pH of 6; P8, control at pH of 6; SWW, Synthetic wastewater; STWW, synthetic textile wastewater; GCMS, gas chromatography mass spectrometry; HPLC, high-performance liquid chromatography; UV, ultraviolet; SPSS, statistical package for social sciences

Chapter 2
Critical Literature Review

2.1 Overview

This chapter introduces the processes of the textile industry and includes the effluent characteristics of the real, typical and synthetic wastewaters. In addition, dye classifications and the environmental challenges related to textile dye effluents are discussed in this chapter. A critical literature review is presented regarding the reported chemical constituents used to prepare the synthetic textile wastewater containing dyes. Also, a critical review is given about the constructed wetland systems, including the wetland types for dye wastewater treatment and the mechanism of pollutants removal. This chapter is divided into thirteen sections as follows: Section 2.1 presents an overview of the chapter; Section 2.2 explains the types of textile industry and the main processes of textile production; Section 2.3 characterises typical and real textile industry effluents; Section 2.4 presents the synthetic textile wastewater inputs and some corresponding characteristics; Section 2.5 defines and classifies textile dyes; Section 2.6 defines the importance of azo dyes; Section 2.7 describes the environmental pollution problems associated with textile effluents and azo dyes. The development of wetland systems for treating wastewater, and constructed wetlands classification are both highlighted in Sections 2.8 and 2.9, respectively. Section 2.10 defines the algae based systems and their efficiency for dye remediation; Section 2.11 demonstrates the composition of wetlands. Lastly, the process of pollutants removal within wetland systems is shown in Section 2.12, and the chapter summary is presented in Section 2.13. Figure 2.1 below shows the different constructs of the literature review.

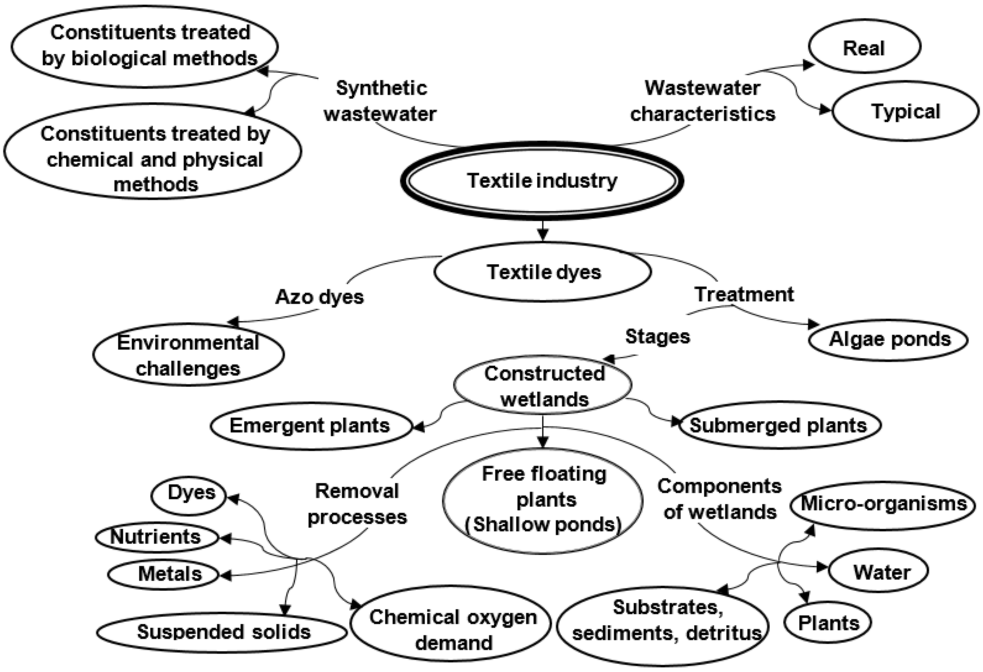


Figure 2.1 Constructs of the literature review

2.2 Textile industry

The textile industry is one of the largest and oldest sectors present globally, which provides career opportunities with no required specific skills or expertise and consequently positively affects the economy worldwide (Ananthashankar, 2012; Sekomo, 2012; Upadhye & Joshi, 2012; Ghaly et al., 2014; Suresh, 2014), more so in the developing countries. In Europe, for example, there are around 110,000 textile production companies, which provides around €200 billion as income per year (Morali, 2010). China is the most important exporter of all types of textiles, followed by the European Union, India and then the United States (Saranraj, 2013; Ghaly et al., 2014). Figure 2.2 shows the major exporters for textiles, the percentage of market share and the related yearly income. One of the key issues associated with textile factories is the undesirable dye effluents, which are difficult to degrade (He et al., 2004; Nguyen & Juang, 2013).

The classification of textile industries depends on the type of fabrics they produce, including cellulosic materials obtained from plants (e.g., cotton, rayon and linen), protein fabrics which come from animals (e.g., wool, silk and mohair), and synthetic fabrics produced artificially (e.g., nylon, polyester and acrylic) (Bledzki & Gassan, 1999; Ananthashankar, 2012; Ghaly et al., 2014). In the textile industry, the final fabric is manufactured by converting the fibres into yarn and then to fabric or other related products that pass through wet processing, ending with the dyeing and finishing stages (Ghosh & Gangopadhyay, 2000; Babu et al., 2007; Upadhye & Joshi, 2012). The wet process uses a considerable quantity of potable water and releases highly contaminated liquid waste due to the addition of a wide range of chemicals, such as dyes, soaps, starch, metals, acids and alkalis (Mishra & Tripathy, 1993; Banat et al., 1996; Vandevivere et al., 1998; Talarposhti et al., 2001; Paul et al., 2012; Holkar et al., 2016). This process mainly consists of sizing, de-sizing, sourcing, bleaching, mercerising, dyeing, printing and finishing techniques (United State Environmental Protection Engineering (USEPA), 1997; Babu et al., 2007; Kumar et al., 2009; Liu et al., 2010; Vigo, 2013). The sizing process is the first step for preparation of the fabric and involves adding sizing agents, such as starch, polyvinyl alcohol and carboxymethyl cellulose (Liu et al., 2010, Carmen & Daniela, 2012) to make the fibre stronger and reduce ruptures. However, these agents hinder other processes such as dyeing, printing and finishing. For example, starch impedes dye penetration into the fibre. Therefore, de-sizing is required as a second step for eliminating all materials added during the sizing step by applying hydrolysis or an oxidation agent (Babu et al., 2007) such as enzymes, sodium bromide, sodium chlorite and hydrogen peroxide. The scouring step is the process of adding an alkali solution, such as sodium hydroxide, potassium hydroxide or sodium carbonate to remove the oils and waxes, and suspend contaminations from the fibres. After that, the bleaching process is used for removing the undesirable colour or creamy appearance, improving the whiteness and brightness of the fibres by adding bleaching chemicals, such as hydrogen peroxide, sodium hypochlorite, hypochlorite and peracetic acid (Rott & Minke, 1999; Babu et al., 2007). The mercerising process follows, increasing the fibres' appearance, strength, and dye uptake by adding a concentrated alkaline solution (sodium hydroxide) and acid solution. The application of neutralisation agents, including acetic acid or formic acid (Bradbury et al., 2000) is required to neutralise the fibres after the scouring, bleaching, reduction and mercerising processes. The dye process is an important step in textile manufacturing; during this stage, the colour is added as a solution to the fibres (Kanawade et al., 2010; Sivakumar et al., 2013). To improve the binding between the dyes and the fabric, other different chemicals are added, such as metals, salts, surfactants, organic processing aids, sulphide and formaldehyde. Regarding the printing step, this involves the same reactions as the dye stage, however in printing, the colour is added as a thick paste. The final part of the textile process is the finishing step, where some properties are imparted to the fabric, such as softening, waterproofing, antibacterial and ultraviolet protection, by adding formaldehyde-based agents (Babu et al., 2007; Holkar et al., 2016). Thus, during the wet process many of these chemicals and additives become part of the final product, while the rest are released as waste into textile effluents (Dos Santos et al., 2007; Carmen & Daniela, 2012). This release is attributed to the impact of the washing, rinsing, and drying steps required between the main stages of wet processing. Consequently, a large amount of contaminated wastewater is generated from these stages forming one of the main problems related to the textile industry. Figure 2.3 shows the steps of the wet process and the main pollutants in the wastewater discharge from each step, as indicated by Kant (2012) and Holkar et al. (2016).

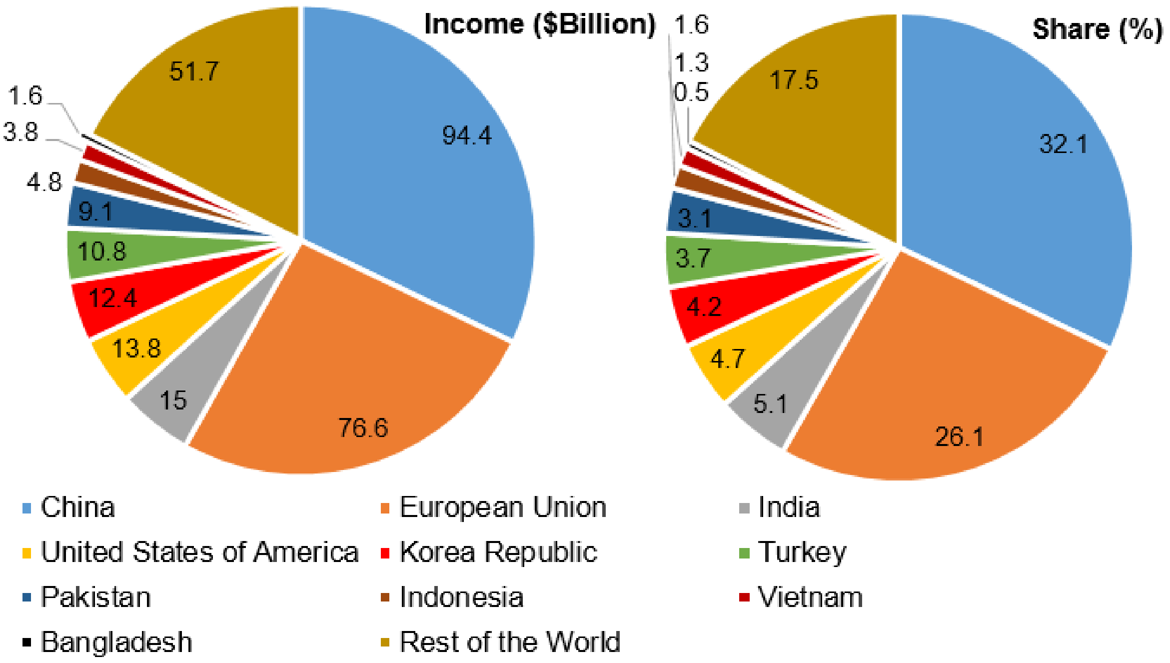


Figure 2.2 Major exporters for textiles, market share and income (after Ghaly et al., 2014)

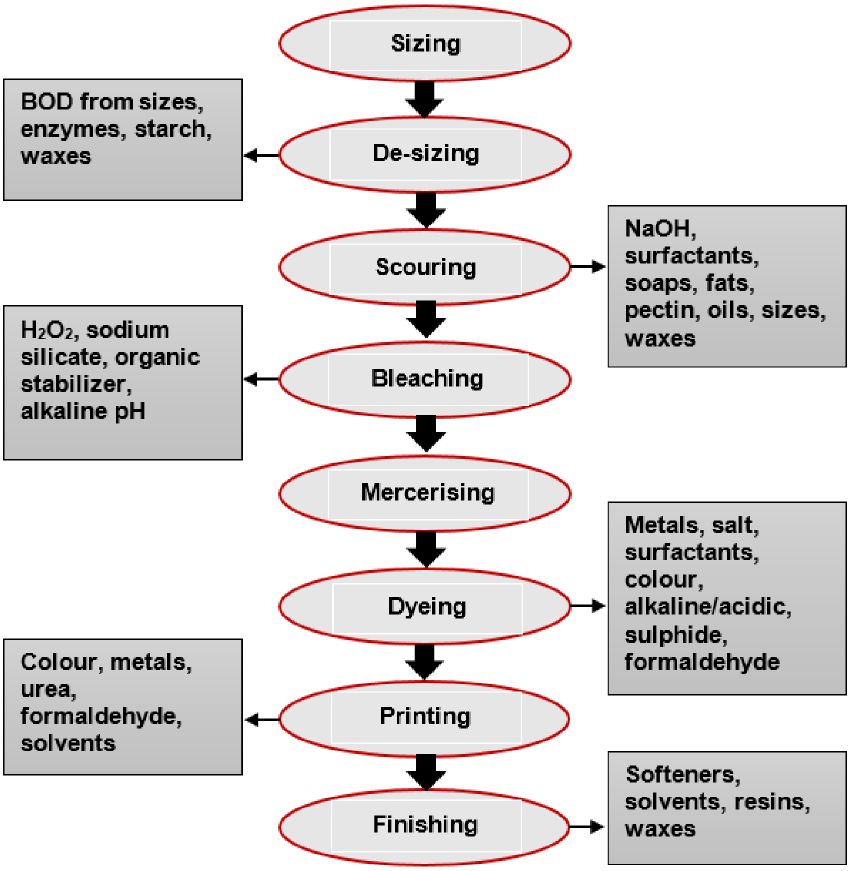


Figure 2.3 Main pollutants discharged from each step of textile wet processing (after Kant, 2012; Holkar et al., 2016)

2.3 Textile wastewater characteristics

The effluents discharged from textile factories are heavily polluted with a mixture of organic (e.g., carbohydrates, fats and oils, dyestuffs, phenols, and detergents) and inorganic (e.g., acids, alkalis, metals, other salts, phosphates, nitrates, and sulphides) compounds that are applied during the textile manufacturing processes and then released as unused substances to the receiving watercourses (Brik et al., 2006; Hassani et al., 2008; Palácio et al., 2012; Upadhye & Joshi, 2012; Gupta et al., 2015; Verma et al., 2015). Many authors have confirmed that the textile effluents containing dyes are characterised by high values in the main water quality parameters, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), colour, total dissolved solids (TDS), suspended solids (SS), turbidity, salinity, heavy metals, and pH values (Globo et al., 2005; Dos Santos et al., 2007; Kabra et al., 2012; Shah et al., 2013; Daud, 2014; Suresh, 2014). However, the most important parameters in textile wastewater are COD, BOD, pH, SS, fats, oil, nitrogen, sulphate and phosphorus (Tufekci et al., 2007). Therefore, during the treatment processes, it is important to monitor and compare most of these parameters with the standard concentrations before discharging the effluent to the receiving watercourse.

The composition of textile wastewater varies from mill to mill and from country to country, which consequently affects the wastewater characteristics, dependent on the process, the equipment used in the factory, type of fabric produced, chemicals used, the weight of the fabric, colouring materials, as well as the dye constituents, season and the trend in fashion (Brik et al., 2006; Sekomo, 2012; Hussein, 2013; Kehinde & Aziz, 2014). Table A.1 (Appendix A) summarises the typical characteristics of textile wastewater and Table A.2 (Appendix A) presents the wastewater characteristics of the main processes used in textile manufacture. These characteristics exhibit differences among their values from one reference to another, which was confirmed by Khandare et al. (2013), indicating that typical textile wastewater is difficult to define because the textile application methods, even in the same process, are different from one industry to another. Table A.3 (Appendix A) shows the reported characteristics of actual textile effluent before treatment, belonging to different sources and countries. Some of these real effluent characteristics are not within the typical range of values given in Table A.1 (Appendix A), demonstrating the wide range of variety in real wastewater. This may be because these effluents are related to a particular step in the textile industrial processes (Almazan-Sanchez et al., 2016; Tomei et al., 2016) or passed from a specific treatment stage (Nopkhuntod et al., 2012; Qian et al., 2013) to the next.

It is reported that around 93% of the water used in the textile production industry discharges as dyeing wastewater (Wijannarong et al., 2013; Gupta et al., 2014; 2015), and may contain considerable concentrations of heavy meals as well (Tsezos, 2001; Cheng et al., 2002; Merzouk et al., 2009; Fu & Wang, 2011; Sekomo, 2012). These two pollutants (the dyes and metals) require more attention due to their toxic impact in the receiving streams. Metal contamination in textile effluents occurs due to the presence of the dyes and the additives used, e.g., caustic soda, sodium carbonate and salts, during the textile manufacturing steps. The main metals, which cause environmental challenges, are copper, chromium, zinc, iron, mercury, and lead (Hussein, 2013). However, the main metals found within the dye chromophores in textile effluents are zinc, cobalt, copper, and chromium (Adinew, 2012; Kaur & Sharma, 2015). The concentrations of the main metals (elements) in real textile wastewater are presented in Table A.4.(Appendix A). Dye concentrations in textile wastewater are reported over a wide range of values. Laing (1991) indicated that the dye level in the textile effluent is 10–50 mg/l. However, the reactive dyes in cotton factories are reportedly discharged at a concentration of 60 mg/l (Pierce, 1994; Shelley, 1994), and a concentration between 100 and 200 mg/l (Gahr et al., 1994). A concentration of the reactive dye effluent of 7000 mg/l was reported by Koprivanac et al. (1992). This concentration is extremely high, compared to other references, and may refer to effluent discharge from a specific textile industry (O'Neill et al., 1999). Vandevivere et al. (1998), Jadhav et al. (2007) and Saratale et al. (2011) referred to dye effluents ranging between 600 and 800 mg/l. Sivakumar (2014) mentioned that the outflow concentration of the dye acid orange 10 from the final clarifier of the textile industry in India is 45 mg/l, which is within the range of concentrations reported previously by Abid et al. (2012), that is, between 20 and 50 mg/l, from the 14-Ramadhan textile industry in Iraq. However, Ghaly et al. (2014) mentioned that the dye concentrations discharged from dye houses ranged from 10–250 mg/l, and this range covers most of the above values which were referred to by the authors, except the extreme concentrations indicated by Koprivanac et al. (1992) and Vandevivere et al. (1998). The concentrations of textile dyes in real wastewater are presented in Table A.5 (Appendix A). Thus, all available literature regarding the treatment of real textile effluents, as well as the reported outflow dye and heavy metal concentrations, have a wide variation of textile wastewater characteristics (Al-Kdasi et al. 2004), which makes the composition used for preparing synthetic textile wastewater containing dye varied as well (Ong et al., 2010; Punzi et al., 2015).

2.4 Synthetic textile wastewater

Many authors have examined treatment technologies and corresponding dye removal efficiencies from either an aqueous solution or a prepared synthetic textile wastewater. This is firstly due to the common absence of raw textile wastewater as a local and permanent source, and secondly to keep the composition of the untreated wastewater constant to make valid comparisons of treatment system evaluations (O'Neill et al., 1999). A wide range of investigations have been carried out regarding the removal efficiency of different textile dyes from aqueous solutions prepared by mixing dye with tap water, deionised water or distilled water (Ncibi et al., 2007; Mehta et al., 2011; Mezenner et al., 2013; Rajkumar et al., 2013; Sun et al., 2013; Aysu & Kucuk, 2015; Castaneda-Diaz et al., 2017; Dehghani et al., 2017). Below are some of the reported methods used in this field.

Davies et al. (2005, 2006, 2009) assessed the performance of constructed wetland (CW) using *Phragmites australis* for the treatment of wastewaters prepared by dissolving the dye acid orange 7 with tap water (dye inflow concentrations were 130 and 700 mg/l). Chatzisymeon et al. (2006) prepared synthetic wastewater to investigate the electrochemical oxidation method for dye wastewater treatment. The composition contained 16 dyes and the total dye concentration was equal to 361 mg/l. Kashefialasl et al. (2006) treated the dye acid yellow 36 in aqueous solution by the electrocoagulation method using iron electrodes. Noonpui and Thiravetyan (2011) examined the performance of CW vegetated by *Echinodorus* spp. Richard ex Engelmann in A. Gray, 1848 (burhead) under both soil and soil-free conditions for the treatment of three reactive azo dyes of different molecule sizes: reactive red 2, reactive red 120 and reactive red 141 in aqueous solution. Shirzad-Siboni et al. (2014) studied the removal of acid blue 113 and reactive black 5 in aqueous solution using activated red mud as a cheap adsorbent material. Other adsorbents have been used for dye removal such as fly ash (Sun et al., 2013) and activated carbon (Aysu & Kucuk, 2015). Textile dye removal from aqueous solution was also evaluated using plant species in batch test studies such as *Posidonia oceanica* (L.) Delile (Neptune grass) (Ncibi et al., 2007), *Calotropis procera* (Aiton) W.T.Aiton (apple of Sodom) (Ali & Muhammad, 2008) and *Lemna minor* (Reema et al., 2011; Movafeghi et al., 2012; Salman et al., 2016).

The treatment of dyes in aqueous solutions has been applied using a wide range of different methods. However, studies using this type of wastewater are unable to provide a full understanding of the treatment performance regarding the impact of other missing contaminants (nutrients, chemicals and additives) on the removal efficiency (Gupta et al., 2015) since, as mentioned by Bulc and Ojstrsek (2008), the pollutants' reduction in treatment systems depends mostly on composition of textile wastewater, and the chemical constitution of applied organic substances in addition to other factors. This has led many authors to prepare synthetic textile wastewater contaminated with textile dyes, which have been arranged by mixing the dye with a water source and nutrients or different chemicals in specific concentrations. This mixture aims to, according to Verma et al. (2012, 2015), match the wastewater characteristics with those of real effluents containing various chemicals, auxiliaries and dyes added during the textile manufacturing steps. A wide variety of compositions have been suggested in the literature for the simulation of textile effluents, to mimic the effluent either in a particular country or a specific textile factory. These synthetic effluents, which have been treated by different chemical, physical and biological methods, consist of some common chemicals corresponding to specific steps in the textile manufacturing processes (Panswad & Luangdilok, 2000; Ojstrsek et al., 2007; Avlonitis et al., 2008; Cumnan & Yimrattanabovorn, 2012; Mountassir et al., 2015). The following sections will highlight the available reported constituents for preparing synthetic textile wastewater according to the method of treatment.

2.4.1 Constituents of wastewater treated by chemical and physical methods

The chemicals, concentrations and methods of preparation for the simulated textile wastewater treated by chemical and physical methods, are described clearly by many researchers. Regarding the chemical approaches used for treating simulated wastewater, Alaton et al. (2002) focused on studying the efficiency of different advanced oxidation processes (O_3/OH^-, H_2O_2/UV-C and TiO_2/UV-A) for treatment of simulated wastewater comprising a mixture of five reactive dyes and different auxiliaries in concentrations, which matched those of the reactive dye effluents discharged from a cotton factory. The chemicals used in the mixture were acetic acid, sodium chloride, sodium carbonate, sodium hydroxide, polyether based co-polymer micro-dispersion, acryl co-polymer-phosphor mixture and alcyl phenol polyglycol ether. The mixture of the dyes and auxiliaries was first dissolved in boiling deionised water to ensure that all the chemicals hydrolysed, and then stored for 12 hours in a cool room before use. Kang et al. (2002) studied the ‘Fenton Process’ (involving oxidation and coagulation) for the treatment of synthetic textile wastewater containing polyvinyl alcohol mixed with reactive blue dye R94H to simulate COD and colour, respectively. Sakkayawong et al. (2005) focused on chemical adsorption by chitosan for the treatment of synthetic wastewaters containing a mixture of the dye reactive red 141 at a concentration of 400 mg/l, sodium carbonate and sodium sulphate, which was prepared according to the dye procedures in the textile factory. Bali and Karagozoglu (2007) studied the Fenton process (involving oxidation and coagulation), ferric coagulation and the H_2O_2/pyridine/Cu(II) system for the removal of colour from a synthetic textile wastewater containing polyvinyl alcohol and a reactive dye (remazol turquoise blue G-133). Verma et al. (2012) studied the effectiveness of magnesium chloride and ferrous sulphate as chemical coagulant materials with lime as a coagulant aid for treating synthetic textile wastewater. They introduced a composition for a synthetic textile wastewater to simulate the characteristics of the real textile effluents in Tirupur (India) reported on by Eswaramoorthi et al. (2008). The composition consisted of starch, acetic acid, sucrose, sodium hydroxide, sulphuric acid, sodium carbonate, sodium chloride, sodium lauryl sulphate and dye, which were mixed using tap water. The dyes used were reactive black 5, congo red and disperse blue 3 at total concentrations of 200 mg/l. Later, Verma et al. (2015) used an anaerobic sludge blanket reactor as a pre-treatment step of the same synthetic textile wastewater using a magnesium chloride and aluminium chlorohydrate mixture. Mountassir et al. (2015) treated synthetic textile effluents by an electro-coagulation method with an aluminium electrode in the presence of clay particles. The wastewater was prepared by mixing the dye reactive violet 4, starch, ammonium sulphate and disodium phosphate in deionised water. Punzi et al. (2015) proposed a novel method for treating synthetic textile effluents using an anaerobic biofilm reactor followed by ozonation. The synthetic effluent contained starch dissolved in heated water and mixed with sodium hydroxide, sodium chloride and the azo dye remazol red.

Concerning the reported constituents treated by physical treatment technology, Marquez and Costa (1996) prepared synthetic textile effluent for evaluation by the powdered activated carbon treatment process. The mixture contained peptone, meat extract, urea, potassium hydrogen phosphate, sodium chloride, calcium chloride dihydrate, magnesium sulphate heptahydrate and the dye acid orange 7. Avlonitis et al. (2008) examined the impact of various operational conditions concerning the performance of a nanofiltration membrane technique for treatment of three synthetic textile dyeing wastewaters. The synthetic wastewaters were prepared by dissolving salts and the dye reactive black 5 using tap water at concentrations higher than the typical ranges found in the effluents of the cotton dye industry. However, Hassani et al. (2008) studied the impact of different TDS values (1000, 2000, and 3000 mg/l) on dye removal efficiency by mixing the dyes with sodium chloride (salt source) using the nanofiltration technique as well. Aouni et al. (2012) evaluated ultrafiltration and nanofiltration methods for the treatment of synthetic dye wastewater in Spain. The synthetic reactive dye wastewater was prepared by mixing 15 gram from each dye separately (everzol black, everzol blue, and everzol red) with 2 litres ultra-pure water. This was followed by mixing with 12.5 gram sodium chloride and ultra-pure water to prepare 25 litres of dye solution in total. Sodium hydroxide was added for pH adjustment to reach a pH value of 10. The dye concentration was 600 mg/l. Nopkhuntod et al. (2012) conducted a batch test using shale as an adsorbent material before the main experiment to examine the operational parameters for removing the dyes reactive red, blue and yellow separately by preparing synthetic dye wastewater. The synthetic effluents contained reactive dye and sodium chloride in a specific concentration dissolved with distilled water. The mixture was heated to 60°C for two hours and sodium carbonate was added within the first hour. After that, sodium hydroxide was added for preparing 1 litre of wastewater solution.

All these compositions aimed to simulate the real textile effluents, however most of them did not contained the main chemicals used in a textile factory and the related synthetic wastewater was prepared to remove specific parameters (in most cases, the colour and COD), which limits the full simulation of textile effluents, as not all the main parameters are covered, such as ammonium-nitrogen (NH_4–N), nitrate-nitrogen (NO_3–N), and ortho-phosphate-phosphorus (PO_4–P) (Kang et al., 2002; Bali & Karagozoglu, 2007; Aouni et al., 2012). Regarding the composition reported by Verma et al. (2012, 2015), this was the most appropriate one compared to other reported compositions, due to the presence of most of the chemicals used in textile processing. However, a source of metals is still required in this mixture to fully simulate the synthetic wastewater compounds.

2.4.2 Constituents of wastewater treated by biological methods

Many authors concentrated on preparing synthetic textile wastewater to examine different biological approaches, in terms of the treatment of textile pollutants. The compounds and the related concentrations, as well as the process of preparation, are documented in detail.

Regarding the anaerobic/aerobic or anoxic/aerobic sequential batch reactor methods, Basibuyuk and Forster (1997) operated four up-flow aerated biofilters in sequence for treatment of simulated textile wastewater using activated sludge. The synthetic textile wastewater contained the dye maxilon red at two concentrations (25 mg/l and 50 mg/l), starch, lab lemco, diammonium phosphate, magnesium sulphate heptahydrate, calcium chloride, iron(II) sulphate heptahydrate, nickel(II) sulphate heptahydrate, manganese(II) chloride tetrahydrate, zinc sulphate heptahydrate, boric acid, cobalt(II) chloride hexahydrate and copper(II) sulphate pentahydrate. Panswad and Luangdilok (2000) investigated the efficiency of an anaerobic/aerobic sequential batch reactor system for treatment of simulated dye wastewater. The synthetic wastewater contained 20 mg/l of four dyes (reactive black 5, reactive blue 19, reactive blue 5 and reactive blue 198) mixed with glucose, acetic acid, urea, potassium dihydrogen phosphate, sodium hydrogen carbonate, magnesium sulphate heptahydrate, calcium chloride and iron(III) chloride hexahydrate. Later, Panswad et al. (2001) evaluated the treatment of synthetic wastewater contaminated with remazol black B using an anaerobic/aerobic sequential batch reactor with sludge. The wastewater consisted of nutrient broth, sodium acetate, glucose, urea, potassium dihydrogen phosphate, sodium bicarbonate, iron (III) chloride hexahydrate, magnesium sulphate heptahydrate and calcium chloride. The authors mixed all chemicals with nutrient broth and sodium acetate in different concentrations, providing in total around 500 mg/l COD. Mohan et al. (2002) used *Spirogyra* spp. (O.F.Müller) Dumortier (water silk) for the treatment of synthetic effluents containing the azo dye reactive yellow 22. The simulated wastewater was prepared by mixing a proportion of the stock solution for reactive yellow 22 with D-glucose, sodium chloride and 1 ml/l trace element solution with distilled water. Tap water was used for further dilutions. The trace element solution consisted of different chemicals as reported previously by Aziz and Ng (1988). Khehra et al. (2006) operated a sequential anoxic/aerobic bioreactor for the treatment of simulated wastewater containing the dye acid red 88. The synthetic wastewater consisted of 100 mg/l of the dye mixed with yeast extract, glucose and mineral salts. The mineral salt medium consisted of disodium phosphate, ammonium sulphate, potassium dihydrogen phosphate, magnesium sulphate heptahydrate, ammonium ferric citrate, calcium chloride dihydrate and trace elements. The trace element solution comprised of zinc sulphate heptahydrate, manganese(II) chloride tetrahydrate, cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate, sodium molybdate dihydrate, boric acid and copper(II) chloride dihydrate. Muda et al. (2010) used granular sludge in a single sequential batch reactor with anaerobic and aerobic conditions for treatment of simulated textile effluents. The synthetic wastewater consisted of glucose, ethanol, sodium acetate, ammonium chloride, potassium dihydrogen phosphate, dipotassium phosphate, calcium chloride dihydrate, magnesium sulphate heptahydrate, ethylenediaminetetraacetic acid, dye mixture (sumifix black EXA, sumifix navy blue EXF and synozol red K-4B) and 1 ml/l of trace element solution. The composition of the trace elements solution, as reported by Smolders et al. (1995), included among other ingredients boric acid, zinc chloride ($ZnCl_2$), manganese(II) chloride tetrahydrate, copper(II) chloride dihydrate, sodium molybdate, cobalt(II) chloride hexahydrate and potassium iodide. Aldoury et al. (2014) studied the ability of sequential anaerobic/aerobic reactors for treatment of synthetic wastewater contaminated with two dyes (acid orange 12 and disperse red 17). The wastewater was prepared by mixing potassium hydrogen phosphate, calcium chloride, magnesium sulphate heptahydrate, zinc sulphate, iron(III) chloride, sodium bicarbonate, ammonium chloride, peptone, dyes and tap water. Al-Amrani et al. (2014) examined the performance of an anoxic/aerobic sequential batch reactor system for azo dyes (acid orange 7, acid orange 10, acid yellow 9 and acid red 14) and COD removal. A suitable amount of dye solutions mixed with sucrose, bacto-peptone, iron (III) chloride hexahydrate, calcium chloride, magnesium sulphate, sodium hydrogen carbonate, ammonium chloride and potassium dihydrogen phosphate was used to prepare the synthetic dye wastewater. Dhaouefi et al. (2018) examined the suitability of using an anoxic/aerobic photobioreactor for carbon and nutrient removal from synthetic textile wastewater. The mixture of synthetic wastewater contained soaping agent, anticrease, dispersing agent, electrolyte, alkaline agent, reducing agent, acid generation, as well as the dyes disperse blue 1 and disperse orange 3.

The above constituents, except those reported by Basibuyuk and Forster (1997), Nopkhuntod et al. (2012), and Punzi et al. (2015), simulated the textile effluents as the mixtures included most of the chemicals used in textile processes.

Based on biological treatment using CWs, it was documented that some compositions of synthetic textile wastewater can be treated by this system. For example, Mbuligwe (2005) investigated the potential of two types of aquatic plants using engineered wetland systems for the treatment of synthetic effluents. The dye-rich synthetic wastewater was prepared by mixing caustic soda, sodium hydrosulphate and dye powder with tap water. However, this new composition was linked with a COD concentration lower than the typical values. Keskinkan and Lugal Goksu (2007) treated synthetic wastewater contaminated with basic blue 41 using CW technology vegetated by submerged plants *Myriophyllum spicatum* L. (Eurasian watermilfoil) and *Ceratophyllum demersum* L. (hornwort). The mixture contained 11 mg/l of the dye with 5% Arnon-Hoagland nutrients, which included chemicals, such as potassium nitrate, calcium nitrate, ammonium dihydrogen phosphate, magnesium sulphate heptahydrate, boric acid, manganese(II) chloride tetrahydrate, molybdenum (VI) acid monohydrate and iron(II) sulphate heptahydrate. However, this composition contained very low chemical concentrations. Ojstrsek et al. (2007) operated CW systems for the treatment of three dyebath wastewaters. These baths contained the dyes reactive red 22, reactive black 5 and vat red 13 (one for each bath) mixed with sodium hydroxide, sodium chloride and auxiliaries (irgapadol MP, alvirol AGK, cibaflow PAD and alviron VKSB) to simulate dye-rich textile wastewater. The dyebaths were prepared by dissolving the dye and the auxiliaries with tap and distilled water. Then, Bulc and Ojstrsek (2008) investigated the ability of a wetland system using the same chemicals and auxiliaries except for alviron VKSB. The dyes used in each bath were reactive black 5, disperse yellow 211 and vat yellow 46. The compositions used by Ojstrsek et al. (2007) and Bulk and Ojstrsek (2008) provided COD values within the typical range in all dye baths. Nevertheless, the authors examined the system without vegetation and the wastewater impact on aquatic plants is unknown, which limited the use of these chemicals with planted systems. Noonpui and Thiravetyan (2011) examined the potential of *Echinodorus* spp. in a CW system for removing reactive red 141 at a concentration of 20 mg/l. They used the same synthetic wastewater proposed by Sakkayawong et al. (2005). However, the authors focused on including salt sources only in their mixture. Ong et al. (2009a, 2010), and Cumnan and Yimrattanabovorn (2012) studied the ability of a CW system planted with emergent macrophytes for the treatment of synthetic textile wastewater consisting of ammonium nitrate, magnesium chloride hexahydrate, calcium chloride dihydrate, sodium benzoate, sodium acetate, potassium phosphate and sodium chloride mixed with dye. The dye concentration used by Ong et al. (2009a, 2010) was 50 and 100 mg/l. However, Cumnan and Yimrattanabovorn (2012) reduced the concentration of the dye to 11.5 mg/l, and the sodium benzoate by half.

The compositions suggested by Ong et al. (2009a, 2010) and Cumnan and Yimrattanabovorn (2012) are rich in salts, nutrients, sodium and carbon sources. The inflow parameters were within the typical range of textile industry effluents (Ghaly et al., 2014), except for NO_3–N and NH_4–N concentrations which were high. In addition, Ong et al. (2009a, 2010) clearly documented the characteristics of the main parameters present in the related synthetic wastewater. Thus, the two compositions reported by Ong et al. (2009a, 2010), and Cumnan and Yimrattanabovorn (2012) typically simulate the textile effluents compared with other reported compositions. The chemical constituents of synthetic textile wastewaters prepared by various researchers which were treated by chemical, physical, and biological methods are summarised in Table A.6 (Appendix A), and the corresponding water quality characteristics are shown in Table A.7 (Appendix A).

2.5 Textile dyes

Dyes are natural and synthetic compounds used to add colour to products or goods, helping to make things more attractive. The history of natural dyes goes back over hundreds of years, since 2600 BC, when dyes were extracted by the ancient Chinese from the natural resources of plants and animals (Carmen & Daniela, 2012; Saranraj, 2013; Ghaly et al., 2014). In 1856, the English student William Henry Perkin, at the Royal College of Chemistry, discovered the first synthetic dye accidently (Welham, 2000; Zollinger, 2003; Saranraj, 2013; Kamat, 2014; Shertate & Thorat, 2014) and by the beginning of the 20th century, more than ten thousand synthetic dyes were used in industries (Robinson et al., 2001; Deniz & Karaman, 2011; Saratale et al., 2011; Ventura-Camargo & Marin-Morales, 2013). Thereafter, the available commercial synthetic dyes increased to around 100,000, and the annual production of dyes was greater than 7×10^5 tonnes worldwide (Nigam et al., 1996; Selvam et al., 2003; Zonoozi et al., 2008; Blanco-Flores et al., 2014; Castaneda-Diaz et al., 2017).

The synthetic dyes are cheap, produced easily, formed in different colours, and characterised by their fastness when exposed to water or sunlight, which makes them extensively used in preference to natural dyes (Robinson et al., 2001; Khehra et al., 2006; Gupta & Suhas, 2009; Samanta & Agarwal, 2009; Da-Silva et al., 2010; Kant, 2012; Kamat, 2014). They are widely used as colouring agents in a variety of technological fields, such as the textile dye industry (Gupta et al., 1992; Shukla & Gupta, 1992; Sokolowska-Gaida et al., 1996; El Haddad et al., 2014; Castaneda-Diaz et al., 2017), the paper production industry (Diyanati et a., 2013), the leather tanning industry (Tunay et al., 1999; Forgacs et al., 2004; Joghatayi et al., 2015), hair and cosmetics colouring (Scarpi et al., 1998; Dehghan et al., 2017), food industry (Bhat & Mathur, 1998; Slampova et al., 2001), and colour photography (O'Mahony et al., 2002; Selvam et al., 2003; Reema et al., 2011). However, it is worth noting that a significant proportion of these dyes are extensively used in textile mills for dyeing the fabrics (Walker & Weatherley, 1997; Pandey et al., 2007; Zonoozi et al., 2008; Shah et al., 2013; Gupta et al., 2015).

The synthetic textile dye is defined as a coloured substance which is able to resist fading when applied to fibres, thus providing a stable colour (Saratale et al., 2011). Textile dye molecules consist of two components: Chromophore groups, which are responsible for the colour of the dye, and auxochrome groups, which are responsible for the intensity of the colour (Gupta & Suhas, 2009; Salleh et al., 2011). The main chromophore configurations include azo (-N=N-), ethylene (=C=C=), carbonyl (=C=O), methane (-CH=), carbon-nitrogen (=C=NH; CH=N-), carbon-sulphur (=C=S; ≡CS-S-C≡), nitro (-NO2; -NO-OH), and nitroso (-N=O; =N-OH) groups. The most important auxochrome groups are amino (-NH_2), carboxyl (-COOH), sulphonate (-SO_3H) and hydroxyl (-OH) (Welham, 2000; Suteu et al., 2011; Carmen & Daniela, 2012; Singh et al., 2012; Saranraj, 2013; Ventura-Camargo & Marin-Morales, 2013; Daud, 2014; Ghaly et al., 2014; Suresh, 2014; Tahir et al., 2016). In the main, synthetic textile dyes are classified either according to their mode of application, (e.g., acid, basic, direct, disperse, reactive, mordant, sulphur, pigment, and vat dyes) or according to their chemical structure (e.g., nitro, azo, anthraquinone, diphenylmethane, phthalocyanine, and triarylmethane) as discussed previously (Saleh, 2005; Demirbas, 2009; Abid et al., 2012; Carmen & Daniela, 2012; Somasekhara Reddy et al., 2012; Suresh, 2014; Adegoke & Bello, 2015; Salman et al., 2016). Furthermore, these dyes are also classified based on the degree of dissociation in aqueous solution, into anionic (e.g., direct, acid, and reactive dyes), non-ionic (e.g., disperse dyes), and cationic (e.g., basic dyes) as indicated by Robinson et al. (2001), Salleh et al. (2011), Balarak et al. (2015), and Tahir et al. (2016). Among all these dye types, the azo compounds are pervasively used in the textile industry compared with other dye classes (Asamudo et al., 2005; Zille et al., 2005; Adinew, 2012) worldwide.

2.6 Azo dyes

Azo dyes are aromatic compounds, and one of the main groups of dyes that are widely used in the textile industry, due to an increasing demand for non-fading colours (Forgacs et al., 2004; Davies et al., 2009; Daud, 2014; Yaseen & Scholz, 2016). This group of dyes have a complex structure and xenobiotic nature, which enhances their resistance to degradation (Stolz, 2001; Maddhinni et al., 2006; Savin & Butnaru, 2008; Pahlaviani et al., 2011; Solis et al., 2012; Saranraj, 2013; Tahir et al., 2016). The structure of these dyes possesses at least one azo group, which is demonstrated by two bounded nitrogens (-N=N-), and the categorisation of these dyes is considered according to the number of these azo linkages. They are classified into monoazo, diazo, triazo, and polyazo dyes that bear one azo group, and two, three, and four or more azo groups, respectively (Wallace, 2001; Zollinger, 2003; Hunger, 2007; Gupta & Suhas, 2009; Chacko & Subramaniam, 2011).

In textile dyebaths, most dyes cannot bind with textiles entirely, which leads to the residuals being released into watercourses as waste (Pearce et al., 2003; Van Der Zee & Villaverde, 2005; Ratna, 2012; Yaseen & Scholz, 2016, 2017b). The amount of dye lost usually depends on the type of azo dye used, the degree of shading wanted, and the method of application (O’Neill et al., 1999; Pandey et al., 2007; Carmen & Daniela, 2012). It is estimated that the textile industry discharges around 280,000 tonnes of synthetic dyes per year, and the main concern is related to these azo dyes and their adverse impact on the environment (Pearce et al., 2003; Maas & Chaudhari, 2005; Jin et al., 2007; Solis et al., 2012). Table 2.1 shows the typical degree of losses in the effluents and the main pollutants associated with different textile dyes, as indicated by the European Water Association (EWA) (2005). Although a high proportion of dye effluent is associated with reactive dyes, ranging between 10% and 50%, as shown in Table 2.1, these dyes make up approximately 30% of the market share, due to their fading resistance (Pearce et al., 2003). In contrast, basic dyes have lower effluent levels compared with other dye classes. Some of the commercial dyes that are extensively used in the textile industry are reactive blue 198 (Chen et al., 2003; Kim et al., 2004; Nguyen, 2014), reactive yellow 84, reactive red 120 (Neamtu et al., 2003), basic red 46 (Deniz & Karman, 2011; Sheshdeh et al., 2014), acid blue 113 (Surana et al., 2010; Mehta et al., 2011; Balarak et al., 2016b), direct orange 39 (Chen et al., 2003), methyl orange (Mehra & Sharma, 2012) and methylene blue (Mehra & Sharma, 2012; El-Ashtoukhy & Fouad, 2015).

Table 2.1 Fixation and loss degree of different textile dyes (after EWA, 2005)

Dye class	Fibre	Typical fixation (%)	Losses to the effluent (%)	Main pollutions
Acid	Wool and nylon	80–95	5–20	Unstable dyes, organic material containing acids and the colour
Basic	Acrylic and some polyesters	95–100	0–5	N/V
Direct	Cellulosic including cotton and rayon	70–95	5–30	Unstable dye, salt and colour
Disperse	Polyester	90–100	0–10	Unstable dyes, organic material containing acids, and phosphate
Reactive	Wool and cellulosic including cotton	50–90	10–50	Unstable dye, colour, alkali, and salts
Sulphur	Cellulosic including cotton	60–90	10–40	Unstable dye, colour, alkali, oxidising and reducing agent
Vat	Cellulosic including cotton	80–95	5–20	Alkali, colour, oxidising and reducing agent

Note: N/V, not available.

2.7 Textile dyes and environmental challenges

The impact of coloured effluents from textile factories on environmental sustainability is objectionable, because most textile dyes with a rather low concentration of 1 mg/l, treated or not, can be detected by the human eye, and could increase community complaints and concerns (Hussein, 2013; Dhaouefi et al., 2018). Therefore, this aesthetic problem is one of the major challenges for receiving watercourses, especially for the non-acceptable colours of river water such as red or purple compared to more accepted colours such as green or blue (Kadirvelu et al., 2005; Pandey et al., 2007; Zaharia et al., 2009; Suresh, 2014; Yaseen & Scholz, 2017b). In addition, a high concentration of these dyes in the receiving watercourse will inhibit sunlight penetration and respiration activities, consequently upsetting the biological and photosynthesis processes in the aquatic environment (Vandevivere et al., 1998; O'Mahony et al., 2002; Canizares et al., 2006; Kadirvelu et al., 2003; Arami et al., 2006; Dincer et al., 2007; Reema et al., 2011; Saratale et al., 2011). It also contributes to overloading chemical oxygen demand, suspended solids, total dissolved solids, nutrients etc. in the receiving water streams. Moreover, these effluents pass through soil layers and may contaminate nearby surface water and groundwater which consequently affects fisheries, agriculture, farm animals and the food chain (Lin et al., 2008; Sivakumar et al., 2013; Sivakumar, 2014; El-Ashtoukhy & Fouad, 2015). The presence of these dyes over a long period of time (several years) in watercourses leads to toxic effects, due to dye accumulation in sediments, fish and other organisms. Also, dye decomposition and corresponding hazardous compounds may also have a toxic impact on aquatic life (Van Der Zee & Villaverde, 2005; Carmen & Daniela, 2012; Zhang et al., 2012), although some authors reported that the toxicity of dyes at concentrations below 1 mg/l did not inhibit the growth of different organisms, such as fish, rats, algae, bacteria, protozoans etc. (Greene & Baughman, 1996; Suresh, 2014). Moreover, azo dyes, which are widely used in textile manufacturing and their daughter products (aromatic amine) can cause allergies, dermatitis, skin irritation, carcinogenic and mutagenic actions as well as acute and chronic toxicity (Jain et al., 2003; Kadirvelu et al., 2003; Pinheiro et al., 2004; Megateli et al., 2009; Carmen & Daniela, 2012; Mesquita et al., 2012; Suresh, 2014; Almazan-Sanchez et al., 2016; Yaseen & Scholz, 2017a). Accordingly, human exposure to these azo dye effluents may lead to immunological, neurological and circulatory disorders, irritation to lung oedema, eye and skin infections and allergy problems (Morikawa et al., 1997; Weisburger, 2002; Shen et al., 2009; Foo & Hameed, 2010; Adegoke & Bello, 2015).

Therefore, due to these environmental challenges, the textile industries are obligated by environmental legislation to remove the colour from their effluents, before disposal into receiving watercourses (McKay et al., 1985; Lee et al., 2006). For instance, the environmental policies in the UK have required concentrations of synthetic dye wastewaters to be discharged to the aquatic environment to be zero since September 1997 (Willmott et al., 1998; O'Neill et al., 1999; Robinson et al., 2001; Pearce et al., 2003), to ensure that dye effluents are treated effectively before discharge.

2.8 Wetlands definition and treatment stages

Pollutants elimination (or reduction to within the allowable ranges that cause no harmful impact on life forms) is the main aim of wastewater treatment systems. Wetlands, both natural and constructed, are preferred to conventional techniques of contaminant remediation, due to their features of being environmentally friendly, requiring low energy usage and having minimal capital cost (Sekomo, 2012). Wetlands are land areas in which the permanent shallow or temporary standing water plays a significant role, with the help of vegetation, animals, and micro-organisms for controlling the environment of wetland systems (Ramsar, 2010; Al-Isawi, 2016), and consequently interact in the presence of light, soil and air, to enhance the quality of the water (Eke, 2008). The broader definition of wetlands, which is well accepted, was stated in the Ramsar Convention in 1980, by the International Union for the Conservation of Nature and Natural Resources (Scholz, 2006). It defined wetlands as "*any areas of marsh, fen, peat land, or water, whether natural or artificial, permanent or temporary, fresh, brackish or salty, including areas of marine water, the depth of which at low tide does not exceed six metres*" (Mitsch & Gosselink, 1993, 2000; Ramsar, 2010). This description covers a variety of coastal, marine, and inland environments, such as marshes, lagoons, springs, ponds, channels, marine mud-flats, estuaries, deltas, shallow and deep lakes, swamps, reservoirs, and beds of marine algae or seagrasses, in addition to estuaries and rivers comprising aquatic plants (Eke, 2008).

Although this technology was poorly documented during the early stages of development, some authors mentioned the applications and types of wetland that have been used in the last six decades, and this provided good historical information about wastewater remediation and disposal using this system (Moshiri, 1993; Kadlec & Knight, 1996; Vymazal et al., 1998; Vymazal, 2010, 2011). Wetlands can be natural or artificial, and they are different according to some main factors, which include weather conditions, water chemistry, macrophyte type and soil type, as well as human participation (Eke, 2008). Natural wetlands are found in different regions with a wide range of weather conditions and in all continents except Antarctica (Vymazal et al., 1998). Vymazal (2011) pointed out that, for centuries, natural wetlands have been used in some sites for wastewater disposal rather than treatment as a suitable technology that easily serves the receiving effluents. However, this uncontrolled release of contaminated water led to full degradation of many wetland areas. The oldest applications of wetland sites for wastewater treatment, were in North America between 1912 and 1939 (Kadlec & Knight, 1996; Kadlec & Wallace, 2009). The practical examination of natural wetlands has been applied for more than 100 years to treat wastewater in the United Kingdom (UK) (Cooper & Boon, 1987). Since 1950, knowledge regarding natural wetlands has increased, and consequently the amount of polluted water being discharged, for disposal rather than treatment purposes, has reduced in some regions of the world (Vymazal, 2011). The natural wetland systems are still operated under controlled conditions (Kadlec, 2009), however, the operation of CWs has been favourable in recent years (Kadlec & Wallace, 2009; Vymazal, 2008, 2011). Eke (2008) defined constructed wetland as "*a man-made system designed to imitate the optimal treatment conditions found in natural wetlands, which filter out pollutants and act as sinks for nutrients by purifying the water through physical (sedimentation and filtration), physical-chemical (adsorption on plants, soil and organic substrates) and biochemical (biochemical degradation, nitrification, denitrification, decomposition and plant uptake) processes*". This artificial eco-system has proven to be inexpensive, green, and simple to use and operate.

The early stage of CWs for purifying water was in 1901 by Cleophas Monjeau, as documented by Wallace and Knight (2006). However, the first application of planted CWs that mimic the natural wetlands was conducted in the 1950s by Kathe Seidel in Germany. She conducted many experiments during the period between 1952 and 1956 to treat phenol, dairy, and livestock wastewaters using wetland macrophytes (Seidel, 1955, 1961, 1965a, 1966), as described by Vymazal (2008). In the early 1960s, Seidel worked on growing different plants in polluted water, and focused on developing and improving the performance of some ponds and ineffective septic tanks to treat wastewater in rural areas (Seidel, 1965b). In addition, she started to use sandy soils to improve her system, as mentioned by Vymazal (2011). By the mid-1960s, Seidel was working in collaboration with her student Reinhold Kickuth to develop horizontal flow CWs, a system known as the Root Zone Method (RZM). The system was brought into operation in 1974 using Common Reed plants, and was the early wetland system in Germany built with heavy soil media to treat municipal sewage (Kickuth, 1977, 1978, 1981), as reported by Vymazal (2005, 2009) and Sekomo (2012). The reported information about CWs technology for the period between 1970 and 1980 has spread gradually. Thereafter, CWs have been known as a reliable system for wastewater treatment (Vymazal, 2011), resulting in the establishing of around 200 systems to treat municipal and industrial effluents (Bastian & Hammer, 1993) in Europe. In the mid-1980s, CWs were well accepted and became more popular in the UK (Cooper et al., 1996). In 1987, a hybrid system of vertical flow-horizontal flow CW was introduced in the UK at Oaklands

Park (Burka & Lawrence, 1990). Regarding the United States of America (USA), the exploration of CWs was increased during the 1970s and 1980s. After that, in the 20th century, European countries used all the types of CWs, although some countries concentrated on using vertical flow and hybrid wetlands due to the high removal requirements for ammonia. Engineered wetlands were accepted officially in some countries, such as Austria in 1997, Denmark in 1999, Australia and the United States in 2000 (Vymazal, 2011). Sekomo (2012) and Vymazal (2009) reported that the early stages of using CWs for textile wastewater treatment were in Germany and Australia between the end of 1980 and beginning of 1990 using horizontal flow CW systems. The studied CWs were a promising method and cost-effective option for coloured textile wastewater treatment.

Today, the application of CW systems to treat wastewater is being recommended and disseminated worldwide (Vymazal, 2011, 2014). However, the practical operation of this technology is not common in developing countries, especially in tropical and subtropical regions, such as Nigeria and Tanzania, due to the lack of knowledge about the features and importance of wetland systems for protecting the environment and solving the water pollution problems economically (Sani, 2015). Despite this, a number of research studies have been reported in recent years regarding CWs in some developing countries (Blackwell et al., 2002; Mitsch & Jorgensen, 2004; Zedler & Kercher, 2005), such as Egypt (Abou-Elela & Hellal, 2012; Abou-Elela et al., 2013) and Kenya (Kimani et al., 2012). On the other hand, it is worth noting that CWs applications in other developing countries, such as China and India began in 1990 and the research studies in this area have continued (Sheoran & Sheoran, 2006; Xinshan et al., 2010; Zhang et al., 2012), as these countries have gained a sufficient awareness concerning the benefits of applying CWs technologies.

Although many authors reported the use of CWs applications to treat diverse wastewater (Kadlec & Knight, 1996; Cooper et al., 1996; Vymazal et al., 1998; Eke, 2008; San, 2015; Mohammed, 2017), the experience in treating textile effluents is limited (Sekomo, 2012) and the internal processes applied during the treatment are not discussed in detail. Therefore, it is necessary to observe the performance of experimental wetlands in order to increase understanding, and consequently increase the awareness of wetland technology as an effective method for treating polluted water and saving the environment with low input cost (Kadlec & Wallace, 2009; Vymazal, 2014; Sani, 2015; Scholz, 2015; Al-Isawi, 2016).

2.9 Configurations of constructed wetlands

Constructed wetlands for wastewater treatment are classified according to several design criteria which mainly include: hydrology (free water surface flow and subsurface flow), the type of plant used (emergent, submerged, and free-floating), and the direction of flow (horizontal and vertical), as described by Kadlec and Knight (1996), the International Water Association (IWA) (2000), Kadlec et al. (2000), and Vymazal (2005, 2008, 2011, 2010, 2014). Further types of engineered wetlands are: the hybrid systems, which are created for enhancing the removal efficiency of contaminants by combining two types of CWs as a single system (Vymazal, 2013b; Sani, 2015); and the intensified systems, which deal with highly loaded effluents and are designed for achieving high removal efficiency (Al-Isawi, 2016). Recently, a new classification of wetlands has been reported in some studies, which depends on the purposes for applying these systems, such as habitat creation, flood control, and wastewater treatment (Vymazal 2013a, 2014; Sani, 2015).

Due to the practicability of this study, the applications of using CWs to treat textile dyes are considered according to the type of main macrophytes utilised in the treatment system, which include emergent, submerged, and free-floating macrophytes. Macrophytes are aquatic plants capable of eliminating or diminishing nutrients and other substances from polluted water through the absorption process (Keskinkan & Lugal Goksu, 2007). Figure 2.4 below shows the main categories of CWs.

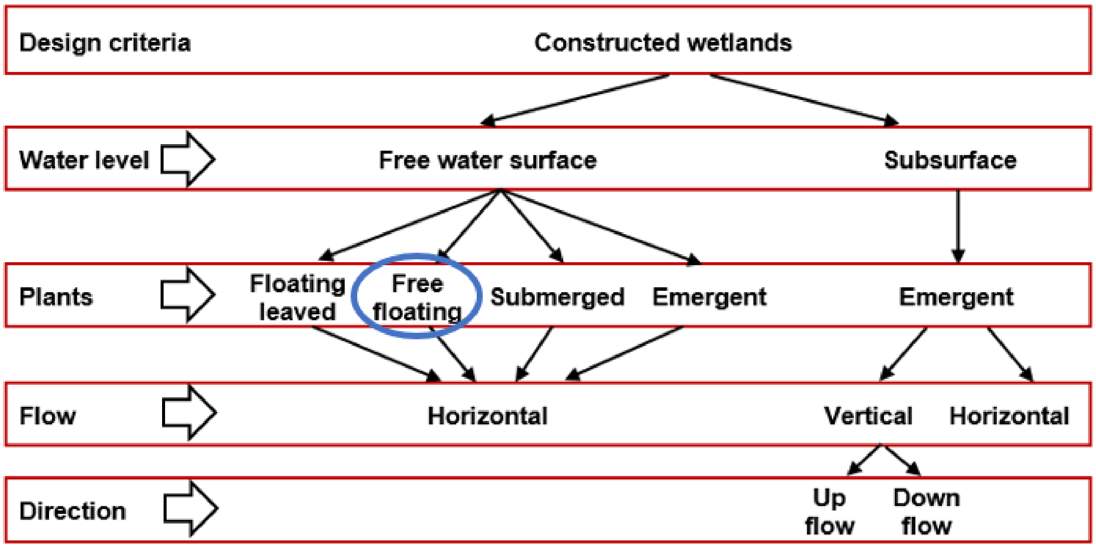


Figure 2.4 Main categories of constructed wetlands (after Vymazal, 2008; Kadlec & Wallace, 2009). Note, blue ellipse identifies the system used in this study.

2.9.1 Emergent macrophyte-based treatment systems

These systems describe the CWs that are vegetated with rooted emergent aquatic plants for pollutants purification. These aquatic plants are found at the banks of rivers and lakes and their roots are attached to the substrate media, which allows them to absorb pollutants and nutrients from the soil sediment. It has been reported that the potential of emergent plants to assimilate the contaminants and nutrients is greater than the floating macrophytes, because they have more supportive tissue and their roots are deeper within the soil (Sekomo, 2012). However, these plants are not harvested and so they act as a temporary storage for nutrients which are released to the water system after the plants' decay (Kadlec & Wallace, 2009). Generally, emergent macrophytes are the largest aquatic plants growing in CWs and include: Common Reed (*Phragmites australis*), Manchurian wild rice (*Zizania latifolia*), Burhead (*Echinodorus cordifolius* L.) and Narrow-leaved cattails (*Typha angustifolia* Linn.). Constructed wetlands planted with emergent plants are filled with soil, gravel or sand as a substrate media to support the growth of the emergent plant roots (Kadlec & Wallace, 2008; Vymazal, 2013a). This type of wetlands has proven to be highly effective in the removal of diverse pollutants, such as suspended solids, biochemical oxygen demand, nitrogen, heavy metals and other contaminants, except phosphorus removal which is limited in all wetland systems (Kadlec & Knight, 1996; Vymazal, 2007; Kadlec & Wallace, 2009; Yaseen & Scholz, 2018). Emergent plants-based systems are divided into four main groups based on the flow pattern: free water surface (FWS) CWs; horizontal subsurface flow (HF) CWs; vertical subsurface flow (VF) CWs, which are divided further based on the flow direction into up flow (UF) CWs and down flow (DF) CWs; and finally hybrid or combined CW systems. The configuration and flow direction of each kind of CW planted with emergent macrophytes are shown in Figure 2.5.

In the past few years, the literature indicates promising results for textile dye and other contaminants removal using free water surface, vertical, horizontal and hybrid wetland systems vegetated with different emergent plants (Bulc & Ojstrsek, 2008; Ong et al., 2009a; Cumnan & Yimrattanabovorn, 2012). These studies provide good knowledge about the efficiency of each system, the potential of different emergent plants, the impact of different contact times using a wide range of dye concentrations and under natural, greenhouse or laboratory conditions, although the results rarely cover all seasons (Vymazal, 2014) and all the main water quality parameters (Davies et al., 2005).

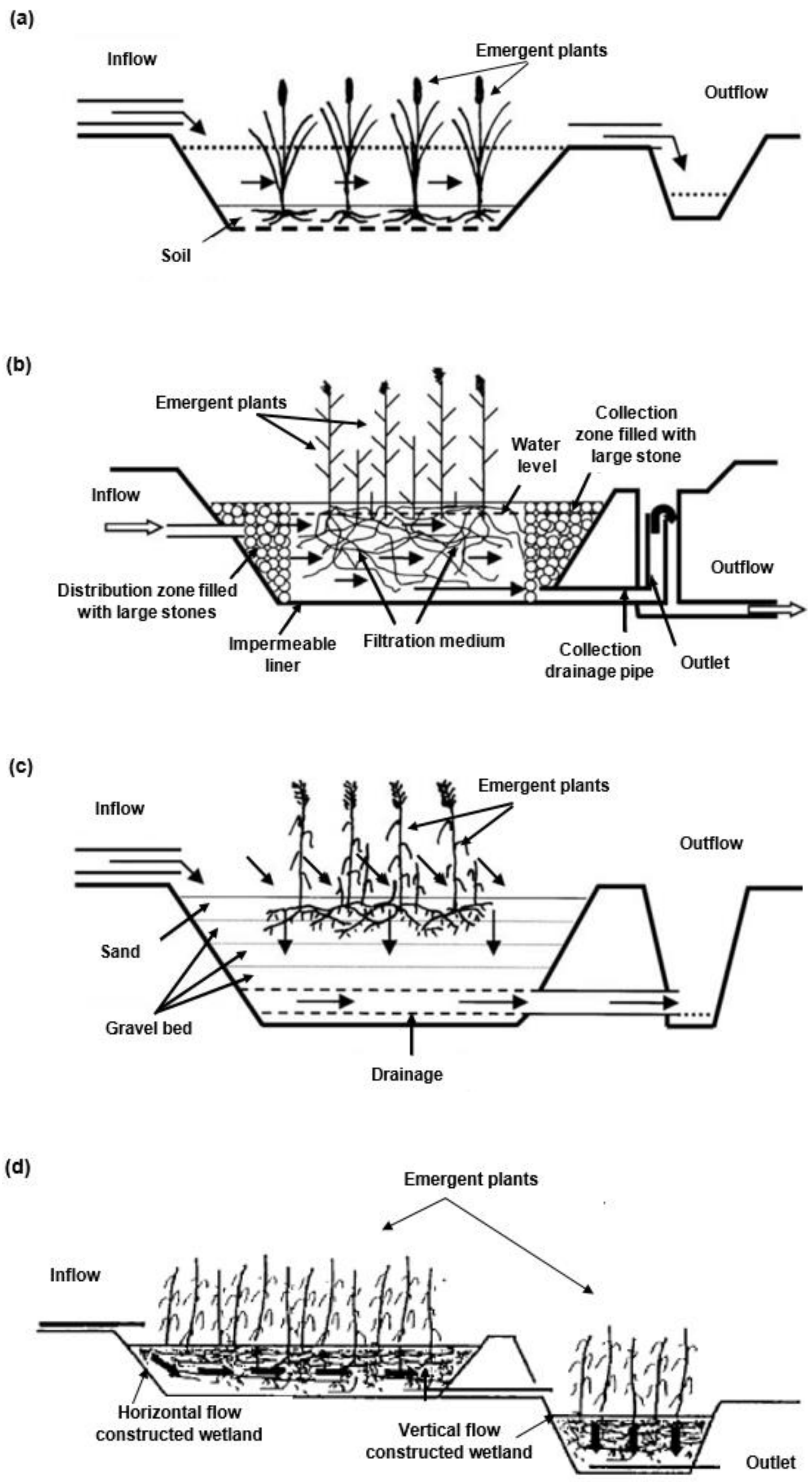


Figure 2.5 Schematic illustration of different types of rooted emergent plants-based constructed wetlands. Note: (a), free water surface; (b), horizontal subsurface flow; (c), vertical subsurface flow; (d), hybrid system (after Vymazal, 2007).

The performance of an aerated VFCW using a gravel-sand bed and planted with *P. australis* has been investigated by Pervez et al. (2000) to treat synthetic wastewater containing the azo dyes reactive blue 171 and acid blue 113. The results of ten weeks of operation showed high removal efficiency for both dyes of around 98%. However, the study did not focus on the COD and nutrients removal in wastewater.

Davies et al. (2005) assessed the performance of DFCWs to remove the azo dye acid orange 7 using *P. australis* in an outdoors experiment. The system was filled with a gravel layer topped by sandy-clay soil. The results indicated that the plant produced enzymes capable of degrading the dyes with a removal efficiency of 69% and 74% for a dye inflow concentration of 130 and 700 mg/l, respectively. Furthermore, Davies et al. (2006) studied the aerobic degradation of acid orange 7 and the removal efficiency was 99% using 127 mg/l of the dye in a VFCW. Moreover, Davies et al. (2009) studied the phytoremediation of acid orange 7 and the reaction between *P. australis* and the dye molecules. The results indicated that the dye acted as a chemical stressor agent for *P. australis* by activating the gene expression and enzymatic activities of plants with a removal efficiency of 68% for dye inflow concentration of 748 mg/l in a VFCW. However, these experiments operated for a short duration of a few months and the authors focused on the plant enzymatic activities and the removal efficiency of the dye, COD and total organic carbon (TOC) only.

The performance of UFCWs planted with *P. australis* and filled with a small glass layer topped by gravel to remove the dye acid orange 7 has been reported (Ong et al., 2009a, b; Ong et al., 2010). The system operated under laboratory conditions with different concentrations, contact times (HRT - hydraulic retention time) and artificial aeration through three experiments using synthetic textile wastewater. The first experiment (Ong et al., 2009a), was to compare the removal efficiency between two species of emergent plants *P. australis* and *Zizania latifolia* and the effect of supplementary aeration on the system performance using 50 mg/l dye concentration for three days. The investigations showed that the aerated reactor was better than the non-aerated reactor for acid orange 7 and COD removal with no difference between the plant efficiencies, except for the COD removal in the aerated reactor being higher with *P. australis* than with *Zizania latifolia*. After that, the effect of increasing acid orange 7 concentration (Ong et al., 2009b) was evaluated by using 100 mg/l with the same contact time. The results showed a high percentage of dye removal, whereas the supplementary aeration boosted the efficiency of aromatic amine removal. These results match with Ong et al.'s (2011) outcomes when using the same system under semi-batch operations for two days of contact time. Finally, in the last stage, HRT was increased to six days (Ong et al., 2010). The overall results presented the fact that the system using this type of plant was efficient for the removal of acid orange 7 and COD by more than 96%. In addition, a high concentration of acid orange 7 reduced the dye removal under aerated conditions and decreased the COD removal under non-aerated conditions. However, the high contact time only boosted the removal efficiency in terms of COD removal. The authors performed these three experiments to investigate the main factors affecting the system performance, such as the type of the plant, contact time, dye concentration, and the additional aeration. They recorded the main water quality parameters including COD, NH_4–N, NO_3–N, nitrite nitrogen (NO_2–N) and PO_4–P. However, each experiment operated for three months only.

Further investigation of the performance of UFCWs has been conducted by Nilratnisakorn et al. (2009) for the treatment of synthetic reactive dye wastewater (SRDW) vegetated with *Typha angustifolia* Linn. They studied the effect of the plant and/or the substrate media on the removal efficiency of reactive red 141. The system was operated in a greenhouse and, after an experimental period of 15 days, their findings showed that the colour removal using the plant was only 49% at best, whilst it was very low at 2.82% with sand and 1.95% with gravel. Overall, the results showed that the system using both the plant and matrix was able to remove colour from the SRDW by up to 58%. Thereafter, Noonpui and Thiravetyan (2011) examined the performance of HFCWs vegetated by *Echinodorus cordifolius L.* using both soil and soil-free (hydroponic) conditions for the treatment of three reactive azo dyes in different molecular sizes: reactive red 2, reactive red 120 and reactive red 141 in aqueous solution. The results within one week of experimental work in a greenhouse demonstrated that the *Echinodorus cordifolius L* was efficient for dye removal in both soil and soil-free conditions and the values were 100% and 99%, respectively, though the dye removal efficiency for the soil condition was noticeably higher than the hydroponic condition within the first four days of the system operation. Regarding the molecular weight, dye removal increased with the reduction of it. Although, these research studies were performed for short periods under greenhouse conditions, the results provided an attractive conclusion about the very small impact of the matrix bed (gravel, sand, or soil) compared with plant efficiency in terms of dye removal. The low impact of a gravel bed in terms of dye removal by CWs was also confirmed by Ong et al. (2009a). This could help to save the extra cost required for the substrate media in CWs, as one of the main objectives required in developing countries during treatment systems design.

Yalcuk and Dogdu (2014) assessed the performance of VFCWs consisting of gravel, sand, and zeolite beds to compare the potential of two species of emergent plants (*Canna idica* L and *Typha angustifolia* L) to treat the dye acid yellow 2G E107. The results showed a high percentage of colour removal of around 98% for planted CWs and 87% for unplanted CWs, and the authors discussed the removal efficiencies of other main parameters, such as ammonia-nitrogen and ortho-phosphate-phosphorus. However, the system operated over three months only.

Hussein and Scholz (2017) operated a DFCW planted with *P. australis,* and filled with gravel layers for the treatment of the dyes acid blue 113 and basic red 46 at high and low concentrations. Results of thirteen months of operation showed that the wetland was able to improve the water quality parameters and to remove the dye basic red 46 and the dye acid blue 113 at both studied concentrations. This system, however, operated under greenhouse conditions. The operation was for a long term and the results cover all the main water quality parameters.

The performance of FWS engineered wetlands operated under outdoors conditions and backed with a river sand bed has been assessed by Mbuligwe (2005) to compare dye-rich wastewater treatment using different emergent plants (Cattail and Cocoyam). The evaluation indicated that the colour removal was between 72 and 77% in the planted wetland and only 14% in the control one. In addition, a Cocoyam bed upgraded the colour removal efficiency to 7.6% more than a Cattail bed. Although the study covered the colour, COD, and sulphate removal as well as the pH variations, the authors mentioned that the limited operation time of 75 days could affect the results of this investigation, and in addition nutrients removal was not recorded.

With regard to the hybrid systems, Bulc and Ojstrsek, (2008) operated a pilot-scale system as a vertical horizontal sub-surface flow constructed wetland in order to treat real textile wastewater contaminated with dyes under natural conditions. The results proved that the hybrid system with the *P. australis* was efficient for improved water quality parameters. The removal efficiency in VFCW and HFCW was similar in spite of the mass load being different. In addition, Cumnan and Yimrattanabovorn (2012) suggest that, the integrated FWS and subsurface flow (SSF) wetland system planted with *P. australis* could treat synthetic textile wastewater contaminated by azo dye with a high percentage of removal, although the CW of SSF-FWS outperformed the FWS-SSF in terms of colour removal. It is obvious that the hybrid systems are very efficient, since they achieve higher removal efficiency of contaminants compared with the single wetland systems (Vymazal, 2008). However, it incurs additional costs compared to other CW types, as it is a combination of two or more wetland systems.

Emergent macrophytes-based treatment systems are normally used as a secondary treatment stage and the authors in this area showed attractive results that encourage operating all types of this system. However, there are some limitations related to each type of CW system vegetated with emergent plants. For example, because the processes of water treatment in these wetlands occur when the plant and the substrate are in contact with the wastewater, FWS wetlands require more surface area than SSF systems (Vymazal, 2006), thus FWS systems can be expensive, especially in regions characterised by high land costs. In addition, authors suggest operating FWS systems planted with emergent plants in tropical and subtropical regions only, to avoid freezing problems associated with these wetlands during the winter season (Vymazal, 2007), which affect the system performance (Al-Isawi, 2016). Furthermore, there is a possibility of mosquito growth in FWS systems planted with emergent plants compared with SSF wetlands (Vymazal, 2013a). On the other hand, the capital cost incurred for SSFCWs is more than that for FWSCWs because the soil required to support the emergent vegetation in FWS systems is very low and limited within the root zone only if compared with the soil required in SSFCWs (Vymazal, 2010). Therefore, the higher cost of the emergent plants-based treatment systems compared with other wetlands, such as free-floating plants-based treatment systems (Kadlec & Wallace, 2009; Chen et al, 2016), may limit the utilisation of these systems, especially in low-income developing countries.

2.9.2 Submerged macrophyte-based treatment systems

Submerged macrophytes are completely submerged within the CW system which, as a result, means that both the roots and the leaves of these plants have a shared responsibility for pollutant removal (Sekomo, 2012), examples include: Water Milfoil (*M. spicatum*), Coontail (*C. demersum*) (Keskinkan & Lugal Goksu, 2007), Hydrilla (*Hydrilla verticillata*) (Sekomo, 2012), Dense waterweed (*Egeria densa*), Waterweed (*Elodea canadensis* and *Elodea nuttallii*) and Pondweed (*Pamogeton* spp.) (Moshiri, 1993). These aquatic plants are found in ponds, lakes, reservoirs, small rivers, estuaries and bays.

A comparative study established by Keskinkan and Lugal Goksu (2007) used a lab-scale CW system filled with a sand bed and planted with *M. spicatum* and *C. demersum* for the removal of basic blue 41. Results showed that the mean dye removal was around 95%, 94%, and 71% for *M. spicatum, C. demersum,* and control wetlands, respectively. The authors pointed out that the studied CW during the operation period of 40 days was able to remove the dye effluents effectively at contact times of 9 and 18 days without any adverse effect on the plants' survival. However, at HRT of three or six days, the plants did not survive and the dye removal efficiency was lower. This result led to the conclusion that the application of submerged plant treatment systems require a long HRT.

This technology deals with pollutants at low concentrations as a polishing treatment stage (Vymazal, 2008) and therefore it is not suitable to treat water at high turbidity level because the high turbidity prevents light penetration and consequently affects the photosynthesis process of the plants (Vymazal et al., 1998). The problem linked with submerged plants-based systems for dye removal is that the submerged plants cannot grow well in water characterised by long term anoxic or anaerobic conditions (Vymazal et al., 1998; Vymazal, 2008), and therefore bacterial degradation of coloured effluents, which mostly requires anoxic or anaerobic conditions (Ong et al., 2009a), may not be achieved by this system. Figure 2.6 shows a schematic illustration of a submerged macrophyte-based constructed wetland system.

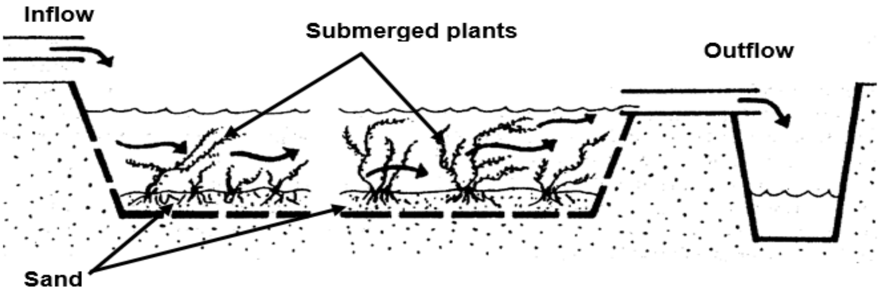


Figure 2.6 Schematic illustration of a submerged plants-based constructed wetlands (after Vymazal et al., 1998)

2.9.3 Free-floating macrophyte-based treatment systems (shallow ponds)

In these systems, the whole body of the free-floating plants is above the water except for the roots. These macrophytes are found in various habitats and different forms, as large floating leaves with deep submerged roots, or tiny floating fronds without or with few small roots. The main kinds of free-floating aquatic plants are Water hyacinth (*Eichhornia crassipes*), Water lettuce (*Pistia stratiotes*), Pennywort (*Hydrocolyle umbellate*) (Vymazal et al., 1998) and Common Duckweed (*Lemna minor* L.) (Sivakumar, 2014). Constructed wetlands with free-floating macrophytes, also known as shallow ponds systems (Vymazal, 2008; Chen et al., 2016), are one or more shallow ponds containing plants floating on the system surface (Kadlec et al., 2000). These wetlands depend on the natural chemical, physical, biological and microbial processes in the presence of the plants to treat the wastewater (Sivakumar, 2014). A schematic illustration of free-floating macrophyte-based treatment system is shown in Figure 2.7.

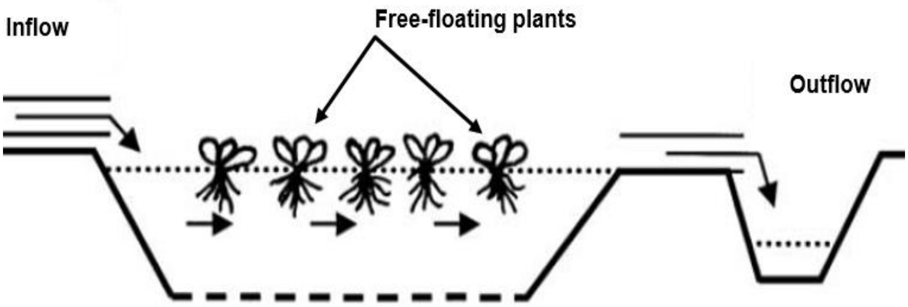


Figure 2.7 Schematic illustration of a free-floating macrophyte-based constructed wetlands (after Vymazal, 2007)

The potential of an *Eichhornia crassipes*-based treatment system to treat two textile dyes in outdoor experiments using plastic containers was assessed by Muthunarayanan et al. (2011). The plant biomass was 400 g, which equals 12 plants in each pond. The experiment was conducted in aqueous solution for 168 hours at concentrations of 10–50 mg/l. The best removal efficiency was 95% for red RB and 99.5% for black B after 6 days in 10 mg/l concentration. Although the dye removal was very high, this study operated for one week only. In addition, there is no mention of the potential of the control (without plants) systems and water quality parameters.

Previous studies regarding dye removal using *Eichhornia crassipes*-based CW systems are very limited, although the performance of other contaminants removal, such as suspended solids, biochemical oxygen demand, nitrogen and phosphor are well documented. In addition, the main recommended design criteria, such as contact time, hydraulic load, and water depth are reported according to the type of treatment (e.g., secondary, advanced secondary and tertiary) (Vymazal et al., 1998). The main drawbacks of CWs planted with *E.*

crassipes is the operation, which is restricted to greenhouse or outdoors summer conditions only (i.e. tropical and subtropical regions). This is because plants' survival is strongly dependent on the climate temperature, as they are damaged by frost and their growth is reduced at temperatures below 10˚C. Furthermore, this system requires supplementary aeration to prevent mosquitos' growth and odour problems (Vymazal et al., 1998). Thus, in addition to the cost required for this extra aeration, it is not desirable in dye effluent applications which, according to Ong et al. (2009a, 2011), require anaerobic or anoxic conditions to achieve the first stage of dye removal (decolourisation) by microbes.

Alkhateeb et al. (2005) operated an experiment using plastic containers simulating a pond system planted with *L. minor* to assess the ability for textile effluents decolourisation under sun, shadow, and dark conditions. The results showed that the percentage of colour removal was more than 70% under sunlight conditions. However, the colour reduction rate was very low for systems under dark, shadow, and without plants conditions, being 3.13%, 3.33% and 3.5%, respectively. These results showed a good potential of *L. minor* for colour removal in a pond system. However, the plant was examined for a limited period of 20 days only, and the system performance under natural conditions is unknown as the experiment was operated under laboratory conditions only.

Sivakumar (2014) assessed the role of *L. minor* in constructed wetlands using plastic containers, and reported that *L. minor* was efficient in COD and colour removal. The experiment was, however, operated over a short period of time and conducted in plastic tanks treating acid orange 10 at a concentration of 45 mg/l. The results concluded that the percentage of colour removal in an aqueous solution was 86% higher than the removal in textile industry wastewater (83%) under optimum operational parameters; i.e. nutrient dosage (50 g of activated sludge), dilution ratio (8), pH (8) and contact time (4 days).

The ability of pond systems to remove colour and other pollutants from textile industry wastewater in Bursa city (Turkey) was assessed using *L. minor* under controlled conditions by Uysal et al. (2014). The experiment was operated using different contact times (3, 7 and 10 days). High removal was observed for a contact time of three days, and the results were similar (56% to 66%) during the run period (15 days). The percentage of removal decreased continuously from 34% to 12% and from 62% to 3% for contact times of 7 and 10 days, respectively. This work tested *L. minor* efficiency and mentioned dye and COD removal, pH values, and the relative growth rate of the plant. However, it did not mention the other water quality parameters and it was operated for a short time. Furthermore, both treatment systems operated by Alkhateeb et al. (2005), Sivakumar (2014) and Uysal et al. (2014) were conducted only under laboratory conditions, and the mixed dyes, which exactly simulate the textile dye effluents (Turabik & Gozme, 2013), were not considered.

L. minor is more efficient compared with *E. crassipes* as it is easily harvested (Bejarano, 2005), and can survive and grow within a wide temperature range. In addition, further aeration of the *L. minor* system is not required due to the full cover of the water surface by the plant, which prevents mosquitos' penetration (Vymazal et al., 1998) and leads to odour reduction (Babu, 2011).

These systems, free-floating plants-based treatment systems, require less capital cost compared with other wetlands planted with emergent and submerged plants that are especially linked with SSFCWs systems (Kadlec & Wallace, 2009; Chen et al., 2016). This draws attention towards operating such systems in low-income developing countries as cheap, effective, and environmentally friendly techniques (Bejarano, 2005; Yaseen et al., 2017). Therefore, information regarding the operating conditions and design variables must be considered carefully in order to achieve optimum performance. However, it is clear that the investigations using these systems for textile dyes removal are very limited and still require more attention and clarification (Alkhateeb et al., 2005). Table 2.2 shows a comprehensive summary of the available research and the dye removal findings using CWs systems.

Table 2.2 Summary of the available research and the dye removal findings using CWs systems

Reference	Wetland type	Wetland plant	Matrix bed (From the bed to the top)	Additive	Period of operation	Parameters monitored	Type of dye	Dye concentration	Dye/colour removal	Mechanism
Pervez et al. (2000)	Emergent plants-based system (VFCW) (Experiment)	*Phragmites australis*	Gravel, sand	Fertiliser	40 days	COD	AB 113	50,100, 200, 300, 400 mg/l	Reduction of AB113 was higher than RB171, higher removal was at lower dye concentration, maximum removal achieved for both dyes was 98%, and addition of peat to the substrate enhanced the removal efficiency	Adsortionp
							RB 171	50, 100, 200 mg/l		
Alkhateeb et al. (2005)	Free-floating plants-based system (Pond) (Experiment)	*Lemna minor L.*	Without	Without	20 days	Colour	N/V	N/V	Colour reduction of 70%, 3.13%, and 3.33% in planted ponds under sunlight, shadow, and dark conditions, respectively, and 3.5% under sunlight conditions without plant	Phytoextraction, phytodegradation, Phytovolatisation
Davies et al. (2005)	Emergent plants-based system (DFCW) (Pilot scale)	*Phragmites australis*	Gravel, sandy-clay soil	Without	54 days 16 days	Plant tissue sampling, peroxidases activity, COD, TOC, NO_3, SO_4	AO7	130 mg/l 700 mg/l	Dye removal of 69%, 74% at concentration of 130 mg/l and 700 mg/l, respectively	Degradation
Mbuligwe (2005)	Emergent plants-based system (FWSCW) (Pilot scale)	Cattails sp., Cocoyam	River sand bed, at inlet and outlet zones gravel added	Without	75 days	COD Sulphate pH	N/V	100.22 Pt Co	Dye removal of 72%, 77%, 14% for Cattail bed, Cocoyam bed, and Control CWs, respectively	Uptake and chemical decolourisation
Davies et al. (2006)	Emergent plants-based system (VFCW) (Pilot scale)	*Phragmites australis*	Gravel, sandy-clay soil	Without	48 days	COD, TOC, NO_3, pH, ORP, $SO_4{}^{2}$, plant growth, soil temperature	AO7	127 mg/l	Dye removal rate 99%	Degradation
Keskinkan and Lugal Goksu (2007)	Submerged plants-based system (FWSCW) (Experiment)	Water Milfoil, Coontail	Sand bed	Nutrient	40 days	pH, dissolved oxygen	BB41	11 mg/l	Dye removal of 95%, 94%, 71% for Water Milfoil, Coontail, Control CWs, respectively, at contact time of 18 days	N/V
Ojstrsek et al. (2007)	(DFCW) (Experiment)	Without	Gravel, sand, washed zeolite	Without	24 hours	pH, COD, TOC, EC	RR22, RB5, VR13	33.3 mg/l	Dye reduction of 40%, 70%, 43% for RR22, RB5, VR13, respectively	Filtration and/or adsorption

Table 2.2 (Continued)

Bulc and Ojstrsek, (2008)	Emergent plants-based system (VHFCW) (Pilot scale)	*Phragmites australis*	Coarse sand, fine sand	Without	5 months	COD, BOD, TOC, *N* total, *N* organic, NH_4-N, TSS, DO, temperature, EC, pH SO_4, Anionic surfactant	RB5, DY211, VY46	30 mg/l	Colour reduction rate 90% and the removal efficiency in VF was similar to HF bed, although the loading rates were lower in HF than the VF beds	Filtration and/or adsorption
Davies et al. (2009)	Emergent plants-based system (VFCW) (Pilot scale)	*Phragmites australis*	Gravel, sandy-clay soil	Without	60 days	Gene-expression analyses, COD, TOC	AO7	748 mg/l	Dye removal rate 68%	Degradation
Nilratnis-akorn (2009)	Emergent plants-based system (UFCW) (Experiment)	*Typha angustifolia* Linn.	Gravel, sand	Without	15 days	pH, COD, TDS, plant tissue analysis	RR141	20 mg/l	Colour removal was 49 %, 2.82%, 1.95%, 58% for CW containing only planted, only sand, only gravel, plant with sand and gravel bed, respectively	Absorption
Ong et al. (2009a)	Emergent plants-based system (UFCW) (Experiment)	*Phragmites australis, Zizania latifolia*	Small glass, gravel	Sludge	75 days	COD, NH_4-N, NO_3-N, NO_2-N, TN, TP, ORP, DO, plant growth	AO7	50 mg/l	Dye reduction was similar for planted and control CWs and for both plants, 96% for non-aerated CWs, 98% for aerated CWs at HRT of 3 days	Degradation
Ong et al. (2009b)	Emergent plants-based system (UFCW) (Experiment)	*Phragmites australis*	Small glass, gravel	Sludge	6 months	ORP, COD, aromatic amine	AO7	100 mg/l	Dye removal 96% for both aerated and non-aerated CWs	Degradation
Ong et al. (2010)	Emergent plants-based system (UFCW) (Experiment)	*Phragmites australis*	Small glass, gravel	Sludge	Three experiments within 1 year	COD, NH_4-N, NO_3-N, NO_2-N, TN, TP, ORP, DO, plant growth	AO7	50 mg/l 100 mg/l	Reduction rate of 98% and 96% at HRT of 3 days and dye concentration of 50 mg/l, 94% and 96% at HRT of 3 days and concentration of 10 mg/l, and 98% and 98% at HRT of 6 days and concentration of 100 mg/l within the aerated and non-aerated CWs, respectively	Degradation
Ong et al. (2011)	Emergent plants-based system (UFCW) (Experiment)	*Phragmites australis*	Gravel	Sludge	27 days	COD, NH_4-N, DO, plant growth	AO7	50 mg/l	Dye reduction 94.3%, 99.6%, 95.4% for control, aerated, non-aerated CWs at HRT of 2 days	Degradation

Table 2.2 (Continued)

Noonpui and Thiravetyan (2011)	Emergent plants-based system (HFCW) (Experiment)	*Echinodorus cordifolius L.*	With Soil Without Soil	Without	7 days	pH, EC, TDS, plant growth	RR2, RR120, RR141	20 mg/l	Dye removal 97%, 92%, 88% for RR2, RR120, RR141, respectively in CW without soil, and 100% and 99% for SRRW141 in CWs with and without soil, respectively	Uptake
Muthunarayanan et al. (2011)	Free-floating plants-based system (Pond) (Experiment)	*Eichhornia crassipes*	Without	Without	6 days	Functional group analysis for plant tissues	RR198, RB5	10-50 mg/l	Higher removal was 95% for RR198 and 99.5% for RB5 at concentration 10 mg/l	Bio-sorption
Cumnan and Yimrattanabovorn (2012)	Emergent plants-based system (FWS and SSFCW) (Pilot scale)	*Phragmites australis*	Shale	Without	N/A	COD, ORP, pH, DO, temperature	Azo dye	11.5 mg/l	SSF-FWS outperformed FWS-SSF in colour removal	Adsorption
Shehzadi et al. (2014)	Emergent plants-based system (VFCW) (Experiment)	Typha domingensis	Coconut shavings, gravel, sand, soil	Without	3 days	pH, colour, EC, COD, BOD, TDS, TSS, TOC	N/V	42,53, 47,61 cm^{-1}	The presence of the plant only and the plant with bacteria enhanced constructed wetland efficiency in terms of textile effluent degradation	Degradation
Sivakumar (2014)	Free-floating plants-based system (CW) (Experiment)	*Lemna minor L.*	N/V	Sludge	7 days	COD	AO10	45 mg/l	Maximum reduction was 86% in real effluent, and 83% in an aqueous solution at pH of 8, dilution ration 8, nutrient dosage of 50 g, HRT of 4 days	N/V
Uysal et al. (2014)	Free-floating plants-based system (Pond) (Experiment)	*Lemna minor L.*	Without	Without	45 days	pH, COD, plant growth, MLSS	N/V	2200 Pt Co	Results were similar (56% to 66%) during the run period (15 days). The percentage of removal decreased continuously from 34% to 12% and from 62% to 3% for contact times of 7 and 10 days, respectively	N/V
Yalcuk and Dogdu (2014)	Emergent plants-based system (DFCW) (Experiment)	*Canna idica* L., *Typha angustifolia* L	Gravel, sand, zeolite	Without	79 days	COD, NH$_4$–N, PO$_4$–P, pH, ORP, EC	AY 2G	22900 Pt Co	Colour removal of around 98.6%, 98.2%, 87% for *Typha angustifolia*, *Canna idica*, and control CWs, respectively	N/V

Table 2.2 (Continued)

Chandanshive et al. (2016)	Free-floating plants-based system (constructed lagoon) (Experiment)	*Salvinia molesta*	Without	Without	8 days	pH, COD, BOD, TDS, TSS, heavy metals	Rubine GFL	100 mg/l	Colour reduction rate 97%	Phytotransformati-on
Hussein and Scholz (2017)	Emergent plants-based system (DFCW) (Experiment)	*Phragmites australis*	Washed gravel	Fertiliser	13 months	COD, NH_4-N, NO_3-N, PO_4–P, pH, ORP, EC, SS, TBD, plant growth	AB113	6.6 mg/l	Higher removal for both dyes was at low inflow concentration. For BR46, reduction rate was 97% and 96% at low concentration planted and control CWs, respectively. For AB113 reduction rate was 86% and 71% at low concentration planted and control CWs, respectively.	N/V
								222 mg/l		
							BR46	6.9 mg/l		
								209 mg/l		

Note: RB171, reactive blue 171; AB113, acid blue 113; AO7, acid orange 7; BB41, basic blue 41; RR22, reactive red 22; RB5, reactive black 5; VR13, vat red 13; RR2, reactive red 2; RR120, reactive red 120; RR141, reactive red 141; RR198, reactive red 198; AY 2G, acid yellow 2G; COD, chemical oxygen demands; NH_4–N, ammonium-nitrogen; NO_3–N, nitrate-nitrogen; NO_2–N, nitrite nitrogen; TN, total nitrogen; TP, total phosphorus; CW, constructed wetland; VF, vertical flow; VHF, vertical and horizontal flow; UF, up flow; HF, horizontal flow; SSF, subsurface flow; FWS, free water surface; TOC, total organic carbon, EC, electrical conductivity; TSS, total suspended solids; TDS, total dissolved solids; MLSS, mixed liquor suspended solids; DO, dissolved oxygen; TBD, turbidity; PO_4–P, ortho-phosphate-phosphorus; SS, suspended solids; ORP, oxidation redox potential; $SO4^{2-}$, sulphate; Pt Co, Platinum-Cobalt; N/V, not available.

2.10 Algae ponds

Algae are micro-organisms and photosynthetic by nature. They are diverse microscopic organisms and can be found in waters, such as freshwater, oceans, ponds, ice, and hot springs, as well as in soil and other exposed locations (Mohan et al., 2002). Moreover, they are mostly used to treat wastewater in natural oxidation ponds. Algae are found in different colours, such as green, yellow, orange, blue, red, or brown and classified accordingly. However, the green phytoplankton algae are the predominant species that are responsible for the green colour in aerobic and facultative ponds (Gray, 2000). Many authors confirmed the capability of algae species to treat heavy metals (Gupta et al., 2006; Sekomo et al., 2012) and to decolourise and mineralise azo dyes (Yan & Pan, 2004; Daeshwar et al., 2007) from textile wastewater. For example, the decolourisation of more than thirty azo dye at concentration of 20 mg/l has been assessed by Jinqi and Houtian (1992) using three species of algae (*Chlorella vrenoidosa, Chlorella vulgaris* and *Oscillateria tenuis*). The authors concluded that the degree of dye decolourisation by algae depends on the structure of the azo dye, the species of algae and the environmental conditions. Other parameters which affect dye decolourisation by algae have been pointed out by Daneshvar et al. (2007), including the algae concentration, dye concentration, temperature and pH value. Chia and Musa (2014) found that high concentrations of dyes decrease the algae growth in treatment systems, and accordingly negatively affect the system performance and the dye degradation, due to several reasons: the eutrophication state, prevention of light penetration to the water, and the changes in the pH value (Elumalai & Saravanan, 2016). Karacakaya et al. (2009) pointed out that the colour removal increased when algae growth was enhanced by adding a plant growth regulator such as triacontanol hormone.

Algae ponds or algae waste stabilisation ponds which mimic the natural biological processes of natural wetlands are the most common systems used to treat the contaminated effluents in low-income developing countries, particularly in tropical and subtropical locations (Babu, 2011). The waste stabilisation ponds, however require a large area, but are characterised as a simple and cheap method (Kadlec & Wallace, 2009; Chen et al., 2016) in terms of the design, construction, operation and maintenance. The United Nations Environment Programme (UNEP) (1999) reported that these systems are still the cheapest method of wastewater treatment applied, as mentioned by Babu (2011). Algae systems consist of single or several large artificial shallow basins which are constructed to achieve anaerobic, facultative, or maturation (aerobic) conditions dependent on the oxygen content in the system (Gray, 2000). The anaerobic ponds are deep basins used as a main treatment stage, constructed in depths ranging between 2.5 m and 4.5 m, and characterised by the absence of oxygen within the whole system except for the surface layer, therefore being more applicable for industrial wastewater treatment (USEPA, 2011). Facultative ponds normally contain aerobic and anaerobic bacteria as a comprehensive treatment method. The organic loads applied to the facultative ponds is lighter compared with the anaerobic ponds, which enhance the growth of algae in the top layers of the system. These algae are a source of oxygen (aerobic conditions) in the surface layers, however, below these layers and in the sediment zone, anaerobic conditions are present (Gray, 2000). Facultative ponds are typically constructed in depths ranging between 0.9 m and 2.4 m (USEPA, 2011). Aerobic ponds refer to a polishing treatment stage (Gray, 2000), and are constructed in shallow depths ranging between 0.18 m and 0.3 m (USEPA, 2011) to ensure that the dissolved oxygen is present within the entire system depth (Gray, 2000). Algae ponds have proven to be effective in treatment of organic matter and pathogens (Zimmo, 2003). However, there are some drawbacks related to these systems, such as mosquito breeding, bad odour, limited nitrification zone, short-circuiting, and the outflow characterised by high total suspended solids. (Babu, 2011). In general, algae and bacteria are the main treatment organisms in algae ponds, the mechanisms of treatment in the ponds are the absorption of organic and inorganic pollutants by the basin, settling of suspended solids by sedimentation process, and organic contaminants degradation to simple compounds by aerobic and anaerobic bacterium (Gray, 2000). Then, algae uptake these compounds and produce the oxygen required for aerobic bacteria survival (USEPA, 2011).

The mechanism of dye removal by both live and non-viable algae includes the bio-sorption and the biodegradation processes (Lim et al., 2010). Bio-sorption occurs when the dye moves from the water phase to the solid phase (the bio-adsorbent). However, the biodegradation (bioconversion or biotransformation) process occurs when algae species break down the azo dye to other simple compounds (conversion of dyes to colourless intermediates or CO_2 and H_2O) (Holkar et al., 2016). Marungrueng and Pavasant (2006), and Aravindhan et al. (2007) have studied the bio-sorption process of basic dyes using the live algae *Caulerpa lentillifera* and *Caulerpa scalpelliformis*, respectively. Thereafter, Khalaf (2008) investigated the potential of non-viable algae spirogyra as a good bio-sorbent biomass to remove the colour of the reactive dye Synazol. Acuner and Dilek (2004) confirmed the bioconversion of the azo dye tectilon yellow 2G by *Chlorella vulgaris* species. They noticed that the algal conversion of the studied azo dye to aniline after colour removal ranged between 63% and 69%. Other mechanism of dye degradation by live algae occurs through the consumption of dyes for their growth (Holkar et al., 2016).

Algae ponds were evaluated as a polishing step to remove heavy metals from a textile factory (Sekomo, 2012). In addition, Chlorella ponds used for textile azo dyes removal, treated with a high rate algae pond, showed around 50% of colour removal (Lim et al., 2010). The role of algae for dye removal in stabilisation pond systems requires further investigation, as suggested by Jinqi and Houtian (1992).

2.11 Components of wetland systems

2.11.1 Overview

All wetland systems are designed as a basin that normally contains water/wastewater, vascular plants, sediments, detritus and substrate, which are considered as the main components that characterise the wetland systems and affect the purification efficiency. However, other important components that grow naturally in wetland systems, both natural and constructed, are microbes and aquatic invertebrates. All these components interact with each other in wetland systems to improve the water quality (Scholz, 2006; Kadlec & Wallace, 2009), and therefore can be manipulated appropriately to enhance the internal processes within the system to improve the treatment efficiency (Scholz, 2010, 2015).

2.11.2 Water

The wetlands environment is controlled by the water as a main factor that also has an effect on aquatic plant and animal life (Al-Isawi, 2016). Wetland systems are constructed using relatively impermeable underlying strata to prevent water seepage, and can be established anywhere in the landscape by designing the system (basin) to collect and retain the water (Eke, 2008). Hydrology is one of the key factors in constructed wetland design as it links all the functions in a wetland system, and is responsible for the success or failure of treatment efficiency by wetlands. It is related to the water transportation through the air, above the land and within the earth layers. There are three main hydrological considerations which strongly characterise the hydrological behaviour of any wetland: water level, hydroperiod and HRT (USEPA, 2008), in addition to other factors, such as hydraulic loading rate, meteorological conditions, evapotranspiration losses, and overall water balance.

The wetland water level is the elevation of water relative to the ground surfaces of the constructed wetland system. Normally, this hydrological descriptor is used to indicate the type of macrophytic vegetation that is suitable or likely to grow in wetland systems (USEPA, 2008). The water level in wetland systems is normally at or near the surface of the land or above the land at a shallow depth.

The hydroperiod is the pattern of variations in wetland water level over time and space (USEPA, 1995), and it results from the balance between the inflow, outflow and the storage water in a wetland system. The wetland hydroperiod commonly refers to the timing, duration and distribution of the water level in a wetland system, together. It determines some of the environmental conditions that strongly affect wetland efficiency and can be anticipated, such as the dry and wet conditions, yearly water variation, precipitation and the evapotranspiration (USEPA, 2008).

Hydraulic retention time is the average period of time that water remains in a treatment system and it is calculated by dividing the average volume of water in a wetland by the mean outflow rate (USEPA, 2008). HRT is a very important parameter in designing, operating, and evaluating the efficiency of a constructed wetlands system, as the plant uptake, sedimentation of solids and biochemical processes occur during this period (Kadlec & Knight, 1996; Sani, 2015).

2.11.3 Macrophytes

Macrophytic vegetation is an important component that affects the performance of natural and constructed wetland systems (Scholz, 2010; Vymazal, 2011, 2013a), and it is more likely that the CWs have been described as a green technology due to the presence of these aquatic plants (Eke, 2008; Stefanakis et al., 2014), which enhance the aesthetic value of CW systems (Sani, 2015).

Both higher plants (vascular plants) and algae (non-vascular plants) play a prominent role in CWs treatment. The functions of higher plants in CWs are attributed to their life cycle (growth and death) during the treatment process: plant growth increases the vegetative mass which provides more tissue for nutrients and pollutants assimilation, and provides more attachment areas for micro-organisms to enhance their growth and their activities for organic matter degradation (Eke, 2008; Al-Isawi, 2016); plant decay generates detritus and is a source of organic carbon, which is used as a fuel for bacterial metabolism (Mohammed, 2017). With regard to the algae, they play a vital role during the photosynthesis activity by enhancing the dissolved oxygen level of the water in wetlands (Vymazal, 2011). However, in CWs that are fully covered by *L. minor*, algae growth, if found, is very limited (Yaseen & Scholz, 2016), and therefore the main source of oxygen in these shallow systems is from the atmospheric diffusion (Yaseen & Scholz, 2018) and the oxygen transport from the air to the water via the plant roots (Sekomo et al., 2012). The ability of higher plants to transport oxygen is not specified exactly, as a significant impact was assumed by some wetland researchers and a negligible impact was reported by others (USEPA, 1999; Eke, 2008; Yaseen & Scholz, 2017a).

Macrophytes are the common species of plants that are widely used in treatment wetlands (Scholz, 2006; Vymazal, 2011; Sani, 2015; Zheng et al., 2016). The common macrophytes found in eutrophic water are the emergent (*P. australis*, *Scirpu Efeocharis*, *Typha*, *Echinodorus cordifolius* L.), submerged (*Egeria densa*, *Pamogeton* spp, *Elodea nuttallii*) and free-floating (*Eichhornia crassipes*, *Hydrocolyle umbellate*, *L. minor*) plants, which specify the type of CWs system accordingly (Vyamzal et al., 1998), as mentioned in Section 2.9. Plants assays showed that most of these macrophytes were effective in removing diverse pollutants including textile dyes (Davies et al., 2006; Keskinkan & Lugal Goksu, 2007; Ong et al., 2011; Yalcuk & Dogdu, 2014). However, Vymazal et al. (1998) pointed out that Duckweed (e.g., *L. minor*) "*has several characteristics which place them in a high potential category for use in wastewater treatment*".

Despite the widespread use of *L. minor* and other macrophytes in treatment wetlands, Scholz (2006) pointed out that the role of these plants in wastewater treatment within wetlands systems is still a questionable issue, as several authors have reported high contaminants removal efficiency with planted wetlands compared with unplanted ones (Cooper et al., 1996; Kadlec et al., 2000; Kaseva, 2004; Mbuligwe, 2005; Iamchaturapatr et al., 2007; Keskinkan & Lugal Goksu, 2007; Yaseen & Scholz, 2018), while other researchers found no significant improvement in some water quality parameters in the case of vegetated or non-vegetated wetlands (Huang et al., 2000; Scholz & Xu, 2002; Calheiros et al., 2007; Ong et al., 2009a). For example, Ong et al. (2009a) found a negligible impact from the plant (*P. australis*) in terms of dye, COD, and NH_4–N reduction using vertical CW filters. However, Keskinkan and Lugal Goksu (2007) found a higher dye reduction rate of more than 94% by a planted CW compared with an unplanted system which showed a reduction rate of around

71%. In addition, Mbuligw (2005) found that the CWs planted with Cattail and Coco yam significantly removed the dye by about 72% and 77%, respectively, and the COD by about 68% and 72%, respectively, compared with a control system which showed dye and COD removal efficiency of around 15% and 51%, respectively.

2.11.4 Micro-organisms

Many researchers have confirmed the presence of various micro-organism populations within the aerobic and anaerobic zones of wetland systems, including bacteria, yeasts, fungi, protozoa and rind algae (Moshiri, 1993; Kadlec & Knight, 1996; Scholz et al., 2001; Meng et al., 2014). These organisms and their metabolism largely regulate the functional characteristics of wetlands, and play a vital role in wastewater treatment, as they, due to their microscopic size, can attach and feed on the contaminants directly using their enzymatic activities (Truu et al., 2009; Sani, 2015).

In wetlands, the microbial biomass acts as a sink for many organic and inorganic matters, by processing them into harmless or insoluble matters. In addition, microbial activities affect wetland processing capacity by changing the oxidation redox potential conditions of the matrix bed, and involve nutrients recycling (USEPA, 1995; Truu et al., 2009; Sani, 2015). Organic matter degradation occurs in wetlands under aerobic and anaerobic conditions by, in most cases, autotrophic and heterotrophic bacteria, and, in certain cases, protozoa and fungi (Kadlec & Wallace, 2009; Meng et al., 2014). Organic pollutants serve two purposes for the organisms: they provide a source of carbon, which is one of the basic building blocks of new cell constituents, and they provide electrons, which the organisms can extract to obtain energy (Al-Isawi, 2016). However, chemical compounds biodegradation by microbial activities consists of several complex biochemical interactions, and the process varies according to the microbes used (Sani, 2015). The microbial transformation of nutrients includes aerobic transformation (taking place in the presence of oxygen), anaerobic transformation (taking place in the absence of oxygen) and facultative aerobes (occurring in the presence or absence of oxygen). Micro-organisms live and grow naturally in soil, water, and on macrophyte roots of wetland systems (Al-Isawi, 2016) under favourable conditions with presence of sufficient nutrients. However, under unsuitable environmental conditions, many organisms enter a dormant period, which could last for several years until these conditions become suitable again (USEPA, 1995). In wetland treatment systems, microbes can be affected by toxic pollutants, thus care must be taken regarding the toxic chemicals in wastewater which must be maintained at concentrations which prevent damage to the microbial community (USEPA, 1995).

Recently, many research studies have confirmed the capability of diverse microbes for decolourisation and mineralisation of a wide range of textile dyes. These diverse microbes include bacteria (Jadhav et al., 2007; Dawkar et al., 2008), fungi (Fournier et al., 2004; Saratale et al., 2006), and yeasts (Lucas et al., 2006; Jadhav et al., 2007). Researchers found that the microbial effectiveness for dye removal is basically based on the adaptability and the activity of the chosen microbes (Pandey et al., 2007; Saratale et al., 2011).

2.11.5 Substrate, sediments and detritus

Constructed wetland beds normally include substrates, sediments and detritus (USEPA, 1995). Substrates (also called aggregates) are used according to the treatment target in CW systems, and include soil, sand, gravel, rock and organic matter (Zhao et al., 2016). The presence of substrates in wetlands is important for several reasons: they support the vegetation (rooted submerged and emergent plants) and provide appropriate sites for the living organisms, which enhance the chemical and biological (mainly microbial) transformation processes; they affect the water pathway due to the substrate permeability; and they act as a storage for many wastewater pollutants (USEPA, 1995) as substrates are able to adsorb, filtrate and sediment most of these pollutants (Al-Isawi, 2016).

Substrates characteristics change when they are saturated because water replaces the atmospheric gases in the pore spaces and the available oxygen consumed by microbial metabolism. Since oxygen is consumed more rapidly than it can be replaced by diffusion from the atmosphere, substrates enter anoxic conditions. These anoxic conditions are necessary in the removal of some pollutants such as dyes, nitrogen and metals (USEPA, 1995). Soil is the main type of substrate used in wetland systems as it supports plant growth and the microbial biofilms, effectively (Meng et al., 2014; Stefanakis et al., 2014). However, soil is an expensive material compared with sand (USEPA, 1995), and it is also associated with clogging problems in SSFCWs due to the soil characteristics of small pore sizes and low permeability for the hydraulic and organic load (Wallace & Knight, 2006). The International Water Association (IWA) (2000) reported that the mixture of both gravel and sand as a filter media is recommended to improve contaminants removal and hydraulic conditions, however, these two materials dry out quickly and therefore the water level in the wetland should be maintained, especially during the period of plant growth (USEPA, 1995).

Substrates affect the total capital costs of CW systems, and therefore the applications of FWSCWs is more preferable than SSFCWs systems as the cost required for the substrate is limited to rooting soil on the bottom of the beds (Vymazal, 2010). Therefore, CWs planted with free-floating plants are the cheapest in terms of required substrate as these plants float on the top surface and their very small roots do not need any support by the substrates (Chen et al., 2016). In terms of the treatment of dyes using CWs, many authors have used substrates in their treatment systems, such as soil (Mbuligwe, 2005), gravel (Hussein & Scholz, 2017), sand (Keskinkan & Lugal Goksu, 2007) without mention of the exact impact of these materials for dye removal. However, Nilratnisakorn et al. (2009) pointed out that the sand and gravel beds improved the dye adsorption capacity in CWs by only 2.82% and 1.95%, respectively. In addition, Noonpui and Thiravetyan (2011) reported that the impact of a soil filter is negligible as their results showed that the dye removal was 100% and 99% in CWs with and without soil, respectively. Furthermore, Ong et al. (2009a) reported that the impact of a gravel bed in CWs is negligible (0.5% of total dye removal). Therefore, the impact of substrates in terms of dye elimination is not significant.

Organic materials have been used as organic substrates, such as activated carbon, zeolite, sawdust and compost. These materials are used as a source of carbon, which supports the microbe’s activities, consumes oxygen, and thus creates anoxic conditions which are required for specific pollutants removal (USEPA, 1995). However, Scholz and Xu (2002) mentioned that there is no benefit in using costly organic materials (e.g., granular activated carbon) for enhancing the adsorption processes in CWs.

Sediments accumulate, with time, in wetlands that are characterised by low water flows and a high rate of productivity or in stagnant systems. The presence of sediments in the wetland bed is important to provide more supportive area for the living organisms (USEPA, 1995).

The term “detritus” is used to describe the small materials which accumulate in a CWs bed as result of plant decay and/or stem breakdown (USEPA, 1995). In wetlands, plants assimilate and accumulate nutrients and other pollutants in their tissues, and because the direct consumption of these plants by animals is very low, during the plant life cycle, most of the biomass and energy assimilated by the plants is converted to detritus or senesced plant litter (Magee, 1993). Webster and Benfield (1986) pointed out that detritus is one of the main substrates and energy sources in wetlands. In addition, Wallace and Knight (2006) stated that detritus serves as a source of carbon to wetland microbes for denitrification and helps with long-term phosphorus accumulation. These materials are enriched by nutrients which are released, after detritus decomposition, to the wetlands water and soil, to be a good source of food for micro-organisms, algae, and small aquatic animals. Detritus decay occurs, basically, due to organic matter oxidation by microbial activates. The decayed detritus becomes a good site for supporting and feeding many invertebrates that in turn provide fodder for vertebrates, e.g., fishes and waterfowl (Magee, 1993). Through this cycle, energy is transferred from detritus to other wetland biotic components (Magee, 1993). The continuous accumulation of plants detritus or litter in the wetland bed increases the amount of organic matter and consequently provides more attachment sites for micro-organisms and for material exchange, as well as providing more carbon, the energy source responsible for enhancing the biological reactions (USEPA, 1995).

2.12 Processes of pollutant removal in wetlands

2.12.1 Overview

The applications of CWs aim to utilise cost-effective and eco-friendly alternatives to treat contaminated effluents by mimicking the natural processes that occur in natural wetlands between the substrates, plants, and associated microbes, but with controlled conditions. Pollutant reduction by CWs can possibly be classified based on three processes encompassing physical (abiotic), chemical (abiotic), and microbial and phytological (biotic) processes, which are accomplished by several treatment pathways. Physical treatment processes in wetlands include: the trapping of sediments by the plant, as the water moves rather slowly due to the resistance from the wetland plants and from the uniform flow conditions; and the settling of the sediments due to gravity and low flow velocity (Interstate Technology and Regulatory Council Wetlands Team (ITRC), 2003). Concerning chemical treatment processes in wetlands, they include: the sorption process, occurring by moving the charges from liquid to solid phase, via either the adsorption process (transferring of ions to soil particles) or the precipitation process (converting metals to insoluble forms); the photo-oxidation process, which utilises the sunlight for pollutants degradation and oxidation; and the volatilisation process, which occurs by expelling the degraded pollutant into the atmosphere as a gas (DeBusk, 1999). The last and main processes in wetlands are biological treatment processes, which include: the phytodegradation process, as a main way for pollutants reduction that occurs when the plant roots directly uptake pollutants (DeBusk, 1999); the rhizodegradation process, which occurs when plants secrete contaminants that add to biological degradation; the phytovolatilisation process, which occurs when the pollutants transpire through the macrophyte leaves after entering the macrophyte biomass (ITRC, 2003); the microbial metabolism process for inorganic nitrogen removal, and nutrient uptake by soil bacteria, which converts the energy source, carbon, found in organic matter to carbon dioxide or methane depending on whether the wetland conditions are aerobic or anaerobic, respectively (DeBusk, 1999). The treatment processes are specified relying upon some factors in the wetland including wetland characteristics, interaction within the wetland system, the quantity of wastewater, the type of wastewater, and the type of pollutant, in addition to the system hydrology and environmental conditions (Eke, 2008). Table 2.3 summarises the treatment processes of specific contaminations in wetland systems, as indicated by Cooper et al. (1996), the United Nations Human Settlements Programme (UN-HABITAT) (2008), and Kagalkar (2011). The following sections will highlight the pollutant removal processes by constructed wetlands with the main focus on dye, organic matter, nitrogen and heavy metals which are normally found in textile effluents.

Table 2.3 Contaminant removal processes in constructed wetlands

Pollutant	**Removal process**
Suspended solids	Sedimentation Filtration
Soluble organics	Aerobic microbial degradation Anaerobic microbial degradation
Nitrogen	Ammonification followed by microbial nitrification Denitrification Plant uptake Matrix adsorption Ammonia volatilisation (mostly in FWS system)
Phosphorous	Matrix sorption Plant uptake Microbial assimilation precipitation
Metals	Adsorption and cation exchange Complexation Precipitation Plant uptake Microbial Oxidation/reduction
Dye	Microbial degradation/transformation Adsorption by microbes Adsorption and/or accumulation by plant Rhizodegradation Phytodegredation or phytotransformation Phytovolatilisation Adsorption by substrate (matrix bed)

2.12.2 Processes of suspended solids removal

Wetland systems receive effluents contaminated with organic and inorganic solids particles that are diverse in their sizes and compositions (Sani, 2015). Constructed wetlands are able to produce and remove suspended solids (SS) due to the natural processes that occur in these treatment systems. The production of SS in wetland systems is attributed to invertebrate death, plant detritus, microbe production (within the water or attached to the plant surfaces), and formation of chemical precipitates (USEPA, 1999). However, in terms of SS elimination, researchers have confirmed the potential of CWs in removing solids and particulate matter (Kadlec & Knight, 1996; ITRC, 2003) by different pathways, which include: settling and sedimentation, adsorption, and microbial degradation, although sedimentation and filtration are the predominant effective (physical) pathways for suspended solids removal in constructed wetlands (Kadlec, 2009; Abou-Elela et al., 2013, Sani, 2015). Most of the solids particles within the inflow water are filtered out and settled within the nearest few metres outside the inlet zone in all wetland systems. However, accumulation of entrapped solids leads to the occurrence of clogging problems which affect wetland performance, especially in subsurface flow wetland systems (Vymazal et al., 1998).

In FWSCWs, flocculation/sedimentation and filtration/interception, are the main removal processes of SS. Regarding the settling pathway by gravity, it is typically categorised into discrete settling, when the particles settle independently without any effect from other particles or conversion of their size or density, and flocculent settling, when the particles interact with each other resulting in conversion of their size and characteristics. Both these settling processes are impacted by some factors, such as the characteristics of particle size, specific gravity, shape, and fluid specific gravity and viscosity. The filtration process does not play an important role in SS removal in FWSCWs as it depends on the plant's ability to trap the particles found in inflow wastewater. However, an important process for SS removal is the interception by and adhesion to plant surfaces, as the wetland macrophytes are coated by a biofilm layer that absorbs colloidal and soluble matter, which after their metabolism are converted to gases or biomass. Similar reactions may occur in the surface detritus (USEPA, 1999).

In particular, in wetland systems with free-floating plants, suspended solids removal basically occurs through gravity sedimentation in the layer below the surface of the floating mat (Vymazal, 2008). *L. minor*-based systems are characterised by low SS outflows compared with algal-based systems (Bejarano, 2005; Zimmo et al., 2005). Because algae die-off enhances the SS concentrations in the system, whereas, *L. minor* is normally harvested from the wetland systems. However, in the case of *L. minor* being allowed to die in the system, the SS content will increase as well. In addition, the full coverage of *L. minor* inhibits algae growth in wetlands, causing the existing algae to die and consequently increasing the level of SS within the outflow water (Dalu & Ndamba, 2003). The main factors influencing SS removal in Duckweed-based systems are the algal growth, biodegradation of organic matter and sedimentation of particles, as reported by Dalu and Ndamba (2003), and Yaseen and Scholz (2017a).

2.12.3 Processes of organic matter removal

Constructed wetlands receive textile effluents containing a wide variety of organic matters that could be settleable and soluble compounds. The removal of these organics is assessed by the changes in COD, BOD and TOC concentrations before and after treatment (Eke, 2008). Organic matter comprises around 45–50% carbon, which is used as a source of energy for microbial activities, and then converted into carbon dioxide which is utilised by the plants to produce the oxygen required in the treatment process (DeBusk, 1999).

The main processes of organic matter elimination in CWs are, volatilisation, sorption, and aerobic and anaerobic microbial degradation (ITRC, 2003). Settleable compounds removal in wetlands occurs quickly by gravity, under quiescent conditions, and the mechanism includes deposition and filtration processes (USEPA, 1993; Vymazal et al., 1998; UN-HABITAT, 2008). The biological degradation of soluble organic matter is accomplished by the growth of the attached and suspended microbes (Sani, 2015). Organic substances decompose aerobically and anaerobically depending on the oxygen content and the presence of heterotrophic microbes in wetlands (Ong et al., 2009a, 2010). Aerobic degradation of soluble organics is achieved by the aerobic heterotrophic bacteria, when oxygen is supplied directly from the atmosphere by diffusion or from vegetative roots by leakage. The degradation of organic compounds containing nitrogen under aerobic conditions is governed by autotrophic bacteria called nitrifying bacteria, which are responsible for the nitrification process in CWs. However, with the absence of oxygen, under anaerobic conditions, the facultative or obligated anaerobic heterotrophic bacteria are responsible for the anaerobic degradation of soluble organic substances (Vymazal et al., 1998; Ong et al., 2009a). The accretion of organic matter in wetlands provides a long-term source of carbon and a sustainable source of nutrients, which are required to support the microbial activities for the denitrification process. Cooper et al. (1996) and Vymazal et al. (1998) pointed out that uptake of organic matter by the wetlands macrophytes is negligible compared to the biological degradation processes.

A further process for organic matter degradation in CWs is biochemical conversion, as a result of mineralisation and the formation of organic matter by synthesis of fresh biomass (DeBusk, 1999; Sani, 2015). In addition, adsorption/absorption processes are able to remove organic substances, however, USEPA (1999) noted that the capacity of the adsorption process depends on some factors in wetland systems related to the surface media, vegetation, detritus and organic matter characteristics. The phytovolatilisation process of organic matter in CWs is accomplished as a direct process by uptake and translocation of the organic compounds and then volatilisation of them from the macrophyte stems or leaves, and indirect phytovolatilisation when organic compounds volatilise from soil due to the activities of macrophyte roots (Limmer & Burken, 2016). Further to phytovolatilisation, Sani (2015) and Al-Isawi (2016) implied that some macrophytes in CWs have the ability to absorb pollutants via their roots and release them to the water stream by the transpiration process. Specifically, organic matter in free-floating macrophytes-based systems are basically eliminated by the bacterial metabolism of both attached and free-living bacteria. The large surface area provided by the roots of free-floating plants increases the attached micro-organisms, and consequently improves the potential for decomposition of organic matter (Vymazal, 2008).

2.12.4 Processes of nutrients removal

The discharge of uncontrolled quantities of nutrients, nitrogen and phosphorus, into watercourses can cause many environmental problems, such as eutrophication, damaging the aquatic life, as ammonia and nitrite (even at low concentrations) are toxic to fish and other living organisms, reducing the DO content to unacceptable levels in the receiving watercourses, and supporting algal blooms, which all makes the removal of nutrients a very important parameter for wastewater treatment systems (Kadlec & Wallace, 2009; Yaseen & Scholz, 2018). Numerous research studies have proven the removal of various forms of nitrogen (organic and inorganic) by different types of CWs systems. However, in some cases, the system ability for meeting the high standards for nutrient discharges is limited (Al-Isawi, 2016). According to Vymazal (2007), the removal efficiency of total nitrogen in all CW types ranges between 40% and 50% with the removed load ranging between 250 and 630 g nitrogen/m^2 year. Nitrogen reduction in CWs occurs by different processes, such as ammonification, ammonia volatilisation, nitrification, denitrification, fixation, plant and microbial uptake, substrate adsorption, organic nitrogen burial, and anaerobic ammonia oxidation (Vymazal et al., 1998; Kadlec & Wallace, 2009), although many researchers have confirmed that the nitrification and denitrification processes are the dominant pathways responsible for nitrogen removal in most CWs systems (Lee et al., 2009). In wetland systems with free-floating plants, nitrogen reduction is potentially accomplished by three processes which are nitrification-denitrification processes, plant uptake, and ammonia volatilisation (Vymazal, 2008).

The ammonification process, does not remove nitrogen, it just converts the organic nitrogen to the inorganic form of nitrogen, which occurs when the pH levels in the water range between 6.5 and 8.5, and depends on some other factors, such as the carbon/nitrogen ratio in the residue, temperature, and nutrient contents, as well as the soil conditions (Vymazal et al., 1998; Vymazal, 2007). Once organic nitrogen is transformed to ammonium by ammonification, nitrification can take place.

Nitrification is the process of oxidising the ammonium biologically to nitrite (first step) and then nitrite to nitrate (second step) in the presence of oxygen and nitrifying bacteria (Vymazal et al., 1998). Therefore, this process is similar to ammonification as it does not remove nitrogen from the wastewater in the wetland system but acts as a pathway for nitrogen transformation (Vymazal, 2007). The first step of nitrification occurs strictly via aerobic bacteria that rely on ammonia oxidation as a source of energy to grow, including *Nitrosomonas*, which is found in soil and fresh water, in addition to other types found in soil only (*Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus*, and *Nitrosococcus*). However, the second step occurs via facultative bacteria that depend on nitrite oxidation and organic compounds as a source of energy to grow, including *Nitrobacter* and *Nitrospina*, which are found in both water and soil (Vymazal et al., 1998; Vymazal, 2007; Kadlec & Wallace, 2009). In both steps, carbon dioxide is utilised as a source of carbon. The nitrification process in wetlands with free-floating macrophytes occurs in the water column when the nitrifying bacteria can grow attached to the plant roots that supply oxygen, and when the DO contents in the water are suitable for supporting the nitrifying bacteria activity. It is worth noting that these conditions, as a result of the nitrification process, are associated with systems that are partially covered by plants and are at low plant densities (directly after harvest) (Vymazal, 2008).

Denitrification, (USEPA, 1999), is the process of nitrate reduction to molecular nitrogen or nitrogen gases under anoxic conditions by heterotrophic bacteria, such as the genera of *Bacillus, Micrococcus, Pseudomonas* (which are identified in soils), *Aeromonas,* and *Vibrio* (which are found in water) as mentioned by Vymazal et al. (1998). In free-floating plants-based treatment systems, the anoxic zone occurs when the plant grows well as a dense floating mat that covers all of the water surface, which, firstly, inhibits algae growth due to prevention of sunlight penetration, and, secondly, makes the DO content in the water very low due to the limited oxygen diffusion into the water. This resulting zone (layer) is suitable for the nitrate denitrification process, which may also occur beneath the anoxic zone in the case of the presence of a sufficient source of organic carbon (Vymazal, 2008). Therefore, nitrification and denitrification processes in systems with free-floating macrophytes are accomplished as a result of the regular growth and

harvest cycle of these plants.

The uptake pathway of nutrients (ammonium-nitrogen and nitrate-nitrogen) by plants occurs effectively when these plants are characterised by their rapid growth rate (high productivity with regular harvest), high tissue nutrient level, and high ability for biomass accumulation per unit area. The capacity of nutrients assimilation by macrophytes, if the biomass is regularly harvested, indicates the nutrients removal efficiency. The higher capacity of plant tissue for uptake of nitrogen is linked with the highly productive free-floating macrophytes followed by the emergent plants, and then the submerged ones. However, it is worth noting that the nitrogen removal by plant harvesting, especially the emergent plants, in constructed wetland systems designed as a secondary treatment unit is very low compared with the high load of nutrients entering these systems. Therefore, this process plays a key role in wetlands operated as a polishing stage (Vymazal et al., 1998), such as free-floating plants-based systems. Brix (1996) pointed out that the nutrients assimilated by wetland plants return to the water column by decomposition processes if the plants are not harvested, and these nutrients could be stored in the wetland system over the long term in the case of plant detritus and the other types of undecomposed wetland litter.

Ammonia volatilisation is the process of aqueous ammonia losses through volatilisation and release into the atmosphere as an ammonia gas. Nitrogen reduction by this process highly depends on pH values in the system, as it is not significant at pH levels of below 7.5, and not very significant at pH below 8. However, it is potentially effective at pH values of 9.3 and above The presence of algae in constructed wetlands increases the pH level due to the photosynthesis process and consequently enhances the ammonia volatilisation process

(Vymazal et al., 1998). Wetlands with free-floating macrophytes are able to reduce nitrogen by the ammonia volatilisation pathway, as reported by Vymazal et al. (1998), however, this occurs in the case of the photosynthesis process by this plant creating high pH values. Vymazal (2007) summarised the potential of free-floating plants-based treatment systems in terms of each process for nitrogen transformation, which are as follows: nitrification, volatilisation, and microbial uptake are low; ammonification is high; ammonia adsorption is zero; denitrification, and plant uptake by harvesting are medium; and organic nitrogen burial is very low.

It worth noting that the nitrogen removal efficiency using constructed wetlands is affected by the type of wetland systems, environmental conditions (such as pH, dissolved oxygen, and temperature) and operational parameters (such as loading rate of pollutants, hydraulic retention time, influent feed mode, and organic carbon sources availability) (Al-Isawi, 2016). For example, Kadlec (1999a) pointed out that the annual nitrogen removal is affected by environmental parameters such as temperature, humidity, and precipitation. In addition, the IWA (2000) confirmed that the activities of nitrifying bacteria are highly influenced by environmental conditions such as pH, dissolved oxygen, and temperature. Furthermore, Vymazal (2007) stated that the reduction rate of total nitrogen was very limited in a single stage wetland, except in the case of a large treatment surface area.

Phosphorus is found in diverse wastewaters and used as a macronutrient for several organisms within the ecosystem (Al-Isawi, 2016). In wetlands, it presents as orthophosphate, dehydrate orthophosphate and organic phosphorus (Vymazal et al., 1998). However, the general form of phosphorus considered in wetlands is orthophosphate, as it is directly used by wetland plants to be the main link between the organic and inorganic phosphorus cycling. The range of phosphorus removal in all kinds of CWs is very low varying between 40% and 60% with removed load ranging between 45 g and 75 g phosphorus/m^2 year (Vymazal, 2007). Phosphorus reduction in wetland systems is accomplished by different physical, chemical, and biological processes (Moshiri, 1993; Kadlec & Knight, 1996; Kadlec & Wallace, 2009). In particular, the main process of phosphorus removal by free-floating plants-based CW systems occurs by microbial assimilation, precipitation with divalent and trivalent cations, adsorption onto clays or organic matter, and plant uptake. However, many authors confirmed that plant uptake and their consequent harvesting, is the appropriate pathway for long-term phosphorus removal, as reported by Vymazal (2008). Plants uptake phosphorus by their roots, and also absorb some phosphorus in their leaves and other parts during the growing season. When the plants die and decompose the stored phosphorus is returned to the wetland water. Therefore, the regular harvesting of wetland macrophytes maximises phosphorus reduction within the treatment system as it consequently reduces the releases of phosphorus to the wetland after plant decay. It is worth noting that some authors (Kadlec, 1999b) confirmed that long-term storage of phosphorus can also occur by plant parts that uptake phosphorus and withstand decay. Certain micro-organisms in wetlands, such as algae and bacteria, are able to quickly remove phosphorus by the assimilation process as these organisms grow rapidly, however, they are able to store only low quantities of phosphorus, and act as a temporary solution because once organisms decay, phosphorus is returned to the water (Vymazal, 2007).

In CWs, several parameters require accurate selection to improve low phosphorus removal in the system, such as plant, water depth, contact time (Al-Isawi, 2016), and substrates, which must have at high sorption capacity (Vymazal, 2007). However, it is important to realise that the wetland systems are unable to meet primary phosphorus removal standards.

2.12.5 Processes of heavy metals removal

Textile effluents contain different toxic metals, which are quite dangerous and can cause health hazards to humans (Yapoga et al., 2013) either at high concentrations only, such as copper, selenium, and zinc, or even at low concentrations, such as cadmium, mercury, and lead (DeBusk, 1999). Metals occur in both soluble and particulate associated forms (Vymazal et al., 1998). CWs are widely used for the treatment of heavy metals from diverse wastewaters (Vymazal et al., 2007; Sekomo et al., 2012; Sukumaran, 2013) under hydroponic, laboratory (Ladislas et al., 2013) and real environmental (Ladislas et al., 2015) conditions. The main pathways of heavy metals removal by CWs involve filtration and sedimentation, precipitation, uptake by micro-organisms and plants, and adsorption to substrate, sediments and organic matter (DeBusk, 1999; Stottmeister et al., 2003). All these processes react with each other as a complicated process (Sheoran & Sheoran, 2006), which finally results in metals accumulation within the wetland substrate (Mohammed, 2017).

Filtration is a simple physical process which occurs in CWs for heavy metal removal when the wastewater passes through the treatment system, which relies on the ability of plant roots and substrate pores to trap metals suspended on solids and retain them in wetlands (ITRC, 2003). This pathway is efficient for treatment of different metals, such as cadmium, lead, silver and zinc (Al-Isawi, 2016). Sedimentation is also physical method widely recognised as the main pathway for heavy metal removal in both natural and CWs systems. It is effective for the removal of metals associated with particulate matter large enough to settle in the wetland bed (Kadlec & Knight, 1996; ITRC, 2003) and therefore sedimentation follows other chemical processes (e.g., precipitation, co-precipitation or floc-formation), to aggregate heavy metals, as reported by Sheoran and Sheoran (2006). In wetlands with a floating mat of vegetation, these plants, may help somewhat as sediment traps due to the water conditions, of slow movement or in a calm state, within these systems. Once wastewater enters the wetland system, metals transport from the wastewater to plants and animals (biota) or to the substrate or vice versa, even when the water is stagnant, leading to the filtering and trapping of these metals in CWs. In calm water conditions, particles which are heavier than the water will settle down in the system bed. However, in the presence of light particles that are less dense than water, floc-formation is necessary before the sedimentation process. Flocs are able to settle faster than other single particles, and absorb other suspended particles including heavy metals (Sheoran & Sheoran, 2006).

Plant uptake is a biological pathway for the removal of dissolved heavy metals from wastewater by CWs (Vymazal et al., 1998). The reduction rate of metals by this process varies according to the macrophyte growth rate and metals concentration in the plant tissue. In wetlands with emergent plants, heavy metals are taken up through plant roots and then passed, in small portions, to the other parts. However, in the case of systems with free-floating or submerged macrophytes, heavy metals uptake occurs by both leaves and roots (Sheoran & Sheoran, 2006). Many researchers confirmed that the higher contribution of plant parts in heavy metals removal is by plant roots followed by the rhizomes and then, the lowest contribution comes from the above-ground parts (Vymazal et al., 1998). Therefore, harvesting of the above-ground parts slightly enhances metals removal in wetlands (Cheng et al., 2002). However, harvesting of the full biomass (e.g., *L. minor*) improves metals removal, to some extent, more so than the case of above-ground biomass harvesting. It is reported (Stottmeister et al., 2003; Mohammed, 2017) that the potential of wetland plants to assimilate heavy metals is 5% of the total removal of metals in CWs. However, many authors confirmed a higher impact of planted wetlands versus unplanted ones in terms of metal removal. In addition, plants, after decay, act as a source of organic matter (Stottmeister et al., 2003) that, over time, provides good sites for metals sorption and precipitation to enhance the long-term performance (Mohammed, 2017). Another biological process for heavy metals removal by wetlands is microbial uptake. Micro-organisms can uptake and store considerable concentrations of metals from wastewater due to their metabolic processes. It is also reported that algae species (such as Chara and Nitella) are effective as a long-term method for heavy metals removal (Sheoran & Sheoran, 2006).

Chemical processes for heavy metals removal in CWs are adsorption and precipitation. Adsorption is the process of ions transformation from the soluble to the solid phase. In this process metals are adsorbed to soil or sediment, or may be chelated with organic matter (Mohammed, 2017). Precipitation is a main adsorptive pathway in CWs sediments. This method depends on the solubility product of the metals, wetlands pH, concentration of metal ions and relevant anions (Al-Isawi, 2016).

2.12.6 Processes of azo dye removal

Textile wastewater contains high concentrations of various dyes which are organic contaminants and not easily biodegradable (Kagalkar, 2011). In wetlands, biological processes are the predominant pathway for dyes elimination, which occurs, according to Khelifi et al. (2008) and Reddy and Lee (2012), via the plants and micro-organisms. Basically, dye removal processes in wetlands include: adsorption and/or accumulation on and/or into plant tissues, phytodegradation or phytotransformation, phytovolatilisation, rhizodegradation, microbial degradation/transformation (Kagalkar, 2011; Tahir et al., 2016), microbial adsorption (Pearce et al., 2003), and adsorption and/or filtration by substrate (Ojstrsek et al., 2007).

Macrophytes play an important role in dye removal by wetland technology (Mbuligw, 2005). Diverse plants possess the ability to adsorb, absorb, accumulate, detoxify and metabolise (degrade) a wide range of synthetic dyes and colorants (Nilratnisakorn et al., 2007; Kagalkar, 2011). In wetlands, the binding of xenobiotic dyes to the plant roots is accomplished by the occurrence of adsorption subsequent to the absorption processes. The dyes, after active uptake, are transferred either into the cell vacuole or into the extracellular space. Thereafter, these dyes are combined into cell membrane components which leads to these dyes being non-extractable from the macrophyte roots and shoots due to the covalent associations with macromolecules (lignin, hemicellulose, protein, cellulose or pectin) in the cell wall (Davies et al., 2005). Diverse plants in constructed wetland, such as *P. australis* and *E. crassipes* showed their ability for dye removal by the adsorption pathway (Pervez et al., 2000; Muthunarayanan et al., 2011). However, it is reported that many factors could affect adsorption capacity by plants, such as initial dye concentration, pH, adsorbent dosage, contact time and temperature (Kagalkar, 2011; Sharma et al., 2016). For example, with increasing dye concentration, the available adsorption sites become fewer which in turn reduces the dye removal efficiency (Sharma et al., 2016). Regarding the impact of pH, in acidic conditions, the adsorption of cationic dyes is less and of anionic dyes is greater, and vice versa, because as mentioned by Salleh et al. (2011), in acidic conditions, the positive charge in the solution increases and the adsorbent surface appears positively charged. Therefore, in the case of cationic dyes, the concentration of this dye will increase leading to a reduction of the adsorbent. However, for anionic dyes, the concentration will decrease leading to an increase of the adsorbent. The adsorption capacity for dye removal increases significantly with increasing adsorbent dosage (Khataee et al., 2012). Increasing the contact time up to a certain point (equilibrium) increases the dye adsorption, however, after the equilibrium point, the dye removal is not affected due to the lack of available adsorption sites (Sharma et al., 2016). In terms of temperature impact, dye adsorption improves with increasing temperature (Movafeghi et al., 2013; Yaseen & Scholz, 2016). Dyes also accumulate in the plant roots and can be further translocated to shoots and leaves (Kagalkar, 2011), this pathway has been confirmed by many authors for dye removal over a wide range of concentrations of different plants, such as *L. minor* (Reema et al., 2011), and *Rheum rabarbarum* and *Rumex hydrolapatum* (Aubert & Schwitzguebel, 2004). The plant potential for dye accumulation is enhanced by increasing the plant biomass (Kagalkar, 2011). Both adsorption and accumulation processes are efficient for the removal of different dyes, however, these pathways contribute to, only, concentrating the dye contaminations onto and/or into plant surfaces and do not lead to the complete dye eradication from the treatment system (Kagalkar, 2011). Thus, the removal of dyes by these processes requires regular harvesting of plants, that must be grown easily and rapidly in the treatment system, such as *L. minor* (Reema et al., 2011; Movafeghi et al., 2013).

Phytodegradation (also called phytotransformation) is the main pathway observed for dye removal by plants. In this process, dye molecules are either converted into harmless substances that are safely released to the environment (which refers to the breakdown and conversion of the dye molecules surrounding the plant through enzyme activities, which are produced by the plants) or completely degraded and eliminated from the system (which refers to the breakdown of dyes that are taken up by the plants through metabolic processes within the plants) (Kagalkar, 2011; Tahir et al., 2016). The phytotransformation process is accomplished when the plants develop detoxifying mechanisms as protection against the polluted water (Davies et al., 2005). This process helps to eliminate some of the pollutants (e.g., aromatic compounds) that are difficult to degrade microbially (Aubert & Schwitzguebel, 2004). The elimination is achieved by the production of enzymes which are able to accept these aromatics as a substrate and process them into simpler compounds that are integrated with the plant tissue, which in turn, fosters plant growth. Enzymatic activities for textile dye degradation by plants occur either intracellularly, involving enzymes inside the plant tissues, or extracellularly with the help of plant enzymes in rhizosphere regions (Kagalkar, 2011). Authors have reported that different plants, such as *P. australis* (Davies et al., 2005), *Aster amellus* Linn. (Khandare et al., 2011), and *L. minor* (Movafeghi et al., 2013), through their enzymatic mechanisms showed their efficiency in phytotransformation of diverse dyes and conversion of the daughter products to less toxic compounds. Davies et al. (2005) also confirmed the potential of the peroxidases enzyme produced from *P. australis* for degradation of acid orange 7 by a CW system. However, Ong et al. (2009a) pointed out that the plants in a wetland system slightly enhanced the degradation of aromatic amine, which was due to mineralisation by aerobic biomass growth near to the macrophyte roots or due to uptake by the plants followed by metabolic processes within the plant.

Phytovolatilisation is the process of uptake and transpiration of the dye pollutants by a macrophyte, and then release of these dyes, or the modified form of the dyes, from the plant to the atmosphere. This pathway is accomplished by passing the dyes through the plant to the leaves and then evaporation or volatilisation to the atmosphere. Another pathway for dye removal in wetlands occurs as a contribution from plant roots and microbes called the rhizodegradation process. This pathway is slower than the phytodegradation process and refers to dye degradation by microbial activities in the soil surrounding the roots of plants, as their presence helps to enhance these microbial activities (Alkhateeb et al., 2005).

Microbial adsorption of azo dyes is the simplest process for dye removal by micro-organisms onto their biomass. However, this pathway is not suitable for long-term treatment systems, such as wetland systems, as the biomass becomes saturated by dyes that are concentrated on it over time, during the adsorption process, and thus the saturated biomass must be disposed of (Pearce et al., 2003).

Microbial degradation (biodegradation) processes of azo dyes are the main pathway for dye removal by microbes which refers to either the conversion of the dyes into non-toxic substances (biotransformation) by chemical modification produced from living organisms or to complete degradation and elimination of dye molecules from the system (Tahir et al., 2016). It is reported that different microbes possess the potential to degrade diverse dyes, which differ in their structure and properties (Kagalkar, 2011; Holkar et al., 2016). The biodegradation pathway of dye removal in constructed wetlands occurs via two stages. The first stage is the process of reductive or decolourisation of the azo dye, when the double bond between the nitrogen atoms is broken by enzymatic activities and daughter products, such as aromatic amines, are produced, which occurs under anaerobic conditions (within the anaerobic zone in wetlands) as stated by Ong et al. (2009a, 2010) and Kagalkar (2011). However, many authors have confirmed that this step could occur under anoxic or aerobic conditions as well, including Davies et al. (2006) who mentioned that the azo bond cleavage of acid orange 7 occurs in aerobic conditions, as these conditions were dominant in their constructed wetland. Stolz (2001) and Pearce et al. (2003) reported that under aerobic conditions, aerobic bacteria adapted for long-term aerobic growth with some structurally simple azo dyes and consequently produced an azoreductase enzyme, able to reductively cleave the azo bond. However, under anaerobic conditions, bacterial reduction is unspecific with regard to the type of azo dye involved, and therefore it is widely used for the decolourisation of azo dye. The second stage is the process of aromatic amine mineralisation, which occurs easily under aerobic conditions (within the aerobic zone in wetlands) (Ong et al., 2009a, 2010), or anoxic conditions (within the anoxic zone) by bacteria only (Pandey et al., 2007). Kagalkar (2011) confirmed the presence of different enzymes in different microbe species such as lignin peroxidase, laccase, tyrosinase, and azoreductase, which are able to breakdown the azo bonds in the dye and convert it into a biological form that is easily degraded by the plant.

The degradation of textile dye achieved by an individual plant or microbe system could lead to a partial removal, and their combined synergistic approach is important for achieving a high degree of dye mineralization (Kagalkar, 2011). Therefore, the combination of the microbial and plant enzymes in wetland systems could efficiently enhance the dye molecules removal (Davies et al., 2009). It is worth noting that the microbial removal of dyes is affected by the dye characteristics. Studies have indicated that dyes characterised by low molecular weight, simple chemical structure (Pearce et al., 2003), and absence of sulpho or nitro groups (Saratale et al., 2011) are more effectively removed than dyes which have high molecular weight or a complex structure.

The adsorption process of dye molecules by the substrate in CWs is reported as a negligible pathway by many authors (Nilratnisakorn et al., 2009; Ong et al., 2009a; Noonpui &

Thiravetyan, 2011). This process is not effective for long-term dye removal as the matrix bed reaches equilibrium after a limited period leading to dye adsorption onto the matrix bed being negligible. This was confirmed by Davies et al. (2006, 2009); the authors demonstrated that acid orange 7 adsorption onto gravel topped by a sandy clay matrix bed reached equilibrium after 16 days and 30 days in CWs fed by 127 mg/l (Davies et al., 2006) and 748 mg/l (Davies et al., 2009), respectively, and the adsorption by the substrate became negligible after these periods. However, Ojstrsek et al. (2007) observed 70% dye removal by unplanted wetland filled with gavel, sand and zeolite, and therefore it is expected that this high removal was either because equilibrium was not reached within the 24 hour experimental operation period or due to the impact of zeolite, although these reasons were not specified by the authors.

2.13 Chapter summary

As the problems associated with dye wastewater from textile factories have highlighted the importance of treating these effluents before discharge, constructed wetlands have been recommended worldwide as a green alternative, characterised by low cost of construction, operation and maintenance, for the treatment of diverse wastewaters over the past three decades (Scholz & Xu, 2002; Vymazal, 2007; Kadlec & Wallac, 2009; Vymazal, 2011, 2014; Adhikari et al., 2015). *L. minor*-based constructed wetlands have proved to be efficient for treatment of dye wastewater in recent years (Alkhateeb et al., 2005; Sivakumar, 2014; Uysal et al., 2014). These systems are the cheapest compared with other surface flow and subsurface flow wetlands planted with emergent or submerged plants (Kadlec & Wallace, 2009; Chen et al., 2016). Furthermore, the features of *L. minor* within the treatment system compared with other free-floating plants make its utilisation promising. These features include: the plant fully covers the system surface providing the main (aerobic, anaerobic and anoxic) zones necessary for the treatment of most wastewater contaminants (Ozengin & Elmaci, 2007); it is harvested and adapted easily; it grows rapidly (Adhikari et al., 2015); it is able to grow under a wide range of temperatures; it can control algae growth, and prevent bad odours and mosquito growth, etc. (Vymazal et al., 1998; Bejarano, 2005).

No long-term study treating dye wastewater using an *L. minor*-based treatment system was found in the literature. In addition, no previous study assessed the performance of identical systems for treating the same dye wastewaters under both semi-natural and controlled laboratory conditions. Furthermore, no previous work treated the dyes used in this study as either individual dyes or as a mixture using this type of wetlands. Moreover, there is a lack of discussion of basic mechanisms regarding the treatment performance in terms of dye removal and other water quality parameters. The aim of this thesis was to assess in detail the potential of *L. minor*-based constructed wetlands as shallow ponds, using different design variables and operational conditions, and to investigate the treatment performance (as a post treatment stage) of synthetic wastewater contaminated by textile dyes, with the main focus on dye removal under hydroponic conditions as a cost-effective technology for helping low-income developing countries. The treatment performance of algae stabilisation ponds was also investigated, and therefore the term ‘pond’ is considered in this thesis for both *L. minor*-based constructed wetland systems and algae stabilisation ponds as *L. minor* ponds and algae ponds, respectively. Table 2.4 identifies the differences between the research of this thesis and other publications.

Table 2.4 Differences between the research of this thesis and other publications

Reference	Type of dye	Type of plant			System					Conditions			Matrix bed		Operation period		Type of wastewater		
		Emergent	Free-floating	Submerged	VFCW	HFCW	FWS	Pond	Hybrid	Laboratory	Semi-natural	Green-house	With	Without	More than 6 months	Less than 6 months	Synthetic	Aqueous	Real
Pervez et al. (2000)	AB 113, RB 171	√			√					√			√			√	√		
Alkhateeb et al. (2005)	N/V		√					√		√			√	√		√			√
Davies et al. (2005, 2006, 2007)	N/V	√			√						√		√			√		√	
Mbuligwe (2005)	AO7	√				√	√				√		√			√	√		
Keskinkan and Lugal Goksu (2007)	BB41			√			√			√			√			√	√		
Ojstrsek et al. (2007)	RR22, RB5, VR13				√					√			√			√	√		
Bulc and Ojstrsek, (2008)	RB5, DY211, VY46	√			√	√			√		√		√			√	√		√
Nilratnisakorn (2009)	RR141	√			√							√	√			√	√		
Ong et al. (2009a, 2011)	AO7	√			√					√			√			√	√		
Ong et al. (2009b, 2010)	AO7	√			√					√			√		√		√		
Noonpui and Thiravetyan (2011)	RR2, RR120, RR141	√				√						√	√	√		√	√	√	

Table 2.4 (Continued)

Muthunarayanan et al. (2011)	RR198, RB5		√					√			√			√		√		√	
Cumnan and Yimrattanabovorn (2012)	N/V	√			√		√		√		√		√			N/V	√		
Shehzadi et al. (2014)	N/V	√			√					√			√			√			√
Sivakumar (2014)	AO10		√				√			√				N/V		√		√	√
Uysal et al. (2014).	N/V		√					√		√				√		√			√
Yalcuk and Dogdu (2014)	AY 2G	√			√					√			√			√		√	
Chandanshive et al. (2016)	Rubine GFL		√					√		√				√		√		√	
Hussein and Scholz (2017)	AB113 BR46	√			√							√	√		√		√		
This study	AB113 RB198 BR46 DO46		√				√			√	√			√	√		√		

Note: RB171, reactive blue 171; AB113, acid blue 113; AO7, acid orange 7; BB41, basic blue 41; RR22, reactive red 22; RB5, reactive black 5; VR13, vat red 13; RR2, reactive red 2; RR120, reactive red 120; RR141, reactive red 141; RR198, reactive red 198; AY 2G, acid yellow 2G; RB198, reactive blue 198; DO46, direct orange 46; N/V, not available.

Chapter 3
Materials and Methods

3.1 Overview

The materials used and methods applied in this research are presented in this chapter. Section 3.1 provides an overview of the chapter. A description of the materials selected and used in each experiment, including the dyes, the synthetic wastewater, the plants, and other materials is discussed in Section 3.2. Section 3.3 explains the preparation procedure of solutions and inflow wastewater used to supply the treatment systems. Section 3.4 discusses the set-up and operation of each experiment conducted in this research. Plant growth monitoring and measurement, as well as the environmental conditions monitoring is described in Sections 3.5 and 3.6, respectively. Section 3.7 illustrates the sampling and analysing of water quality parameters including the dyes, as well as the plant tissues. Statistical analysis applied for data interpretation is explained in Section 3.8. The research limitations are stated in Section 3.9.

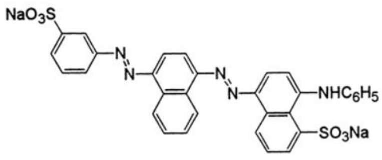
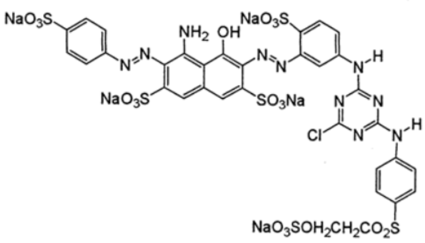
3.2 Materials

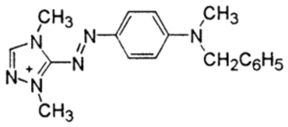
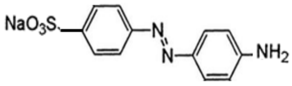
3.2.1 Dyes

Four commercially available azo dyes were used in this study: acid blue 113 (AB113), reactive blue 198 (RB198), basic red 46 (BR46) and direct orange 46 (DO46), which were supplied by Dystar UK Limited (Colne Side Business Park, Huddersfield, United Kingdom), except for AB113 which was obtained from Sigma-Aldrich Company UK Limited (The Old Brickyard, New Road, Gillingham, United Kingdom). The selected dyes are different in their modes of application and, due to the impact of the number of azo groups on treatment performance (Pearce et al., 2003; Saratale et al., 2011), these dyes are also different in the azo bond numbers: AB113 and RB198 comprise two double bonds between nitrogen atoms and belong to the diazo group, and BR46 and DO46 contain one double bond and belong to the monoazo group. Dyes characteristics and structures are shown in Table 3.1. These dyes were used as example azo dyes to assess the removal of xenobiotic organic compounds and were selected for the following reasons:

- They belong to the azo group of dyes, which are widely used in the textile industry (Forgacs et al., 2004; Davies et al., 2009; Daud, 2014) and are associated with a wide range of environmental challenges (Carmen & Daniela, 2012; Sivakumar, 2014), as mentioned previously in detail in Chapter 2, Sections 2.6 and 2.7.
- They are among the commercial dyes used in textile industries (Chen et al., 2003; Kim et al., 2004; Nguyen, 2014; Deniz & Karman, 2011; Sheshdeh et al., 2014; Surana et al., 2010; Mehta et al., 2011; Balarak et al., 2016b).
- There is no previous literature about assessing the removal efficiency of these dyes with simulated shallow pond systems.

Table 3.1 Characteristics and structures of the dyes used

Dye	**Acid Blue 113**	**Reactive Blue 198**
Molecular formula	$C_{32}H_{21}N_5Na_2O_6S_2$	$C_{41}H_{30}C_{l4}N_{14}Na_4O_{14}S_4$
Molecular weight (g/mol)	681.6	1304.8
λmax (nm)	566	625
Chemical class	Diazo (anionic)	Diazo/Oxazine (anionic)
Dye content (%)	Approximately 50	50–80
CAS number	335–05–1	124448–55–1
Chemical structure		

Dye	**Basic Red 46**	**Direct Orange 46**
Molecular formula	$C_{18}H_{21}N_6$	$C_{12}H_{10}N_3NaO_3S$
Molecular weight (g/mol)	321.4	299.2
λmax (nm)	530	421
Chemical class	Monoazo (cationic)	Monoazo (anionic)
Dye content (%)	70–80	60–80
CAS number	1222−69–1	1222–37–6/50814–31–8
Chemical structure		

Note: CAS, chemical abstracts survey registry; λmax, wavelength at maximum absorption; C, carbon; H, hydrogen; N, nitrogen; Na, sodium; O, oxygen; S, sulphur; Cl, chlorine.

3.2.2 Plants

The aquatic macrophytes, *Lemna minor* L., were collected from a small pond close to Cowpe Reservoir, Rossendale, Lancashire (Greater Manchester, United Kingdom), which had no connection with any textile wastewater discharge point. The plants were placed in plastic tanks containing the same pond water (Figure B.1, Appendix B) and moved to the project location, which was Salford University (Greater Manchester, United Kingdom). The plants were washed carefully with dechlorinated tap water before use in the experiments. This plant was collected from the pond twice for use in the first and second experiments. Thereafter, the plant was kept under laboratory conditions to grow for using in the third and fourth experiments. Figure 3.1 shows a picture of *L. minor* macrophyte. *L. minor* is a rather small free-floating macrophyte, of size 1–3 mm and root depth 1–3 cm (Al-Qutob & Nashashibi, 2012), belonging to the Lemnaceae family. This plant is recommended widely for use in wastewater treatments (Vymazal et al., 1998; Bejarano, 2005; Sekomo, 2012; Movafeghi et al., 2013). *L. minor* was selected in this research due to its characteristics, including:

- It is among the faster-growing plants in the world, frequently doubling its biomass in an exponential growth (Vymazal et al., 1998; Bekcan et al., 2009).
- It adapts easily to diverse aquatic conditions in stagnant ponds or slow-flowing streams (Azeez & Sabbar, 2012; Movafeghi et al., 2013; Selvarani et al., 2015), is able to grow over a wide temperature range between 1°C (Vymazal et al., 1998) and 35°C (Ozengin & Elmaci, 2007; Yaseen & Scholz, 2018), and is sensitive to a wide variety of pollutants (Bejarano, 2005; Movafeghi et al., 2013).
- It reduces mosquito growth and controls algae growth in the system, as the water surface is fully covered by the plants (Bejarano, 2005), as well as controlling odour release from the treatment system (Steen et al., 2003).
- It has the ability to accumulate and assimilate pollutants from diverse wastewater (Zimmo, 2003; Zimmo et al., 2005; Bekcan et al., 2009; Vanitha et al., 2013; Selvarani et al., 2015). In addition, it is used for the removal of dyes and heavy metals (Sekomo et al., 2012) from textile wastewater using living (Reema et al., 2011; Sivakumar, 2014) and dried (Balarak et al., 2016a, b; Salman et al., 2016) plants.
- The floating mat of *L. minor* on the wetland surface offers three zones which are normally required in treatment processes: an aerobic zone near to the top layer, an anoxic zone in the middle of the treatment system, and an anaerobic zone within the

system bed (Ozengin & Elmaci, 2007).

- The cost of a treatment system planted with *L. minor* is less compared with other wetlands (Kadlec & Wallace, 2009; Chen et al., 2016), and an *L. minor*-based system is constructed without substrate as the plants float on the top of the system surface and do not require substrate to support their roots (Chen et al., 2016).
- It is harvested easily (Bejarano, 2005; Adhikari et al., 2015), and the harvest biomass can be used as a good source of fodder, because it has high concentrations of protein and low fibre content (Bekcan et al., 2009), and for composting and soil amendments (Adhikari et al., 2015).

Figure 3.1 Picture of Common Duckweed (*Lemna minor* L.)

3.2.3 Wastewater

Two types of prepared wastewater compositions were used in this research, which were as follows:

3.2.3.1 Synthetic wastewater

The TNC complete fertiliser composition selected to prepare synthetic wastewater simulates the real effluents at low contaminant concentrations, it is an aquatic plant nutrient supplied by TNC Limited (Spotland Bridge Mill, Mellor Street, Rochdale, United Kingdom). The corresponding constituents were 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%). Ethylenediaminetetraacetic acid, which is used as a source for copper, iron, manganese and zinc, was also provided by TNC

Complete. The fertiliser (1 ml per 10 l dechlorinated tap water weekly) was applied to simulate synthetic wastewater sufficiently rich in nutrients and trace elements to support plant growth. The characteristics of the prepared synthetic wastewater were lower than the typical range (except for colour and pH values) for most textile effluents (Ghaly et al., 2014), and consequently simulate wastewater already treated in the preliminary and secondary treatment stages to, thereafter, pass through the polishing stage which deals with low concentrated effluents (Reed et al., 1995).

3.2.3.2 Synthetic textile wastewater

The chemicals selected to prepare synthetic textile wastewater simulate the real textile effluents from textile factories, and contained a mixture of organic carbon, nutrients and buffer solution. The corresponding constituents and concentration (mg/l) of the composition, as reported by Ong et al. (2009a, 2010), were as follows: sodium benzoate (C_6H_5COONa, 107.1), sodium acetate (CH_3COONa; 204.9), ammonium nitrate (NH_4NO_3; 176.1), sodium chloride ($NaCl$; 7), magnesium chloride hexahydrate ($MgCl_2.6H_2O$; 3.4), calcium chloride dehydrate ($CaCl_2.2H_2O$; 4) and potassium phosphate dibasic trihydrate ($K_2HPO_4.3H_2O$; 36.7). All chemicals were of analytical grade and applied without any further purification. The supplier was Scientific Laboratory Supplies Limited (Wilford Industrial Estate, Ruddington Lane, Wilford, Nottingham, England, United Kingdom).

This synthetic textile wastewater was utilised to simulate the real textile effluents, and because the contaminations and their corresponding concentrations in wastewater could affect the survival of the plants (Stottmeister et al., 2003; Vymazal, 2009; Shehzadi, 2014) and the microbes in the treatment system. The constituents reported by Ong et al. (2009a, 2010) were appropriate for this research, as their synthetic textile wastewater had been previously examined in a biological treatment experiment using a planted wetland system and most of the characteristics of this wastewater were within the range of characteristics of typical textile wastewater.

3.2.4 Other chemicals

The other analytical grade solutions used were hydrochloric acid solution (1 M HCl) and sodium hydroxide solution (1 M $NaOH$), which were used for pH adjustment as a strong acid and base, respectively. Nitric acid S.G. 1.42 (>68%) was used in heavy metal analysis. Acetonitrile (C_2H_3N) was used for high performance liquid chromatography analysis. All these chemicals were purchased from Scientific Laboratory Supplies Limited (Wilford Industrial Estate, Ruddington Lane, Wilford, Nottingham, England, United Kingdom).

3.2.5 Containers

The experiments were conducted using impermeable plastic containers (length, 33 cm; width, 25.5 cm; depth, 14 cm), to simulate a small part (in terms of width and length) of shallow artificial or real pond system sealed by an impermeable plastic-based liner and located above the ground. The containers were supplied by Home Bargains (Salford, Greater Manchester, United Kingdom).

3.3 Solutions and inflow wastewater preparation

The dyes stock solutions were prepared, for each dye, by dissolving 5 g of a dye in 1 litre of distilled water using a magnetic stirrer (IKA, Hotplate Stirrer, RCT basic safety control IKAMAG, IndoGama.com) for 1 hour to guarantee that all dye powder dissolved, and stored in the dark at 4ºC. The stock solution of synthetic textile wastewater (Section 3.2.3.2) was prepared by mixing all the chemicals with dechlorinated boiled tap water using a magnetic stirrer with a heating plate at 150 rpm for 1 hour to guarantee that all chemicals dissolved. Both stock solutions were prepared under non-sterilised conditions at a laboratory temperature of around 25°C.

The synthetic wastewater containing dye was used as an inflow of the pond systems in the first experiment, and prepared by mixing the solution of each dye separately with dechlorinated tap water and the TNC Complete fertiliser (1 ml: 10 l dechlorinated tap water), providing a 5 mg/l concentration for each dye. However, the synthetic textile wastewater containing dye was used as an inflow of the treatment systems in the second, third and fourth experiments by mixing the dye solutions of each dye separately with the solution of the synthetic textile wastewater and dechlorinated tap water, providing a 10 mg/l concentration for each dye. Diluted synthetic textile wastewater, with a dilution ratio of 1 part synthetic textile wastewater to 24 parts raw water, was prepared for use in the third and fourth experiments. Such a high dilution was required, because all plants in the second experiment eventually died due to the high concentrations of some chemicals in the synthetic textile wastewater.

3.4 Set-up and operation of pond experiments

The four experiment set-ups in this research were designed to cover the study objectives. The outdoor experiment operated for more than one year to cover the system performance over all seasons of the year. However, the indoor experiments operated for at least nine months which was sufficient for monitoring the plant survival during several life cycles, and to enable the collected data to become stable, except for the second experiment which was operated for three months only due to the death of *L. minor* in the pond systems. The contact time used for all experiments was 7 days, which was similar to those commonly published in the literature regarding *L. minor* (Kilic et al., 2010; Movafeghi et al., 2013; Sivakuma, 2014) used for the treatment of textile dyes. In addition, Reema et al. (2011) and Sharma et al. (2016) stated that the potential of *L. minor* for uptake of dyes accelerates with an increase in contact time for all initial dye concentrations. The set-ups were as follows:

3.4.1 First experiment: set-up for impact of design variables and environmental conditions on pond efficiency

The experiment set-up was located in Newton Yard, Salford University (Greater Manchester, United Kingdom), in the North West of England (53° 28′ 59″ N, 2° 17′ 35″ W), and performed using 98 plastic containers simulating shallow ponds. Twenty containers were allocated for each dye, and an additional 18 containers without dyes were also monitored. The containers were manually operated. The set-up of this experiment consisted of three phases. In the first phase between 10 July 2014 and 11 August 2014, plants were collected, as mentioned in Section 3.2.2, and then each container was filled with tap water to the desired level of 6.9 cm depth, which is equivalent to 5 litres. Thereafter, 200 green and washed *L. minor* plants (about 2.600±0.0292 gram) containing four fronds were added to each container, and the system was fed weekly with water and (TNC Complete) fertiliser (1 ml per 10 l dechlorinated tap water). In the second phase between 11 August 2014 and 15 December 2014, the system was kept outside for acclimatisation and monitoring purposes. During this period, the plants grew very well, and some algae started to develop in most systems naturally, From 9 September 2014, dyes at a concentration of 5 mg/l were added to examine plant survival. Figures B.2a and b (Appendix B) show pictures of the first experiment set-up during the second phase. On 15 December 2014, the third (main) phase was started. The experiment was performed by moving 69 containers to the Maxwell

Building (The University of Salford, Greater Manchester, United Kingdom), under controlled laboratory conditions (indoor), and the remaining 29 were moved to the roof of the Newton Building (The University of Salford, Greater Manchester, United Kingdom), under semi-natural uncontrolled (outdoor) conditions. The indoor experiment comprised 14 ponds for each dye (AB113, RB198, BR46, and DO46) and 13 ponds without any dye. The set-up consisted of four treatment groups (design variables). The first group comprised *L. minor* and algae (P1), the second one used only algae (P2), the third group used only *L. minor* (P3) and the fourth group represented the control (unplanted) (P4). The outdoor experiment comprised 6 ponds for each dye (AB113, RB198, BR46, and DO46) and 5 ponds without any dye. The set-up consisted of two treatment groups. The first group comprised *L. minor* (P3) and the second group represented the control (unplanted) (P4). Four replicates for each group planted with *L. minor* and/or algae (P1, P2, and P3), and two replicates for the control group (P4) were used. However, only one replicate was use for control ponds which were without any dye in both indoor and outdoor set-ups.

The group of simulated ponds containing only *L. minor* as well as the control group without any plants were kept free of algae. Considering that *L. minor* grows very rapidly, the surface areas of the ponds were frequently covered preventing sunlight from reaching any traces of algae in the *L. minor* ponds. However, any visual traces of algae were removed manually. This was only the case for *L. minor* ponds at the start of the experiment before *L. minor* got fully established. Furthermore, control ponds were free of any algae from the start. The experiment was conducted in a batch mode. The first dose of synthetic wastewater containing dyes was added to the pond systems after the transfer date on 23 December 2014. All doses had concentrations of 5 mg/l. The corresponding contact time was 7 days. The dosages were added weekly by topping up each pond to the same desired water level equivalent of 5 litres as required to address water loss due to evapotranspiration for both set-ups. For the outdoor set-up, sometimes due to the effect of rainfall, the excess water was removed carefully until the level was equivalent to 4 litres, and 1 litre was added as a new dose in case the water level was higher than the equivalent of 5 litres. Note that removal efficiencies were calculated for loads. All water volumes added or removed were considered in these calculations. The samples were collected by syringes regularly each week (before adding new dosages) to monitor the system performances. The indoor experiment operated till 15 September 2015. However, the outdoor experiment was conducted till 2 February 2016 to cover all seasons of the year. Figure 3.2 is a schematic diagram for the indoor and outdoor experiment set-ups. Table 3.2 shows a summary for the experimental phases. Figures 3.3 and 3.4, show photograph pictures of the first experiment set-up during the third phase under controlled (indoor) and semi-natural (outdoor) condition, respectively.

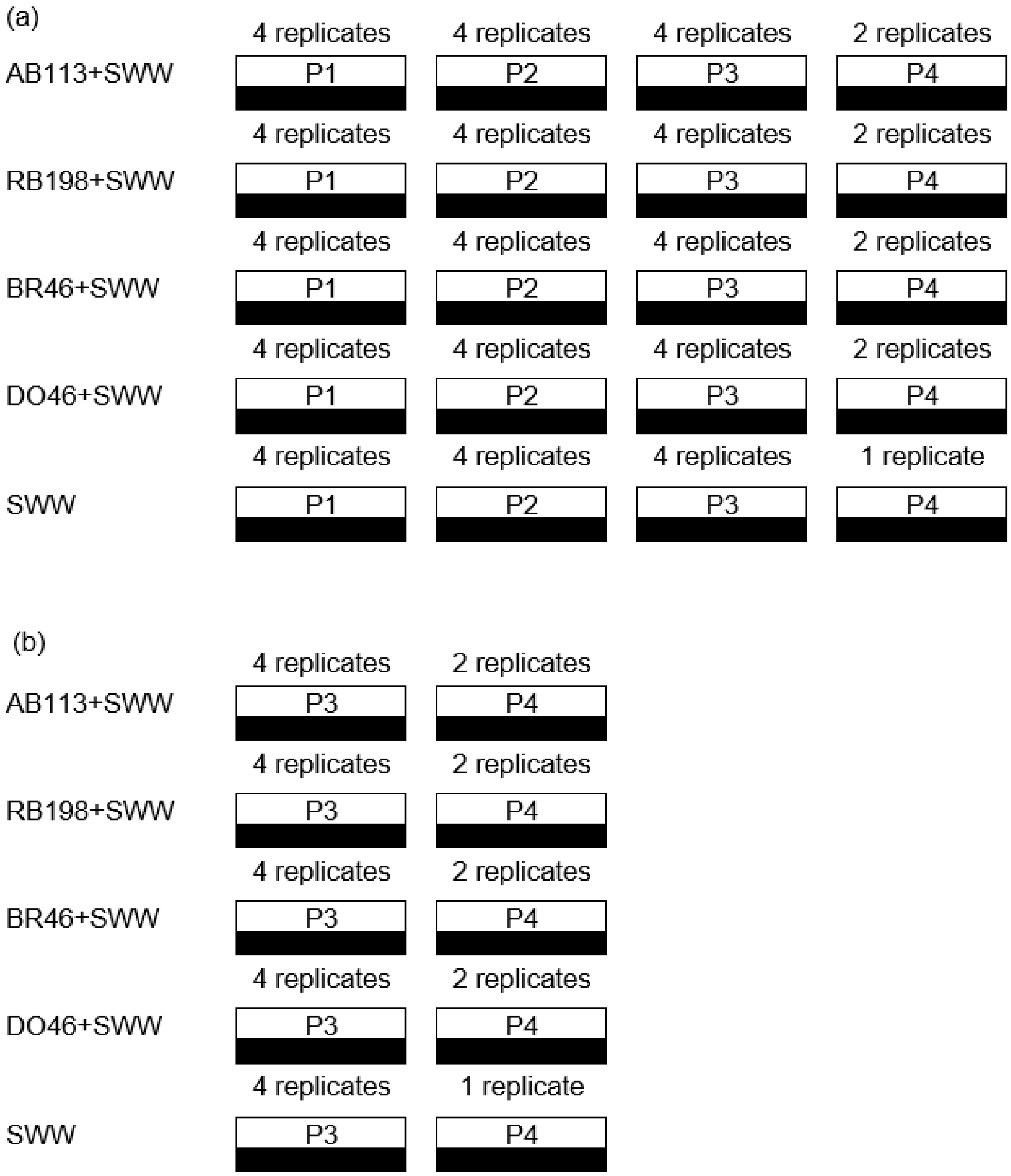


Figure 3.2 Schematic diagram of the first experiment set-up. Note: (a), indoor set-up; (b), outdoor set-up; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds.

Table 3.2 Experimental phases

Phase number	Range of dates	Notes
1	10/07/2014 to 11/08/2014	Set-up period: Plants were added to the containers and fed with fertiliser weekly.
2	11/08/2014 to 15/12/2014	Monitoring period: The plants acclimatised. Algae started to grow as well. Dyes were added to the system and plants were monitored.
3	15/12/2014 to 15/09/2015[a] 15/12/2014 to 02/02/2016[b]	Main experiment: Systems were operated under laboratory and semi-natural conditions (data recorded).

Note: a, indoor set-up; b, outdoor set-up.

Figure 3.3 Indoor set-up of the first experiment of pond systems during the third phase. Note: (1), acid blue 113; (2), reactive blue 198; (3), basic red 46; (4), direct orange 46; (5), without dye.

Figure 3.4 Outdoor set-up of the first experiment of pond systems during the third phase. Note: (1), acid blue 113; (2), reactive blue 198; (3), basic red 46; (4), direct orange 46; (5), without dye.

3.4.2 First experiment: set-up for performance comparison between indoor and outdoor ponds

The set-up phases of the comparison experiment are as discussed in Section 2.4.1. This experiment was performed using 58 containers simulating shallow pond systems. Twenty-nine containers from the indoor ponds of the first experiment are used in comparison with the 29 containers from the outdoor ponds of the first experiment. The indoor set-up was located in the Maxwell Building (The University of Salford, Greater Manchester, United Kingdom), and the outdoor set-up was located on the roof of the Newton Building (The University of Salford, Greater Manchester, United Kingdom). The set-up for each dye under both environmental (operational) conditions was comprised of two treatment groups: the first one comprised *L. minor* (P3; four replicates) and the second one denoted the controls without using *L. minor* (P4; two replicates). Further, ponds without dyes were also included. Operation of pond systems, doses added and contact time are as mentioned in Section 2.4.1. In this experiment, the period of operation for both indoor and outdoor experiments extended till 15 September 2015. Figure 3.5 is a schematic diagram for the comparison of indoor and outdoor experiment set-ups.

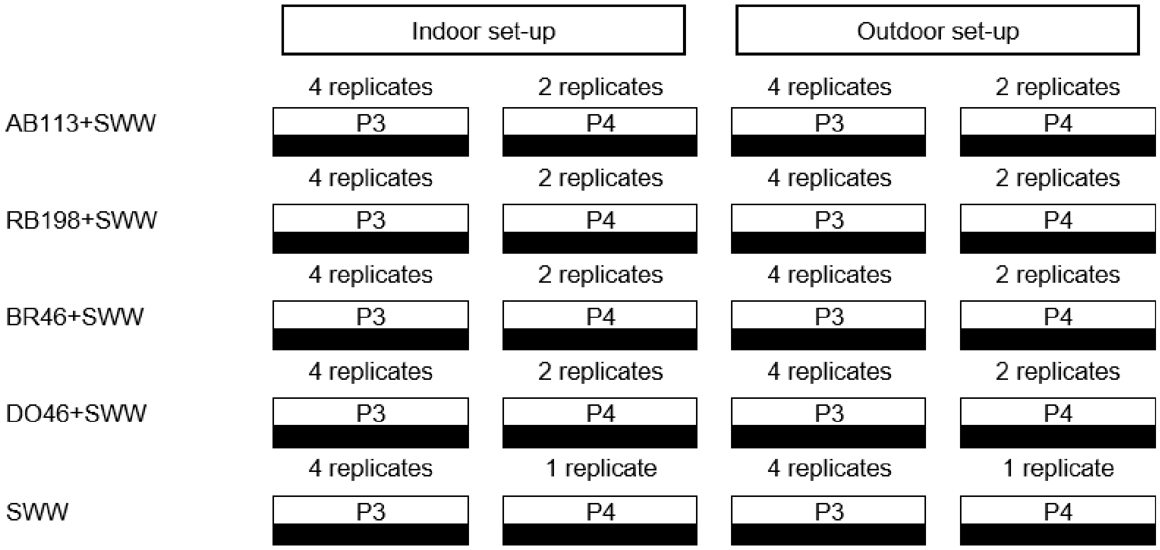


Figure 3.5 Schematic diagram of the comparison experiment set-up. Note: AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; P3, *Lemna minor* L. ponds; P4, control ponds.

3.4.3 Second experiment: set-up for short-term impact of pH on pond efficiency

The second experiment was conducted under controlled laboratory conditions in the Maxwell Building (The University of Salford, Greater Manchester, United Kingdom), between 1 October 2015 and 19 January 2016 using plastic containers. The plants were collected on 1 of October 2015, washed and then kept for acclimatisation under laboratory conditions for 12 days. The experiment comprised 78 shallow pond simulations. Eighteen containers were used for each dye and a further six systems had no dyes. The set-up for each dye (either AB113, RB198, BR46 or DO46) consisted of two groups of treatment; the first group contained *L. minor* (planted ponds P3, 5 and 7) and the second group had no plants and functioned as controls (control ponds P4, 6 and 8). Three replicates were assessed for each group. The system was fed with four synthetic textile wastewaters (one for each dye) containing dye at initial dye concentrations of 10 mg/l. Three different wastewater inflows for each dye were assessed: ponds with wastewater without any hydrolyses at normal pH (inflows of P3 and P4), ponds with wastewater adjusted to pH 9 (inflows of P5 and P6), and ponds with wastewater adjusted to pH 6 (inflows of P7 and P8). The extra six ponds were as follows: three planted ponds (P3) receiving synthetic textile wastewater only without dye and three planted ponds (P3) receiving only tap water for comparison reasons. All pond systems were filled with synthetic textile wastewater containing dye up to a depth of 6.9 cm.

This level equates to a corresponding volume of 5 litres on 13 October 2015 as a first dose. Subsequently, 200 healthy *L. minor* plants (about 2.6±0.03 gram) with approximately equal frond numbers were introduced to each system. After adding the first dose, the system was fed weekly by removing the water solution in each pond (manually using suction pump) until the level was equivalent to three litres (4.4 cm depth) and two additional litres were supplied to keep the water level equivalent to five litres again. This is because the depth equivalent to three litres was enough to keep the root of *L. minor* without any contact with the base of the pond, and to mimic the natural systems when the treated water is discharged to the receiving watercourses and new doses are added. The corresponding contact time was seven days. Figure 3.6 presents the experiment set-up diagram, and Figure 3.7 presents some photographs of the experimental set-up.

The pH values of 6 and 9 were selected, because they equate to common lower and upper pH discharge thresholds (Carmen & Daniela, 2012), and are within the typical range of textile effluents (Ghaly et al., 2014). The selected values are also within the tolerable pH range (4 to 9) for *L. minor* growth according to Movafeghi et al. (2013). Furthermore, Saratale et al. (2011) mentioned that higher colour removal requires pH values between 6 and 10 as optimum. Note that the removal efficiency of dyes in strongly acid or alkaline environments noticeably drops in biological systems used for treatment purposes.

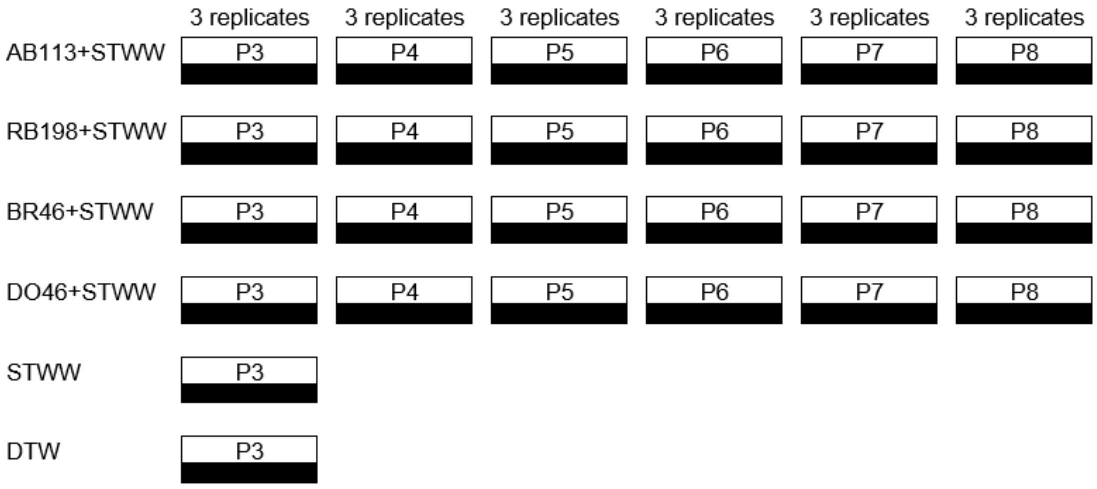


Figure 3.6 Schematic diagram of the second experiment set-up. Note: AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; STWW, synthetic textile wastewater; DTW, dechlorinated tap water; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

Figure 3.7 Set-up of the second experiment of pond systems. Note: (1), acid blue 113; (2), reactive blue 198; (3), basic red 46; (4), direct orange 46; (5), without dye.

3.4.4 Third experiment: set-up for long-term impact of pH on pond efficiency

The experiment was operated under laboratory-controlled conditions in the Maxwell Building (The University of Salford, Greater Manchester, United Kingdom) in two phases (Figure 3.8). The first phase was between 20 January 2016 and 18 October 2016. The set-up consisted of 42 containers simulating shallow ponds. Eighteen containers were applied for each dye and a further six systems were maintained without the presence of dyes. The set-up for each dye (RB198 and BR46) consisted of two groups of treatment; the first group contained *L. minor* (planted ponds P3, 5 and 7) and the second group had no plants and functioned as controls (control ponds P4, 6 and 8). Three replicates were assessed for each group. The system was fed with two diluted synthetic textile wastewaters containing dye at initial dye concentrations of 10 mg/l (one for each dye). Three different wastewater inflows for each dye were assessed (similar to the second experiment): ponds with wastewater without any hydrolyses at normal pH (inflows of P3 and P4), ponds with wastewater adjusted to pH 9 (inflows of P5 and P6), and ponds with wastewater adjusted to pH 6 (inflows of P7 and P8). The extra six ponds were as follows: three planted ponds (P3) receiving synthetic textile wastewater only without dye and three planted ponds (P3) receiving only tap water for comparison reasons. All pond systems were filled with diluted synthetic textile wastewater containing dye up to a depth of 6.9 cm. This level equates to a corresponding volume of 5 litres on 26 January 2016 as a first dose. Subsequently, 200 healthy *L. minor* plants (about 2.6±0.03 gram) with approximately equal frond numbers were introduced to each system. After adding the first dose, the system was fed weekly by removing the water solution in each pond (manually using suction pump) until the level was equivalent to three litres (4.4 cm depth) and two additional litres were supplied to keep the water level equivalent to five litres again, as explained previously (Section 3.4.3). The corresponding contact time was seven days.

The second phase was operated between 18 October 2016 and 30 June 2017. The influent pH was normal. The system comprised 20 containers: 6 pond systems for each dye and a further 8 systems without dye. The set-up for each dye comprised of three groups: the first group contained *L. minor* and algae (P1). The second used only algae (P2). The third was without *L. minor* and/or algae as a control (P4). However, the ponds without dye consisted of two treatment groups: one used *L. minor* and algae, and the other contained only algae. The experiment operated in two replicates. This phase was conducted to evaluate the impact of the algae *Oedogonium*, which grew naturally in all planted ponds during the last three months of the first phase. The first planted pond (P3, first replicate) in the first phase was divided into two ponds and labelled *L. minor* and algae ponds (P1). Then, the second planted pond (P3, second replicate) in the first phase was divided into two ponds after completely removing *L. minor*, and the new system was labelled as algae ponds (P2), where only the algae were kept in the system. Regarding the control ponds, this phase was conducted by continuing to use two control ponds, which were already operated in the first phase (P4, first and second replicate of first phase). Note that the target concentration of each pond was established before adding the first dose of the second phase, which compensated for the water level until a volume equivalent of 5 litres was reached. The same procedure was undertaken for the ponds without dyes. Then, the system was fed weekly as explained earlier in phase one. Note that the volume of water removed or added as well as their corresponding concentrations were considered in the calculations. Table 3.3 provides an overview of the operation. Set-up pictures for the third experiment of both phases are shown in Figure 3.9.

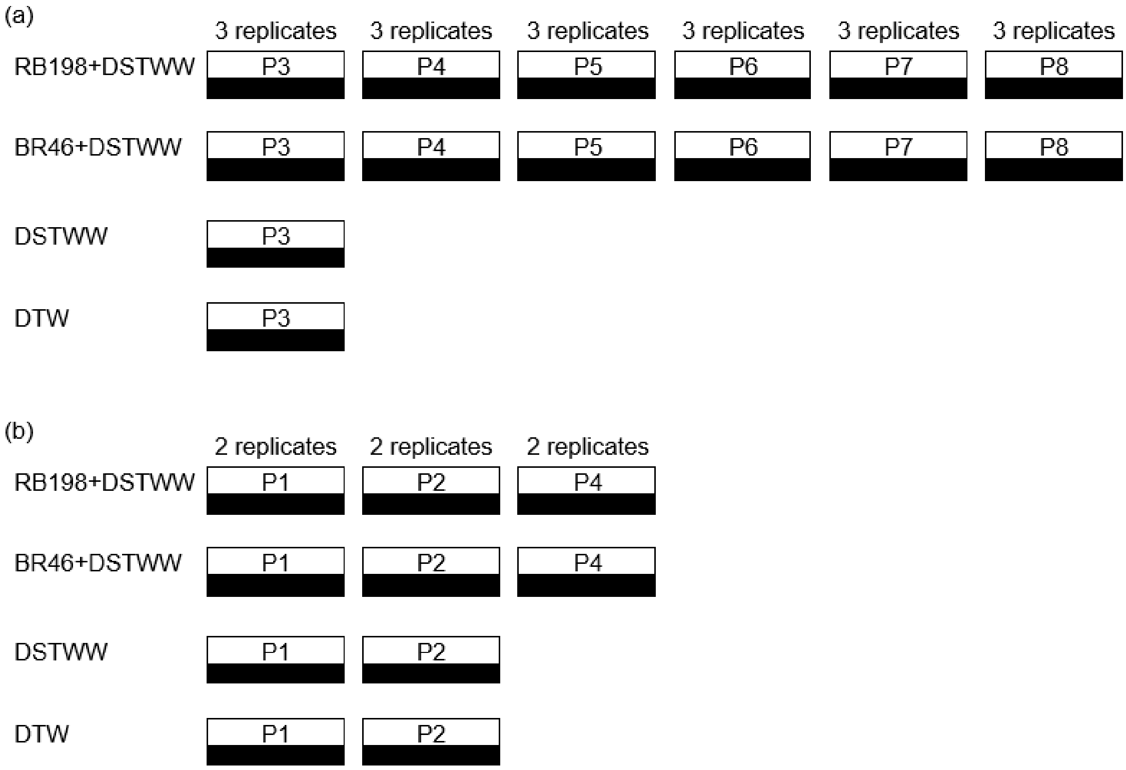


Figure 3.8 Schematic diagram of the third experiment set-up. Note: (a), first phase; (b) second phase. AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water; P1, *Lemna minor* L. and algae ponds receiving inflow at normal pH; P2, algae ponds receiving inflow at normal pH; P3, *Lemna minor* L ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

Table 3.3 Experimental operation periods and details

Phase number	Duration	Notes
1	20/1/2016 to 18/10/2016	Forty-two ponds for the treatment of the dyes RB198 and BR46 at normal, basic and acidic conditions using synthetic textile wastewater after and clean water at a ratio of 1:24.
2	18/10/2016 to 30/6/2017	Twenty ponds for the treatment of the dyes RB198 and BR46 at normal conditions using synthetic textile wastewater and clean water at a ratio of 1:24.

Note: AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46.

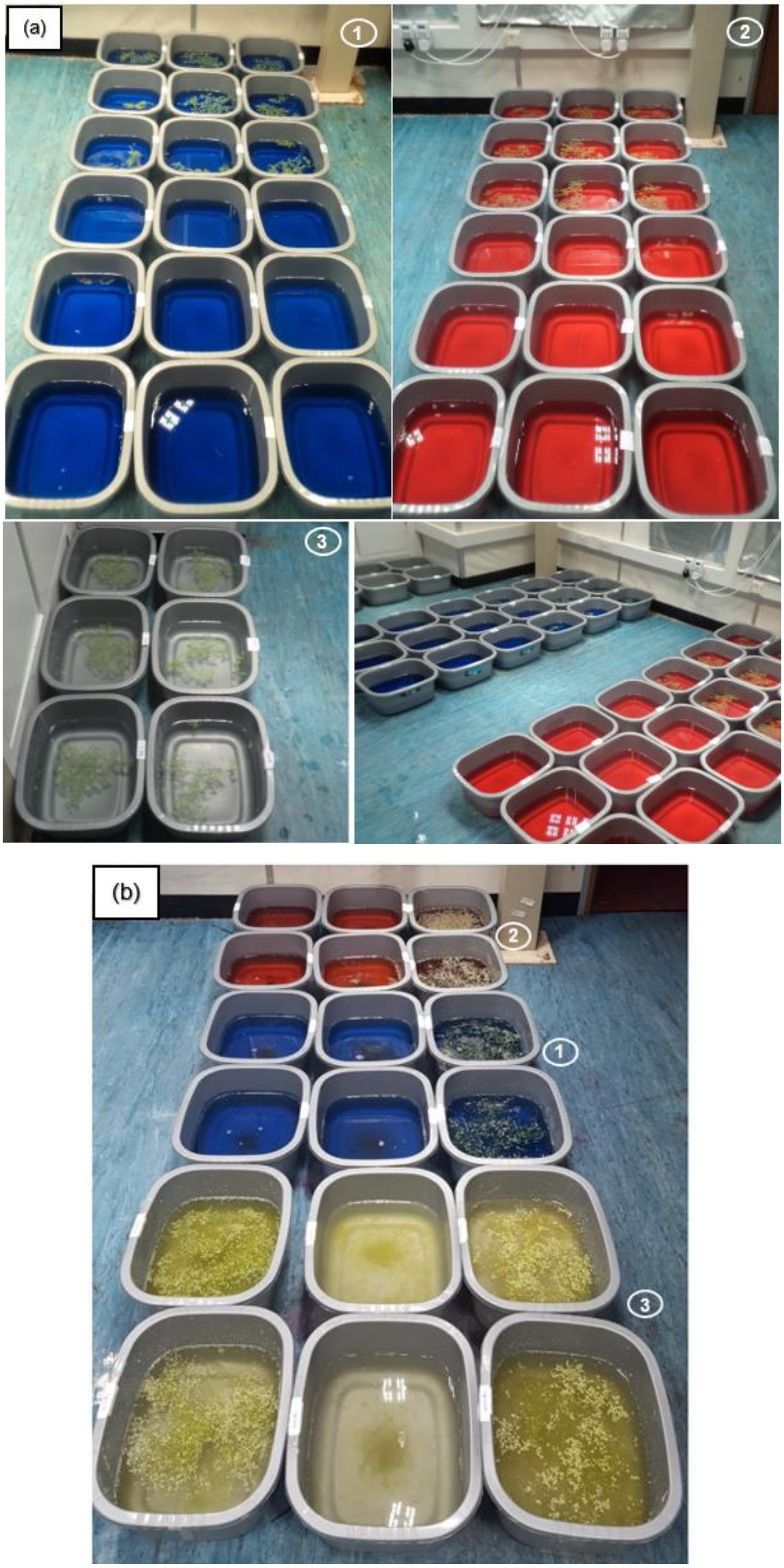


Figure 3.9 Set-up of the third experiment of pond systems. Note: (a), first phase; (b), second phase; (1) reactive blue 198; (2), basic red 46; (3), without dye.

3.4.5 Fourth experiment: set-up for performance of *L. minor* ponds treating wastewater containing dye mixtures

The experiment was operated between 14 October 2016 and 27 June 2017 under controlled laboratory conditions in the Maxwell Building (The University of Salford, Greater Manchester, United Kingdom) using plastic containers. The experiment consisted of 22 shallow pond simulations. Six ponds were used for each mixture of dyes and an extra four containers had no dyes. The set-up for each mixture of dyes involved two treatment sets; one contained *L. minor* (P3; four replicates) and the other group had no plants as controls (P4; two replicates). The remaining four ponds were as follows: two planted ponds receiving synthetic textile wastewater only without dye and two planted ponds receiving only tap water for comparison reasons.

All pond systems were filled with prepared wastewater up to a depth of 6.9 cm, which was equal to 5 litres on 18 October 2016 as a first dose. Subsequently, 200 healthy *L. minor* plants (about 2.6±0.03 gram) with approximately equal frond numbers were introduced to each system. After adding the first dose, the system was fed weekly by removing the water solution in each pond (manually using suction pump) until the level was equivalent to three litres (4.4 cm depth). Two additional litres were supplied to keep the water level equivalent to five litres again, as mentioned in Section 3.4.4. The retention time was seven days. The pond systems were fed by three mixtures, which were prepared by combining the solutions of each dye, according to the required concentrations as mentioned below, together with the solution of the diluted artificial textile wastewater and dechlorinated tap water. Each mixture had a total concentration of 10 mg/l. The initial (inflow) dye concentrations in each mixture were as follows: mixture 1 (2 mg/l of RB198 and 8 mg/l of BR46), mixture 2 (8 mg/l of RB198 and 2 mg/l of BR46) and mixture 3 (5 mg/l of RB198 and 5 mg/l of BR46).

A schematic diagram of the experiment set-up is shown in Figure 3.10. Pictures of the experimental set-up are shown in Figure 3.11.

Figure 3.10 Schematic diagram of the fourth experiment set-up. Note: mixture 1, (8 mg/l of basic red 46 + 2 mg/l reactive blue 198); mixture 2, (2 mg/l of basic red 46 + 8 mg/l reactive blue 198); mixture 3, (5 mg/l of basic red 46 + 5 mg/l reactive blue 198); DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water; P3, *Lemna minor* L. ponds; P4, control ponds.

Figure 3.11 Set-up of the fourth experiment of pond systems. Note: (1), mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); (2), mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); (3), mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198); (4), without dye.

3.5 Plant growth monitoring

In order to assess the impact of dye accumulation on *L. minor*, the plants were monitored and harvested to avoid over-crowding, which inhibits the optimum growth of *L. minor*. The monitored parameters of plants included fresh weight, dry weight, colour, frond numbers and the coverage area. The growth rate of *L. minor* was calculated based on the fresh weight, and the frond number using the image processing technique. The fresh biomass weights were taken after putting the plants on absorbent paper for five minutes. The dry weights were recorded after the plants were dried in an oven at 105ºC for 24 hours (Thierry et al., 2013). The frond numbers and coverage areas were monitored and recorded using the Aletheia Lemna Edition software (not in the public domain) developed by the Pattern Recognition and Image Analysis research lab at the University of Salford (www.primaresearch.org).

Regarding the Aletheia Lemna Edition Software application, the digital images were taken using an OLYMPUS VH 520, a compact digital camera with 14 megapixels on a full liquid crystal display screen. Natural light was used and the flash was switched off. To obtain a good resolution, the camera was in the automatic option mode (denoted "auto") and the distance was 60 cm above the water level. After taking pictures of the buckets containing *L. minor* from the top and of the relevant colours in the Munsell colour chart, the Aletheia Lemna Edition was applied using the steps below:

- Step 1: Opening the combined image of Munsell colour chart and *L. minor* bucket in the Aletheia software, after pasting the relevant picture of the Munsell colour chart together with the picture of each bucket (pond) into Microsoft Paint, then specifying the bucket width and the frond size in Aletheia setting.
- Step 2: Specifying the border of the water surface using a polygon tool.
- Step 3: Modifying the leaf section by setting three parameters: hue (colour tone), saturation (greyness), and brightness/value (HSV colour model). Each point (pixel) of the image has a specific colour defined by those three values, which were set to 20 for each one.
- Step 4: Activating add by colour tool (or remove by colour for editing cases), then clicking on the specific colour in the Munsell colour chart; the Aletheia software will count the percentage of the coverage area in the pond, matching this colour and calculating the total fronds number.
- Step 5: Repeating the last step (step 4) until all leaves (except died/white leaf) in the

pond are highlighted by orange colour Figure C.1 (Appendix C) shows a picture related to each step.

For more details about the Aletheia Lemna Edition software see Appendix C. The relative growth rate (RGR) based on the frond number and the fresh weight were calculated according to Equations (3.1) and (3.2), and the relative frond number (RFN) of the plants was assessed according to Equation (3.3) as an indicator of possible toxicity (Radic et al., 2009; Horvat et al., 2007). The colour of the fronds was determined with the help of the Munsell colour chart (Munsell, 1977) using the above software.

$$\mathrm{RGR\ (per\ day)} = \ln(\mathrm{F.at\ day\ n} - \mathrm{F.at\ day\ 0})/(\mathrm{day\ n} - \mathrm{day\ 0}) \tag{3.1}$$

$$\mathrm{RGR\ (per\ day)} = \ln(\mathrm{FW.at\ day\ n} - \mathrm{FW.at\ day\ 0})/(\mathrm{day\ n} - \mathrm{day\ 0}) \tag{3.2}$$

$$\mathrm{RFN} = (\mathrm{F.at\ day\ n} - \mathrm{F.at\ day\ 0})/\mathrm{F.at\ day\ 0} \tag{3.3}$$

Where, F is the frond number or the fresh weight; day 0 is the initial time; and day n is the finial time; RGR, relative growth rate; RFN, relative frond number.

3.6 Algae identification

Algae were growing naturally in the pond systems during the second phase (under natural weather conditions) of the first experiment, and were detected using a Leica DM750 LED Biological Microscope with ICC50W Camera Module-5.0 Mega Pixel (New York Microscope Co., Lauman Lane, Hicksville, New York, USA). Algal species were identified with the help of standard textbooks Nakadaand and Nozaki (2015), as *Oedogonium*, *Scenedesmus* and *Cosmarium* species (Figure D.1, Appendix D). However, within the third experiment (under laboratory conditions), algae started to appear in the ponds planted with *L. minor*. Filamentous algae were the dominating group in all ponds and belonged to the *Oedogonium* species. These were identified by the Culture Collection of Algae and Protozoa (CCAP), Research services Limited (The Scottish Association for Marine Science, Scottish Marine Institute, Oban, United Kingdom). Pictures of the algae were provided by CCAP, after sending the aqueous samples which were taken from ponds comprising RB198, BR46, and synthetic wastewater without dye (Figure D.2, Appendix D), and these are shown in Figure D.3, Appendix D.

3.7 Environmental monitoring

For the first experiment under controlled laboratory conditions, OSRAM HQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights supplied by OSRAM (North Industrial Road, Foshan, Guangdong, China) and supported by a H4000 Gear Unit, which was provided by Philips (London Road, Croydon, United Kingdom), were used in the laboratory during the third phase. For the other experiments under laboratory conditions, Budmaster Osram Delux (OD) led lamps (204 W) were used as grow lights. They were supplied by Budmaster LED (Unit 4, QHEP, Glan y Wern Road, Colwyn Bay, Gwynedd, Wales, United Kingdom).

Light was controlled by a timer (electronic digital mains Timer Socket Plug-in with LCD Display) purchased from amazon (Amazon.co.uk), simulating the daylight conditions in Salford, with the help of the website Time and Date (2014). The relative humidity and temperature readings of the laboratory environment for all experiments were measured using the Thermometer-Hygrometer-Station supplied by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). For the outdoors experiment (third phase), the temperature readings were measured using the mercurial thermometer at about 10:45 A.M. (United Kingdom time). Light measurements were performed by using the lux meter ATP-DT-1300 for the range between 200 lux and 50000 lux (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, United Kingdom). Readings were taken directly above the plants for both indoor and outdoor experiments.

3.8 Samples analysis

3.8.1 Water quality analysis

In all experiments, a sample was taken from each prepared inflow wastewater, to measure all water quality parameters directly after adding it in the pond systems; these water quality parameters were also measured for the effluent samples, which were collected weekly after a contact time of 7 days. All of the water samples were transported to the laboratory for analyses directly. The procedure for water quality sampling and the appliances used for water quality parameter measurements were performed according to the American Public Health Association standard (APHA) (2005), unless stated otherwise. According to standard laboratory methods, all meters and their sensors were regularly calibrated to be ready for measurements accordingly. Calibration for all equipment used in water quality measurement was performed when necessary, as stated in the user manuals.

Water quality sampling (50 ml) was carried out to monitor the water quality and examine the performance of the treatment system. The spectrophotometer DR 2800 Hach Lange (Hach Lange, Willstätter Strasse, Düsseldorf, Germany, www. hach.com) was used for standard water quality analysis for variables including COD, NH_4–N, NO_3–N, PO_4–P, SS, absorbance and apparent colour. Apparent colour (unit: Pt Co) was measured at 455 nm when dissolved and suspended matter was present. The turbidity was measured with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, The Tintometer Limited, Lovibond House, United Kingdom, www.lovibond.com). The redox potential and pH for all water samples were measured using a VARIO meter (Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany). Dissolved oxygen was measured with a Hach Lange HQ30d dissolved oxygen meter (Hach, Pacific Way, Salford, England, United Kingdom). Both electrical conductivity (EC) and total dissolved solids (TDS) for all water samples were recorded using the METTLER TOLEDO Education Line Conductivity Meter (The Mettler Toledo Limited, Boston Road, Leicester, United Kingdom). Furthermore, elements analyses were conducted according to USEPA (1994) for aqueous samples using a Varian 720-ES Inductively Coupled Plasma-Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK Ltd, Wharfedale Road, Wokingham, Berkshire, UK). The analysis was undertaken to determine nutrient and trace element concentrations. The samples of 15 ml were filtered using a Whatman filter paper (diameter of 0.45 µm), which was purchased from Scientific Laboratory Supplies Limited (Wilford Industrial Estate, Nottingham, United Kingdom), then acidified using nitric acid S.G. 1.42 (> 68), to dissolve any suspended material in order to extract heavy metals and to reduce the pH to below 2, which was required for analysis. Thereafter, the samples preserved in centrifuge tubes at 4ºC.

3.8.2 Dye analysis

The analysis of the single dyes was performed for 12 ml samples, which were filtered through a 0.45 µm pore diameter Whatman filter paper, for assessing the system performance. The filtered water sample was then analysed with a UV–vis spectrophotometer (DR 2800 Hach Lange) having a range between 400 and 800 nm at the maximum absorption wavelengths for each dye for the measurement of the dye absorbance. The maximum absorption wavelength of each dye was determined for an aqueous solution using a scanning

UV–vis spectrophotometer WPABio Wave II (Biochrom, Cambourne Business Park, Cambourne, Cambridge, United Kingdom). The corresponding wavelengths were 566, 625, 530 and 421 nm for AB113, RB198, BR46 and DO46, respectively. The concentrations of dyes were determined depending on standard calibration curves, which were computed for each dye by plotting the linear correlation line between known concentrations (mg/l) versus the absorbance at maximum absorption wavelength. The correlation coefficients were all around 0.99. Dye concentrations in the standard curves ranged from 0 to 20 mg/l using stock solutions (20 mg/l, three replicates), which were prepared by weighing exactly 20 mg for each dye and then dissolving the dye in 1 l of distilled water using magnetic stirrers at 1200 rpm in volumetric flasks. Further dilutions were undertaken to obtain the desired concentrations in steps of 0.25 mg/l between 0 and 20 mg/l for each dye (Noonpui & Thiravetyan, 2011), as shown in Figure 3.12.

Dye mixture assessments, which were applied according to the absorbance values, were performed after filtering the water samples (12 ml each) using a 0.45 μm diameter Whatman filter paper. The filtered liquid was subsequently analysed with a UV–vis spectrophotometer (DR 2800 Hach Lange) at the maximum absorption wavelengths for each mixture of dye as discussed by Tony et al. (2009) for the measurement of the absorbance concerning the mixed dye as a new dye or new solution. The corresponding maximum absorption wavelengths were 528 nm, 524, and 524 nm for the first, second, and third mixture, respectively.

The ultraviolet visible scans of the aqueous samples were carried out using a Varian Cary 300 UV–vis spectrophotometer (www.varianinc.com) with a range between 200 and 800 nm. A quartz cuvette of 10 µl was applied to analyse the samples.

High performance liquid chromatography (HPLC) as a biotransformation analysis was used for confirming organic molecule (dyes and their metabolites) separation by monitoring the peak area, to find out if it disappeared or shifted toward lower retention time. The tests were applied using Agilent 1260 on a HiChrom excel C18 column (4.3 mm to 250 mm; 1.7 µm particles), which was supplied by Hichrom Limited (The Markham Centre, Reading, Berks, United Kingdom). Acetonitrile (70%) and water (30%) were used at a flow rate of 1.3 l/min. The UV detector was kept at the maximum wavelength for each individual dye (first, second, and third experiments) and for each mixture (fourth experiment). Aqueous samples of 20 µl were injected manually into the injector port using a microliter syringe, which was purchased from Hichrom Limited.

A gas chromatography-mass spectrometry (GC-MS) test was conducted after sending the aqueous samples (one litre each in glass bottles) to Concept Life Sciences Analytical & Development Services Limited (Concept Life Sciences, Hadfield House, Hadfield Street, Cornbrook, Manchester, England, United Kingdom) for external analysis. Figure D.4 (Appendix D) shows some photographs of the samples sent.

The dye and other pollutant removals were calculated according to Equations (3.4) to (3.6).

$$\mathrm{R}(\%) = ((\text{Load influent} - \text{load effluent})/\text{Load influent}) \times 100 \tag{3.4}$$

$$\text{Load influent}\ (\mathrm{mg/m^2\ week}) = (\mathrm{V1} \times \mathrm{C1} + \mathrm{V2} \times \mathrm{C2})/(\mathrm{SA} \times \mathrm{HRT}) \tag{3.5}$$

$$\text{Load effluent}\ (\mathrm{mg/m^2\ week}) = (\mathrm{V3} \times \mathrm{C3})/(\mathrm{SA} \times \mathrm{HRT}) \tag{3.6}$$

Where, R: Removal efficiency in week n (%); V1: volume of water remained in the ponds in the end of week n-1, after evaporation and (removing the excess water due to the rain to keep the remaining water level at 4 litres for the outdoor run only, or removing the extra water to keep the remaining water level at 3 litres for the second, third and fourth experiments) (l); C1: concentration of last grab sample in the end of week n-1 (mg/l); V2: volume of the dose added in the beginning of week n (l); C2: concentration of the dose added in the beginning of week n (mg/l); V3: volume of water remaining in the pond at the end of week n, (before removing any water) (l); C3: concentration of grab sample at the end of week n (mg/l); SA, surface area of pond (cm^2); HRT, hydraulic retention time (contact time; week). Note that the dye mixture removal in the fourth experiment was performed using the absorbance (Abs) values Abs1, Abs2, and Abs3 instead of C1, C2, and C3, respectively.

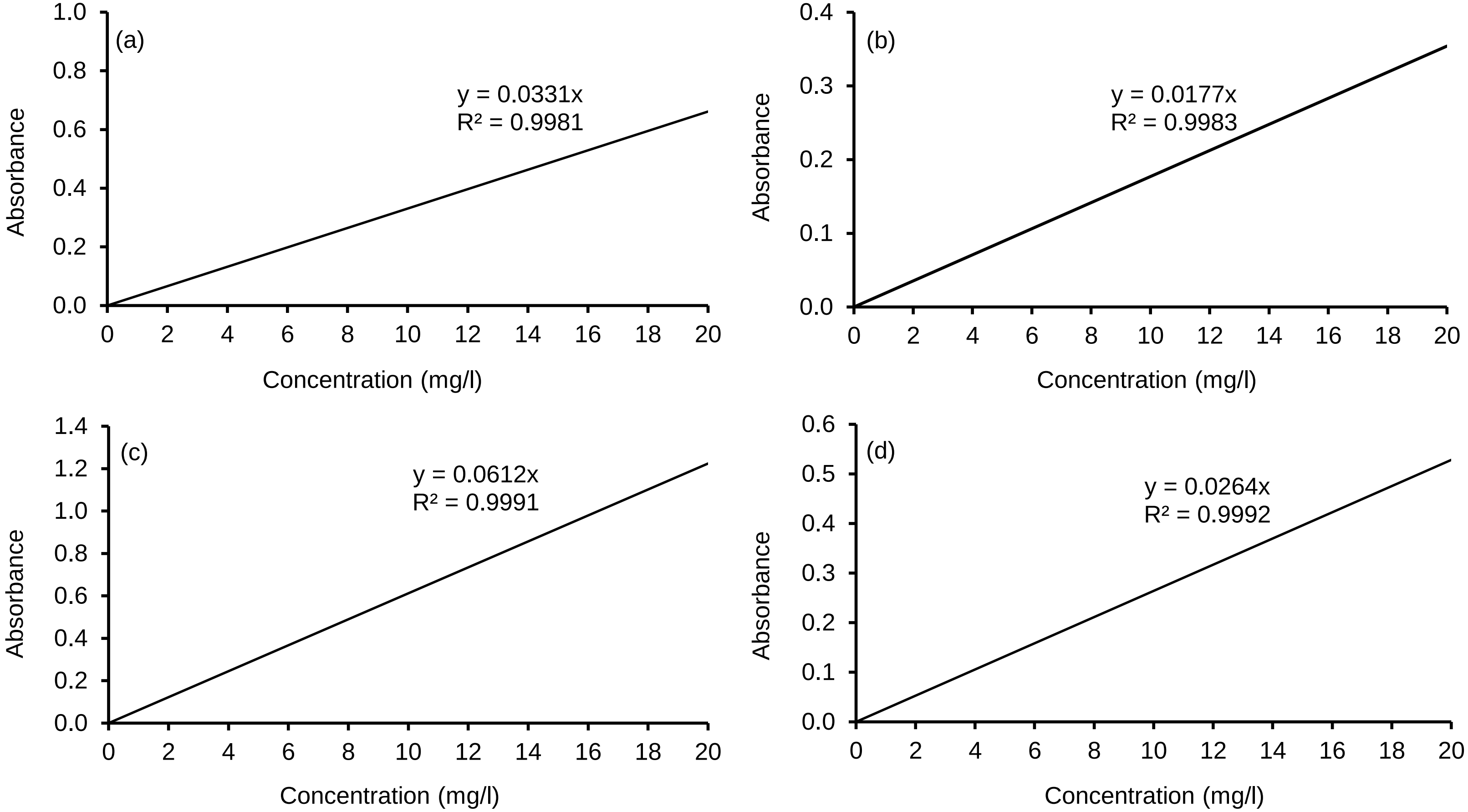


Figure 3.12 Standard calibration curve of dyes. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46.

3.8.3 Plant tissue analysis

Element contents within plant tissues were analysed as mentioned by Plank (1992) using a Varian 720-ES Inductively Coupled Plasma-Optical Emission Spectrometer. The fresh plants and the dried ones, located on the pond sides, were harvested when the plants fully covered the pond surface, as well as when the experiment was finished, and then dried in the oven at105ºC for one day (Sekomo et al., 2012). The dried plant was ground and sieved through a 2-mm diameter sieve. After that, around 0.2 g of each sample was added to a microwave tube with 10 ml of nitric acid. The samples plus an extra blank without plants were digested in the microwave (CEM Mars Xpress Microwave Digestion Oven) for 90 minutes until they were cooled-down. The samples were purified applying a Whatman filter paper (diameter of 0.45 μm) and diluted by deionised water up to volumes of 25 ml into volumetric flasks. The stock digest solutions were stored at 4ºC. Finally, further dilutions were conducted by adding 2.5 ml of the stock digest samples to 7.5 ml of deionised water in 12 ml centrifugal tubes, making in total four dilutions of the stored digest solution that was ready for analyses. The capacity of *L. minor* for phytoremediation of heavy metals was calculated according to the bioaccumulation factor (BCF), which was calculated according to Equation 3.7, as mentioned by Hegazy et al. (2011) and Sukumaran (2013).

$$\mathrm{BCF} = \frac{\text{Element concentration in plant tissue (mg/kg)}}{\text{Element concentration in the inflow water (mg/l)}} \tag{3.7}$$

3.9 Data analysis

Microsoft Excel (www.microsoft.com) was used for all standard analysis of data, unless stated otherwise. The IBM SPSS Statistics Version 22 (www.ibm.com) was used to assess if data were normally distributed or not, using the Shapiro-Wilk test, due to the data variability. Then, it was applied to calculate the non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests) for the non-normally distributed variables and the parametric tests (one-way and univariate analysis of variance (ANOVA) using the Tukey HSD post hoc multiple comparison test, and the t-test) for computing the normally dependent variables. The non-parametric tests were applied for the data which were still non-normally distributed even after transformations were applied with transformers such as arc sine, square root, log, ect. The Person and Spearman tests were used to calculate the correlation coefficients of different parametric and non-parametric variables, respectively.

3.10 Experimental research limitations

The small-scale experiments of wetland systems used in this research were very small compared with the large-scale treatment systems used in the field, and do not take into account the impact of numerous animals and other micro-organisms that inhabit very large-scale constructed wetland systems. However, previous findings based on similar studies using pot-scale (Nilratnisakorn et al., 2009) or lab-scale (Bulc & Ojstrsek, 2008) wetlands confirmed that the results achieved were appropriate at the field scale and therefore have been fully accepted by the scientific public.

The second, third and fourth experimental wetlands, which were studied under controlled conditions, may not correspond with other wetlands operated under natural conditions at the field scale due to variable environmental factors. However, the results of the first experimental comparison, which operated under both semi-natural and controlled conditions in this research, provides insight on the impacts of environmental factors and could serve as a guide in designing and up-scaling field scale wetlands operated in different climate conditions, especially given that the results of a system operated under a semi-natural environment may strongly mimic the operations under typical field (natural) conditions.

Despite the insufficient space in the lab, the replication of planted or unplanted wetland systems containing dyes was achieved. In addition, other parameters, such as the impact of other dye concentration was considered in this research, although the space was limited, by operating further experiments. Moreover, despite the limitations in financial support, some parameters linked with expensive reagents were tested monthly. And in addition, some external analysis, such as GC-MS analysis for aromatic amine detection, and algae species identification, were taken in this research for some samples. Finally, although algae concentrations in treatment systems were not taken into account, the effect of this parameter was not the main aim of the research compared with the *L. minor*.

Chapter 4

Impact of Design Variables and Environmental Conditions on System Efficiency

4.1 Overview

The overall results and discussions of the first experiment, for treating synthetic wastewater (SWW) containing dyes, are documented in this chapter. The set-up and operational processes of this experiment are explained in Chapter 3 (Sections 3.4.1 and 3.4.2). Section 4.1 provides an overview of the chapter. Sections 4.2, 4.3 and 4.4 present the overall results of pond performance using different design variables for treating synthetic wastewater containing 5 mg/l of dyes under indoor and outdoor conditions, as well as the comparison between them, respectively. The chapter summary is presented in Section 4.5.

4.2 Performance of *L. minor* and/or algae ponds under controlled conditions

4.2.1 Inflow water quality parameters

The inflow water quality parameters, including the elements detected through the Coupled Plasma-Optical Emission (ICP-OES) of the prepared SWW, which include fertiliser and textile dye are shown in Table 4.1 and Figure 4.1. These parameters compared with the typical characteristics of textile wastewater (Upadhye et al., 2012). The pH and the colour values were within the typical range of 6 to 10 and 50 to 2500 Pt Co, respectively. Also, zinc, iron, boron and copper values were within the typical range of textile wastewater discharge of less than 10 mg/l (Ghaly et al., 2014). The dye inflow concentration was 5 mg/l lower than the typical range, but it compared well with concentrations used in the literature for the treatment of brilliant blue R (Kilic et al., 2010), basic red 46 (Movafeghi et al., 2013) and acid blue 92 (Khataee et al., 2012). In addition, because duckweed systems are used as a polishing treatment stage to treat effluents which pass from preliminary or secondary treatment stages, they are associated with concentrations lower than the textile effluents.

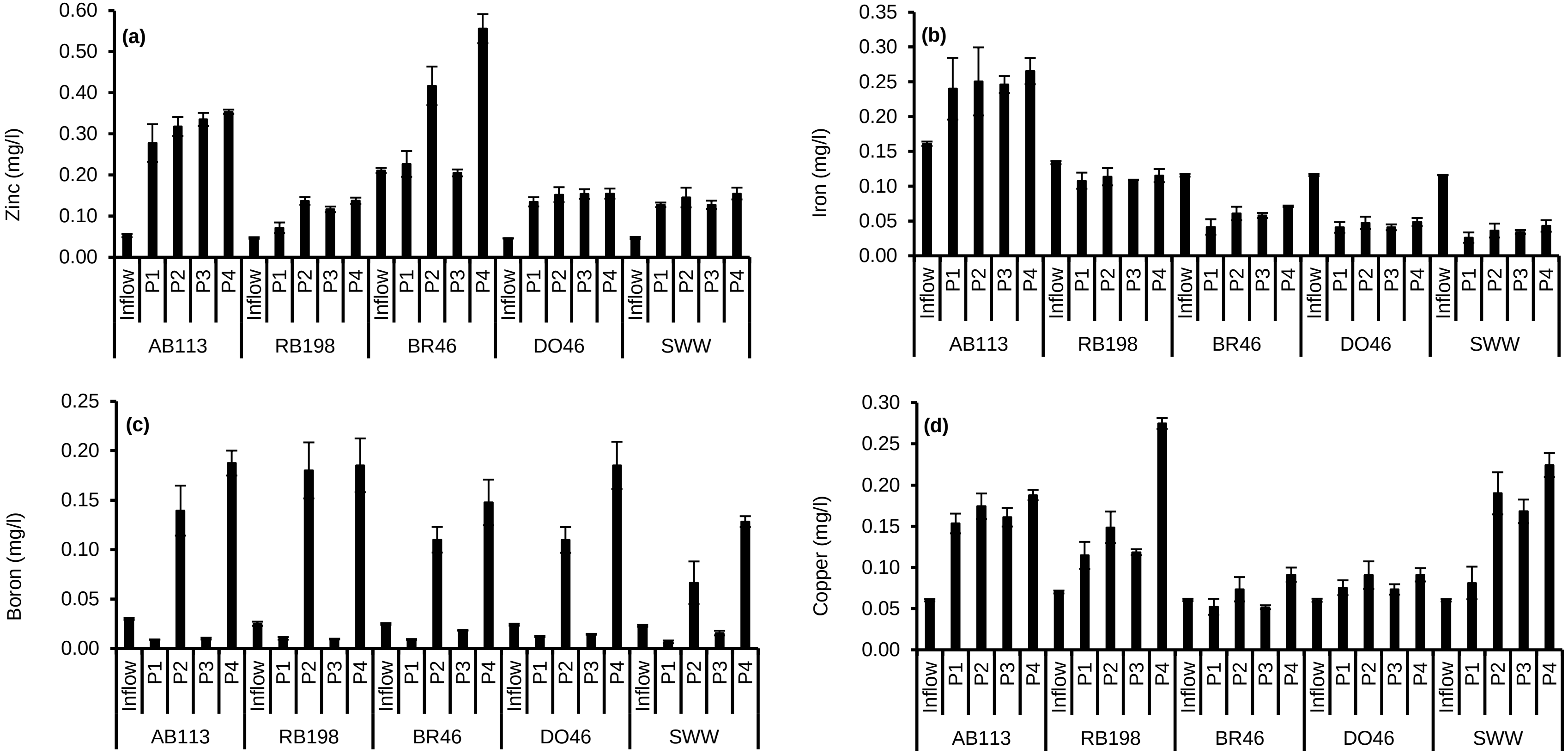
Table 4.1 Inflow water quality parameters for each system between 15 December 2014 and 15 September 2015

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue 113 +Synthetic wastewater						
Dye concentration	mg/l	5	0.26	4.5	5.6	38
pH	-	7.3	0.11	7.1	7.5	38
Redox	mV	-25.6	6.76	-37	-14	38
Dissolved oxygen	mg/l	9.6	0.48	8.6	10.8	38
Electrical conductivity	µS/cm	115.9	4.49	108	125	38
Total dissolved solids	mg/l	57.95	2.245	54	62.5	38
Suspended solids	mg/l	3.6	1.06	1	6	38
Turbidity	NTU	1.4	0.4	0.3	2.1	38
Colour	Pt Co	447.9	30.13	416	484	10
Chemical oxygen demand	mg/l	25.2	0.59	24.3	26	8
Reactive blue 198 + Synthetic wastewater						
Dye concentration	mg/l	5.1	0.39	4.5	5.8	38
pH	-	7.4	0.09	7.2	7.5	38
Redox	mV	-33.9	5.52	-39	-26	38
Dissolved oxygen	mg/l	9.3	0.09	9	9.4	38
Electrical conductivity	µS/cm	121.8	5.17	111	133	38
Total dissolved solids	mg/l	60.9	2.585	55.5	66.5	38
Suspended solids	mg/l	5.5	0.82	4	7	38
Turbidity	NTU	1.9	0.33	1.2	2.6	38
Colour	Pt Co	171.2	35.43	123	204	10
Chemical oxygen demand	mg/l	9.3	0.32	9.2	10.1	8
Basic red 64 + Synthetic wastewater						
Dye concentration	mg/l	5	0.32	4.5	5.7	38
pH	-	7.3	0.07	7.2	7.4	38
Redox	mV	-27.8	3.86	-35	-22	38
Dissolved oxygen	mg/l	9.3	0.22	8.9	9.7	38
Electrical conductivity	µS/cm	115.1	5.23	104	126.9	38
Total dissolved solids	mg/l	57.55	2.615	52	63.45	38
Suspended solids	mg/l	2.9	0.58	2	4	38
Turbidity	NTU	2.4	0.3	1.9	2.8	38
Colour	Pt Co	408.3	11.79	393	424	10
Chemical oxygen demand	mg/l	13.3	0.79	12.2	14.8	8
Direct orange 46 + Synthetic wastewater						
Dye concentration	mg/l	5.1	0.43	4.4	5.7	38
pH	-	7.4	0.1	7.2	7.5	38
Redox	mV	-31.9	5.32	-38	-25	38
Dissolved oxygen	mg/l	9.4	0.13	9	9.5	38
Electrical conductivity	µS/cm	116	2.53	111	120	38
Total dissolved solids	mg/l	58	1.265	55.5	60	38
Suspended solids	mg/l	3.3	0.71	2	4	38
Turbidity	NTU	1.5	0.26	1.2	1.9	38
Colour	Pt Co	676.6	35.12	626	722	10
Chemical oxygen demand	mg/l	14.4	1.02	13.8	16.7	8

Table 4.1 (Continued)

Synthetic wastewater						
pH	-	7.3	0.08	7.16	7.3	9
Redox	mV	-26.2	4.78	-32	-20	9
Dissolved oxygen	mg/l	9.1	0.28	8.9	9.7	9
Electrical conductivity	μS/cm	85.8	1.11	83	86.6	9
Total dissolved solids	mg/l	42.9	0.555	41.5	43.3	9
Suspended solids	mg/l	1.8	1.66	0	3	9
Turbidity	NTU	3.5	0.55	2.9	4.1	9
Colour	Pt Co	3	0.67	2	4	9
Chemical oxygen demand	mg/l	4.2	0.27	3.6	4.4	8

Note: NTU, nephelometric turbidity unit.


(a)
Zinc (mg/l)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
(b)
Iron (mg/l)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
(c)
Boron (mg/l)
0.25
0.20
0.15
0.10
0.05
0.00
(d)
Copper (mg/l)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Inflow
P1
P2
P3
P4
AB113
RB198
BR46
DO46
SWW

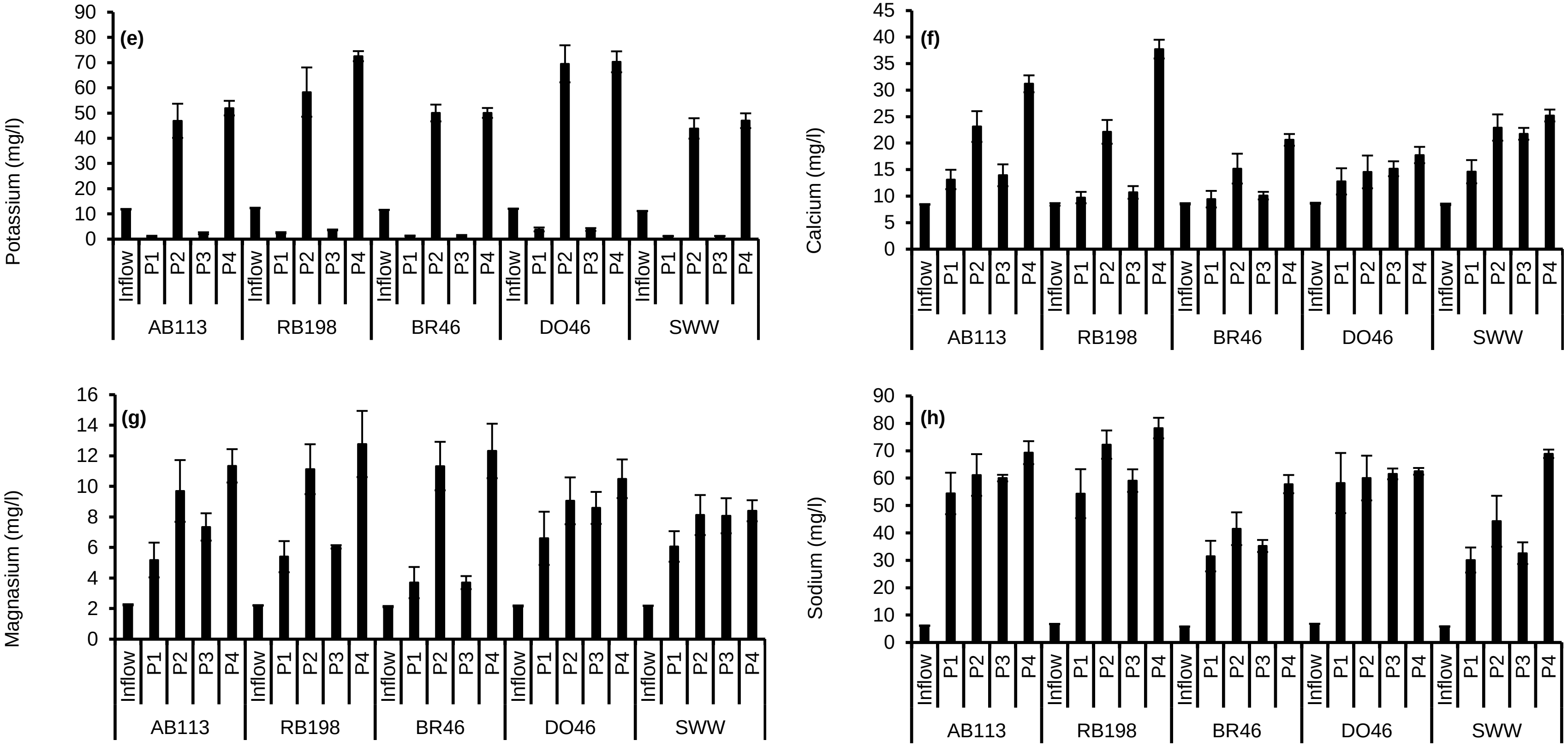


Figure 4.1 Mean inflow and outflow concentrations of the detected trace elements during the experiment between 15 December 2014 and 15 September 2015. Note: (a), zinc; (b), iron; (c), boron; (d), copper; (e), potassium; (f), calcium; (g), magnesium; (h), sodium; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds; AB113, acid blue113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dye.

4.2.2 Treatment performance

4.2.2.1 Dye and apparent colour

Generally, the two parameters dye and apparent colour are used to assess the potential of the pond for removing dyes and consequently producing a colourless product. In general, very low dye removal efficiency (Table 4.2) was observed for acid blue 113 (AB113), reactive blue 198 (RB198) and direct orange (DO46). Statistically, no difference (Table 4.3) was found for the mean values of dye removal efficiency among the design variables for ponds containing A113, RB198 and DO46. This indicates that the biological treatment method of using shallow ponds with *L. minor* and/or algae, which is associated with high dissolved oxygen (DO) values and the presence of anoxic conditions, as discussed later, inhibits the removal of dye molecules. However, pond systems treating basic red 46 (BR46) show mean removal efficiencies, which are highest for *L. minor* ponds (69%) followed by *L. minor* and algae ponds (67%), and then algae ponds (53%). The lowest mean removal (31%) is linked to the control ponds. These results indicate that the impact of *L. minor* and algae on BR46 removal are 38% and 22%, respectively. These data resemble outcomes using submerged plants for handling basic blue 41 (Keskinkan & Goksu, 2007). Statistically, no difference in mean BR46 removal was found between ponds containing *L. minor*, and *L. minor* and algae (Table 4.3), which indicates that the presence of a limited amount of algae does not affect the removal in *L. minor* and algae ponds. This is because *L. minor* covers the surface area, preventing light penetration, and consequently inhibits the growth of algae biomass in the system (Sekomo et al., 2012). In addition, ponds with *L. minor* as well as ponds with *L. minor* and algae had significantly higher mean removal efficiencies than those without *L. minor* (algae and control ponds), which confirmed the impact of *L. minor* in removing BR46.

The longitudinal profile of the dye removal is shown in Figure 4.2. The maximum and minimum removal were as follows: 26% and -4% for *L. minor* and algae ponds, respectively, and 39% and -5% in algae ponds, respectively, 26% and -6% for *L. minor* ponds in that order, and 29% and 11% in control ponds in that order for the treatment of AB113 (Figure 4.2a). However, these values were 21% and 5%, 29% and 1%, 28% and 3%, and 34% and -10% for *L. minor* and algae ponds, algae ponds, *L. minor* ponds, and control ponds, respectively, for the treatment of RB198 (Figure 4.2b). For BR46 (Figure 4.2c), the maximum and minimum removals were as follows: 85% and 26% in *L. minor* and algae ponds, 84% and 19% in algae ponds, 88% and 24% in *L. minor* ponds, and 60% and 3% in control ponds. The DO46 removal ranged between 26% and -6%, 22% and -5 %, 24% and -9%, and 16% and -7% in *L. minor* and algae ponds, algae ponds, *L. minor* ponds, and control ponds, respectively (Figure 4.2d). Negative dye removal efficiencies can be explained by water surface evaporation and pond edge effects. Multiple comparisons of removal efficiencies among dyes showed no difference between AB113 and RB198. However, BR46 was significantly higher and DO46 was significantly lower than other dyes (Table 4.4), and the corresponding dye values were ranked as follows: BR46 > RB198 > AB113 > DO46. This can be explained by the simple structure and small molecular weight of BR46. These findings match related ones by Noonpui and Thiravetyan (2011). Furthermore, the neutral pH in the system was suitable for BR46 uptake, due to the electrostatic attraction between the sorbent surface, which is negatively charged, and the dye cation, that has a positive charge (Reema et al., 2011). Similar results have been reported by Movafeghi et al. (2013). Moreover, the absence of sulpho groups in BR46 contributes to a good level of degradation during biological treatment methods (Pearce et al., 2003). The performance of treatment systems was ranked as follows: P3 > P1 > P2 > P4.

The treatment performance in terms of the outflow dye concentrations (Table 4.5) showed that the mean values of the outflow AB113 concentrations were not significantly different (Table 4.3) among the design variables. The ranges were between 6.3 mg/l for the control ponds and 7.6 mg/l for ponds containing *L. minor*. The outflow AB113 concentrations increased gradually over time during all experimental periods, which reflects the low removal efficiency and the accumulation of this dye in the systems due to the weekly doses, except for the control ponds, where they decreased in August and then were sharply enhanced at the end of the experiment (Figure 4.3a). However, for the other dyes, the outflow dye concentrations were higher within the control ponds than the remaining ponds (Figure 4.3b, c and d). No significant difference (Table 4.3) was found between combined *L. minor* and algae ponds and ponds planted by *L. minor* for all dyes, which indicates that the presence of algae does not affect the dye outflow concentrations. The outflow concentrations from algae ponds and control ponds were significantly higher than other design variables in ponds containing BR46, which indicates that the presence of *L. minor* reduces BR46 concentration. The lowest standard deviations were linked to BR46, because the outflow concentrations were stable (Figure 4.3c), except for those of the control ponds, which fluctuated highly.

High performance liquid chromatography (HPLC) results showed a peak at a retention time of 1.711 min for the inflow AB113 (Appendix E, Figure E.1a). However, the outflow samples showed a peak at 1.584, 1.595, 1.606, and 1.963 min for P1, P2, P3, and P4, respectively (Appendix E, Figure E.2). Although peaks changed, the very low dye removal efficiencies (Table 4.2) and the highly coloured outflow samples for all (planted and unplanted) ponds containing AB113 indicated that the dye removal was by microbial activities only. The inflow RB198 sample displayed a peak at 1.505 min (Appendix E, Figure E.1b). Although, the peak for the inflow RB198 sample after treatment shifted to 1.61, 1.607, 1.602, and 1.6 min for P1, P2, P3, and P4, respectively (Appendix E, Figure E.3), the low removal values (Table 4.2) were not achieved by the transformation mechanism as the outflow samples were highly coloured. The inflow BR46 showed peaks at retention times of 1.488, 1.693, 2.569 and 2.405 min (Appendix E, Figure E.1c). However, the outflow samples showed a peak at 2.053, 1.61, 2.089, and 2.114 min for P1, P2, P3, and P4, respectively (Appendix E, Figure E.4). The disappearance of most of the inflow peaks and appearance of one new peak for each treatment system linked with the high percentage of BR46 removal (Table 4.2) confirms the BR46 elimination by adsorption and transformation by plant and microbes for ponds containing *L. minor*, and by bio-sorption and may be the biotransformation process for algae ponds, and control ponds (Khandare et al., 2011; Kabra et al., 2012; Tahir et al., 2016). The inflow DO46 showed a peak at 1.497 min (Appendix E, Figure E.1d). However, the outflow samples (Appendix E, Figure E.5) showed peaks at 1.607, 1.606, 1.607, and 1.606 min for P1, P2, P3, and P4, respectively. In some cases when the removal is very low (AB113, RB198 and DO46), peak changes may be due to the interaction between the dye and the synthetic wastewater.

Colour mean outflow values were higher in control and algae ponds (Table 4.5), which reflects the low dye removal efficiency in these ponds. However, the lowest mean values were in *L. minor* and, *L. minor* and algae ponds for all types of dye. The colour mean outflow values in ponds containing dechlorinated tap water and fertiliser (synthetic wastewater) were ranked as follows: P4 > P1 > P2 > P3. Nevertheless, no differences among design variables for AB113, RB198 and DO46 were noted. In contrast, ponds with *L. minor* only and control ponds were significantly different than other ponds for BR46 (Table 4.3). Correlation analysis results indicate that the colour was significantly ($p < 0.01$) positively correlated with the dye ($r = 0.703$, $p = 0.000$) and chemical oxygen demand ($r = 0.638$, $p = 0.000$) concentrations. Inflow and outflow sample pictures are shown in Appendix F, Figure F.1.

Table 4.2 Dye and chemical oxygen demand removals for each system between 15 December 2014 and 15 September 2015

Parameter	Load in (mg m^{-2} $week^{-1}$)	Standard deviation	Maximum	Minimum	Load out (mg m^{-2} $week^{-1}$)	Standard deviation	Maximum	Minimum	Removal (%)
Acid blue 113 + Synthetic wastewater									
Dye									
P1	399.1	182.7	629.3	58.5	366.2	179.2	591.8	43.9	8
P2	372.1	202.6	650.7	55.0	340.9	199.0	595.2	45.6	8
P3	421.7	194.4	687.8	58.8	388.3	189.4	612.4	43.3	8
P4	353.0	150.7	565.8	68.4	318.8	135.2	505.4	62.2	10
Chemical oxygen demand									
P1	7209.2	914.0	8504.3	6159.2	7734.7	1663.2	10667.9	6201.5	-7
P2	6744.5	2148.4	10413.0	4407.7	6424.1	1526.8	8844.6	4984.1	5
P3	7110.3	811.2	8338.0	5907.7	7428.3	1274.0	9557.6	5792.5	-4
P4	6229.5	1964.6	9612.4	3965.3	6233.2	1328.4	8212.1	4448.1	0
Reactive blue 198 + Synthetic wastewater									
Dye									
P1	478.9	103.8	628.4	277.7	421.6	90.5	557.1	253.7	12
P2	393.9	132.7	630.4	128.5	338.1	110.2	506.2	108.8	14
P3	466.0	79.8	579.9	308.4	411.0	69.4	513.5	278.1	12
P4	475.0	136.1	735.8	231.9	417.3	109.2	579.8	230.4	12
Chemical oxygen demand									
P1	4365.6	736.4	4871.8	2899.8	3580.3	548.5	4170.6	2756.3	18
P2	3326.8	717.4	4015.8	2148.3	2974.4	455.7	3518.3	2168.7	11
P3	4188.2	1050.5	5300.1	2595.6	3461.4	644.9	4349.5	2355.6	17
P4	2914.1	838.5	3833.6	1548.9	2419.5	545.0	3278.4	1588.0	17
Basic red 46 + Synthetic wastewater									
Dye									
P1	58.0	12.4	87.8	24.4	19.3	4.5	31.3	8.3	67
P2	78.4	19.5	142.4	53.6	37.1	19.0	84.0	11.1	53

Table 4.2 (Continued)

P3	56.0	12.0	80.1	17.8	17.2	4.6	28.4	7.6	69
P4	146.7	39.8	241.9	80.9	101.8	41.5	196.4	39.5	31
Chemical oxygen demand									
P1	3905.0	428.8	4651.1	3545.3	3151.5	571.4	3935.4	2187.8	19
P2	3120.5	551.2	3625.6	2102.8	2702.0	480.8	3074.3	1775.0	13
P3	3787.6	715.5	4824.7	2684.5	2941.9	678.8	4068.8	2127.3	22
P4	7240.0	1634.6	9598.3	4730.8	6564.8	1579.8	8640.5	4100.2	9
Direct orang 46 + Synthetic wastewater									
Dye									
P1	353.1	110.2	485.3	107.6	320.6	98.2	437.3	96.2	9
P2	425.1	133.7	601.9	123.9	389.4	121.4	568.5	122.3	8
P3	361.2	115.4	506.6	108.2	328.3	104.2	447.3	96.3	9
P4	517.4	243.3	847.7	139.3	488.9	230.9	828.4	128.6	6
Chemical oxygen demand									
P1	4902.3	2036.0	8692.1	2952.0	4388.6	1834.6	7935.8	2794.4	10
P2	3640.9	396.6	4146.1	2941.8	3050.9	632.9	3789.3	1988.8	16
P3	4412.1	657.1	5612.8	3778.8	3637.9	274.0	4161.0	3359.7	18
P4	3927.4	442.1	4482.4	3245.4	3107.1	530.8	3693.9	2272.2	21
Synthetic wastewater									
Chemical oxygen demand									
P1	3005.6	288.2	3550.0	2745.4	2408.6	244.4	2844.9	2087.9	20
P2	3399.6	864.7	4906.0	2259.6	2898.1	650.7	4076.0	2095.1	15
P3	3514.7	1228.8	5934.3	2548.5	2877.1	960.0	4748.7	2038.0	18
P4	6512.2	1673.7	9130.1	4820.0	5783.7	1545.5	8635.8	4242.4	11

Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds. Note that a negative removal indicates that the system worked as source rather than sink; Number of readings = 38 for dye removal and 5 for COD removal.

Table 4.3 Overview of the statistical analysis for outflow water quality parameters and corresponding removal efficiencies for the period from 15 December 2014 to 15 September 2015

Parameter	Shapiro-Wilk test (p value[1])	Statistical test	p values[2] for different system combinations						
			P1 & P2 & P3 & P4	P1 & P2	P1 & P3	P1 & P4	P2 & P3	P2 & P4	P3 & P4
Dye (mg/l)									
AB 113	<0.001	K-W	0.237	N/A	N/A	N/A	N/A	N/A	N/A
RB 198	0.012	K-W	0.01	0.006	0.301	0.327	0.034	0.006	0.244
BR 46	<0.001	K-W	<0.001	<0.001	0.051	<0.001	<0.001	<0.001	<0.001
DO 46	<0.001	K-W	<0.001	0.006	0.315	0.002	<0.001	0.03	0.003
Dye removal (%)									
AB 113	0.013	K-W	0.78	N/A	N/A	N/A	N/A	N/A	N/A
RB198	<0.001	K-W	0.25	N/A	N/A	N/A	N/A	N/A	N/A
BR46	<0.001	K-W	<0.001	0.003	0.376	<0.001	<0.001	<0.001	<0.001
DO46	<0.001	K-W	0.196	N/A	N/A	N/A	N/A	N/A	N/A
Colour (Pt Co)									
Acid blue 113	0.1	ANOVA	0.146	N/A	N/A	N/A	N/A	N/A	N/A
RB198	0.191	ANOVA	0.3	N/A	N/A	N/A	N/A	N/A	N/A
BR46	<0.001	K-W	<0.001	0.94	0.049	0.001	0.021	0.002	<0.001
DO46	0.797	ANOVA	0.947	N/A	N/A	N/A	N/A	N/A	N/A
SWW	<0.001	K-W	<0.001	0.406	0.406	0.002	0.762	<0.001	<0.001
Chemical oxygen demand (mg/l)									
AB 113	0.455	ANOVA	0.344	N/A	N/A	N/A	N/A	N/A	N/A
RB198	0.202	ANOVA	0.122	N/A	N/A	N/A	N/A	N/A	N/A
BR46	<0.001	K-W	0.37	N/A	N/A	N/A	N/A	N/A	N/A
DO46	<0.001	K-W	0.812	N/A	N/A	N/A	N/A	N/A	N/A
SWW	<0.001	K-W	<0.001	0.199	0.545	0.001	0.496	0.001	0.001
Chemical oxygen demand removal (%)									
AB 113	0.138	ANOVA	0.907	N/A	N/A	N/A	N/A	N/A	N/A
RB198	0.902	ANOVA	0.763	N/A	N/A	N/A	N/A	N/A	N/A
BR46	<0.001	K-W	0.013	0.117	0.602	0.016	0.047	0.076	0.016

Table 4.3 (Continued)

DO46	0.621	ANOVA	0.439	N/A	N/A	N/A	N/A	N/A	N/A
SWW	0.093	ANOVA	0.039	0.235	0.905	0.04	0.572	0.758	0.141
Suspended solids (mg/l)									
AB 113	<0.001	K-W	<0.001	<0.001	0.972	<0.001	<0.001	0.912	<0.001
RB198	<0.001	K-W	<0.001	0.012	0.78	0.001	0.039	0.172	0.002
BR46	<0.001	K-W	<0.001	0.552	0.06	<0.001	0.242	<0.001	<0.001
DO46	<0.001	K-W	0.531	N/A	N/A	N/A	N/A	N/A	N/A
SWW	<0.001	K-W	<0.001	0.289	0.006	<0.001	0.086	<0.001	<0.001
Turbidity (NTU)									
AB113	0.01	K-W	<0.001	<0.001	0.704	<0.001	0.001	0.244	<0.001
RB198	<0.001	K-W	<0.001	0.269	0.708	0.001	0.146	0.006	<0.001
BR46	<0.001	K-W	<0.001	0.529	0.179	<0.001	0.062	<0.001	<0.001
DO46	0.203	ANOVA	0.214	N/A	N/A	N/A	N/A	N/A	N/A
SWW	0.016	K-W	<0.001	0.01	0.001	<0.001	0.401	<0.001	<0.001
Dissolved oxygen (mg/l)									
AB113	0.008	K-W	0.665	N/A	N/A	N/A	N/A	N/A	N/A
RB198	<0.001	K-W	0.752	N/A	N/A	N/A	N/A	N/A	N/A
BR46	0.004	K-W	0.992	N/A	N/A	N/A	N/A	N/A	N/A
DO46	0.161	ANOVA	0.765	N/A	N/A	N/A	N/A	N/A	N/A
SWW	<0.001	K-W	0.011	0.818	0.265	0.006	0.234	0.005	0.044
pH (-)									
AB113	<0.001	K-W	0.169	N/A	N/A	N/A	N/A	N/A	N/A
RB198	<0.001	K-W	<0.001	0.001	0.134	<0.001	0.028	0.793	0.006
BR46	0.003	K-W	<0.001	<0.001	0.007	<0.001	<0.001	0.166	<0.001
DO46	0.005	K-W	0.038	0.027	0.583	0.393	0.008	0.129	0.221
SWW	0.001	K-W	<0.001	0.51	<0.001	<0.001	<0.001	<0.001	<0.001
Redox potential (mV)									
AB113	0.038	K-W	0.006	0.033	0.936	0.006	0.044	0.208	0.006
RB198	0.013	K-W	<0.001	<0.001	0.077	<0.001	0.003	0.682	<0.001

Table 4.3 (Continued)

BR46	0.032	K-W	<0.001	<0.001	0.032	<0.001	<0.001	0.308	<0.001
DO46	0.02	K-W	0.004	0.01	0.303	0.217	0.001	0.082	0.046
SWW	<0.001	K-W	<0.001	0.079	<0.001	<0.001	<0.001	<0.001	<0.001
Electrical conductivity (μS/cm)									
AB113	0.001	K-W	<0.001	<0.001	0.206	0.232	<0.001	0.004	0.301
RB198	<0.001	K-W	<0.001	<0.001	0.407	0.016	<0.001	0.206	0.008
BR46	<0.001	K-W	<0.001	<0.001	0.111	<0.001	<0.001	0.221	<0.001
DO46	<0.001	K-W	<0.001	<0.001	0.678	0.16	<0.001	0.025	0.098
SWW	0.002	K-W	0.001	0.001	0.566	0.015	0.001	0.142	0.028

Note: ^{1}Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); ^{2}p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; K-W, Kruskal-Wallis test; ANOVA, one-way analysis of variance test; N/A, not applicable, because the difference among the variables is not significant.

Table 4.4 Overview of the statistical analysis for dye removal efficiency for the period from 15 December 2014 to 15 September 2015 using a univariate analysis of variance between the type of dye, and the type of treatment system

Treatment comparison based on dyes	*p* value between the dyes	Treatment comparison based on planting regimes	*p* value between the planting regimes
AB113 & RB198	0.521	P1&P2	0.269
AB113 & BR46	<0.001	P1&P3	0.962
AB113 & DO46	0.032	P1&P4	<0.001
RB198 & BR46	<0.001	P2&P3	0.100
RB198 & DO46	<0.001	P2&P4	<0.001
BR46 & DO46	<0.001	P3&P4	<0.001

Note: p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$, the variables are statistically significantly different); AB113, Acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds.

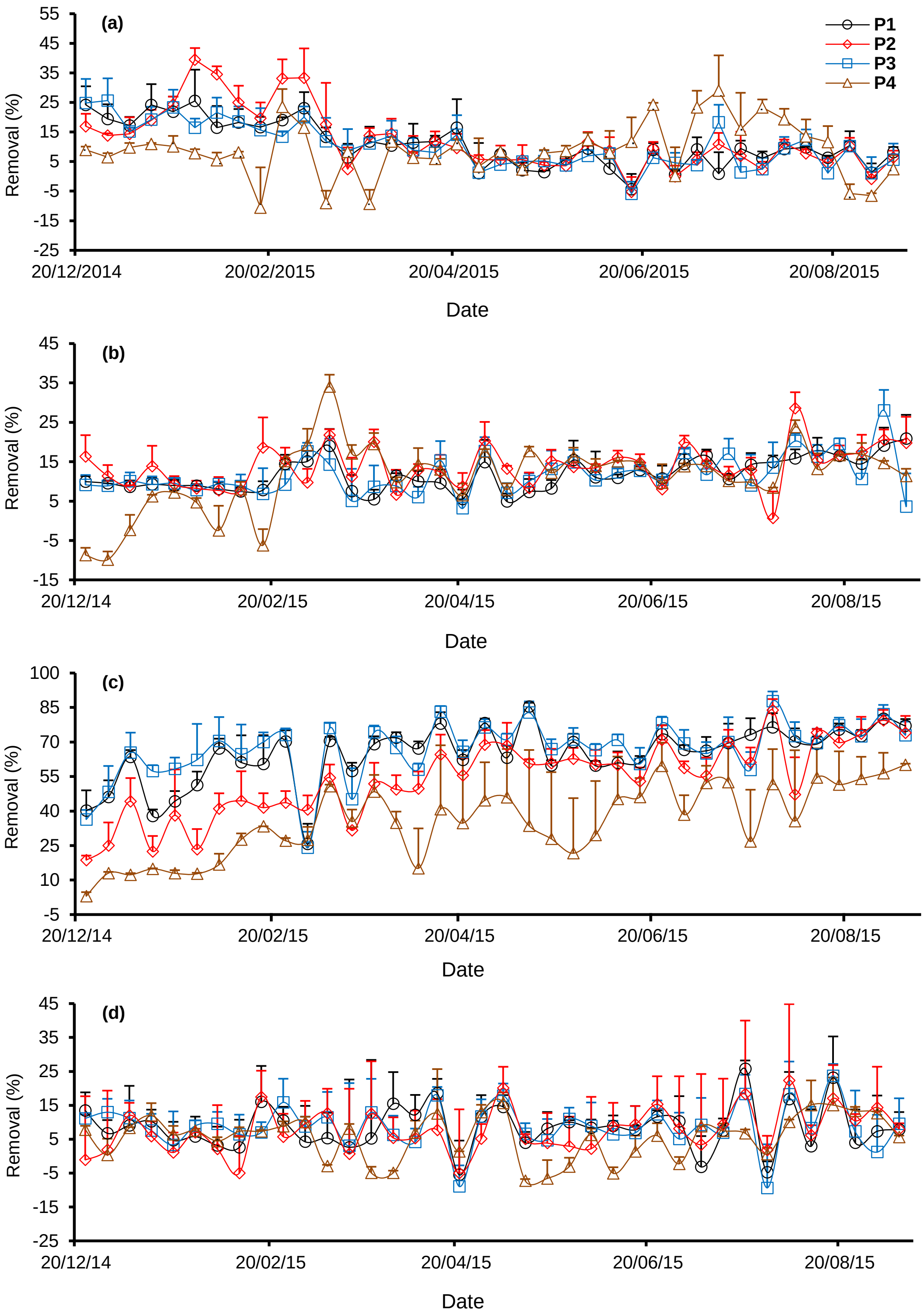


Figure 4.2 Mean values of dye removal profile for the period from 15 December 2014 to 15 September 2015. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds.

Table 4.5 Outflow water quality parameters for each system between 15 December 2014 and 15 September 2015

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue 113 + Synthetic wastewater						
Lemna minor L. and algae ponds						
Dye concentration	mg/l	7.1	3.56	0.6	11.5	39
pH	-	7.7	0.17	7.3	8	39
Redox	mV	-44.6	5.77	-53.5	-30.8	39
Dissolved oxygen	mg/l	8.9	0.33	8.3	9.6	39
Electrical conductivity	µS/cm	314.2	93.32	89.5	496.5	39
Total dissolved solids	mg/l	157.1	46.66	44.75	248.25	39
Suspended solids	mg/l	27.3	10.5	11.8	53.8	39
Turbidity	NTU	9.1	4.73	4.1	22.7	39
Colour	Pt Co	1190.2	750.21	106	2488.3	10
Chemical oxygen demand	mg/l	122.5	54.61	26.4	221	10
Algae ponds						
Dye concentration	mg/l	6.6	4.01	0.7	12.1	39
pH	-	7.7	0.24	7.3	8.2	39
Redox	mV	-48.8	9.29	-67.5	-28.5	39
Dissolved oxygen	mg/l	8.8	0.37	8.1	9.9	39
Electrical conductivity	µS/cm	477.6	209.65	76.6	934.5	39
Total dissolved solids	mg/l	238.8	104.825	38.3	467.25	39
Suspended solids	mg/l	19.1	5.94	11.5	38.8	39
Turbidity	NTU	5.9	3.18	2.3	21	39
Colour	Pt Co	1310	687.02	141.3	2409.8	10
Chemical oxygen demand	mg/l	98	42.37	25.1	181	10
Lemna minor L. ponds						
Dye concentration	mg/l	7.6	3.79	0.4	12.1	39
pH	-	7.7	0.19	7.4	8.2	39
Redox	mV	-44.4	7.33	-57	-25	39
Dissolved oxygen	mg/l	8.9	0.34	8.1	9.8	39
Electrical conductivity	µS/cm	320.2	109.97	76.7	490.5	39
Total dissolved solids	mg/l	160.1	54.985	38.35	245.25	39
Suspended solids	mg/l	27.7	13.33	7.3	69.5	39
Turbidity	NTU	9.3	5.7	3.5	26.6	39
Colour	Pt Co	1133.9	844.49	169.5	2806.8	10
Chemical oxygen demand	mg/l	118	49.23	28.2	198	10
Control ponds						
Dye concentration	mg/l	6.3	2.95	0.8	10.5	39
pH	-	7.8	0.32	7.2	8.5	39
Redox	mV	-51.2	14.23	-76.5	-14.5	39
Dissolved oxygen	mg/l	8.8	0.36	8.1	9.7	39
Electrical conductivity	µS/cm	356.5	187.39	59.6	841.5	39
Total dissolved solids	mg/l	178.25	93.695	29.8	420.75	39
Suspended solids	mg/l	17.5	9.26	1	37	39
Turbidity	NTU	5	2.38	0.9	11.6	39
Colour	Pt Co	2013.9	1180.58	71.5	3800.5	10

Table 4.5 (Continued)

Chemical oxygen demand	mg/l	83.6	55.27	17.6	177.1	10
Reactive blue 198 + Synthetic wastewater						
Lemna minor L. and algae ponds						
Dye concentration	mg/l	8.7	2.12	4.7	11.6	39
pH	-	7.5	0.17	7.1	7.9	39
Redox	mV	-35.1	6.73	-47	-20.5	39
Dissolved oxygen	mg/l	8.8	0.33	8.1	9.5	39
Electrical conductivity	µS/cm	309.2	105.49	95.2	536	39
Total dissolved solids	mg/l	154.6	52.745	47.6	268	39
Suspended solids	mg/l	12.7	7.28	4.3	34	39
Turbidity	NTU	6.1	3.67	2.7	17.3	39
Colour	Pt Co	196.3	55.17	82.8	270.5	10
Chemical oxygen demand	mg/l	62.3	19.26	22.6	87.4	10
Algae ponds						
Dye concentration	mg/l	7.1	2.67	1.9	11	39
pH	-	7.7	0.27	7.2	8.2	39
Redox	mV	-45.6	12.32	-66	-20	39
Dissolved oxygen	mg/l	8.8	0.36	7.9	9.9	39
Electrical conductivity	µS/cm	568.7	270.67	109	1393.8	39
Total dissolved solids	mg/l	284.35	135.335	54.5	696.9	39
Suspended solids	mg/l	9.4	5.07	3.8	27.3	39
Turbidity	NTU	5.2	2.5	2.8	12.9	39
Colour	Pt Co	243.9	39.65	193.5	308.8	10
Chemical oxygen demand	mg/l	52.1	18.68	20	76.9	10
Lemna minor L. ponds						
Dye concentration	mg/l	8.4	1.58	5.2	10.7	39
pH	-	7.6	0.2	7.1	8.1	39
Redox	mV	-38	7.66	-51.5	-16.8	39
Dissolved oxygen	mg/l	8.8	0.34	8	9.5	39
Electrical conductivity	µS/cm	295.2	99.37	117.1	546.3	39
Total dissolved solids	mg/l	147.6	49.685	58.55	273.15	39
Suspended solids	mg/l	13.6	9.83	3.8	46.5	39
Turbidity	NTU	7.3	5.32	2.1	26	39
Colour	Pt Co	206	80.56	79	326.5	10
Chemical oxygen demand	mg/l	59.4	19.88	30.1	93.8	10
Control ponds						
Dye concentration	mg/l	9.1	3.17	3.8	13.6	39
pH	-	7.7	0.25	7.2	8.2	39
Redox	mV	-46.8	10.84	-61.5	-20.5	39
Dissolved oxygen	mg/l	8.9	0.37	8.1	9.9	39
Electrical conductivity	µS/cm	492.7	308.78	74	1439.5	39
Total dissolved solids	mg/l	246.35	154.39	37	719.75	39
Suspended solids	mg/l	7.8	4.67	1	22.5	39
Turbidity	NTU	4	2.3	0.9	10.7	39
Colour	Pt Co	236	62.45	110.5	320	10
Chemical oxygen demand	mg/l	40.6	22.55	10.1	76.4	10
Basic red 46 + Synthetic wastewater						

Table 4.5 (Continued)

Lemna minor L. and algae ponds						
Dye concentration	mg/l	0.4	0.08	0.2	0.6	39
pH	-	7.5	0.23	7.1	8.2	39
Redox	mV	-35.8	10.36	-57	-15	39
Dissolved oxygen	mg/l	8.9	0.35	8.2	9.8	39
Electrical conductivity	µS/cm	188.2	58.87	83.6	373.3	39
Total dissolved solids	mg/l	94.1	29.43	41.8	186.6	39
Suspended solids	mg/l	8.4	4.79	3.3	25.8	39
Turbidity	NTU	4.7	2.63	2.7	13.6	39
Colour	Pt Co	79.4	27.78	35	132	10
Chemical oxygen demand	mg/l	51.7	13.39	30.5	79	10
Algae ponds						
Dye concentration	mg/l	0.7	0.36	0.2	1.7	39
pH	-	7.8	0.35	7.2	8.5	39
Redox	mV	-52.6	17.25	-81	-16	39
Dissolved oxygen	mg/l	8.9	0.31	8.1	9.6	39
Electrical conductivity	µS/cm	351.2	132.73	109.8	720.3	39
Total dissolved solids	mg/l	175.6	66.36	54.9	360.1	39
Suspended solids	mg/l	7.4	3.17	2.8	15.8	39
Turbidity	NTU	4.6	1.71	2.5	9.1	39
Colour	Pt Co	77	18.09	44	110.8	10
Chemical oxygen demand	mg/l	38.4	15.56	17.7	60.7	10
Lemna minor L. ponds						
Dye concentration	mg/l	0.3	0.08	0.1	0.6	39
pH	-	7.3	0.31	6.6	8.1	39
Redox	mV	-27.4	16.82	-66.8	12.5	39
Dissolved oxygen	mg/l	8.9	0.32	8.2	9.7	39
Electrical conductivity	µS/cm	167.6	53.01	92.8	326.2	39
Total dissolved solids	mg/l	83.8	26.51	46.4	163.1	39
Suspended solids	mg/l	6.8	3.68	2.3	20.3	39
Turbidity	NTU	4	1.7	2.2	10.7	39
Colour	Pt Co	53.9	18.16	29.3	86.5	10
Chemical oxygen demand	mg/l	46.6	18.38	11	86.2	10
Control ponds						
Dye concentration	mg/l	2	0.82	0.7	4.1	39
pH	-	7.9	0.42	7.2	8.7	39
Redox	mV	-57.3	21.06	-91	-20	39
Dissolved oxygen	mg/l	8.9	0.46	7.9	9.8	39
Electrical conductivity	µS/cm	313	139.42	80	654	39
Total dissolved solids	mg/l	156.5	69.71	40	327	39
Suspended solids	mg/l	70.1	72.97	1.5	267.5	39
Turbidity	NTU	15.3	13.68	1.4	53	39
Colour	Pt Co	191.3	53.95	57	242.5	10
Chemical oxygen demand	mg/l	80.7	59.49	12.3	179	10
Direct orange 46 + Synthetic wastewater						
Lemna minor L. and algae ponds						
Dye concentration	mg/l	6.2	2.14	1.6	8.6	39

Table 4.5 (Continued)

pH	-	7.8	0.23	7.3	8.3	39
Redox	mV	-49.2	10.41	-68.8	-25.3	39
Dissolved oxygen	mg/l	8.9	0.31	8	9.3	39
Electrical conductivity	µS/cm	285.2	119.41	103.1	613.8	39
Total dissolved solids	mg/l	142.6	59.71	51.55	306.9	39
Suspended solids	mg/l	9.2	4.28	5	26.5	39
Turbidity	NTU	5.5	2.6	2.6	15.4	39
Colour	Pt Co	639.4	273.53	231.5	1081.5	10
Chemical oxygen demand	mg/l	65.1	38.69	21	168	10
Algae ponds						
Dye concentration	mg/l	7.6	2.68	1.6	11.6	39
pH	-	7.8	0.34	7.2	8.4	39
Redox	mV	-59	17.84	-88	-24.5	39
Dissolved oxygen	mg/l	8.9	0.26	8.4	9.5	39
Electrical conductivity	µS/cm	434.6	183.55	76.8	783.5	39
Total dissolved solids	mg/l	217.3	91.78	38.4	391.75	39
Suspended solids	mg/l	7.7	3.79	2.8	19	39
Turbidity	NTU	5.4	2.22	2	11.2	39
Colour	Pt Co	648.2	268.58	202	1104.5	10
Chemical oxygen demand	mg/l	58.9	14.5	30.1	78.5	10
Lemna minor L. ponds						
Dye concentration	mg/l	6.4	2.24	1.5	8.8	39
pH	-	7.7	0.24	7.2	8.1	39
Redox	mV	-46.6	11.15	-69.3	-18	39
Dissolved oxygen	mg/l	8.9	0.29	8.2	9.5	39
Electrical conductivity	µS/cm	275.7	119.62	94	567.8	39
Total dissolved solids	mg/l	137.85	59.81	47	283.9	39
Suspended solids	mg/l	8.3	3.63	2.3	17.3	39
Turbidity	NTU	5.1	1.76	2.7	11.9	39
Colour	Pt Co	593.2	285.81	96.5	1047.5	10
Chemical oxygen demand	mg/l	56.8	21.93	12.2	86.5	10
Control ponds						
Dye concentration	mg/l	9.7	4.9	2	16.3	39
pH	-	7.9	0.34	7	8.4	39
Redox	mV	-51.6	15.02	-81	-11	39
Dissolved oxygen	mg/l	8.9	0.35	8.3	9.8	39
Electrical conductivity	µS/cm	347.4	181.36	77	703.5	39
Total dissolved solids	mg/l	173.7	90.68	38.5	351.8	39
Suspended solids	mg/l	8.2	4.01	2	19.5	39
Turbidity	NTU	6.2	2.46	1.4	10.8	39
Colour	Pt Co	674	332.77	81.5	1240	10
Chemical oxygen demand	mg/l	47.8	22.01	15	73.8	10
Synthetic wastewater						
Lemna minor L. and algae ponds						
pH	-	7.8	0.25	7.2	8.3	39
Redox	mV	-52	10.98	-68.3	-21.3	39
Dissolved oxygen	mg/l	8.9	0.29	8.2	9.5	39

Table 4.5 (Continued)

Electrical conductivity	µS/cm	353.9	123.15	99.3	580.8	39
Total dissolved solids	mg/l	176.95	61.58	49.65	290.4	39
Suspended solids	mg/l	6.2	2.9	2.8	16.5	39
Turbidity	NTU	3.6	1.34	1.6	7.8	39
Colour	Pt Co	50.1	15.88	26.5	80.8	10
Chemical oxygen demand	mg/l	53.2	11.17	31.6	71	10
Algae ponds						
pH	-	7.8	0.32	7.2	8.4	39
Redox	mV	-46.9	15.01	-79	-17.8	39
Dissolved oxygen	mg/l	8.9	0.29	8.2	9.5	39
Electrical conductivity	µS/cm	499.6	221.49	95.8	863.8	39
Total dissolved solids	mg/l	249.8	110.75	47.9	431.9	39
Suspended solids	mg/l	7.1	3.67	2.3	17	39
Turbidity	NTU	4.3	1.47	2.3	9.6	39
Colour	Pt Co	44.9	11.66	25.3	65	10
Chemical oxygen demand	mg/l	45.4	17.94	23.8	84.9	10
Lemna minor L. ponds						
pH	-	7.8	0.27	7.2	8.4	39
Redox	mV	-51	13.53	-80.3	-16.7	39
Dissolved oxygen	mg/l	8.9	0.29	8.2	9.5	39
Electrical conductivity	µS/cm	345.5	159.03	106	698.3	39
Total dissolved solids	mg/l	172.75	79.52	53	349.2	39
Suspended solids	mg/l	8.3	3.97	3.5	20	39
Turbidity	NTU	4.7	1.97	2.6	12.6	39
Colour	Pt Co	44.3	10.19	30	66.3	10
Chemical oxygen demand	mg/l	52.2	23.59	20.3	111	10
Control pond						
pH	-	8.4	0.36	7.6	9	36
Redox	mV	-85.2	18.88	-116	-44	36
Dissolved oxygen	mg/l	9.2	0.48	8	10.1	37
Electrical conductivity	µS/cm	442.4	196.12	120	789	38
Total dissolved solids	mg/l	221.2	98.06	60	394.5	38
Suspended solids	mg/l	139.4	117.67	4	390	34
Turbidity	NTU	34.6	22.58	4.4	92	38
Colour	Pt Co	92	33.66	54	155	10
Chemical oxygen demand	mg/l	128.6	40.26	47	179.5	10

Note: NTU, nephelometric turbidity unit.

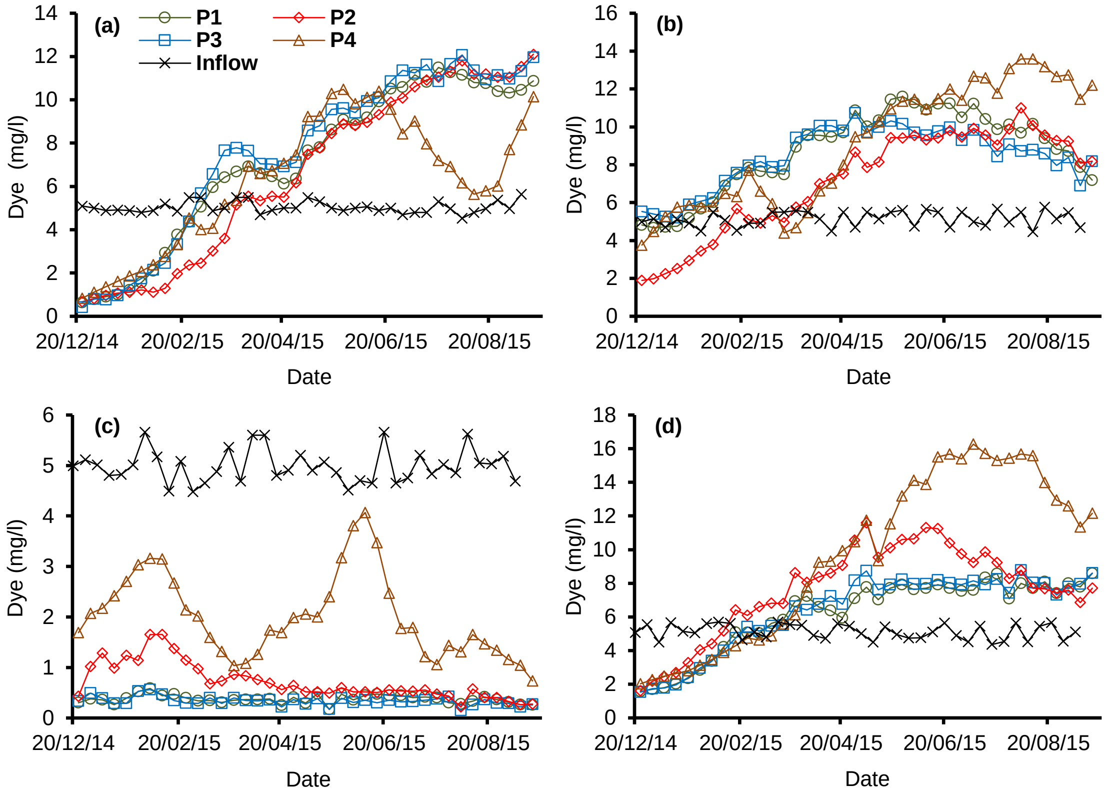


Figure 4.3 Inflow and mean outflow dye concentrations during the experiment between 15 December 2014 and 15 September 2015. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds.

4.2.2.2 Chemical oxygen demand and dissolved oxygen

The average chemical oxygen demand (COD) removal efficiency (Table 4.2) was low for all ponds, which was clearly noticed from the inflow concentrations corresponding to the high outflow concentrations (Table 4.1 and Table 4.5). Low COD removal in all ponds ranged between 22% and -4%, indicating a low level of organic matter degradation occurring in the ponds due to the poor microbial activities. Statistically, no significant difference was found in COD removal among the design variables for ponds containing AB113, RB198 and DO46 (Table 4.3). This is attributed to the low organic matter degradation in these ponds compared with other ponds containing BR46, and only synthetic wastewater. Concerning ponds containing BR46, mean values of COD removal were higher in *L. minor* and algae, and *L. minor* ponds than algae and control ponds: P3 > P1 > P2 > P4. This reflects the high percentage of BR46 degradation in ponds containing *L. minor* (P1 and P3) compared with other design variables (P2 and P4). Statistical analysis showed that the mean COD removal in the control ponds was significantly lower than the removals for *L. minor* and algae, and *L. minor* ponds. Also, *L. minor* ponds were significantly dissimilar to algae ponds. Correlation analysis results indicate that the COD removal was significantly ($r = 0.275$, $p = 0.014$) positively correlated with dye removal.

Based on COD outflow concentrations, the values increased gradually, and were higher than the inflow (Figure 4.4), which indicates low microbial activity for degradation of the organic matter associated with high levels of DO. The COD mean outflow values (Table 4.5) fluctuated and were ranked for AB113 and RB198 as follows: P1> P3 > P2 > P4. The ranking was P4 > P1 > P3 > P2 for BR46, tap water and fertiliser. Finally, the ranking was P1 > P2 > P3 > P4 for DO46. Nevertheless, no differences were found among design variables in systems containing AB113, RB198, DO46 and BR46. The European and international standards (Carmen & Daniela, 2012) set a threshold value for COD of 125 mg/l with respect to the discharge of effluent directly into water bodies. The results have shown that the COD values in *L. minor* and algae containing AB113, *L. minor* with AB113, algae with AB113 and control ponds containing AB113, *L. minor* and algae ponds comprising DO46, control ponds containing BR46, and control ponds with tap water and fertiliser were 6, 6, 2, 3, 1, 4 and 6 times non-compliant, respectively.

Based on dissolved oxygen, the values of outflow DO, which ranged between 7.9 and 10.1 mg/l, were lower than the inflow values, which varied between 8.6 and 10.8 mg/l. In addition, the mean outflow values (Table 4.5) in terms of DO were relatively similar; no differences among the design variables for AB113, RB198, BR46 and DO46 were calculated. In contrast, significant differences were noted for the mean values of control ponds containing tap water and fertiliser (Table 4.3), where the DO was higher compared with ponds containing *L. minor* and/or algae. These findings do not match those published by Sekomo et al. (2012), which are related, however, to the treatment of heavy metals. In their study, higher values of DO were noted for algae ponds than duckweed ponds, because the photosynthetic process in algae ponds happens within the water body, whereas in duckweed ponds, the activities of oxygen production occur at the top layer, where consequently some of the oxygen is lost to the atmosphere and small amounts move to the water via the roots (Sekomo et al., 2012). This difference in results is expected, because the amount of algae in this research is limited and oxygen diffusion by the atmosphere affects the DO level, rather than the impact of the system type. Ong et al. (2011) reported that the biodegradation of organic contaminants in wetlands was boosted remarkably by the presence of a high amount of DO, which can facilitate the growth of aerobic micro-organisms for eliminating organic substances. But high DO inhibited the dye removal mechanism because of the electrons released by microbial cells during the oxidation process utilising oxygen instead of azo dyes during degradation processes (Pearce et al., 2003).

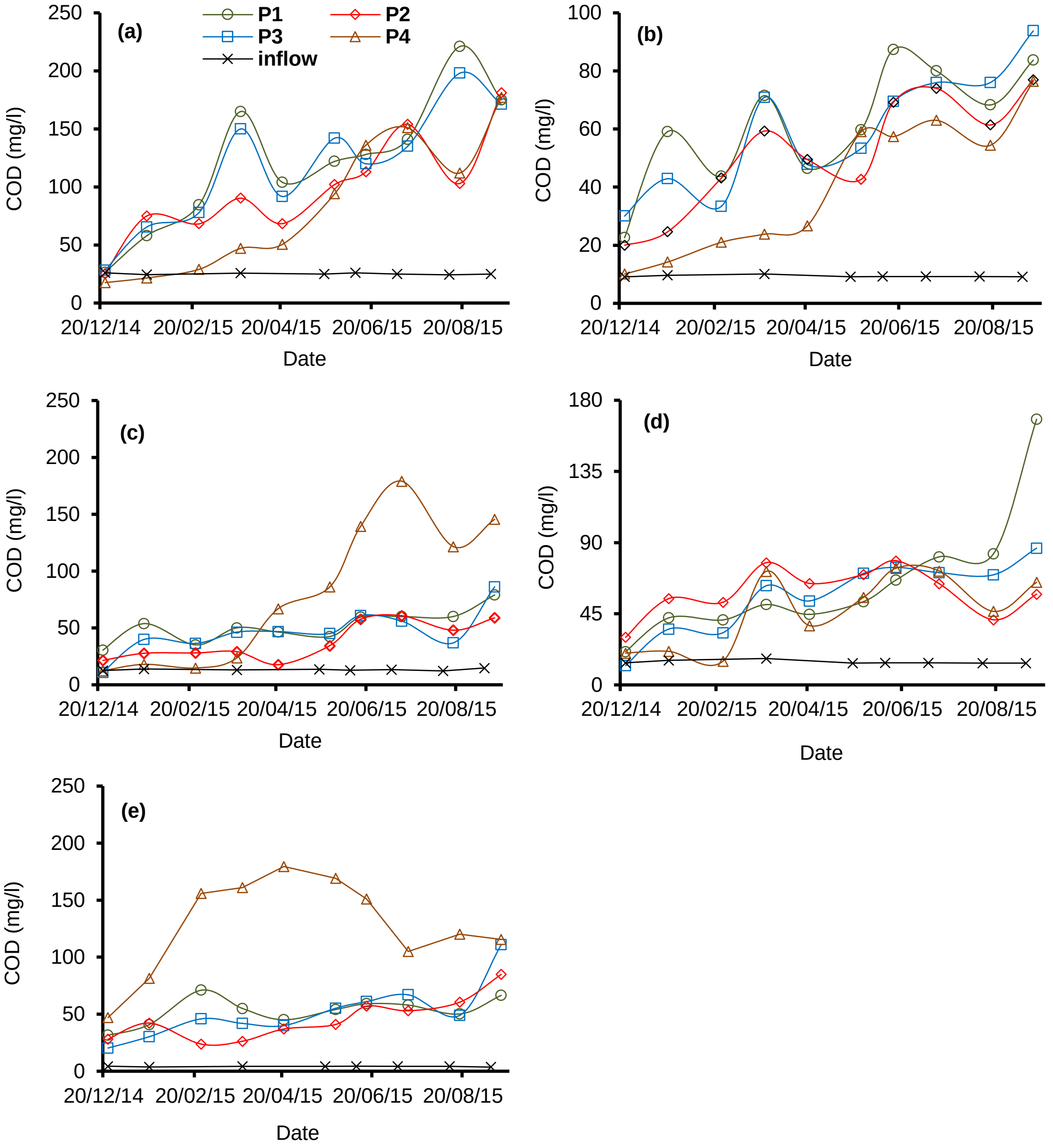


Figure 4.4 Monthly inflow and outflow concentrations of chemical oxygen demand during the experiment between 15 December 2014 and 15 September 2015 for each system. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; (e), synthetic wastewater without dye; COD, chemical oxygen demand; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P3, *Lemna minor* L. ponds; P4, control ponds.

4.2.2.3 pH and redox potential

The mean values of pH obtained from the outflow (Table 4.5) were slightly higher than the inflow values (Table 4.1). Nonetheless, the pH values for all pond outflows were within the European and international standard thresholds (6.5 to 8.5) according to Carmen and Daniela (2012), except the outflow values in the control ponds containing tap water and fertiliser, which were 11 times non-compliant. The highest mean values for the pH outflow were observed in all control ponds, using synthetic wastewater, followed by control ponds comprising dyes. In comparison, the lowest mean values were found in *L. minor* ponds containing BR46. The outflow pH values increased slightly compared to the inflow of all ponds. In addition, pH outflow values for ponds containing *L. minor* (P1 and P3) were slightly lower compared to other ponds without *L. minor* (P2 and P4). This may be because the plants try to gain an equilibrium between chemicals in the cells by proton and ion exchanges from the synthetic wastewater containing dyes (Noonpui & Thiravetyan, 2011). The pH value had an important impact on the capacity of dye uptake and plant growth. The optimum pH to obtain a high removal efficiency depends on the type of dye itself; e.g., 7.0 for methylene blue, 8.0 for basic blue nine (Reema et al., 2011), 6.0–7.5 for BR46 (Movafeghi et al., 2013) and 6.5 for acid blue 92 (Khataee et al., 2012). Saratale et al. (2011) indicated that the optimum pH for high colour removal should be within the range 6–10. The removal efficiency considerably declines at strong acid or alkaline conditions for biological treatment systems. In comparison, the allowable range of pH for growth of *L. minor* is 4.5–8.3 (Reema et al., 2011). Therefore, in this study, the neutral pH outflows seem to be suitable for dye BR46 removal, as mentioned in the previous section.

Based on redox potential monitoring (Table 4.5) as an indicator for the aerobic and anaerobic conditions in the aquatic life (Ong et al., 2009a), no differences were observed for the mean values of the redox potential between algae and control ponds for all dyes. This reflects the very low contribution of algae, in terms of treatment, compared with the control systems. Furthermore, the mean redox potential values in *L. minor*, and *L. minor* and algae ponds were also not different, except for BR46 (Table 4.3). This is possibly because of the treated effluents from ponds containing AB113, RB198 and DO46 being coloured, which interferes with the photosynthesis of algae (Carmen & Daniela, 2012). However, the effluents from ponds comprising BR46 were colourless due to their high removal efficiencies. Regarding ponds containing synthetic wastewater without dye, only algae ponds, and *L. minor* and algae ponds were not different. The maximum and minimum values of redox potential indicated anoxic conditions, except for the control pond containing synthetic wastewater without dye, where the maximum value was -116 mV, indicating anaerobic conditions (Table 4.5).

4.2.2.4 Suspended solids and turbidity

The highest value of mean outflow suspended solids (SS) concentration was noted for control ponds containing tap water and fertiliser followed by the control pond containing BR46. In contrast, the lowest value was observed in *L. minor* and algae ponds fed by tap water and fertiliser followed by *L. minor* ponds containing BR46 (Table 4.5). This indicates that in addition to SS removal by the sedimentation process, the adhesion process to plant and detritus surfaces also contributed to SS elimination in the systems (USEPA, 1999). The European and international standards for SS are 35 mg/l in case of discharge directly into receiving freshwater bodies (Carmen & Daniela, 2012). The results indicate that the *L. minor* and algae ponds, *L. minor* ponds, algae ponds and control ponds containing AB113, *L. minor* ponds comprising RB198, and control ponds fed by BR46, tap water and fertiliser were 10, 9, 1, 1, 3, 22 and 29 times non-compliant, respectively. Statistically, the mean outflow values of SS in the control ponds comprising BR46, tap water and fertiliser were significantly higher than other treatments (Table 4.3). However, for AB113 and RB198, outflow values of SS were significantly higher for ponds containing *L. minor*, and *L. minor* and algae than algae only and control ponds (Table 4.3), indicating the impact of *L. minor*. Also, for AB113 and RB198, no differences were found between the *L. minor*, and *L. minor* and algae ponds, and between algae and control ponds because algae were limited in the systems and had a very small effect on SS in ponds. In terms of DO46, no difference was found among the design variables.

Turbidity is a simple indicator for the clarity of water. The inflow and outflow turbidity values for each system are presented in Table 4.1 and Table 4.5, respectively. The highest and lowest turbidity values mirrored those for SS. A correlation analysis indicated that SS was significantly ($r = 0.773$, $p < 0.001$) positively correlated with turbidity and significantly ($r = -0.132$, $p < 0.001$) negatively correlated with DO. Therefore, high values of DO in the systems may reflect the low micro-organism activities for organic matter degradation, which consequently reduced the SS particles (Sani et al., 2013) and the COD removal (Scholz, 2010). A correlation analysis indicated that COD removal was significantly ($p < 0.05$) negatively correlated with DO ($r = -0.249$, $p = 0.026$).

4.2.2.5 Electrical conductivity and total dissolved solids

The electrical conductivity (EC) is an important indicator to assess indirectly the salinity of a system. An increase in the electrical conductivity values can inhibit the growth of *L. minor* as stated by Wendeou et al. (2013). The optimum growth rate of *L. minor* is associated with EC values ranging between 600 and 1400 µS/cm. However, all minimum outflow values were lower than this range (Table 4.5). All mean outflow EC values increased more than the inflow ones, due to salinity accumulation in all systems after weekly doses. The highest EC mean values were observed for both control and algae ponds, whereas the lowest values were found in *L. minor*, and *L. minor* and algae ponds for all types of wastewater. This may indicate that the presence of plants in the ponds is responsible for the reduction of the EC. In addition, the conductivity mean values for planted ponds containing only water and fertiliser were higher than those for ponds comprising dyes. These findings match observations by Nilratnnisakorn et al. (2009) suggesting that large numbers of dye molecules might be caught in barriers within the vascular plant system. However, salts originate from synthetic wastewater, as the plants are able to remove small molecules of these salts from solutions by passing them through their semi-permeable membrane (Noonpui & Thiravetyan, 2011). Statistically (Table 4.3), the mean outflow values of EC in algae ponds were significantly higher than those values of the other treatments (*L. minor*, *L. minor* and algae, and control ponds) for AB113 and DO46. However, for RB198, BR46, and synthetic wastewater without dye, the mean outflow values of EC in algae ponds and control ponds were significantly higher than those values of the other treatments (*L. minor*, and *L. minor* and algae). In addition, no difference was found between *L. minor*, and *L. minor* and algae ponds, and also no dissimilarity was noted between algae and control ponds for RB198, BR46, and only synthetic wastewater, due to the limited impact of algae.

Total dissolved solids (TDS), which comprise inorganic salt and a small amount of organic matter that are dissolved in water, are a function of EC (Amankwaah et al., 2014) and the relationship between them depends on the type of wastewater. In this research TDS values were equal to half of the EC values. The TDS values for all treatment system outflow waters were compliant with the European standard of 2000 mg/l for discharge directly to receiving freshwater bodies (Carmen & Daniela, 2012).

4.2.2.6 Trace elements

The main source of elements in the outflow samples was the fertiliser in the prepared synthetic wastewater and to a lesser extent some elements in tap water. In addition, zinc ions were present in the dye BR46. Although some of the elements are important for plants as micro-nutrients, these elements could be toxic at high concentrations. The plants play an important role in element phytoremediation due to active and/or passive transport of elements in wetlands (Bonanno & Vymazal, 2017). Figure 4.1 shows the mean inflow and outflow elements. Mean outflow zinc and copper values (Figures 4.1a and d, respectively) were higher than the corresponding inflow concentrations for all ponds, except *L. minor* ponds, and *L. minor* and algae ponds treating BR46, which were slightly lower. Ponds containing *L. minor*, and *L. minor* and algae, which were treating BR46, showed significantly lower zinc (Kruskal Wallis test, $P < 0.05$) and copper (one way ANNOVA, $P < 0.05$) outflows than those in algae and control ponds reflecting the uptake of these metals by plants. All zinc outflows in planted ponds were lower than the values that cause growth reductions in *L. minor*, between 0.5 and 15 mg/l (Khellaf & Zerdaoui, 2009). The standard threshold of zinc for irrigation is 2 mg/l (Metcalf & Eddy, 2003). All outflow values were compliant. Regarding iron, the mean outflow values (Figure 4.1b) were lower than the corresponding inflow concentrations for all ponds except those treating AB113. No significant differences were found for outflow iron values among the design variables (one-way ANNOVA, $P > 0.05$) for all treatment systems, except for those treating BR46 and only synthetic wastewater. For systems comprising BR46, the mean outflow iron values were significantly higher (one-way ANNOVA, $P < 0.05$) in *L. minor*, algae and control ponds compared with corresponding values in *L. minor* and algae ponds. For systems without dye, the mean outflow iron values were significantly higher (one-way ANNOVA, $P < 0.05$) in control ponds compared with corresponding values in *L. minor* and algae ponds. The standard limit of iron for irrigation is 5 mg/l (Metcalf & Eddy, 2003). All outflow values were compliant. Concerning boron and potassium (Figures 4.1c and e, respectively), the outflow values for P1 and P3, which were lower than the inflows, were significantly ($P < 0.05$) lower than the outflow values for P2 and P4, which were higher than the corresponding inflow values, for all systems with and without dyes. These results confirmed the potential of *L. minor* in uptake of boron and potassium. Higher mean calcium, magnesium, and sodium (Figure 4.1f, g and h, respectively) outflow concentrations than inflow ones were found for all treatment systems due to the weekly dosages and low reductions. In addition, the outflow values were lower for P1 and P3 compared with the outflow values for P2 and P4, which may be due to the uptake by *L. minor*.

Regarding elements content in plant tissue, Figure 4.5 provides an overview of the concentrations of all detected elements in plant tissues for all ponds with and without dyes. These results reflect the plant's capacity for element accumulation, which was lower for copper and aluminium (Figure 4.5d and e, respectively) compared with other elements. The levels of zinc and iron in plants (Figure 4.5a and b, respectively) were more than the allowable boundaries of 50 mg/kg and 20 mg/kg, respectively, as mentioned by Nazir et al. (2015).

Bioconcentration factors (Figure 4.6) are indicators of the potential of the plants for accumulating metals. A bioconcentration factor of higher than 1000 indicates that plants can positively accumulate heavy metals as mentioned by Sukumaran (2013). The results in Figure 4.6a, b, and d indicate that plants were positive for phytoremediation of zinc, iron, and boron, respectively. Whereas, the plants were not good accumulators for copper (Figure 4.6c).

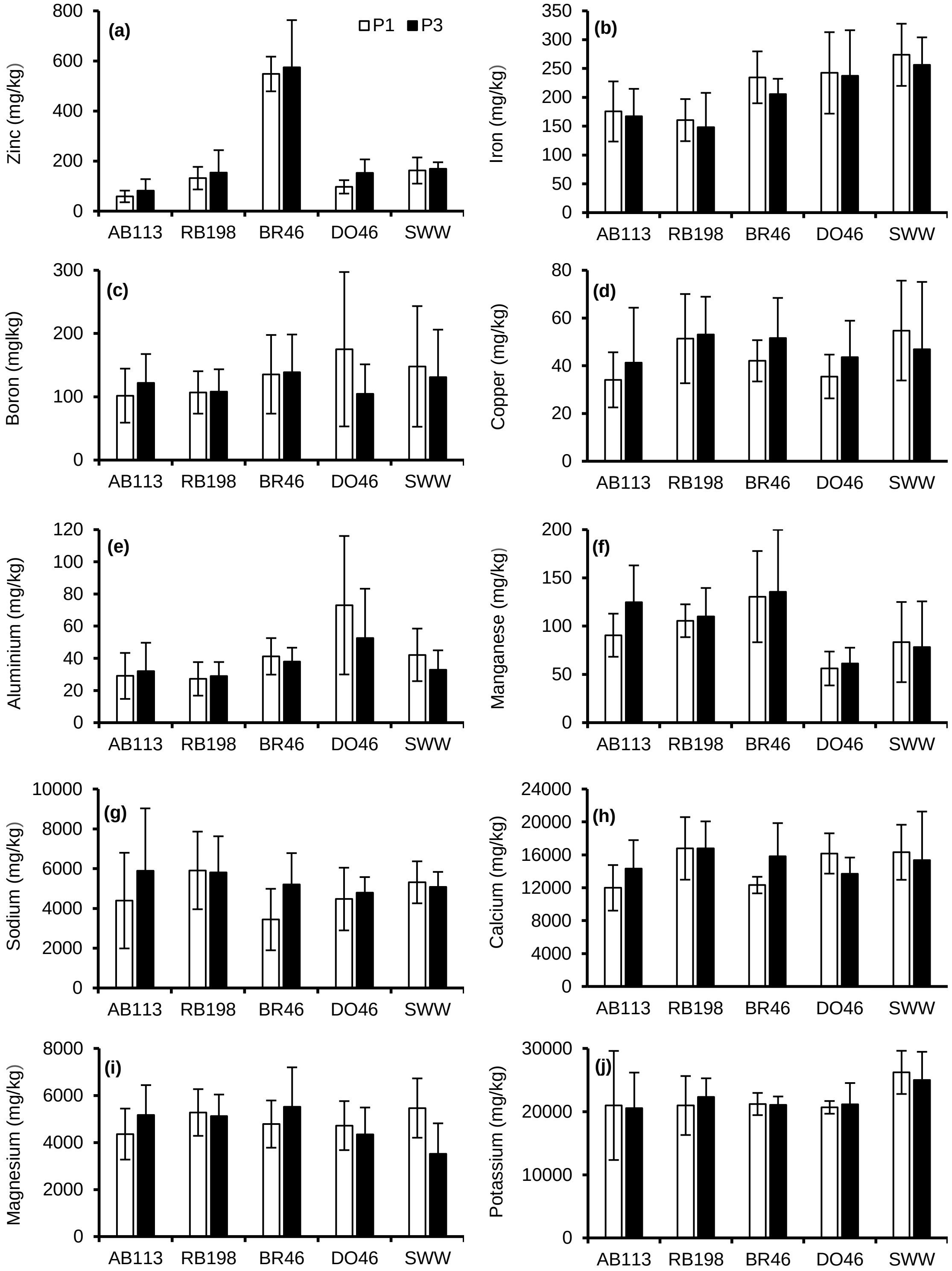


Figure 4.5 Mean concentrations of the detected elements in plant tissue during the experiment between 15 December 2014 and 15 September 2015. Note: (a), zinc; (b), iron; (c), boron; (d), copper; (e), aluminium; (f), manganese; (g), sodium; (h), calcium; (i), magnesium; (j), potassium; P1, *Lemna minor* L. and algae ponds; P3, *Lemna minor* L. ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dye.

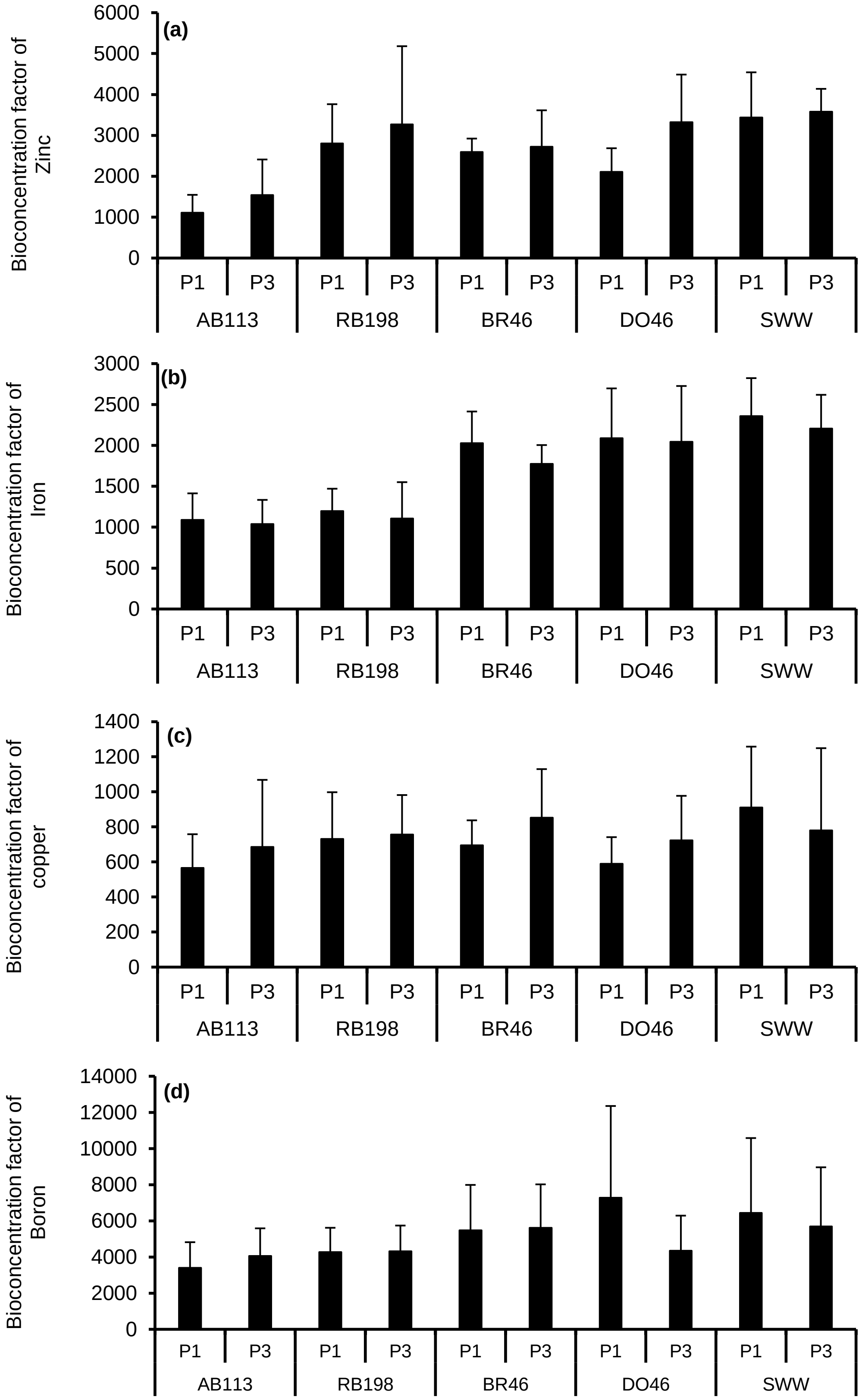


Figure 4.6 Bioconcentration factor during the experiment between 15 December 2014 and 15 September 2015. Note: (a), zinc; (b), iron; (c), copper; (d), boron; P1, *Lemna minor* L. and algae ponds; P3, *Lemna minor* L. ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dyes.

4.2.3 Plant monitoring

Findings show differences in the colour of *L. minor* leaves between ponds with and without dye (Table 4.6). For ponds containing synthetic wastewater (without dyes), the frond colour analysis showed that a high percentage of the area is covered by 7GY (mostly green colour), and a small percentage of the area is covered by 2.5GY (mostly yellow colour), according to Munsell (1977), compared with ponds containing synthetic wastewater with dyes. However, it is important to note that the fronds of colour 2.5GY cover around 1% in ponds without dyes compared with 2.8% minimum and 6.5% maximum in ponds with dyes. These small differences may not negatively affect the photosynthetic pigments as highlighted by Khataee et al. (2012). Acid blue 92 at low concentrations of around 10 mg/l does not significantly affect the chlorophyll content. Figure 4.7 provides an overview of the mean total coverage area including dead and living fronds for all ponds during the experimental period. The results indicate that the total coverage area is higher in ponds containing only synthetic wastewater (without dyes) followed by ponds containing AB113, and then RB198. However, low coverage areas were found in ponds containing DO46 and BR46. Statistically, no difference was found between ponds containing *L. minor* and algae, and ponds comprising only *L. minor* in systems with and without dyes.

The overall mean values of relative growth rate (RGR) (based on the frond number) and relative frond number are shown in Figure 4.8a and b, respectively. These growth parameters have been used as indicators for the toxic effects of dyes on *L. minor* growth. Results clearly indicate that dyes negatively impact on relative frond number and relative growth rates, which had the same trend and were ranked as follows: synthetic wastewater without dye > AB113 > RB198 > DO46 > BR46. This outcome suggests that BR46, which is successfully treated, had a negative effect on the plant growth rate. The same impact was observed for brilliant blue R special, which is a strong inhibitor regarding *L. minor* growth (Khataee et al., 2012). For relative growth rate and relative frond number calculations, no dissimilarities were found between ponds containing *L. minor* and algae, and ponds with *L. minor* only according to a t-test undertaken for all wastewaters (Table 4.7). The correlation analysis resulted in a significantly ($p < 0.01$) positive correlation between coverage area and relative frond number ($r = 0.715$, $p < 0.001$). The plants harvested during the experiment from ponds, which were completely covered by *L. minor*. The fresh and dry weights of plants harvested during the experimental operation period are recorded in Table 4.8.

Table 4.6 Overview of frond colour determinations according to the coverage area (%) using Munsell (1977) and the Eleatheia Lemna edition for each system between 15 December 2014 and 15 September 2015

2.5 GY[a]					5GY				7GY				
8/2[b]	8/4	8/6	8/8	8/10					8/2	8/4	8/6	8/8	
7/2	7/4	7/6	7/8	7/10	7/4	7/6	7/8		7/2	7/4	7/6	7/8	7/10
6/2	6/4	6/6	6/8	6/10	6/4	6/6	6/8	6/10	6/2	6/4	6/6	6/8	6/10
5/2	5/4	5/6	5/8		5/4	5/6	5/8	5/10	5/2	5/4	5/6	5/8	
					4/4	4/6	4/8		4/2	4/4	4/6		
					3/4				3/2	3/4			
Acid blue 113 + SWW (*Lemna minor* L. and algae ponds; P1)													
0.4	0.3	0.4	0.3	0.0					0.2	0.0	0.0	0.9	
2.4	0.0	0.0	0.2	0.0	0.1	0.5	0.0		8.6	24.9	7.2	0.0	1.3
0.2	0.0	0.1	0.0	0.0	2.2	1.3	0.4	0.0	4.3	14.4	9.0	0.6	0.0
0.1	0.0	0.0	0.0		0.0	1.6	0.4	0.0	0.0	1.1	0.5	0.0	
					0.0	0.2	0.1		0.0	0.0	0.0		
					0.0				0.0	0.0			
Acid blue 113 + SWW (*Lemna minor* L. ponds; P3)													
0.2	0.2	0.0	0.0	0.0					0.0	0.3	0.0	0.1	
2.4	0.0	0.3	0.0	0.0	0.6	0.2	0.0		6.5	24.4	5.5	0.6	0.0
0.0	0.4	0.0	0.0	0.0	2.1	1.2	0.0	0.0	5.5	16.6	8.4	0.5	0.0
0.0	0.0	0.0	0.0		0.1	1.9	0.0	0.0	0.0	2.0	2.3	0.0	
					0.0	0.2	0.0		0.0	0.3	0.0		
					0.0				0.0	0.0			
Reactive blue 198 + SWW (*Lemna minor* L. and algae ponds; P1)													
0.2	2.9	0.0	0.5	0.0					0.0	0.0	0.0	0.7	
0.7	0.3	0.0	0.0	0.0	0.4	0.0	1.4		0.4	30.3	11.8	4.4	0.9
0.0	0.0	0.0	0.0	0.0	1.6	0.4	0.0	0.0	0.0	13.0	8.8	2.3	0.0
0.0	0.0	0.0	0.0		0.3	0.0	0.0	0.0	0.0	0.9	2.1	0.0	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			
Reactive blue 198 + SWW (*Lemna minor* L. ponds; P3)													
0.4	2.3	0.0	0.1	0.0					0.0	0.0	0.0	0.8	
0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0		0.7	37.2	9.5	4.9	1.2
0.0	0.1	0.0	0.0	0.0	2.5	1.2	0.4	0.0	0.3	12.6	6.2	1.8	0.0
0.0	0.0	0.0	0.0		1.2	0.0	0.0	0.0	0.0	0.1	1.3	0.3	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			
Basic red 46 + SWW (*Lemna minor* L. and algae ponds; P1)													
0.6	1.6	0.0	0.0	0.0					0.3	0.7	0.2	0.9	
0.2	0.0	0.0	0.0	0.0	0.9	0.1	1.2		3.1	22.7	6.5	5.9	2.6
0.4	0.0	0.0	0.0	0.0	1.4	2.0	0.6	0.0	0.6	11.2	3.9	1.4	0.4
0.0	0.0	0.0	0.0		0.0	0.7	0.0	0.0	0.0	0.0	0.4	0.7	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			
Basic red 46 + SWW (*Lemna minor* L. ponds; P3)													
0.1	0.5	0.0	0.0	0.0					0.0	1.5	0.0	1.2	

Table 4.6 (Continued)

0.4	0.2	0.2	0.0	0.3	2.1	0.7	1.1		0.4	15.9	9.5	7.2	1.7
0.2	0.6	0.0	0.0	0.0	2.3	4.1	0.3	0.4	0.0	12.0	7.4	1.1	0.0
0.0	0.2	0.0	2.0		0.9	0.6	0.0	0.0	0.0	0.9	1.3	0.0	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			

Direct orange 46 + SWW (*Lemna minor* L. and algae ponds; P1)

0.3	2.7	0.0	0.0	0.8					0.0	0.2	0.0	0.6	
0.0	2.5	0.2	0.0	0.0	1.6	2.4	0.8		0.2	19.9	10.0	5.3	3.0
0.3	0.0	0.0	0.5	0.0	2.0	3.6	0.1	0.2	0.0	10.8	4.6	2.5	0.4
0.0	0.0	0.0	0.0		0.7	0.0	0.0	0.0	0.1	0.2	0.0	0.2	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			

Direct orange 46 + SWW (*Lemna minor* L. ponds; P3)

0.2	2.2	0.6	0.4	1.5					0.3	0.4	0.0	0.7	
0.0	1.0	0.0	0.0	0.2	2.7	0.8	3.1		0.0	19.8	6.9	5.6	3.5
0.3	0.0	0.0	0.0	0.0	2.7	4.6	0.3	0.0	0.1	6.8	5.7	1.7	0.8
0.0	0.0	0.0	0.0		0.6	0.3	0.0	0.0	0.1	0.5	0.8	0.0	
					0.0	0.3	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			

SWW (*Lemna minor* L. and algae ponds; P1)

0.3	0.0	0.2	0.0	0.0					0.1	1.9	0.0	0.5	
0.0	0.1	0.0	0.0	0.0	1.2	0.9	0.1		1.2	25.7	17.3	8.8	1.6
0.0	0.4	0.0	0.0	0.0	0.0	1.9	0.2	0.0	2.2	12.0	8.3	2.1	0.0
0.0	0.0	0.0	0.0		0.3	0.0	0.0	0.0	0.0	1.0	0.0	1.2	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			

SWW (*Lemna minor* L. ponds; P3)

0.0	0.6	0.0	0.0	0.1					0.0	1.3	0.0	0.4	
0.0	0.3	0.0	0.0	0.0	1.5	1.6	0.0		1.0	22.0	11.8	8.1	2.7
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	3.1	11.9	7.4	5.4	0.0
0.0	0.0	0.0	0.0		1.6	0.2	0.0	0.0	2.4	3.8	1.2	0.0	
					0.0	0.0	0.0		0.0	0.0	0.0		
					0.0				0.0	0.0			

Note: ^{a} Hue; and ^{b} Value (lightness and darkness of a colour)/Chroma (degree of strength or saturation); Note that the number of replicates is 4 and the pond surface area is 841.5 cm^2; SWW, synthetic wastewater.

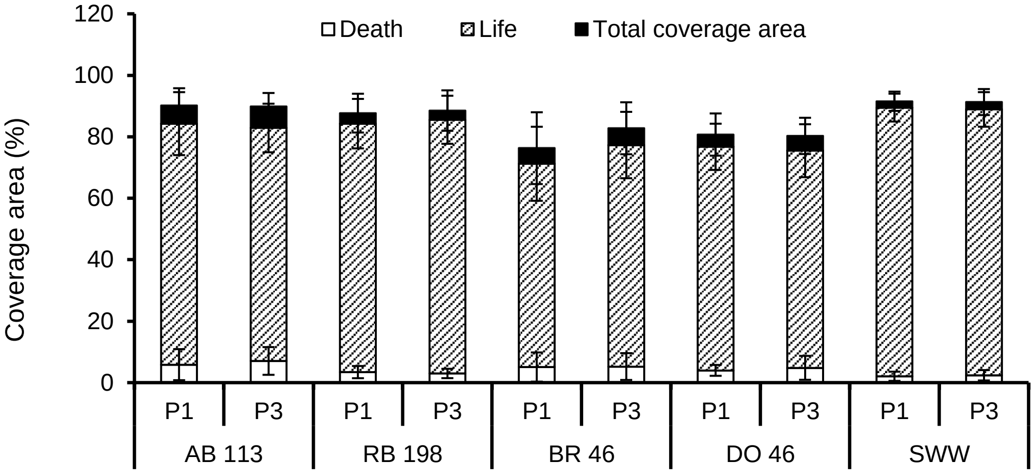


Figure 4.7 Overview of total, living and dead coverage areas (%) for Lemna minor L. fronds using the Eleatheia Lemna edition in each system between 15 December 2014 and 15 September 2015. Note: P1, *Lemna minor* L. and algae ponds; P3, *Lemna minor* L. ponds only; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater.

Table 4.7 Overview of the statistical analysis for the growth parameters between *Lemna minor* L. and algae, and *Lemna minor* L. ponds using the parametric t-test

Parameter	Shapiro-Wilk test (*p* value[1])	*p* value [2] between P1 & P3 ponds
Coverage area (%)		
Acid blue 113	0.401	0.926
Reactive blue 198	0.392	0.938
Basic red 46	0.258	0.275
Direct orange 46	0.728	0.908
Synthetic wastewater	0.865	0.890
Relative frond number (-)		
Acid blue 113	0.064	0.909
Reactive blue 198	0.254	0.932
Basic red 46	0.281	0.972
Direct orange 46	0.604	0.690
Synthetic wastewater	0.377	0.922
Relative growth rate per day (-)		
Acid blue 113	0.848	0.834
Reactive blue 198	0.889	0.948
Basic red 46	0.066	0.948
Direct orange 46	0.597	0.750
Synthetic wastewater	0.312	0.944

Note: [1]Test of normality (if $p > 0.05$, data normally is distributed; if $p < 0.05$, data are not normally distributed); and [2]Probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different).

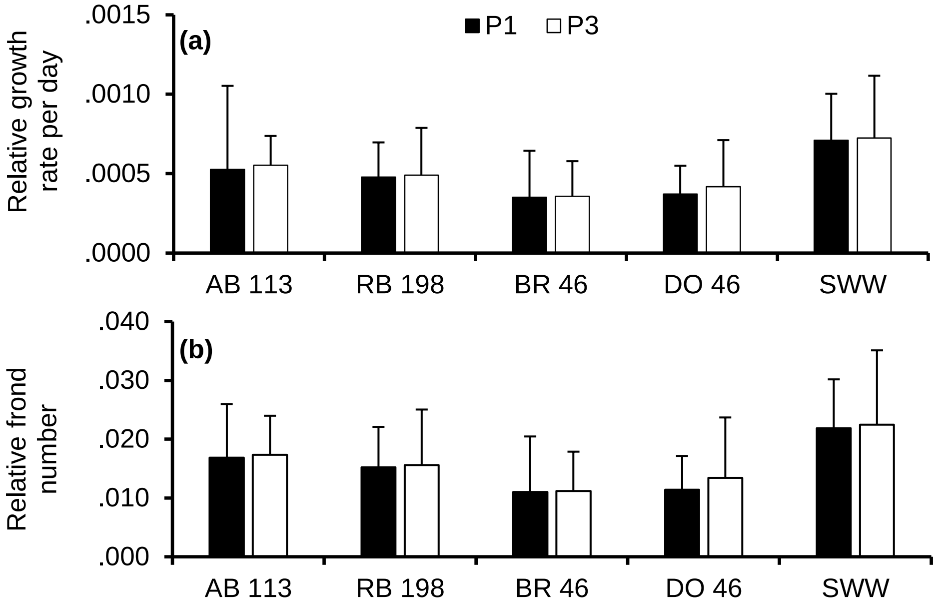


Figure 4.8 Overview of growth parameters using the Eleatheia Lemna edition for each system between 15 December 2014 and 15 September 2015. Note: (a), relative growth rate based on the frond number; (b) relative frond number; P1, *Lemna minor* L. and algae ponds; P3, *Lemna minor* L. ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater.

Table 4.8 Mean of monthly values of the fresh and dry weights of the plants harvested for each system between 15 December 2014 and 15 September 2015

Date	Acid blue 113		Reactive blue 198		Basic red 46		Direct orange 46		Synthetic wastewater	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
Lemna minor L. and algae ponds										
10/02/15	14.52	0.73	13.55	0.70	13.12	0.68	13.55	0.72	12.28	0.62
12/03/15	15.01	0.75	14.01	0.70	13.46	0.68	8.92	0.44	12.22	0.70
16/04/15	14.44	0.73	8.21	0.42	5.30	0.25	6.99	0.35	12.05	0.56
13/05/15	9.92	0.50	8.11	0.41	0.00	0.00	2.44	0.13	13.01	0.67
11/06/15	9.77	0.49	12.11	0.61	4.52	0.26	8.55	0.43	13.44	0.71
15/07/15	9.81	0.49	13.34	0.66	4.12	0.20	5.77	0.29	12.21	0.62
21/08/15	13.01	0.68	13.01	0.56	10.95	0.56	12.06	0.63	11.88	0.57
15/09/15	36.55	1.87	33.00	1.67	27.55	1.40	30.18	1.56	35.66	1.77
Lemna minor L. ponds										
10/02/15	12.20	0.61	11.89	0.60	14.01	0.72	10.56	0.54	13.05	0.70
12/03/15	13.26	0.67	14.15	0.70	14.21	0.71	12.22	0.62	12.87	0.61
16/04/15	16.01	0.80	8.35	0.37	11.21	0.57	5.89	0.30	11.40	0.51
13/05/15	12.21	0.61	7.01	0.35	5.68	0.30	6.09	0.30	12.68	0.63
11/06/15	12.11	0.61	13.51	0.68	8.92	0.51	7.64	0.40	13.10	0.69
15/07/15	9.45	0.47	12.67	0.64	4.47	0.23	5.68	0.28	14.88	0.88
21/08/15	12.77	0.64	14.11	0.67	10.11	0.50	11.66	0.61	11.99	0.61
15/09/15	34.91	1.75	36.12	1.85	29.05	1.43	31.66	1.61	37.05	1.88

Note: FW; fresh weight (gram), DW; dry weight (gram).

4.2.4 Environmental conditions

According to Bekcan et al. (2009), the long-term optimum temperature for *L. minor* growth is 26°C. However, the ranges below 17°C and higher than 35°C reduced the growth of *L. minor* as indicated by Ozengin and Elmaci (2007). The minimum and maximum temperatures records of the laboratory, during the experiment period between 15 December 2014 and 15 September 2015, are 22°C and 30°C, respectively (mean of 27.3 ± 2°C). The temperature records were within the recommended ranges (Ozengin & Elmaci, 2007). Movafeghi et al. (2013) found that dye removal improved with an increase in temperature. They suggested that dye bio-sorption is an endothermic process linked to *L. minor*. Light intensity values ranged between 2023 lux and 2450 lux, which were close to the optimum value of 2400 lux as documented by Kilic et al. (2010). A summary of the environmental boundary conditions in the laboratory is shown in Table 4.9.

Table 4.9 Overview of environmental boundary conditions in the laboratory between 15 December 2014 and 15 September 2015

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	N
Temperature	°C	27.3	2.59	22.4	29.9	127
Temperature (minimum within 24 h)	°C	21.9	2.38	18.0	24.1	126
Temperature (maximum within 24 h)	°C	29.4	2.11	25.0	32.3	126
Relative humidity	%	53.7	4.08	36.0	60.0	127
Relative humidity (minimum within 24 h)	%	47.6	3.56	34.0	52.0	127
Relative humidity (maximum within 24 h)	%	66.7	5.35	45.0	74.0	127
Illuminance (one-off records)	lux	2215.4	145.52	2023.0	2450.0	33

Note: N, number of records; h, hours.

4.3 Performance assessment of *L. minor* ponds under semi-natural conditions

4.3.1 Inflow water quality parameters

Table 4.10 and Figure 4.9 summarise the main inflow water quality parameters of the prepared SWW with and without dyes. The values of pH and apparent colour were within the typical range (Upadhye et al., 2012). In addition, zinc, iron, copper and boron values were within the typical range of textile wastewater discharge (Ghaly et al. 2014). The concentration of the dye was 5 mg/l, which was lower than the typical range of textile factor discharges due to the reasons mentioned previously in Section 4.2.1.

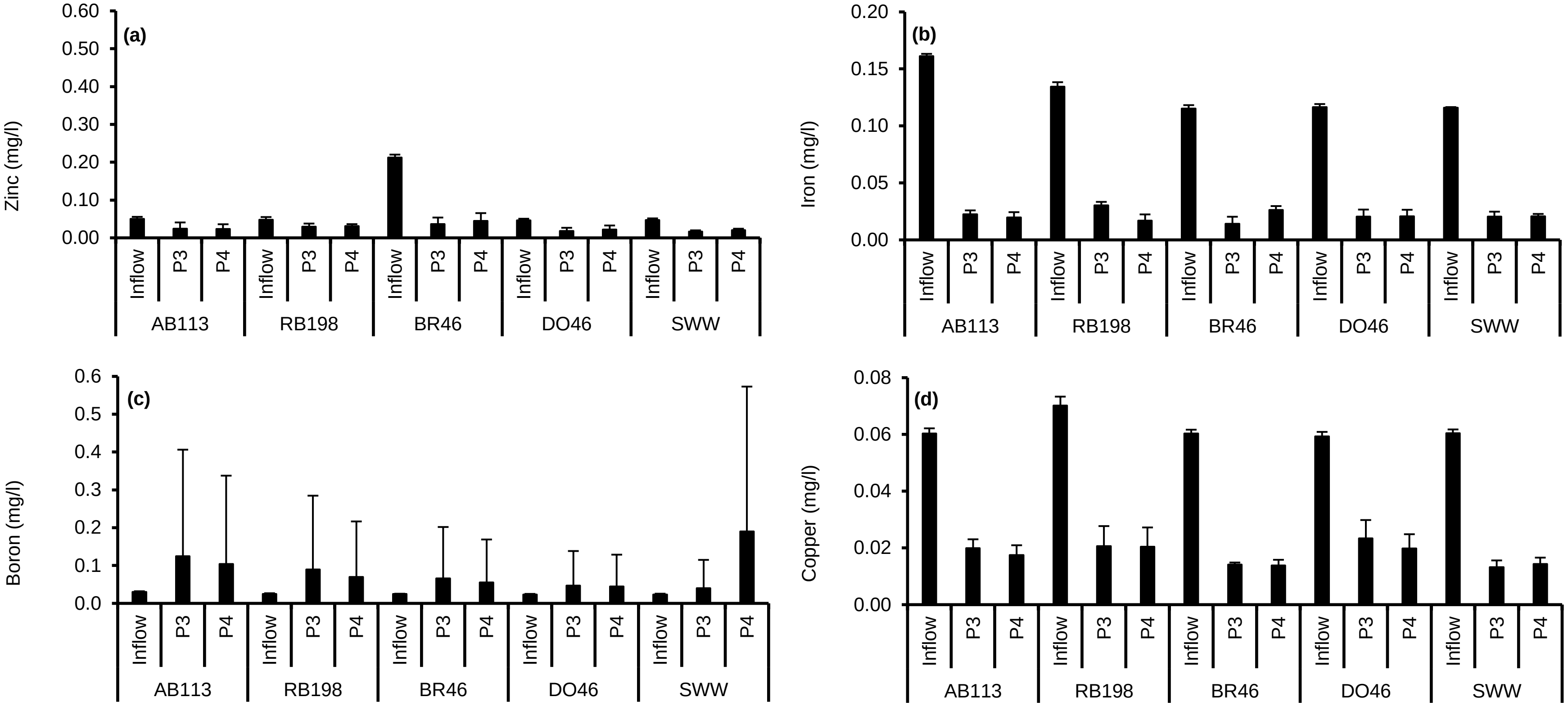
Table 4.10 Inflow water quality parameters for each system between 15 December 2014 and 2 February 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue 113 + Synthetic wastewater						
Dye concentration	mg/l	5	0.4	4.5	5.6	58
pH	-	7.3	0.12	7.1	7.5	58
Redox	mV	-24.4	8.4	-37	-12	58
Dissolved oxygen	mg/l	9.6	0.28	9.2	10.5	58
Electrical conductivity	µS/cm	113.9	6.06	106	133	58
Total dissolved solids	mg/l	56.95	3.03	53	66.5	58
Suspended solids	mg/l	3.7	1.33	2	6	58
Turbidity	NTU	1.6	0.64	0.7	3	58
Colour	Pt Co	445.6	25.14	416	482	14
Chemical oxygen demand	mg/l	24.7	0.96	23	26	13
Reactive blue 198 + Synthetic wastewater						
Dye concentration	mg/l	5.1	0.39	4.4	5.6	58
pH	-	7.3	0.1	7.2	7.5	58
Redox	mV	-31.6	5.24	-38	-23	58
Dissolved oxygen	mg/l	9.6	0.2	9.3	10.5	58
Electrical conductivity	µS/cm	123.7	6.63	112	135	58
Total dissolved solids	mg/l	61.85	3.315	56	67.5	58
Suspended solids	mg/l	4.9	0.82	3	7	58
Turbidity	NTU	2	0.36	1.3	2.7	58
Colour	Pt Co	167.4	38.14	123	201	14
Chemical oxygen demand	mg/l	9.4	0.61	8.5	10.1	13
Basic red 64 + Synthetic wastewater						
Dye concentration	mg/l	5.1	0.3	4.4	5.5	58
pH	-	7.3	0.05	7.3	7.4	58
Redox	mV	-31.7	2.52	-37	-28	58
Dissolved oxygen	mg/l	9.6	0.24	9.2	10.3	58
Electrical conductivity	µS/cm	114.1	4.99	103	121	58
Total dissolved solids	mg/l	57.05	2.495	51.5	60.5	58
Suspended solids	mg/l	3.9	0.95	2	5	58
Turbidity	NTU	3.1	0.83	1.9	6	58
Colour	Pt Co	410.1	12.25	389	424	14
Chemical oxygen demand	mg/l	13.3	0.88	12	14.5	13
Direct orange 46 + Synthetic wastewater						
Dye concentration	mg/l	5	0.35	4.4	5.6	58
pH	-	7.4	0.06	7.3	7.4	58
Redox	mV	-32.2	3.04	-37	-29	58
Dissolved oxygen	mg/l	9.5	0.06	9.5	9.6	58
Electrical conductivity	µS/cm	118.9	10.32	105.4	133	58
Total dissolved solids	mg/l	59.45	5.16	52.7	66.5	58

Table 4.10 (Continued)

Suspended solids	mg/l	3.2	0.98	2	5	58
Turbidity	NTU	2	0.65	0.9	3.5	58
Colour	Pt Co	674.4	38.13	620	721	14
Chemical oxygen demand	mg/l	14.5	0.97	12.5	16	13
Synthetic wastewater (without dye addition)						
pH	-	7.3	0.07	7.2	7.3	13
Redox	mV	-27.2	5.27	-33	-19	13
Dissolved oxygen	mg/l	9.4	0.35	8.9	9.9	13
Electrical conductivity	µS/cm	85.7	0.88	84	87	13
Total dissolved solids	mg/l	42.85	0.44	42	43.5	13
Suspended solids	mg/l	2	1.47	0	4	13
Turbidity	NTU	3.2	0.76	2	4.1	13
Colour	Pt Co	3	0.78	2	4	13
Chemical oxygen demand	mg/l	4.1	0.3	3.6	4.4	12

Note: NTU, nephelometric turbidity unit.


(a)
Zinc (mg/l)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
(b)
Iron (mg/l)
0.20
0.15
0.10
0.05
0.00
(c)
Boron (mg/l)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
(d)
Copper (mg/l)
0.08
0.06
0.04
0.02
0.00
Inflow
P3
P4
AB113
RB198
BR46
DO46
SWW

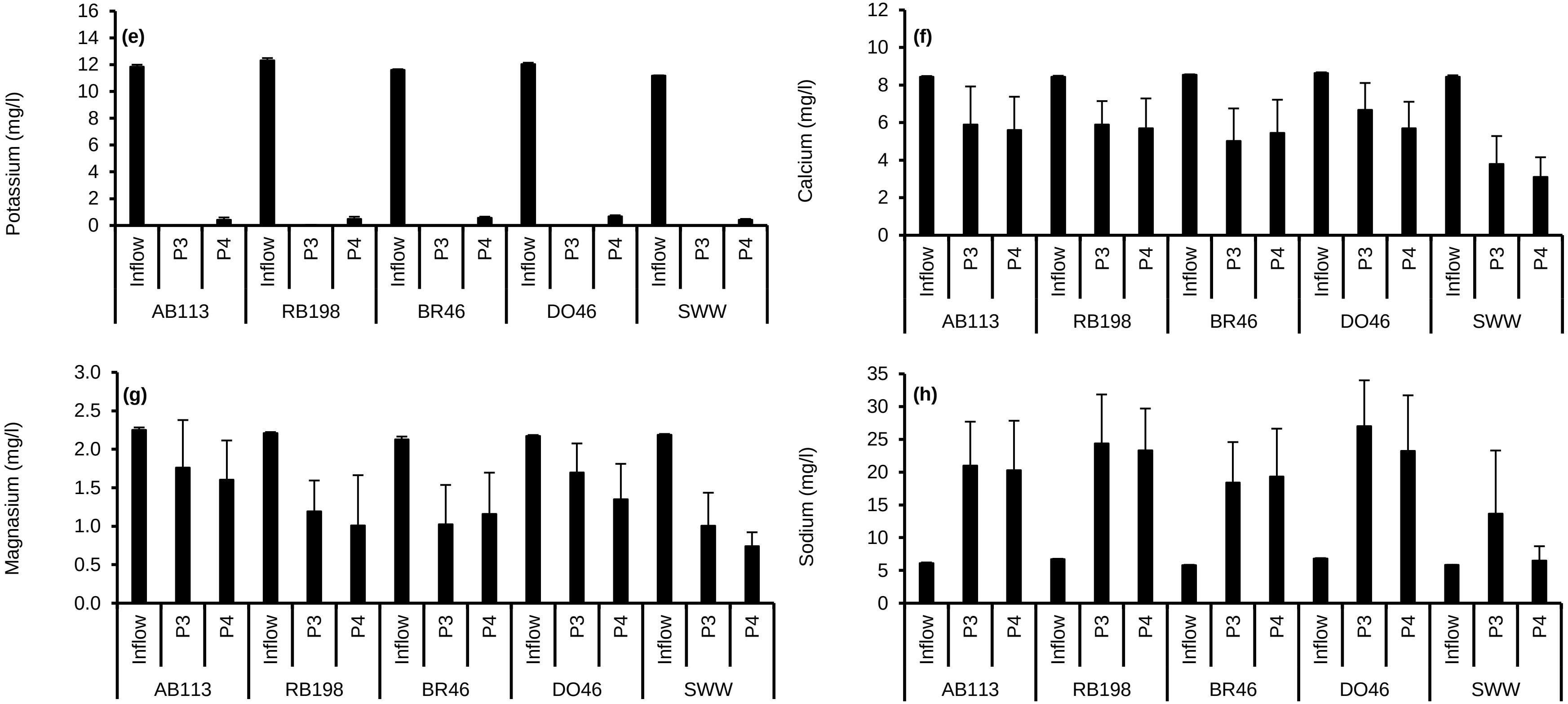


Figure 4.9 Mean inflow and outflow concentrations of the detected trace elements during the experiment between 15 December 2014 and 2 February 2016. Note: (a), zinc; (b), iron; (c), boron; (d), copper; (e), potassium; (f), calcium; (g), magnesium; (h), sodium; P3, *Lemna minor* L. ponds; P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dye.

4.3.2 Treatment performance

4.3.2.1 Dye and apparent colour

The mean removal values were higher within *L. minor* ponds than control ponds for all dyes (Table 4.11). The mean removals of AB113, which are equal to 32% for *L. minor* ponds, and 28% for control ponds, were impacted by the dilution effect considering that rainfall added more water to the ponds than evapotranspiration removed from the ponds, and microbial activity. However, significant differences were found between *L. minor* ponds, and control ponds regarding mean BR46 and RB198 removals (Table 4.12). The removal efficiencies for BR46 and RB198 in *L. minor* ponds were around 51% and 19%, respectively, which were due to the impact of microbes and plants in addition to the adsorption by container walls. The removal efficiencies by plants only due to adsorption and/or transformation processes were only around 13% and 8%, because the control ponds removed these dyes by around to 38% and 11% for BR46 and RB198, respectively. These results resemble outcomes for treatment of basic blue 41 using submerged plants (Keskinkan & Lugal Goksu, 2007). Regarding the treatment performance of ponds containing DO46, although a significant difference in mean removal between design variables was found (Table 4.12), the mean removal values were only 4% and -4% (i.e. system became a source rather than a sink) for *L. minor* and control ponds (Table 4.11), which indicates that these pond systems are unable to break down DO46 molecules.

The longitudinal profile of the dye removal is shown in Figure 4.10. Most of the removal efficiencies in planted ponds were higher than the control ponds for all dyes. In addition, the same longitudinal trend was noted for all dyes throughout the year (Figures 4.10a, b and c), except for some fluctuating within the DO46 profile (Figure 4.10d). The dye removal fluctuated with the variation of rainfall. A considerable increase was noted at the end of the experiment due to the high amount of rainfall in winter. The maximum and minimum removals were 82% and 7% for *L. minor* ponds, and 63% and -1% for control ponds for the treatment of AB11, as well as 43% and 7%, and 34% and -13% for *L. minor* and control ponds, respectively, for the treatment of RB198 (Figure 4.10b). Negative removal efficiencies can be explained by phenomena such as water surface evaporation and pond edge effects. The DO46 removal ranged between 18% and -25%, and between 15% and -41% for *L. minor,* and control ponds, respectively (Figure 4.10d). High removal efficiencies were recorded for BR46 (Figure 4.10c). The minimum and maximum removals were 18% and 86% for *L. minor* ponds, and 14% and 85% for control ponds, respectively. This indicates that BR46 is treated easily by shallow pond systems under natural conditions in Salford. This is due to the simple structure and small molecular weight of BR46 (Noonpui & Thiravetyan, 2011). Moreover, the absence of sulpho groups in BR46 is linked to good degradation during biological treatment (Pearce et al., 2003). Furthermore, the pH values, which ranged from 7 to 10, as indicated later, were suitable for BR46 uptake. Movafeghi et al. (2013) reported that pH values between 5.3 and 9.3 do not noticeably affect the treatment of BR46. They considered that a neutral pH is optimal for decolourisation of this dye. Multiple comparisons of removal efficiencies among dyes showed a significant difference among all dyes. The corresponding dye values were ranked as follows: BR46 > AB113 > RB198 > DO46. The p values were < 0.001 between the dye removal efficiencies using a univariate analysis of variance between the types of dyes for the following dye combinations: AB113 and RB198; AB113 and BR46; AB113 and DO46; RB198 and BR46; RB198 and DO46; and BR46 and DO46.

The treatment performance based on the dye concentrations showed that the outflow concentrations were lower than the inflow concentrations for all dyes except for DO46 during the period between 15 April 2015 and 15 October 2015. Lower outflow values were noticed for *L. minor* and control ponds during periods of relatively high rainfall (Figure 4.11). In addition, the mean outflow dye concentrations for all dyes were higher in the control ponds than in the *L. minor* ponds (Table 4.13). Except for AB113, the mean outflow dye concentrations were significantly higher within the control ponds than the *L. minor* ponds (Table 4.12). Low standard deviations are associated with outflow AB113, RB198 and BR46 concentrations. In contrast, the highest water quality standard deviations were linked to DO46 (Table 4.13).

The HPLC results of inflow samples showed a peak at a retention time of 1.711 min for AB113, 1.505 min for RB198, 1.488, 1.693, 2.569 and 2.405 min for BR46, and 1.497 min for DO46 (Appendix E, Figures E.1a, b, c and d, respectively). However, the outflow samples showed a peak at 1.519 min and 1.91 min for *L. minor* ponds and control ponds containing AB113, respectively (Appendix E, Figure E.6), 1.626, 1.689 min for *L. minor* ponds, and 1.599 min for control ponds containing RB198, respectively (Appendix E, Figure E.7), 2.089 min and 1.607 min for planted and control ponds containing BR46 (Appendix E, Figure E.8), and 1.643 min and 1.653 min for *L. minor* ponds and control ponds containing DO46, in that order (Appendix E, Figure E.9). These changes in peaks were due to the dye removal by both planted and unplanted ponds (Table 4.11) by the adsorption process as the colourless outflow samples weren't achieved for all ponds containing AB113, RB198 and DO46, and due to the interactions with the wastewater. However, the disappearance of most of the inflow peaks and appearance of one new peak for each BR46 treatment system, linked with the higher removal of this dye (Table 4.11) compared with other dyes, confirmed the BR46 elimination. The mechanism of BR46 removal by adsorption and transformation processes by plants and microbes in *L. minor* ponds, and by adsorption and may be biotransformation for control ponds is likely (Khandare et al., 2011; Kabra et al., 2012).

Colour mean outflow values (Table 4.13) were higher for control ponds regarding all types of wastewater except for ponds containing AB113. Overall, the outflow colour values were lower than the inflow colour values for all ponds containing dyes. Inflow and outflow sample pictures are shown in Appendix F, Figure F.1.

Table 4.11 Dye and chemical oxygen demand removals for each system between 15 December 2014 and 2 February 2016

Parameter	Load in (mg m^{-2} $week^{-1}$)	Standard deviation	Minimum	Maximum	Load out (mg m^{-2} $week^{-1}$)	Standard deviation	Minimum	Maximum	Removal (%)	Number of reading
Acid blue 113 + Synthetic wastewater										
Dye										
P3	112.2	23.98	71.8	174.8	76.6	26.79	15.3	146.3	32	58
P4	119.9	27.47	77.2	180.0	86.8	33.74	35.1	159.8	28	58
Chemical oxygen demand										
P3	3107.6	1754.53	809.3	6519.3	2813.5	1699.33	433.2	5876.0	9	9
P4	3158.6	1589.15	1195.5	6566.8	2904.5	1544.67	951.3	6095.9	8	9
Reactive blue 198 + Synthetic wastewater										
Dye										
P3	139.7	27.82	87.9	221.6	112.8	26.73	58.7	181.9	19	58
P4	163.0	39.07	99.3	251.1	145.3	39.71	80.2	258.6	11	58
Chemical oxygen demand										
P3	2382.7	1055.50	594.1	4339.9	2083.0	1078.62	242.8	3835.9	13	9
P4	2515.1	1301.21	608.3	4344.6	2290.1	1303.53	255.5	3946.8	9	9
Basic red 46 + Synthetic wastewater										
Dye										
P3	95.2	23.31	61.4	163.0	46.7	23.85	9.5	98.0	51	58
P4	108.7	30.76	65.2	194.6	66.9	34.28	12.5	162.2	38	58
Chemical oxygen demand										
P3	2509.5	1086.64	593.7	4282.8	1724.4	897.16	215.7	3310.9	31	9
P4	3177.6	1511.97	598.9	5276.3	2384.3	1375.12	254.9	4542.2	25	9
Direct orang 46 + Synthetic wastewater										
Dye										
P3	185.9	66.22	106.9	377.4	178.0	65.18	95.3	411.3	4	58
P4	227.3	79.24	124.8	433.8	236.9	86.57	132.2	461.7	-4	58
Chemical oxygen demand										

Table 4.11 (Continued)

P3	3462.9	2361.97	617.7	7831.3	3260.2	2314.63	276.6	6514.9	6	9
P4	3114.3	1880.33	556.4	5805.1	3090.9	2129.66	283.8	6896.3	1	9
Synthetic wastewater										
Chemical oxygen demand										
P3	3005.3	1524.43	765.3	5279.9	2064.5	1312.14	337.3	4102.7	31	9
P4	4180.2	1160.06	2476.5	6278.1	3652.9	1102.97	2072.5	5934.4	13	8

Note: P3, *Lemna minor* L. ponds; P4, control ponds. Note that a negative removal indicates that the system worked as source rather than sink.

Table 4.12 Overview of the statistical analysis for outflow water quality parameters and corresponding removal efficiencies for the period from 15 December 2014 to 2 February 2016

Type of the dye	Shapiro-Wilk test (*p* value^1)	Statistical test	*p*-values^2 between P3 & P4	Type of the dye	Shapiro-Wilk test (*p* value^1)	Statistical test	*p*-values^2 between P3 & P4
Dye (mg/l)				Dye removal (%)			
AB113	0.034	M-W	0.195	AB113	0.03	M-W	0.201
RB198	0.002	M-W	<0.001	RB198	0.247	t-test	<0.001
BR46	<0.001	M-W	0.004	BR46	0.013	M-W	<0.001
DO46	<0.001	M-W	0.003	DO46	<0.001	M-W	<0.001
Chemical oxygen demand (mg/l)				Chemical oxygen demand removal (%)			
AB113	0.095	t-test	0.826	AB113	<0.001	M-W	0.627
RB198	0.275	t-test	0.777	RB198	<0.001	M-W	0.233
BR46	0.094	t-test	0.531	BR46	0.121	t-test	0.596
DO46	0.094	t-test	0.942	DO46	0.009	M-W	0.691
SWW	0.354	t-test	0.167	SWW	<0.001	M-W	0.043
Colour (Pt Co)				Dissolved oxygen (mg/l)			
AB113	0.046	M-W	0.95	AB113	0.002	M-W	0.625
RB198	0.008	M-W	0.327	RB198	<0.001	M-W	0.838

Table 4.12 (Continued)

BR46	0.003	M-W	0.261	BR46	<0.001	M-W	0.993
DO46	0.318	t-test	0.103	DO46	<0.001	M-W	0.91
SWW	0.019	M-W	<0.001	SWW	<0.001	M-W	0.598
Suspended solids (mg/l)				pH (-)			
AB113	<0.001	M-W	0.897	AB113	<0.001	M-W	0.633
RB198	<0.001	M-W	0.309	RB198	<0.001	M-W	0.262
BR46	<0.001	M-W	0.005	BR46	<0.001	M-W	0.855
DO46	<0.001	M-W	0.332	DO46	<0.001	M-W	0.989
SWW	<0.001	M-W	<0.001	SWW	0.075	t-test	0.001
Turbidity (NTU)				Redox potential (mV)			
AB113	<0.001	M-W	0.473	AB113	0.02	M-W	0.879
RB198	<0.001	M-W	0.196	RB198	<0.001	M-W	0.282
BR46	<0.001	M-W	0.069	BR46	0.059	t-test	0.693
DO46	<0.001	M-W	0.886	DO46	0.008	M-W	0.884
SWW	<0.001	M-W	<0.001	SWW	0.001	M-W	<0.001
Electrical conductivity (μS/cm)							
AB113	0.141	t-test	<0.001				
RB198	0.008	M-W	<0.001				
BR46	<0.001	M-W	0.143				
DO46	0.001	M-W	0.001				
SWW	0.048	M-W	0.081				

Note: ^{1}Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); ^{2}*p* value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; P3, *Lemna minor* L. ponds; P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; M-W, Mann-Whitney U test; K-W, Kruskal-Wallis test.

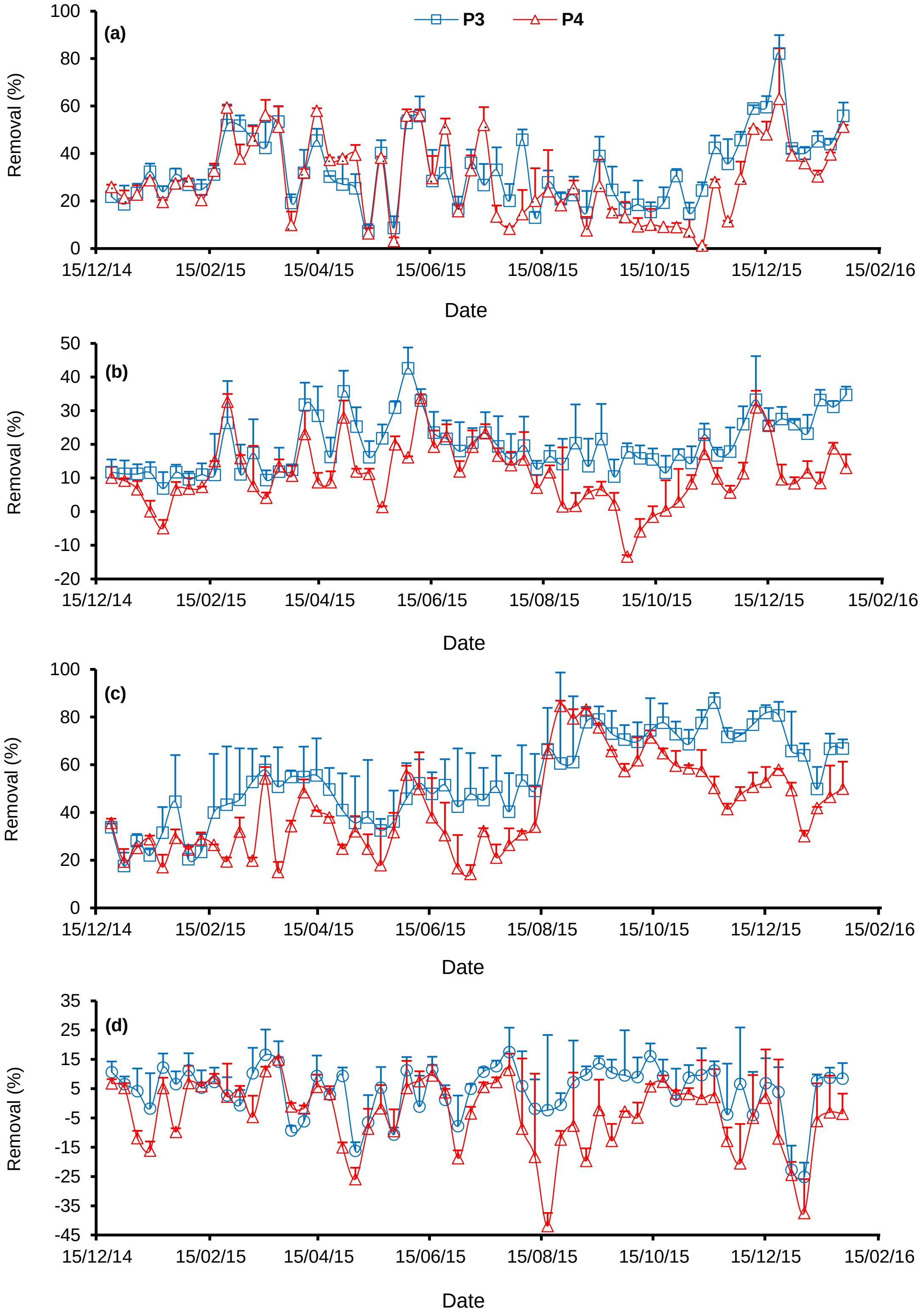


Figure 4.10 Mean values of dye removal profile between 15 December 2014 and 2 February 2016. Note: (a) acid blue 113; (b) reactive blue 198; (c) basic red 46; (d) direct orange 46; P3, *Lemna minor* L. ponds; P4, control ponds.

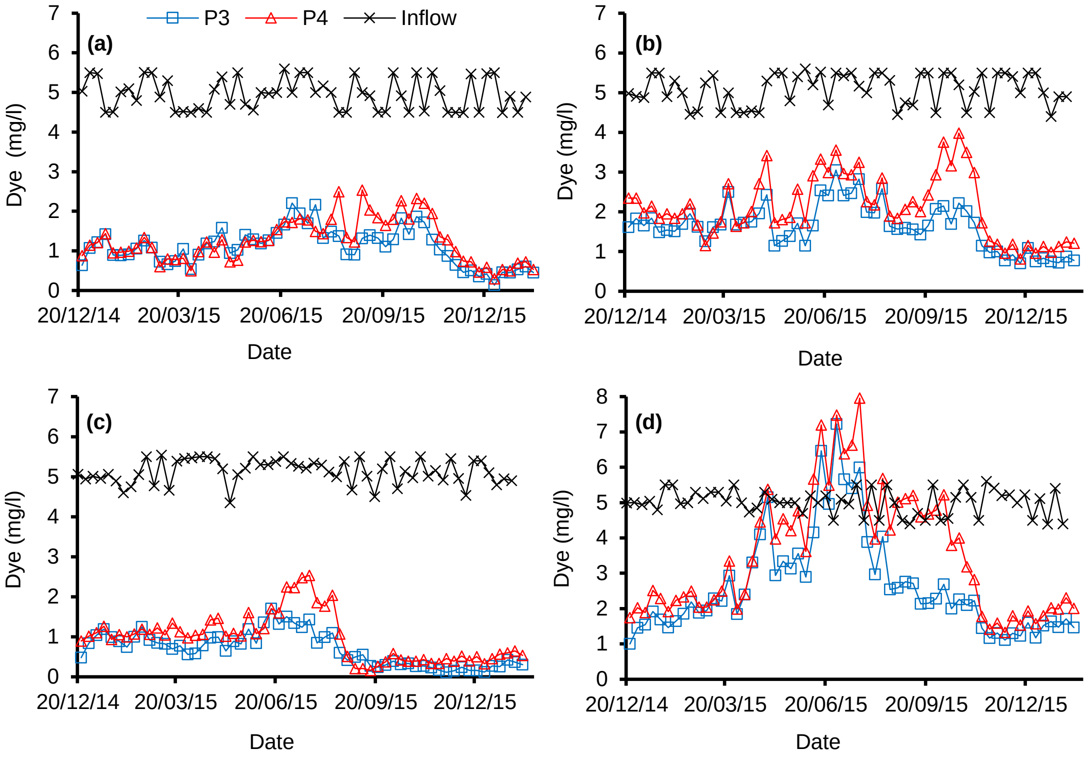


Figure 4.11 Mean inflow and outflow dye concentrations between 15 December 2014 and 2 February 2016. Note; (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; P3, *Lemna minor* L. ponds; P4, control ponds.

Table 4.13 Outflow water quality parameters for each system between 15 December 2014 and 2 February 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue 113 + Synthetic wastewater						
Lemna minor L. ponds						
Dye concentration	mg/l	1.1	0.46	0.1	2.2	59
pH	-	7.9	0.52	7.2	9.1	58
Redox	mV	-50.1	12.3	-71.3	-18.3	58
Dissolved oxygen	mg/l	10.5	1.26	8.2	14.3	58
Electrical conductivity	µS/cm	72.4	20.04	35.5	120	58
Total dissolved solids	mg/l	36.2	10.02	17.75	60	58
Suspended solids	mg/l	24.1	23.6	3.3	94.5	58
Turbidity	NTU	7.2	5.66	2.2	27.4	58
Colour	Pt Co	194	91.97	75.8	355.3	18
Chemical oxygen demand	mg/l	38.4	24.37	4.2	76	14
Control ponds						
Dye concentration	mg/l	1.3	0.55	0.3	2.5	59
pH	-	7.8	0.49	7.1	9.7	58
Redox	mV	-50.8	10.98	-79.6	-16	58
Dissolved oxygen	mg/l	10.5	1.09	8.2	13.4	58

Table 4.13 (Continued)

Electrical conductivity	µS/cm	88.2	22.76	49.8	134.3	58
Total dissolved solids	mg/l	44.1	11.38	24.9	67.15	58
Suspended solids	mg/l	21.2	17.96	2	82	58
Turbidity	NTU	5.8	3.64	1.5	20.6	58
Colour	Pt Co	189.1	98.96	41	381.5	18
Chemical oxygen demand	mg/l	36	24.01	3.7	77.1	14
Reactive blue 198 + Synthetic wastewater						
Lemna minor L. ponds						
Dye concentration	mg/l	1.6	0.56	0.7	3.1	59
pH	-	7.7	0.43	7	9.3	58
Redox	mV	-47.5	9.76	-71.5	-18	58
Dissolved oxygen	mg/l	10.5	1.38	8.2	15.7	58
Electrical conductivity	µS/cm	71.8	20.81	34.5	139.4	58
Total dissolved solids	mg/l	35.9	10.405	17.25	69.7	58
Suspended solids	mg/l	13.2	9.9	1.8	42	58
Turbidity	NTU	5	2.45	2	11.4	58
Colour	Pt Co	61.9	38.72	10.3	135	18
Chemical oxygen demand	mg/l	26.6	18.49	2.5	68.1	14
Control ponds						
Dye concentration	mg/l	2.1	0.79	0.8	4	59
pH	-	7.7	0.57	7.1	9.7	58
Redox	mV	-46.6	11.19	-74.5	-20	58
Dissolved oxygen	mg/l	10.5	1.32	8	14.4	58
Electrical conductivity	µS/cm	95	21.71	64.5	155.5	58
Total dissolved solids	mg/l	47.5	10.855	32.25	77.75	58
Suspended solids	mg/l	11.1	8.6	2	36	58
Turbidity	NTU	4.7	3.03	1	13.5	58
Colour	Pt Co	91.7	67.5	6.5	221.5	18
Chemical oxygen demand	mg/l	29.8	21.03	2.6	72.2	14
Basic red 46 + Synthetic wastewater						
Lemna minor L. ponds						
Dye concentration	mg/l	0.7	0.41	0.1	1.7	59
pH	-	7.7	0.57	7	10	58
Redox	mV	-46.8	12.92	-77.3	-16.3	58
Dissolved oxygen	mg/l	10.5	1.38	8.2	15.9	58
Electrical conductivity	µS/cm	77.5	23.54	32.4	131.6	58
Total dissolved solids	mg/l	38.75	11.77	16.2	65.8	58
Suspended solids	mg/l	31.7	31.36	2.3	100.3	58
Turbidity	NTU	8.9	7.54	1.8	35.6	58
Colour	Pt Co	51.7	35.65	14.3	126.5	18
Chemical oxygen demand	mg/l	30	17.75	2.1	61.1	14
Control ponds						
Dye concentration	mg/l	1	0.59	0.2	2.5	59
pH	-	7.7	0.46	7	9.9	58
Redox	mV	-45.9	11.76	-76.2	-15.5	58
Dissolved oxygen	mg/l	10.4	1.15	8.1	13.4	58

Table 4.13 (Continued)

Electrical conductivity	µS/cm	84.7	30.02	34.1	154.8	58
Total dissolved solids	mg/l	42.35	15.01	17.05	77.4	58
Suspended solids	mg/l	24.6	32.1	1	111	58
Turbidity	NTU	7.4	7.33	1	31.3	58
Colour	Pt Co	63.6	35.94	19	148	18
Chemical oxygen demand	mg/l	35.8	27.82	2.5	88.5	14
Direct orange 46 + Synthetic wastewater						
Lemna minor L. ponds						
Dye concentration	mg/l	2.6	1.43	1	7.2	59
pH	-	7.8	0.57	7.1	9.9	58
Redox	mV	-48.6	12.76	-79	-25.3	58
Dissolved oxygen	mg/l	10.5	1.3	8.2	15.4	58
Electrical conductivity	µS/cm	72.6	19.8	36	131.9	58
Total dissolved solids	mg/l	36.3	9.9	18	65.95	58
Suspended solids	mg/l	12.8	13.28	2	54.3	58
Turbidity	NTU	4.7	2.95	1.7	18.3	58
Colour	Pt Co	177.8	60.61	85.8	303.3	18
Chemical oxygen demand	mg/l	32.5	25.1	2.7	79	14
Control ponds						
Dye concentration	mg/l	3.5	1.72	1.3	8	59
pH	-	7.8	0.56	7.1	10.1	58
Redox	mV	-48.7	12.36	-82	-25.5	58
Dissolved oxygen	mg/l	10.5	1.23	8.1	14.7	58
Electrical conductivity	µS/cm	88	24.94	45.9	144	58
Total dissolved solids	mg/l	44	12.47	22.95	72	58
Suspended solids	mg/l	11.5	8.33	1	34	58
Turbidity	NTU	4.1	1.85	1.3	10.3	58
Colour	Pt Co	230.6	100.3	72.5	398.5	18
Chemical oxygen demand	mg/l	33.4	26.81	2.8	88.8	14
Synthetic wastewater						
Lemna minor L. ponds						
pH	-	7.7	0.39	6.9	8.6	58
Redox	mV	-46.2	11.22	-64.3	-9.5	58
Dissolved oxygen	mg/l	10.5	1.28	7.8	14.6	58
Electrical conductivity	µS/cm	77.1	19.83	34.8	115.8	58
Total dissolved solids	mg/l	38.55	9.915	17.4	57.9	58
Suspended solids	mg/l	8	5.56	2.3	37.8	58
Turbidity	NTU	4.6	1.93	1.2	10.4	57
Colour	Pt Co	36	20.99	8.5	69.8	18
Chemical oxygen demand	mg/l	28.4	20.59	3.3	71	14
Control pond						
pH	-	8	0.49	7.1	8.9	53
Redox	mV	-53.7	12.28	-72	-21	55
Dissolved oxygen	mg/l	10.4	1.34	7.8	14.5	58
Electrical conductivity	µS/cm	84.3	18.92	44	123	58
Total dissolved solids	mg/l	42.15	9.46	22	61.5	58
Suspended solids	mg/l	44.1	22.8	2	82	58

Table 4.13 (Continued)

Turbidity	NTU	8.9	3.49	1	16.1	57
Colour	Pt Co	63.3	11.91	41	85	18
Chemical oxygen demand	mg/l	39.6	18.58	10	86.1	13

Note: NTU, nephelometric turbidity unit.

4.3.2.2 Chemical oxygen demand and dissolved oxygen

The COD removal efficiency (Table 4.11) was low for all ponds, which was evident from the inflow COD concentrations corresponding to the high outflow values, as discussed below. In addition, mean COD removals were higher in *L. minor* ponds than control ones for all systems containing dyes, but the difference was not significant. However, for ponds comprising only synthetic wastewater without dye, outcomes showed that the mean values of COD removal were significantly higher in *L. minor* ponds than in the corresponding control ponds (Table 4.12). These results indicate that the presence of the dyes impacts on the plant performance by reducing their ability to remove COD. Low removal in all ponds ranged between 1 and 31%, indicating a low level of organic matter degradation occurring in the ponds due to poor microbial activities. Correlation analysis results indicate that the COD removal was significantly ($p < 0.01$) positively correlated with dye removal.

Treatment performance according to COD concentrations showed that the mean outflow values were higher in control ponds than *L. minor* ponds for all types of wastewater except for ponds fed by AB113 (Table 4.13). The presence of plants did not significantly affect the COD values. The European and most international (Carmen & Daneila, 2012) standards set threshold values for COD of 125 mg/l. These values are valid for the discharge of effluent directly into waterbodies. Figure 4.12 shows the COD outflow concentration profiles. In general, the outflow COD concentrations fluctuated over time, and were higher than the inflow ones during the period from 20 March 2015 to 10 November 2015 for all types of wastewater. This indicates low microbial activity for degradation of the organic matter associated with high levels of DO, as discussed later. However, during high rainfall periods, particularly in winter at the beginning and the end of the experiment, the outflow concentrations were lower or similar to the inflow values for all dyes (Figures 4.12a to 4.12d), and higher than the inflow values for ponds fed by synthetic wastewater without dyes (Figure 4.12e) in both planted and control ponds.

Regarding the dissolved oxygen, the outflow values ranged between 7.8 mg/l and 13.9 mg/l. However, the inflow values varied between 8.9 and 10.5 mg/l. In addition, the mean outflow concentrations (Table 4.13) in terms of DO for AB113, RB198 and DO46 were similar for *L. minor* ponds, and control ponds. However, the mean outflow values of DO in *L. minor* ponds were slightly higher than those in the unplanted ponds for systems containing BR46, and only synthetic wastewater. These high DO values boosted the biodegradation of organic contaminants in the wetland ponds by facilitating the growth of micro-organisms (Ong et al., 2009a) and on the other hand inhibited the dye removal (Pearce et al., 2003), as mentioned previously in Section 4.2.2.2.

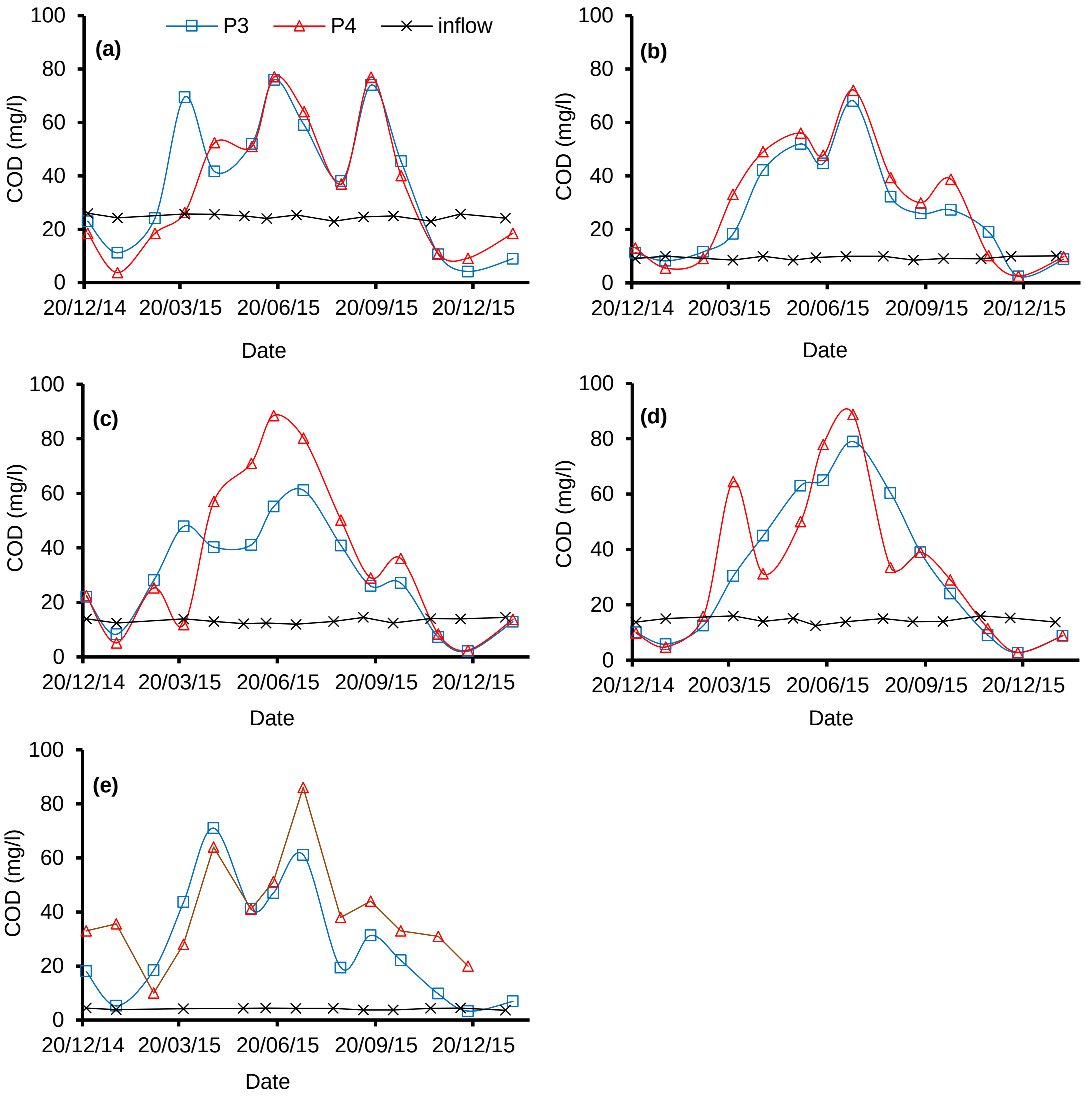


Figure 4.12 Monthly inflow and outflow concentrations of chemical oxygen demand for different treatments between 15 December 2014 and 2 February 2016. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; (e), synthetic wastewater without dye; COD, chemical oxygen demand; P3, *Lemna minor* L. ponds; P4, control ponds.

4.3.2.3 pH and redox potential

The mean outflow pH values (Table 4.13) were slightly higher than the inflow values (Table 4.10) for all ponds. According to the European and international standards (Carmen & Daniela, 2012), samples were 9 and 3, 4 and 6, 4 and 1, 6 and 7, and 2 and 8 times non-compliant for ponds containing AB113, RB198, BR46, DO46, and synthetic wastewater only (without dye) for planted ponds and control ponds, respectively. The pH value influenced the capacity of dye uptake and plant growth, as discussed in Section 4.2.2.3.

In terms of redox potential, the mean outflow values (Table 4.13) were higher in *L. minor* ponds than control ponds for systems containing AB113, DO46, and synthetic wastewater without dye. In contrast, the mean outflows in terms of redox potential for wastewaters containing RB198 and BR46 were higher for control ponds than *L. minor* ponds. Planted ponds containing only synthetic wastewater had significantly higher redox potentials than the control ponds (Table 4.12). As shown in Table 4.13, the minimum and maximum redox potential values indicated anoxic conditions. An aerobic environment is linked to redox potentials higher than 100 mV, while anaerobic conditions are associated with a redox potential of less than -100 mV (Ong et al., 2009a).

4.3.2.4 Suspended solids and turbidity

The mean outflow values for SS (Table 4.13) in the control ponds comprising synthetic wastewater without dye were significantly (Table 4.12) higher than those for *L. minor* ponds which could be reflecting the impact of the plants trapping the SS. In contrast, the mean outflow concentrations of SS for ponds containing BR46 were significantly higher in *L. minor* ponds than control ponds, which is possibly due to higher organic matter degradation in *L. minor* ponds, in addition to the effect of plant decay as it is higher in in *L. minor* ponds containing BR46. The European and many international standards for SS are 35 mg/l in case of effluents discharged directly to receiving freshwater bodies. The results indicate that the *L. minor* ponds and control ponds containing AB113, *L. minor* ponds and control ponds containing RB198, *L. minor* ponds and control ponds containing BR46, *L. minor* ponds and control ponds containing only synthetic wastewater, and *L. minor* comprising DO46 were 13 and 13, 2 and 1, 20 and 17, 1 and 36, and 5 times non-compliant, respectively.

Based on the mean turbidity outflow values (Table 4.13), *L. minor* pond outflows were characterised by higher values than those for control ponds for all dyes. However, the mean outflow turbidity values for *L. minor* ponds were lower than those for the control ponds for systems fed only by synthetic wastewater. This may be attributed to the impact of dyes on the plants' state leading to a high detritus generation in the systems containing dyes compared with those without dyes, enhancing the SS content and consequently the turbidity values. The highest and lowest turbidity values mirrored those for SS. A correlation analysis showed that SS was significantly ($r = 0.917$, $p = 0.000$) positively correlated with turbidity and significantly ($r = -0.471$, $p = 0.000$) negatively correlated with DO. Therefore, high values of DO in the systems may suggest low microbial activities for organic matter degradation, which consequently reduces SS (Sani et al., 2013) and COD removal (Scholz, 2010). A correlation analysis highlighted that the COD removal was significantly ($p < 0.01$) negatively correlated with DO ($r = -0.456$, $p = 0.000$).

4.3.2.5 Electrical conductivity and total dissolved solids

All mean outflow EC values were less than the inflow ones (Table 4.13), and the highest EC mean values were observed for control ponds, whereas the lowest values were found in *L. minor* ponds for all types of wastewater. This indicates that the presence of plants in the ponds is responsible for EC reduction, as explained previously in Section 4.2.2.5. The mean outflow EC values for the control ponds were significantly higher than those for *L. minor* ponds with AB113, RB198 and DO46 (Table 4.12).

The outflow TDS values for all treatment systems (Table 4.13) were compliant with the European and international standards of 2000 mg/l for discharge directly to receiving freshwater bodies and with the threshold of 500 mg/l (class I -natural non-polluted state of water body) (Carmen & Daniela, 2012). All inflow and outflow TDS values were a function (equal to half) of the EC values.

4.3.2.6 Trace elements

All mean outflow values of the detected elements were lower than the corresponding inflows, except for boron and sodium outflows, which were higher than the inflow concentrations (Figure 4.9). The differences between the outflow values of *L. minor* ponds and control ponds for all elements were not significant ($p > 0.05$) for all types of wastewater with and without dyes, except sodium outflows in ponds treated only synthetic wastewater which were significantly higher ($p = 0.03$, Mann-Whitney U test) in planted ponds than those in control ponds. The low differences between the design variables in terms of outflow elements may indicate that the element reduction was due to the impact of rain. However, *L.*

minor also reduced the content of some elements in the system including calcium, magnesium, potassium and sodium by uptake (Figure 4.9) but it seems, after that, the plant acts as a source of these elements due to its decay. Overall, the mean outflow of zinc and iron concentrations (Figures 4.9a and b) were lower than the threshold set of 2 mg/l and 5 mg/l for irrigation, respectively (Metcalf & Eddy, 2003). In addition, all outflow values were within the acceptable limits for *L. minor* (Khellaf & Zerdaoui, 2009). Copper content was also below the threshold limit of 0.2 mg/l for irrigation (Metcalf & Eddy, 2003). In addition, all outflow values were within the tolerance limit of 0.4 mg/l for *L. minor* (Khellaf & Zerdaou, 2009). Khellaf et al. (2008) concluded that copper is a very toxic metal impacting on duckweed at a concentration of 0.5 mg/l. The copper levels in the systems had no adverse effects on the plant growth in this study.

Figure 4.13 shows an overview of the concentrations of elements accumulated in plant tissues for all ponds with and without dyes. The results indicate the plant capacity for sodium, calcium, magnesium and potassium (Figures 4.13g, h, i and j, respectively) accumulation was high. Lower levels of accumulation were indicated for boron, aluminium and manganese (Figures 4.13c, e and f, respectively). The levels of zinc and iron in plants (Figures 4.13a and b, respectively) were more than the allowable boundaries, as mentioned by Nazir et al. (2015). In terms of the bioconcentration factor of metals, results in Figures 4.14a b, c and d indicate that plants were positive for phytoremediation of zinc, iron, copper and boron, respectively. Whereas, the plants were not good accumulators for iron within systems treating AB113 and RB198.

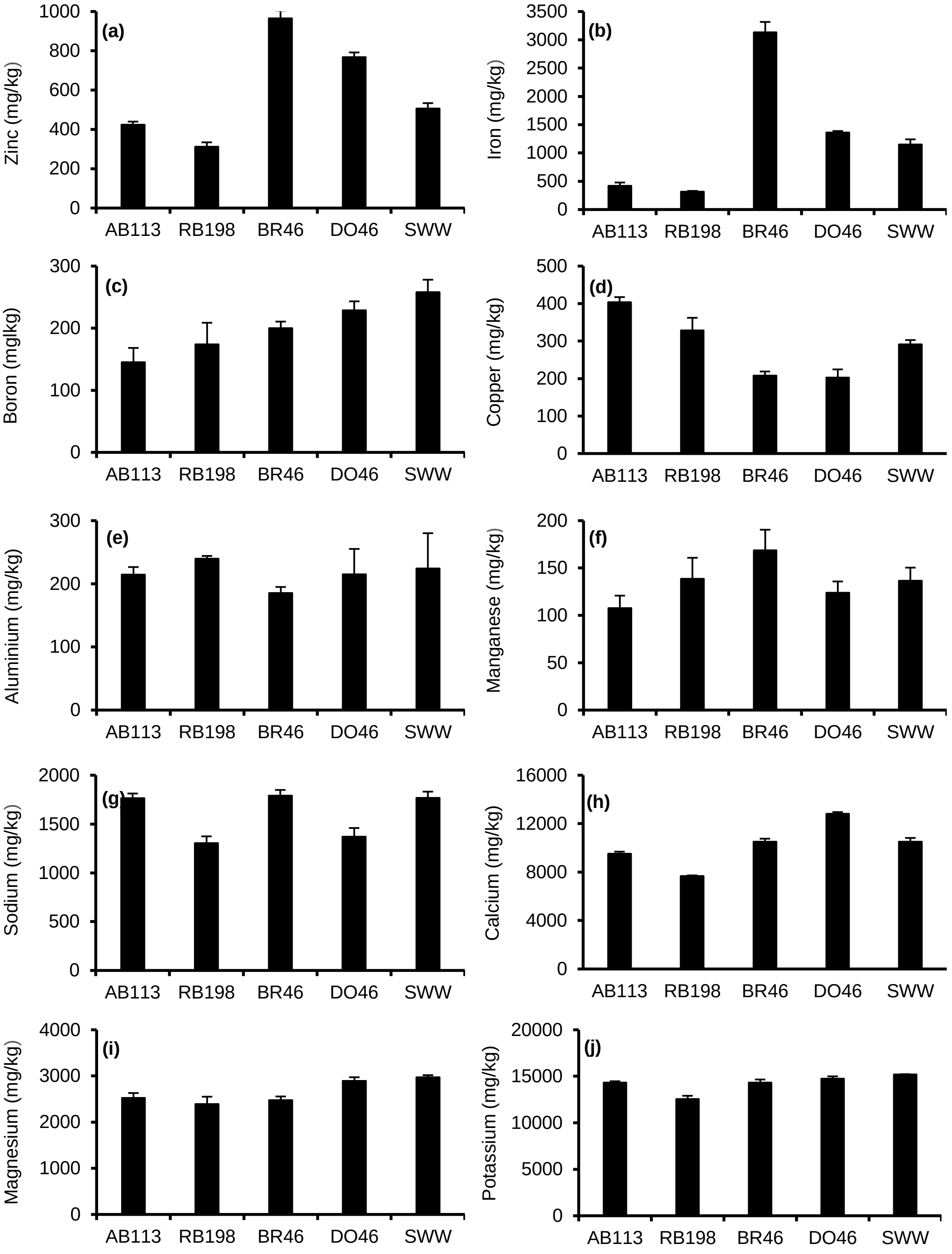


Figure 4.13 Mean concentrations of the detected elements in plant tissue during the experiment between 15 December 2014 and 2 February 2016. Note: (a), zinc; (b), iron; (c), boron; (d), copper; (e), aluminium; (f), manganese; (g), sodium; (h), calcium; (i), magnesium; (j), potassium; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dye.

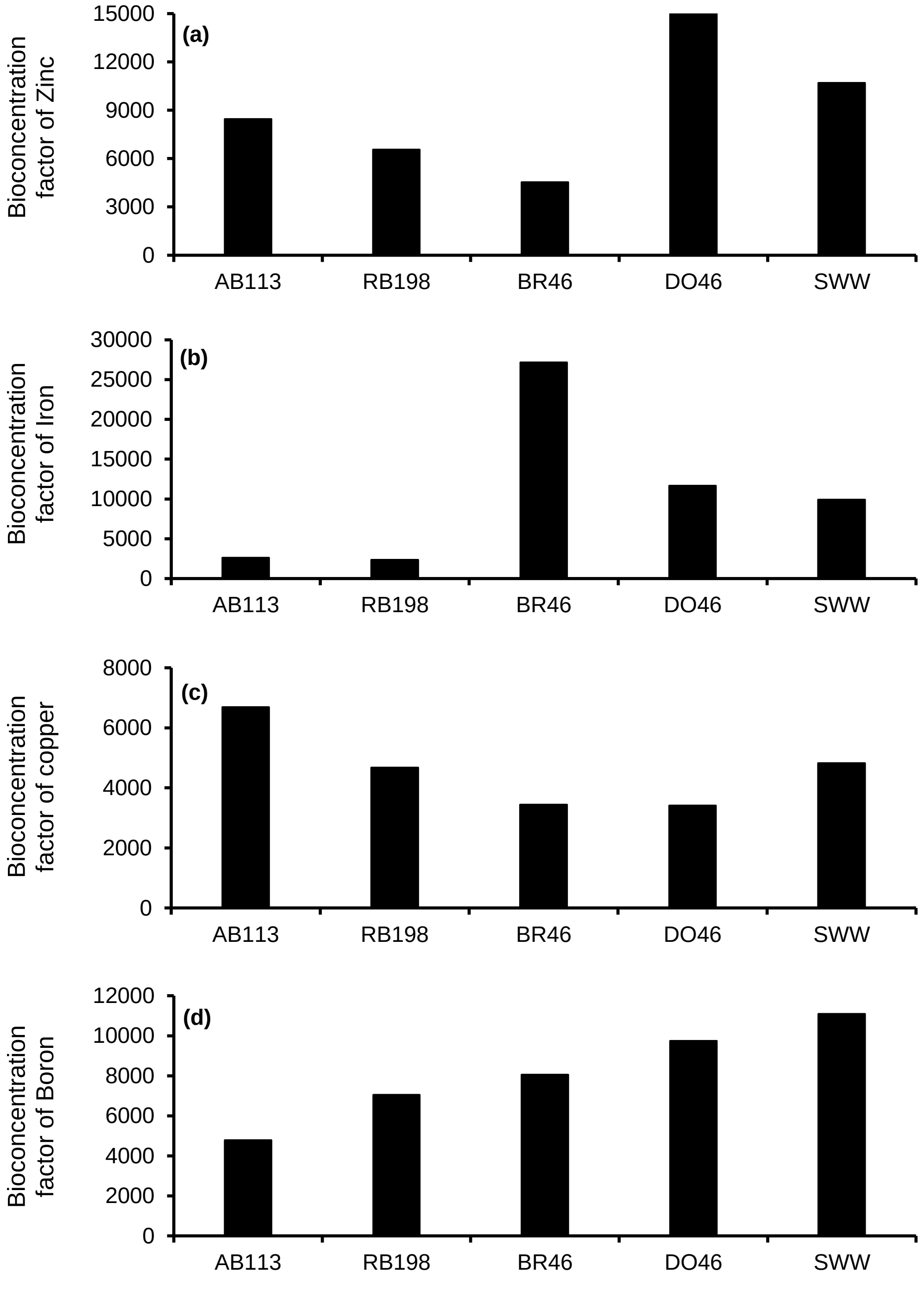


Figure 4.14 Bioconcentration factor during the experiment between 15 December 2014 and 2 February 2016. Note: (a), zinc; (b), iron; (c), copper; (d), boron; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater without dyes.

4.3.3 Plant monitoring

The mean values of RGR, which are based on the fresh weight for plants harvested from each pond are shown in Figure 4.15. This growth parameter has been used as an indirect indicator for the toxic effects of dyes on *L. minor* growth. Results clearly show that dyes negatively influence the RGR, and the values were ranked as follows: synthetic wastewater without dye > AB113 > RB198 > DO46 > BR46. This outcome confirms that BR46 has a more negative effect on the plant growth rate compared with other dyes, as discussed earlier in Section 4.2.3. However, statistical analysis shows that there is no significant difference in RGR values among the ponds containing AB113, RB198, and only synthetic wastewater (Table 4.14). This indicates that the effect of AB113 and RB198 as growth inhibitors was very low. In addition, no significant differences in RGR between ponds comprising BR46 and DO46 were recorded. Moreover, the RGR for BR46 and DO46 were significantly lower than for ponds containing only synthetic wastewater (Table 4.14). The plants harvested in May and July originated only from ponds which were completely covered by *L. minor*. The last harvest took place in February at the end of the experiment (Table 4.15). Zhao et al. (2016) highlighted that temperature and light intensity changes impact on the growth rate of duckweed, which increases during summer and reduces during winter.

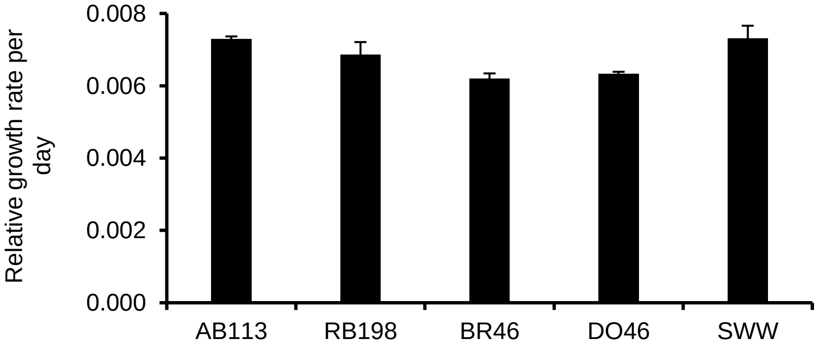


Figure 4.15 Mean relative growth rate for *Lemna minor* L. between 10 July 2014 and 2 February 2016. Note, standard deviations are presented by error bars based on four replicates; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; and SWW, synthetic wastewater.

Table 4.14 Overview of the statistical analysis for the relative growth rate of *Lemna minor* L. ponds between the wastewater types using the parametric one-way ANOVA

Variable	*p* value	Variable combination	*p* value
AB113 & RB198	0.155	RB198 & DO46	0.102
AB113 & BR46	<0.001	RB198 & SWW	0.173
AB113 & DO46	<0.001	BR46 & DO46	0.947
AB113 & SWW	1.000	BR46 & SWW	<0.001
RB198 & BR46	0.027	DO46 & SWW	0.001

Note: *p* value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$, the variables are statistically significantly different); AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater. Test of normality using Shapiro-Wilk: 0.061. Significant value among the wastewater types is < 0.001.

Table 4.15 Mean values of the fresh and dry weights of the plants harvested for each system between 10 July 2014 and 2 February 2016

Type of wastewater	Weight (gram)	Date		
		12/05/15	06/07/15	02/02/16
Acid blue 113	Fresh	18.06	18.05	31.43
	Dry	0.910	0.903	1.570
Reactive blue 198	Fresh	N/A	22.33	31.30
	Dry	N/A	1.121	1.558
Basic red 46	Fresh	N/A	N/A	37.33
	Dry	N/A	N/A	1.900
Direct orange 46	Fresh	N/A	N/A	40.03
	Dry	N/A	N/A	2.002
Synthetic wastewater	Fresh	N/A	14.78	53.50
	Dry	N/A	0.740	2.687

N/A; not applicable.

4.3.4 Environmental conditions

Figure 4.16 shows the environmental conditions in Salford during the experiment period between 15 December 2014 and 2 February 2016. The minimum and maximum temperatures are 4°C and 33°C, respectively. Out of 274 temperature recordings (Figure 4.16), 27 were less than the minimum recommended range (Ozengin & Elmaci, 2007). The mean light intensity value was 12745 lux, and minimum and maximum values ranged between 1079 lux and 46975 lux.

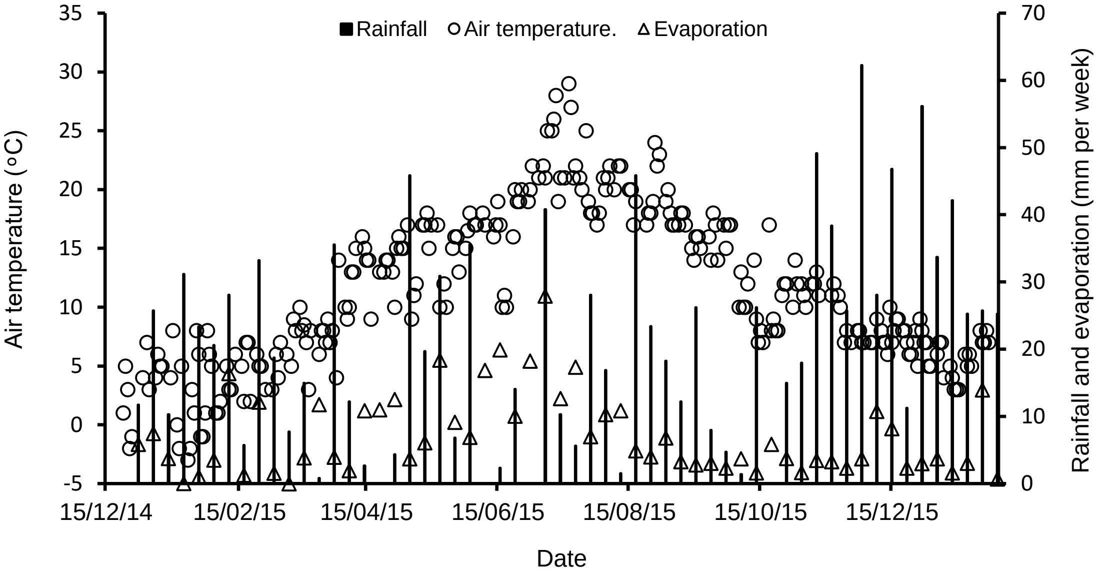
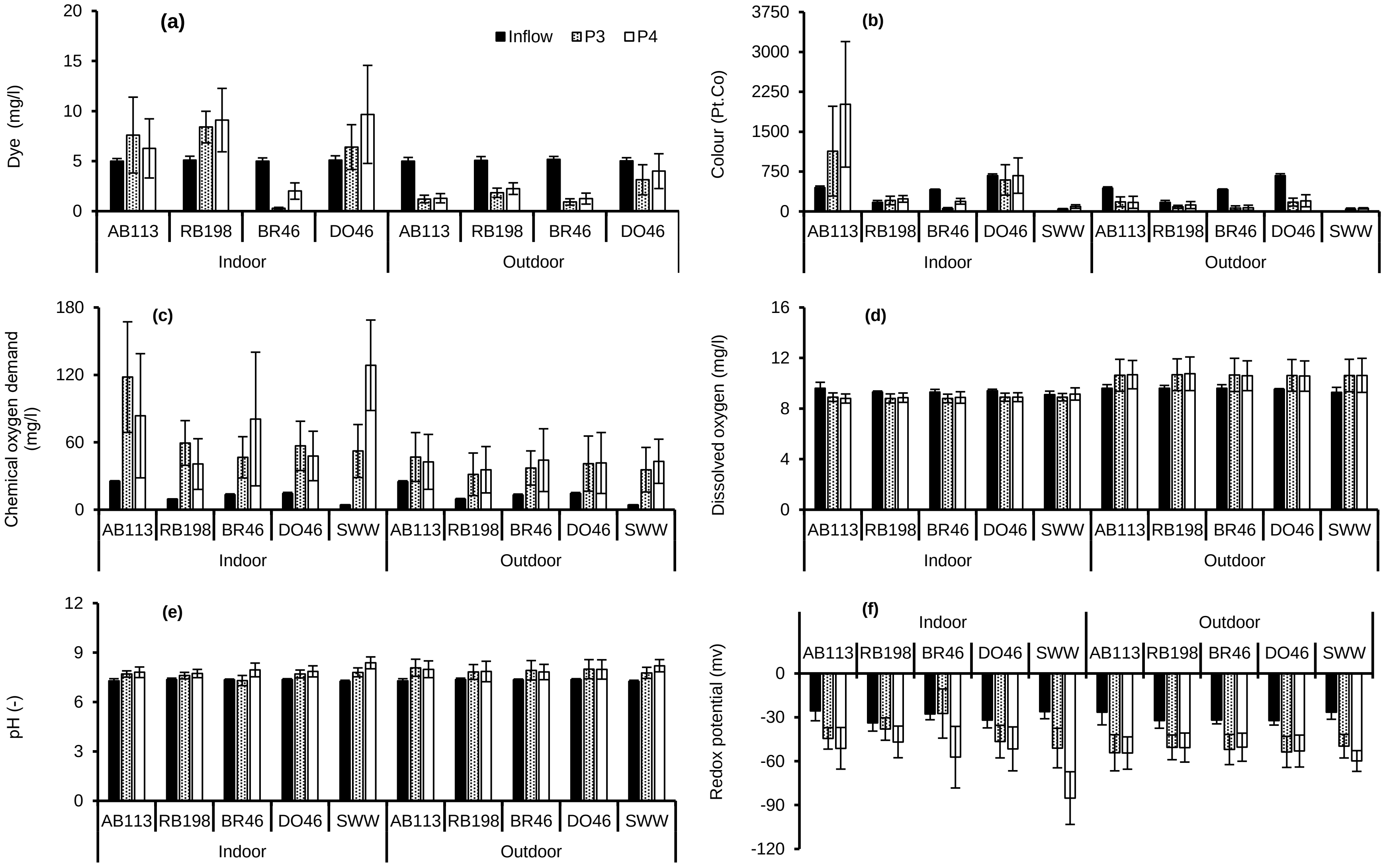


Figure 4.16 Environmental conditions in Salford during the experiment period between 15 December 2014 and 2 February 2016

4.4 Comparison of treatment efficiency between indoor and outdoor ponds

4.4.1 Inflow water quality parameters

The mean values for inflow and outflow water characteristics regarding the indoor (under controlled laboratory conditions) and outdoor (under semi-natural uncontrolled conditions) experiments between 15 December 2014 and 15 September 2015 are shown in Figures 4.17 and 4.18. Although, the inflow wastewater was prepared and mixed in the location of each experiment separately, no significant (t-test, $p > 0.05$) differences were noted between the mean inflow water quality parameters for the outdoor experiment compared with those for the indoor one.


(a)
Inflow
P3
P4
Dye (mg/l)
20
15
10
5
0
AB113
RB198
BR46
DO46
Indoor
Outdoor
(b)
Colour (Pt.Co)
3750
3000
2250
1500
750
0
SWW
(c)
Chemical oxygen demand (mg/l)
180
120
60
(d)
Dissolved oxygen (mg/l)
16
12
8
4
(e)
pH (-)
12
9
6
3
(f)
Redox potential (mv)
-30
-60
-90
-120

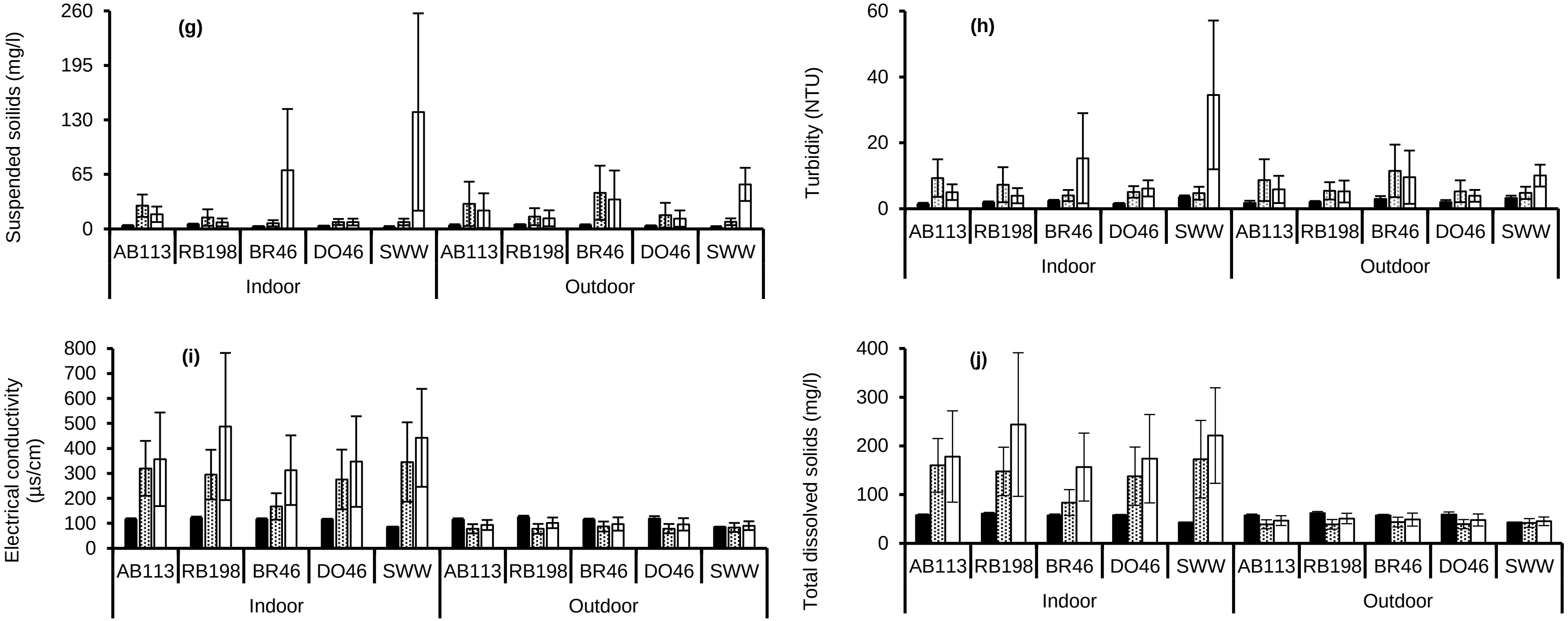
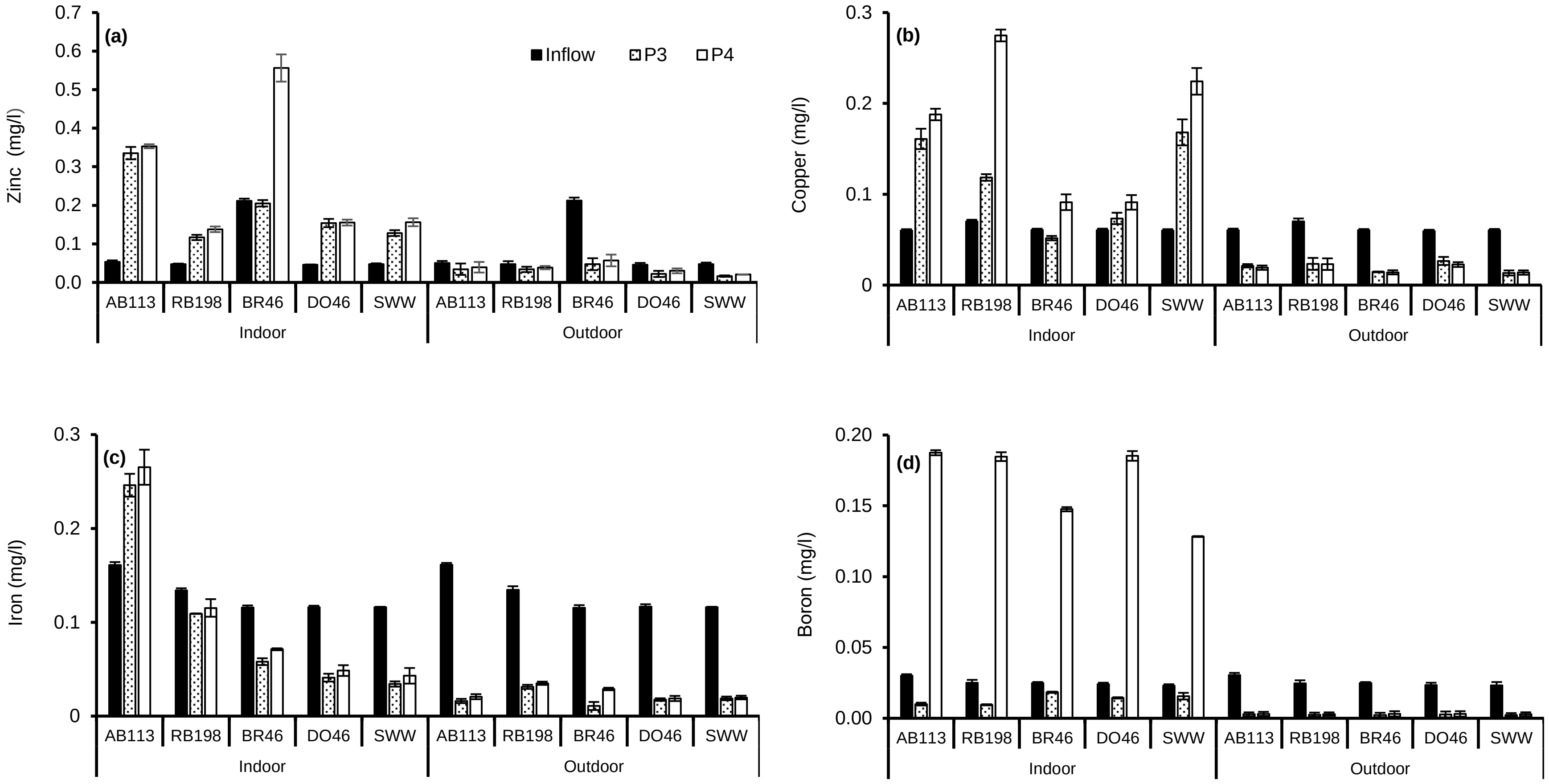


Figure 4.17 Overview of the mean inflow and outflow water characteristics for the indoor and outdoor experiments between 15 December 2014 and 15 September 2015. Note: (a), dye concentration; (b), colour; (c), chemical oxygen demand; (d), dissolved oxygen; (e), pH; (f), redox potential; (g), suspended solids; (h), turbidity; (i), electric conductivity; (j), total dissolved solids; P3, *Lemna minor* L. ponds; P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater.


(a)
Zinc (mg/l)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
■ Inflow
▨ P3
□ P4
(b)
Copper (mg/l)
0.3
0.2
0.1
0
(c)
Iron (mg/l)
0.3
0.2
0.1
0
(d)
Boron (mg/l)
0.20
0.15
0.10
0.05
0.00
AB113
RB198
BR46
DO46
SWW
Indoor
Outdoor

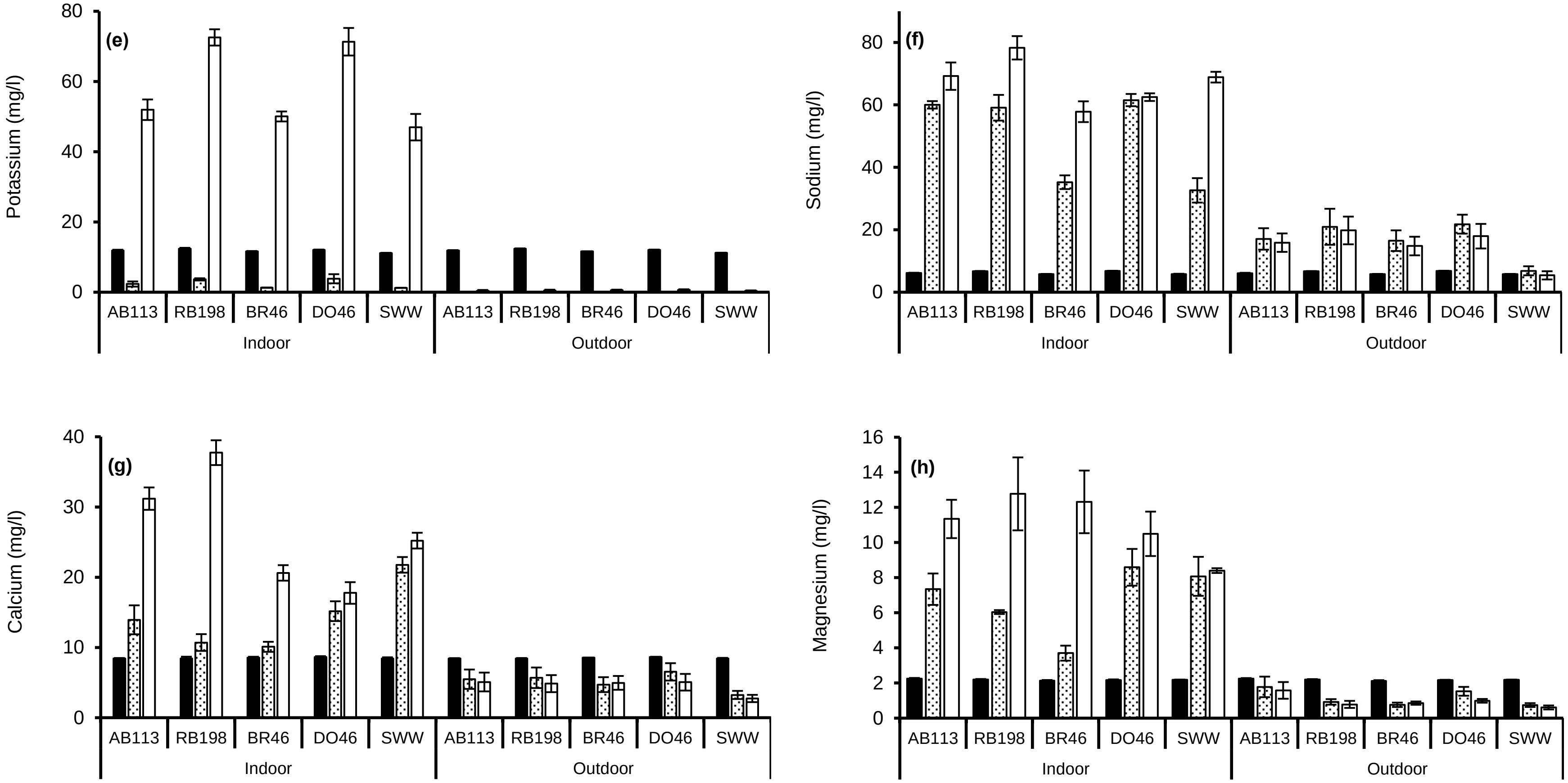


Figure 4.18 Mean inflow and outflow concentrations of the detected trace elements within the indoor and outdoor experiments. Note: (a), zinc; (b), copper; (c), iron; (d), boron; (e), potassium; (f), sodium; (g), calcium; (h), magnesium; P3, *Lemna minor* L. ponds; P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater.

4.4.2 Treatment performance

4.4.2.1 Dye and apparent colour

The dye removal efficiency within the period between 15 December 2014 and 15 September 2015 is shown in Figure 4.19 for both experiments. Table 4.16 summarises the statistical analysis of outflow water quality parameters and the removal efficiencies. AB113 removal for the outdoor experiment was significantly (Table 4.16) elevated compared to the indoor one for both *L. minor* and control ponds. In addition, the mean removal efficiency for the *L. minor* and control ponds was similar (both at around 30%) within the outdoor experiment, and very low (8 and 10%, respectively) for the indoor experiment. This indicates that *L. minor* was unable to break-down AB113 molecules under controlled or semi-natural conditions, and the outdoor removal was due to the dilution effect of rain water and microbes activities. In contrast, Balarak et al. (2016b) reported high removal efficiencies for AB113 using dried *L. minor*. This might be due to the pH of the solution being adjusted to 3, which enhanced the adsorption ability of the plants. However, in this experiment the pH values were without adjustment. In addition, the pH level used by Balarak et al. (2016b) was less than the tolerated values for growth of living *L. minor* which range between 4 and 9 (Movafeghi et al., 2013). Although RB198 removal was small under both conditions, the mean removal efficiency for *L. minor* ponds was significantly elevated for the outdoor (19%) experiment compared to the indoor (12%) one. However, for the control ponds, no significant differences were noted (Table 4.16). The mean removal efficiency of BR46 in *L. minor* ponds was significantly (Table 4.16) reduced for outdoor experiment (43%) compared to indoor one (69%). In addition, the control ponds were able to remove around 33 and 31% (not significant) under semi-natural and controlled conditions, respectively. BR46 was treated better than other dyes in both experiments. The outflow samples were colourless, which is key evidence for the dye having been removed. In addition, during the study period, the maximum value of BR46 removal in *L. minor* ponds was 88% and 86% for the indoor and outdoor experiments, respectively. The impact of *L. minor* in terms of removal efficiency was around 38 and 10% under controlled and semi-natural conditions, respectively, (Figure 4.19). This indicates that the high temperature under control conditions increases the removal efficiency of BR46. This result is matched by findings reported by Khataee et al. (2012) suggesting that the biological removal efficiency of dyes improves with increased temperature as an endothermic process. The mean removal efficiency of DO46 was significantly (Table 4.16) higher within the indoor experiment than the outdoor one for *L. minor* and control ponds. However, this dye exhibited low removal efficiency among the other dyes. Overall, statistical analysis showed that the dye BR46 was removed significantly ($p < 0.05$, Kruskal-Wallis test) more than the other dyes for *L. minor* and control ponds within the indoor system. However, for the outdoor experiment, *L. minor* ponds showed significantly ($p < 0.05$, Kruskal-Wallis test) higher BR46 removal efficiencies compared to the other dyes. In general, the dye BR46 was treated better than the other dyes for both conditions. This may be due to the fact that BR46 is characterised by a simple chemical structure, small molecular weight, and absence of sulpho groups in its structure. In addition, the neutral pH was suitable for BR46 uptake. All these factors enhance the dye removal efficiency as discussed previously in Sections 4.2.2.1 and 4.3.2.1. However, it is worth noting that the dyes DO46, AB113 and RB198 contained one, two and four sulpho groups in their structures, respectively, which may inhibit the degradation efficiency.

Based on dye concentrations, the average outflow dye values concerning the *L. minor* and control pond systems were significantly (Table 4.16) higher for the indoor experiment (see Figure 4.17a) than the outdoor one for all dyes, except for *L. minor* ponds comprising BR46. The mean outflow BR46 concentrations for *L. minor* ponds were significantly lower within indoor experiment than outdoor one, being 0.3 mg/l and 0.9 mg/l for the indoor and outdoor experiments, respectively. Comparing to the inflow dye concentration of 5 mg/l, this was evidence of the high degradation of the dye BR46 under controlled conditions compared to other studied dyes. Overall, the outflow BR46 concentrations in planted ponds were significantly (Table 4.16) reduced compared to the corresponding outflow concentrations for the other dyes under both environmental conditions.

All colour mean outflow values (as illustrated in Figure 4.17b) were significantly higher within the indoor experiment for *L. minor* ponds and also for the control ponds compared to the corresponding outdoor one. However, for the planted ponds comprising BR46 and the planted ponds fed with only synthetic wastewater, no significant differences were noted (Table 4.16) between the two environmental conditions, which were 54 and 67 Pt Co for ponds comprising BR46, and 44 and 46 Pt Co for ponds without dye for indoor and outdoor experiments, respectively. These findings regarding lower outflow values for the dye and colour under semi-natural conditions than the controlled conditions can be explained by the effect of rainfall under semi-natural conditions, which dilutes and subsequently reduces the content of these parameters in the ponds. In addition, the process of removing excess water contributes to the reduction of the load of these parameters. Whereas under control conditions, there is no excess water requiring removal. Figure 4.16 indicates environmental boundary conditions such as rainfall and temperature in Salford during the period between 15 December 2014 and 2 February 2016, which includes the comparison study period.

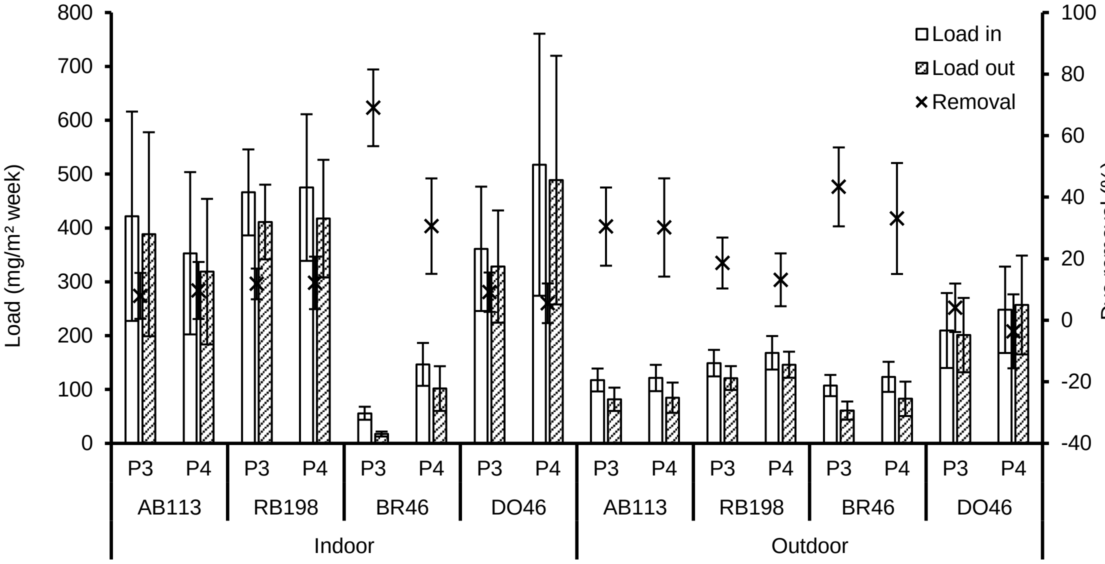


Figure 4.19 Mean values of dye removal efficiency within the period between 15 December 2014 and 15 September 2015 for indoor and outdoor experiments. Note: AB113, Acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; P3, *Lemna minor* L. ponds; P4, control ponds.

4.4.2.2 Chemical oxygen demand and dissolved oxygen

Based on COD removal efficiencies (Figure 4.20), better removal was noted in *L. minor* and control ponds containing AB113, BR46, and only synthetic wastewater within the outdoor experiment compared to the indoor one. However, lower removal efficiencies were noted within the outdoor experiment for *L. minor* and control ponds containing RB198 and DO46. Significant differences (Table 4.16) were found for planted ponds comprising AB113, and planted and unplanted ponds containing DO46. Nonetheless, all COD removal values, which ranged between -4 % and 22 %, and -2 % and 27 % for the indoor and outdoor experiments, respectively, indicated low microbial activities concerning COD mineralisation in these ponds (Figure 4.20). This is evident indirectly by the high DO outflow concentrations. The mean outflow COD concentrations (Figure 4.17c) were higher within the indoor experiment than the corresponding values for the outdoor one. This was due to the rainfall dilution factor for outdoor experiment as discussed above. However, significantly (Table 4.16) higher differences were noted in planted ponds containing AB113, RB198 and the control pond comprising only synthetic wastewater. In addition, all average outflow values were higher compared to the inflow ones, which indicates that the COD concentrations increased with time after each dose for both environmental conditions. This is due to the low COD removal efficiency, and also because some of the plants die-off during their life cycle and increase the organic load in the ponds, which consequently increases the COD concentrations as discussed by Dalu and Ndamba (2003). The findings have indicated that the COD outflow concentrations were 5, 3, 3 and 5 times non-compliant with the standard value for direct discharge (Carmen & Daniela, 2012), for the *L. minor* and control ponds containing AB113, control ponds containing BR46, and control pond comprising synthetic wastewater without dye for the indoor experiment. However, for outdoor experiment, all COD values were below the set threshold.

Regarding the DO concentrations, the mean outflow values were higher than the mean inflow values for outdoor experiment, and slightly lower than the inflow for the indoor one. The mean values of the outflow DO in *L. minor* and control ponds were significantly (Table 4.16) higher within the outdoor experiment than those corresponding to the indoor one for all dyes, as well as the ponds without dye (Figure 4.17d). The minimum and maximum values ranged between 7.9 and 10.1 mg/l, and between 8.5 and 13.9 mg/l for the indoor and outdoor experiments, respectively. This is because the DO is highly affected by temperature variations; colder conditions increase the DO level and vice versa. Note that the temperature records for the outdoor experiment show wide variations compared with the controlled laboratory temperatures, as mentioned previously. The presence of plants also leads to an increase in the DO level in the system due to respiration activities. However, in this study, it seems that temperature and oxygen diffusion by the atmosphere impacted on the DO values rather than the plants.

Table 4.16 Overview of the statistical analysis between the indoor and outdoor experiments for outflow water quality parameters and corresponding removal efficiencies

Parameter	Treatment system	Shapiro-Wilk test (*p* value[1])	*P* values[2]	Parameter	Treatment system	Shapiro-Wilk test (*p* value[1])	*P* values[2]
Dye (mg/l)				Dye removal (%)			
AB113	P3	<0.001	<0.001	AB113	P3	<0.001	<0.001
	P4	<0.001	<0.001		P4	<0.001	<0.001
RB198	P3	<0.001	<0.001	RB198	P3	0.338	<0.001
	P4	<0.001	<0.001		P4	<0.001	0.338
BR46	P3	<0.001	<0.001	BR46	P3	<0.001	<0.001
	P4	<0.001	<0.001		P4	0.230	0.23
DO46	P3	<0.001	<0.001	DO46	P3	0.010	0.013
	P4	<0.001	<0.001		P4	<0.001	<0.001
Chemical oxygen demand (mg/l)				Chemical oxygen demand removal (%)			
AB113	P3	<0.001	0.001	AB113	P3	<0.001	0.008
	P4	0.033	0.095		P4	0.007	0.345
RB198	P3	<0.001	0.003	RB198	P3	0.155	0.111
	P4	<0.001	0.264		P4	0.053	0.345
BR46	P3	<0.001	0.197	BR46	P3	0.205	0.500
	P4	0.030	0.14		P4	0.001	0.155
DO46	P3	<0.001	0.083	DO46	P3	0.334	0.048
	P4	<0.001	0.289		P4	0.949	0.016
SWW	P3	<0.001	0.083	SWW	P3	<0.001	0.155
	P4	0.048	<0.001		P4	0.074	0.345
Colour (Pt Co)				Dissolved oxygen (mg/l)			
AB113	P3	<0.001	<0.001	AB113	P3	<0.001	<0.001
	P4	0.001	<0.001		P4	<0.001	<0.001
RB198	P3	0.023	0.001	RB198	P3	<0.001	<0.001

Table 4.16 (Continued)

	P4	<0.001	0.001		P4	<0.001	<0.001
BR46	P3	0.001	0.335	BR46	P3	<0.001	<0.001
	P4	0.012	<0.001		P4	<0.001	<0.001
DO46	P3	0.010	0.001	DO46	P3	<0.001	<0.001
	P3	0.031	<0.001		P3	<0.001	<0.001
SWW	P4	0.001	0.003	SWW	P4	<0.001	<0.001
	P3	0.007	0.419		P3	<0.001	<0.001
Suspended solids (mg/l)				pH (-)			
AB113	P3	<0.001	0.221	AB113	P3	<0.001	<0.001
	P4	<0.001	0.411		P4	<0.001	0.066
RB198	P3	<0.001	0.393	RB198	P3	<0.001	0.002
	P4	<0.001	0.062		P4	<0.001	0.286
BR46	P3	<0.001	<0.001	BR46	P3	<0.001	<0.001
	P4	<0.001	0.019		P4	<0.001	0.059
DO46	P3	<0.001	0.082	DO46	P3	<0.001	0.049
	P4	<0.001	0.102		P4	<0.001	0.432
SWW	P3	<0.001	0.374	SWW	P3	0.022	0.221
	P4	<0.001	<0.001		P4	0.031	0.008
Turbidity (NTU)				Redox potential (mV)			
AB113	P3	<0.001	0.110	AB113	P3	0.021	<0.001
	P4	<0.001	0.436		P P4	0.001	0.241
RB198	P3	<0.001	0.143	RB198	P3	<0.001	<0.001
	P4	<0.001	0.139		P4	0.002	0.403
BR46	P3	<0.001	<0.001	BR46	P3	0.004	<0.001
	P4	<0.001	0.029		P4	<0.001	0.063
DO46	P3	<0.001	0.328	DO46	P3	0.045	0.003
	P4	0.022	<0.001		P4	<0.001	0.418
SWW	P3	<0.001	0.342	SWW	P3	<0.001	0.238

Table 4.16 (Continued)

	P4	<0.001	<0.001		P4	0.006	<0.001
Electrical conductivity (μS/cm)							
AB113	P3	0.004	0.152				
	P4	0.004	0.001				
RB198	P3	<0.001	<0.001				
	P4	0.005	0.005				
BR46	P3	<0.001	<0.001				
	P4	<0.001	0.025				
DO46	P3	0.003	0.050				
	P4	0.002	0.001				
SWW	P3	0.009	0.014				
	P3	0.022	0.062				

Note: [1]Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); [2]p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$, the variables are statistically significantly different); NTU, nephelometric turbidity unit; P3, *Lemna minor.*L. ponds; P4, control ponds; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; M-W, Mann-Whitney U test which is used when the data are not normally distributed; t-test, independent sample t-test which is used when the data are normally distributed.

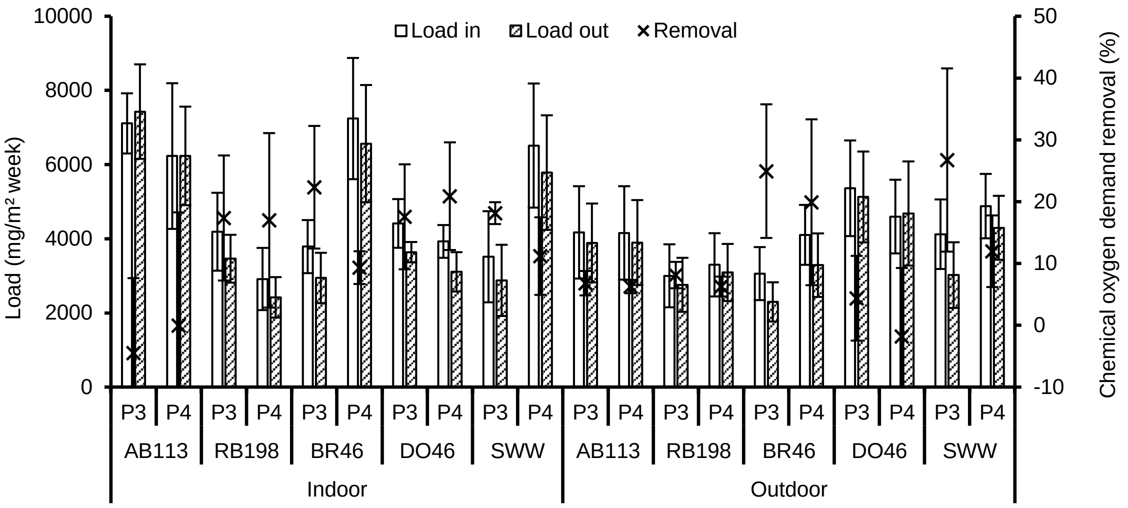


Figure 4.20 Mean values of chemical oxygen demand removal efficiency within the period between 15 December 2014 and 15 September 2015 for indoor and outdoor experiments. Note: AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater; P3, *Lemna minor* L. ponds; P4, control ponds.

4.4.2.3 pH and redox potential

The mean pH outflow values ranged between 7.3 and 8.4 for indoor experiment, and between 7.7 and 8.2 for the outdoor one (Figure 4.17e). The mean outflow pH values for the *L. minor* and control ponds were higher within the outdoor experiment than the corresponding values within the indoor one for all dyes except the control ponds treating BR46. This was due to the impact of lower temperature records within the outdoor experiment compared with the indoor one. Significant differences (Table 4.16) between the indoor and outdoor experiments were found within the planted ponds only. However, the values were lower under semi-natural conditions than the resembling values under controlled conditions for *L. minor* and control ponds comprising only synthetic wastewater, and control ponds treating BR46. Significant (Table 4.16) differences were only recorded for control ponds comprising synthetic wastewater without dye. According to international standards (Carmen & Daniela, 2012) for the discharge of effluents directly to receiving freshwater bodies, the acceptable pH limits are between 6.5 and 8.5. The pH outflow samples were 9 and 3, 4 and 6, 4 and 1, and 6 and 7, and 1 and 8 times non-compliant for systems containing AB113, RB198, BR46, DO46 and synthetic wastewater without dye for planted ponds and control ponds for outdoor experiment in that order. However, for indoor one, only control ponds containing BR46, and synthetic wastewater without dye were 2 and 11 times non-compliant.

Regarding the average values of the outflow redox potential, all values were lower than the inflow for the indoor and outdoor set-ups as shown in Figure 4.17f. Higher mean outflow redox potential values were found for *L. minor* and control ponds regarding systems comprising AB113, RB198 and DO46, and planted ponds containing BR46 concerning the indoor experiment compared to the corresponding outdoor one, with significant (Table 4.16) differences found for planted ponds only. However, the values were lower within the indoor experiment than the outdoor one for ponds with *L. minor*, control ponds comprising synthetic wastewater without dye, and control ponds treating BR46. Significant (Table 4.16) differences were only noted for control ponds comprising synthetic wastewater without dye.

4.4.2.4 Suspended solids and turbidity

The mean SS outflows (Figure 4.17g) for the indoor experiment were lower than the corresponding values for the outdoor one regarding all ponds which could be due to the effect of natural conditions such as dust and rain for the outside experiment, except for control ponds comprising BR46, and control ponds containing only synthetic wastewater, which were significantly higher within the indoor experiment than the corresponding values for the outdoor one (Table 4.16). The minimum and maximum values for control ponds containing BR46, and ponds without dye were 3 mg/l and 390 mg/l, and 2 mg/l and 268 mg/l, respectively. This wide range between the minimum and maximum values may reflect the higher SS of these control ponds within the indoor experiment. The main factors influencing SS removal in free-floating plant-based treatment systems are biodegradation of organic matter, algal growth and sedimentation of particles (Dalu & Ndamba, 2003). In this study, sedimentation and organic matter degradation affected the outflow SS concentrations. The international standards for SS are 35 mg/l in case of discharges directly into watercourses. The samples, for outdoor experiment, were 13 and 11, 2 and 1, 20 and 17, 5, and 30 times non-compliant for planted and unplanted ponds containing AB113, RB198 and BR46, planted ponds comprising DO46, and the control ponds comprising only synthetic wastewater, respectively. However, for indoor experiment, the values were 9, 1, 3, 22 and 29 times non-compliant for planted ponds comprising AB113, unplanted ponds comprising AB113, planted ponds treating RB198, control ponds containing BR46, and control ponds containing only synthetic wastewater, respectively.

Based on the mean turbidity outflow values (Figure 4.17h), no remarkable differences were noted between the indoor and outdoor values except for the control ponds comprising BR46, DO46, and synthetic wastewater without dye, which were significantly higher under controlled than natural conditions. Whereas, planted ponds comprising BR46 were shown to be significantly lower in terms of their mean turbidity outflows under controlled than semi-natural conditions (Table 4.16).

4.4.2.5 Electrical conductivity and total dissolved solids

Mean outflow values for the electrical conductivity (salinity indicator) were significantly higher (Table 4.16) for the laboratory compared to the semi-natural conditions (Figure 4.17i), because of the dilution factor by rain and removing the excess water to achieve the required level under real conditions, which led to a reduction of the EC in the system. The mean outflow values were higher than the mean inflow ones for indoor experiment and slightly lower than the mean inflow values for the outdoor one. This indicates low percentage reductions in EC values under controlled conditions. Moreover, all mean outflow values in control ponds were elevated compared to the planted ponds, which denotes that the plants were able to reduce EC. The minimum and maximum mean outflow values ranged between 168 µS/cm in *L. minor* ponds comprising BR46 and 488 µS/cm in control ponds containing RB198 under laboratory conditions, and between 78 µS/cm in *L. minor* ponds comprising BR46 and 102 µS/cm for control ponds containing RB198 under semi-natural conditions.

The mean TDS (ion concentration) values were half the EC values in this study, and all the inflow and outflow TDS values showed the same trend as the inflow and outflow EC values in terms of the indoor and outdoor experiments (Figure 4.17j). All TDS outflow values for the indoor and outdoor experiments complied with the European and international standards of 2000 mg/l for discharge directly to receiving freshwater and with the threshold of 500 mg/l (class I -natural non-polluted state of water body) (Carmen & Daniela, 2012).

4.4.2.6 Trace elements

Figure 4.18 shows an overview of the mean inflow and outflow concentrations of the elements, which were detected during the ICP-OES analyses for the indoor and outdoor experiments. The mean zinc outflow concentrations (Figure 4.18a) for *L. minor* and control ponds were significantly ($p < 0.05$, Mann-Whitney U test) higher within the indoor experiment than the outdoor one for ponds with and without dye. In addition, for the outdoor experiment, the mean outflow zinc concentrations were less than the inflow ones due to the rain factor. However, the indoor experiment showed that the mean outflow values were higher than the inflow ones except for the *L. minor* ponds treating BR46, which were slightly lower than the inflow. This might be due to the high degradation of this dye. For both experiments, the planted ponds showed mean outflow values slightly lower than the outflows of the corresponding control ponds reflecting the ability of *L. minor* in the uptake of zinc. Overall, for the two experiments, the mean outflow zinc concentrations were lower than the threshold of 2 mg/l set for irrigation (Metcalf & Eddy, 2003). In addition, all outflow values were within the acceptable limits for *L. minor*. Zinc was tolerated by *L. minor* at 15 mg/l without any noticeable toxicity, but a reduction in the plant production rate of between 0.5 and 15 mg/l was reported by Khellaf and Zerdaoui (2009). Obvious damage to duckweed at a zinc concentration of 18 mg/l was reported by Khellaf et al. (2008). Therefore, in this study, zinc concentrations in the system did not impact negatively on the plant growth. Regarding the mean outflow concentrations of copper, all values were significantly ($p < 0.05$, Mann-Whitney U test) higher within the indoor experiment than the outdoor one (Figure 4.18b). The mean copper outflow concentrations, for the outdoor experiment, were lower than the inflow ones, and the differences between the values for *L. minor* and control ponds was very low. However, within the indoor experiment, the mean outflow values were higher than the inflow ones except for the *L. minor* ponds treating BR46. The planted ponds showed mean outflow values lower than the outflow concentrations of the corresponding control ponds indicating that the plants accumulate copper in their tissue, under laboratory conditions. The copper content was below the threshold limit of 0.2 mg/l for irrigation (Metcalf & Eddy, 2003). In addition, all outflow values were less than the tolerance limit of 0.4 mg/l for *L. minor* (Khellaf & Zerdaou, 2009) and less than the limits that cause toxic impact on duckweed at a concentration of 0.5 mg/l (Khellaf et al., 2008). Iron mean outflow concentrations (Figure 4.18c) for indoor experiment were significantly ($p < 0.05$, Mann-Whitney U test) higher than those for the outdoor one, due to the dilution of iron concentrations by the rainfall under semi-natural conditions compared with evaporation only under controlled conditions which led to iron concentration. For both indoor and outdoor set-ups, the mean outflow values were lower than the inflow ones except for the ponds containing AB113, which may affect the iron content in the system. In addition, all planted ponds showed iron levels less than the control ponds which indicated that the plants are responsible for the reduction. Overall, the mean outflow iron concentrations were lower than the threshold of 5 mg/l set for irrigation (Metcalf & Eddy, 2003). The mean outflow concentrations of boron and potassium for the outdoor experiment were significantly ($p < 0.05$, Mann-Whitney U test) lower than the ones for the indoor experiment (Figures 4.18d and e, respectively). Furthermore, the average outflow values for the outdoor set-up were lower than the inflow ones with very low differences between the planted and unplanted ponds. However, under controlled conditions, the mean outflow concentrations were higher than the inflow ones within the control ponds and lower than the inflow ones for the *L. minor* ponds. Sodium mean outflow concentrations (Figure 4.18f) were significantly ($p < 0.05$, Mann-Whitney U test) reduced within the outdoor experiment compared to the indoor one, and all outflow values were higher compared with the inflow concentrations. The indoor set-up shows that the mean outflow elements values were elevated within the control ponds compared to the *L. minor* ponds. However, the outdoor set-up shows that the mean outflow values were lower within the control ponds compared to the *L. minor* ponds. This may be because of the decay of the plants, which consequently increases the sodium content. The mean outflow concentrations for the calcium and magnesium concerning the outdoor experiment were significantly ($p < 0.05$, Mann-Whitney U test) lower than the indoor one (Figures 4.18g and h, respectively). Under controlled conditions, the mean outflow values of these minerals were higher than the inflow ones and the concentrations of planted ponds were lower than the control ones, indicating that the plants slightly reduce the concentrations to support growth. The findings matched those by Patel and Kanungo (2010) indicating that *L. minor* reduces the calcium and magnesium concentrations by 15% and 20%, respectively. However, under semi-natural conditions, the mean outflow values were lower than the inflow and the concentrations of planted ponds were higher than the control, indicating that the dead *L. minor* increased the content of calcium and magnesium.

The comparison between the indoor and outdoor set-ups in terms of metals concentrations in plant tissue, and the bio-concentration factor (BCF) is not applicable. This is because the plants within the indoor experiment were harvested on 15 December 2015, compared with those for the outdoor one which were harvested on 2 February 2016 (when the outdoor experiment was completely finished), and therefore any elements accumulated after 15 December 2015 in outdoor plants affect the comparison accuracy.

4.4.3 Plant monitoring

The relative growth rate of *L. minor* based on the fresh weight was calculated after the experiment was finished and all plants in the ponds were harvested on 15 September 2015 for the indoor experiment, and on 2 February 2016 for the outdoor one, when the outdoor experiment was completely finished, and this may affect the comparison accuracy. For the outdoor experiment, in winter, *L. minor* is dormant, and individual plants settle down to the bottom of the pond system. The plant harvest included all dead, dormant and live plant parts found in the systems. Note that no additional plants were added to the system after set-up. However, for the indoor experiment, the plants were harvested regularly when the surface water was completely covered by *L. minor*. The growth under controlled conditions was clearly higher than in the outdoor experiment due to the impact of environmental conditions in the laboratory, which were suitable for optimum growth, except for plants treating BR46. In addition, Figures 4.21a and b clearly show that the lowest growth rate values were noted in ponds containing BR46 for both experiments. This clearly indicates that the BR46 inhibited the growth of *L. minor*. Furthermore, the plants in synthetic wastewater without dye ponds showed growth rates that were higher compared to other ponds. This indicates that the presence of the dye in the system has a negative impact on the plant growth.

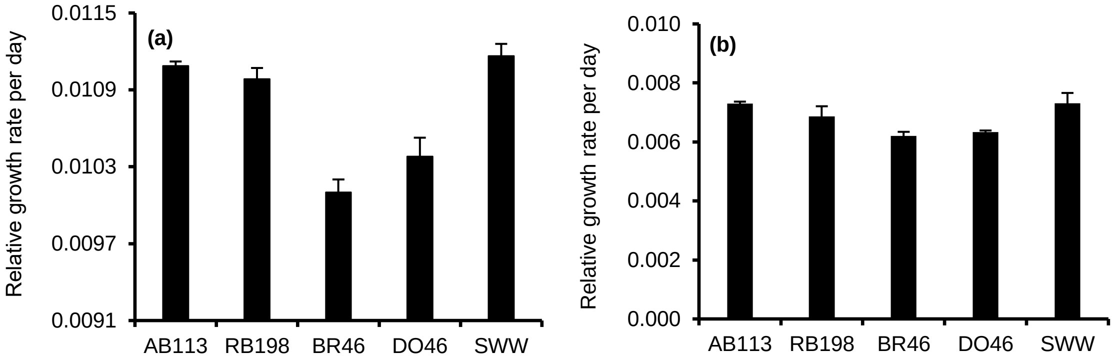


Figure 4.21 Mean relative growth rate for *Lemna minor* L. during the experimental operation period. Note: (a), under controlled conditions; (b), under semi-natural conditions; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; SWW, synthetic wastewater.

4.4.4 Environmental conditions

The mean temperature readings were 27°C and 13°C under controlled and semi-natural conditions, respectively. Light recordings were 2215 lux under controlled conditions and 16507 lux under semi-natural conditions. Mean values of environmental conditions for Salford (outdoor conditions) and the laboratory (indoor conditions) during the study period are presented in Table 4.17.

Table 4.17 Mean values of parameters characterising environmental conditions for both indoor and outdoor experiments between 15 December 2014 and 15 September 2015

Condition	Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Laboratory	Temperature	°C	27.3	2.59	22.4	29.9	127
	Illuminance	lux	2215.4	145.52	2023	2450	33
	Relative humidity	%	53.7	4.08	36.0	60.0	127
Semi-natural	Temperature	°C	12.6	7.3	-3.0	29.0	180
	Illuminance	lux	16506.6	16143.7	2010	49675	35

4.5 Chapter summary

- Planted ponds performed better than control ponds.
- The treatment systems planted with *L. minor* were able (as a polishing stage) to remove the dye BR46 at low concentrations (5 mg/l) from synthetic wastewater significantly ($p < 0.05$) better than the dyes AB113, RB198, and DO46 for both studied environmental conditions, laboratory controlled conditions (69%) and semi-natural uncontrolled conditions (51%).
- The potential of *L. minor* ponds for the treatment of BR46 under controlled conditions significantly ($p < 0.05$) outperformed the corresponding ponds under semi-natural conditions by 23%.
- The outflow values of pH, TDS, COD and SS for the planted ponds containing BR46 under laboratory conditions were within the standard limits for direct discharge. however, all outflow values of TDS and COD for all treatment systems under semi-natural conditions were within the allowable ranges for discharge to the watercourses.
- The outflow values of zinc, iron and copper were below the thresholds set for irrigation purposes, and within the tolerated limits for plants under both controlled and semi-natural conditions.
- The presence of the dyes inhibited the optimum growth of *L. minor*, especially the treated dye BR46 under both controlled and semi-natural conditions. However, the relative growth rate of *L. minor* under controlled conditions was significantly ($p < 0.05$) higher than those under semi-natural conditions.

Chapter 5

Impact of pH Adjustment on System Performance

5.1 Overview

The overall results and discussions of the second and third experiments for treating synthetic textile wastewater (STWW) containing dyes before and after dilution, respectively, are documented in this chapter. The set-up and operational processes of these experiments are explained in Chapter 3 (Sections 3.4.3 and 3.4.4). Section 5.1 provides an overview of the chapter. Sections 5.2 and 5.3 present the overall outcomes related to the short-term and long-term impact of pH factor on system performance in terms of dye removal at concentration of 10 mg/l, and for improvement of other water quality parameters. The chapter summary is presented in Section 5.4.

5.2 Short-term impact of pH on pond performance treating STWW containing dyes

5.2.1 Inflow water quality parameters

The mean values of the inflow characteristics of the synthetic textile wastewater with and without dyes are presented in Table 5.1 and Figure 5.1. The mean inflow values of pH, chemical oxygen demand (COD), colour were within the typical characteristics of textile wastewater ranges (Ghaly et al., 2014). However, ammonium-nitrogen (NH_4–N) and nitrate-nitrogen (NO_3–N) values were higher. The initial dye concentration was 10 mg/l, which was at the lower end of the dye effluents (10 to 250 mg/l) mentioned by Ghaly et al. (2014). Regarding suspended solids (SS) and total dissolved solids (TDS), the mean values were lower than the typical range. In addition, zinc and iron values were within the typical range of textile wastewater discharge of less than 10 mg/l (Ghaly et al. 2014).

Table 5.1 Inflow water quality parameters for each system between 1 October 2015 and 19 January 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue113 + synthetic textile wastewater						
Dye concentration	mg/l	10.0	0.04	9.9	10.1	14
pH	-	7.5	0.11	7.3	7.6	14
Redox	mV	-39.3	4.37	-43.0	-30.0	14
Dissolved oxygen	mg/l	8.6	0.66	7.4	9.1	14
Electrical conductivity	µS/cm	757.9	4.27	751.0	766.1	14
Total dissolved solids	mg/l	379.0	2.13	375.5	383.1	14
Suspended solids	mg/l	12.5	1.12	11.0	14.0	14
Turbidity	NTU	5.8	0.48	5.3	6.4	14
Colour	Pt Co	964.6	14.00	948.0	1005.0	14
Chemical oxygen demand	mg/l	404.0	5.35	397.0	410.0	4
Ammonium-nitrogen	mg/l	31.6	1.40	30.0	33.4	4
Nitrate-nitrogen	mg/l	31.1	0.83	30.0	32.0	4
Ortho-phosphate-phosphorus	mg/l	8.2	0.12	8.0	8.3	4
Reactive blue 198+ synthetic textile wastewater						
Dye concentration	mg/l	10.0	0.08	9.9	10.2	14
pH	-	7.5	0.15	7.2	7.8	14
Redox	mV	-42.8	4.77	-50.0	-31.0	14
Dissolved oxygen	mg/l	8.7	0.41	7.8	9.1	14
Electrical conductivity	µS/cm	762.9	6.32	744.0	771.0	14
Total dissolved solids	mg/l	381.4	3.16	372.0	385.5	14
Suspended solids	mg/l	13.1	0.99	11.0	15.0	14
Turbidity	NTU	5.8	0.51	5.0	7.0	14
Colour	Pt Co	259.0	6.07	245.0	270.0	14
Chemical oxygen demand	mg/l	376.0	3.31	372.0	380.1	4
Ammonium-nitrogen	mg/l	33.6	1.82	31.0	35.0	4
Nitrate-nitrogen	mg/l	31.1	1.61	29.9	33.4	4
Ortho-phosphate-phosphorus	mg/l	7.9	0.22	7.6	8.1	4
Basic red 46+ synthetic textile wastewater						
Dye concentration	mg/l	10.0	0.05	10.0	10.1	14
pH	-	7.4	0.11	7.2	7.6	14
Redox	mV	-38.8	3.91	-50.0	-35.0	14
Dissolved oxygen	mg/l	8.5	0.49	7.8	9.5	14
Electrical conductivity	µS/cm	756.1	6.76	741.0	767.0	14
Total dissolved solids	mg/l	378.1	3.38	370.5	383.5	14
Suspended solids	mg/l	10.3	1.03	8.0	12.0	14
Turbidity	NTU	6.8	0.50	5.8	7.9	14
Colour	Pt Co	775.3	2.60	773.0	781.0	14
Chemical oxygen demand	mg/l	382.0	2.27	379.5	385.0	4
Ammonium-nitrogen	mg/l	33.6	2.73	30.0	36.6	4

Table 5.1 (Continued)

Nitrate-nitrogen	mg/l	31.3	1.72	29.1	33.3	4
Ortho-phosphate-phosphorus	mg/l	7.9	0.09	7.8	8.0	4
Direct orange 46+ synthetic textile wastewater						
Dye concentration	mg/l	10.0	0.12	9.8	10.3	14
pH	-	7.6	0.16	7.3	7.9	14
Redox	mV	-43.9	2.84	-50.0	-40.0	14
Dissolved oxygen	mg/l	8.7	0.34	8.2	9.6	14
Electrical conductivity	µS/cm	751.5	5.75	744.0	768.0	14
Total dissolved solids	mg/l	375.8	2.87	372.0	384.0	14
Suspended solids	mg/l	11.1	1.03	9.0	13.0	14
Turbidity	NTU	6.0	0.68	4.9	7.5	14
Colour	Pt Co	1275.0	4.39	1268.0	1280.0	14
Chemical oxygen demand	mg/l	386.1	1.55	384.2	388.0	4
Ammonium-nitrogen	mg/l	33.7	1.13	32.8	35.3	4
Nitrate-nitrogen	mg/l	31.4	1.19	29.8	32.6	4
Ortho-phosphate-phosphorus	mg/l	8.1	0.09	8.0	8.2	4
Synthetic textile wastewater						
pH	-	7.5	0.11	7.2	7.7	14
Redox	mV	-38.9	3.34	-46.0	-33.0	14
Dissolved oxygen	mg/l	8.5	0.23	8.2	9.3	14
Electrical conductivity	µS/cm	714.8	3.48	711.0	722.8	14
Total dissolved solids	mg/l	357.4	1.74	355.5	361.4	14
Suspended solids	mg/l	7.0	0.93	5.0	8.0	14
Turbidity	NTU	3.5	0.13	3.2	3.8	14
Colour	Pt Co	30.8	1.74	27.0	33.0	14
Chemical oxygen demand	mg/l	366.0	0.82	365.0	367.0	4
Ammonium-nitrogen	mg/l	33.7	0.12	33.5	33.8	4
Nitrate-nitrogen	mg/l	31.1	0.17	30.9	31.3	4
Ortho-phosphate-phosphorus	mg/l	7.5	0.06	7.4	7.6	4
Dechlorinated tap water						
pH	-	7.1	0.16	6.9	7.4	30
Redox	mV	-26.3	9.19	-40.0	-12.0	30
Dissolved oxygen	mg/l	8.8	0.49	8.4	9.8	30
Electrical conductivity	µS/cm	66.4	1.53	64.4	68.8	30
Total dissolved solids	mg/l	32.1	0.8	32.2	34.4	30
Suspended solids	mg/l	1.2	0.69	0.0	2.0	30
Turbidity	NTU	1.2	0.38	0.6	1.7	30
Colour	Pt Co	0.0	0.00	0.0	0.0	30
Chemical oxygen demand	mg/l	2.4	0.93	0.9	3.4	9
Ammonium-nitrogen	mg/l	0.04	0.003	0.04	0.05	9
Nitrate-nitrogen	mg/l	0.38	0.139	0.31	0.40	9
Ortho-phosphate-phosphorus	mg/l	1.03	0.221	0.80	1.44	9

Note: NTU; nephelometric turbidity unit.

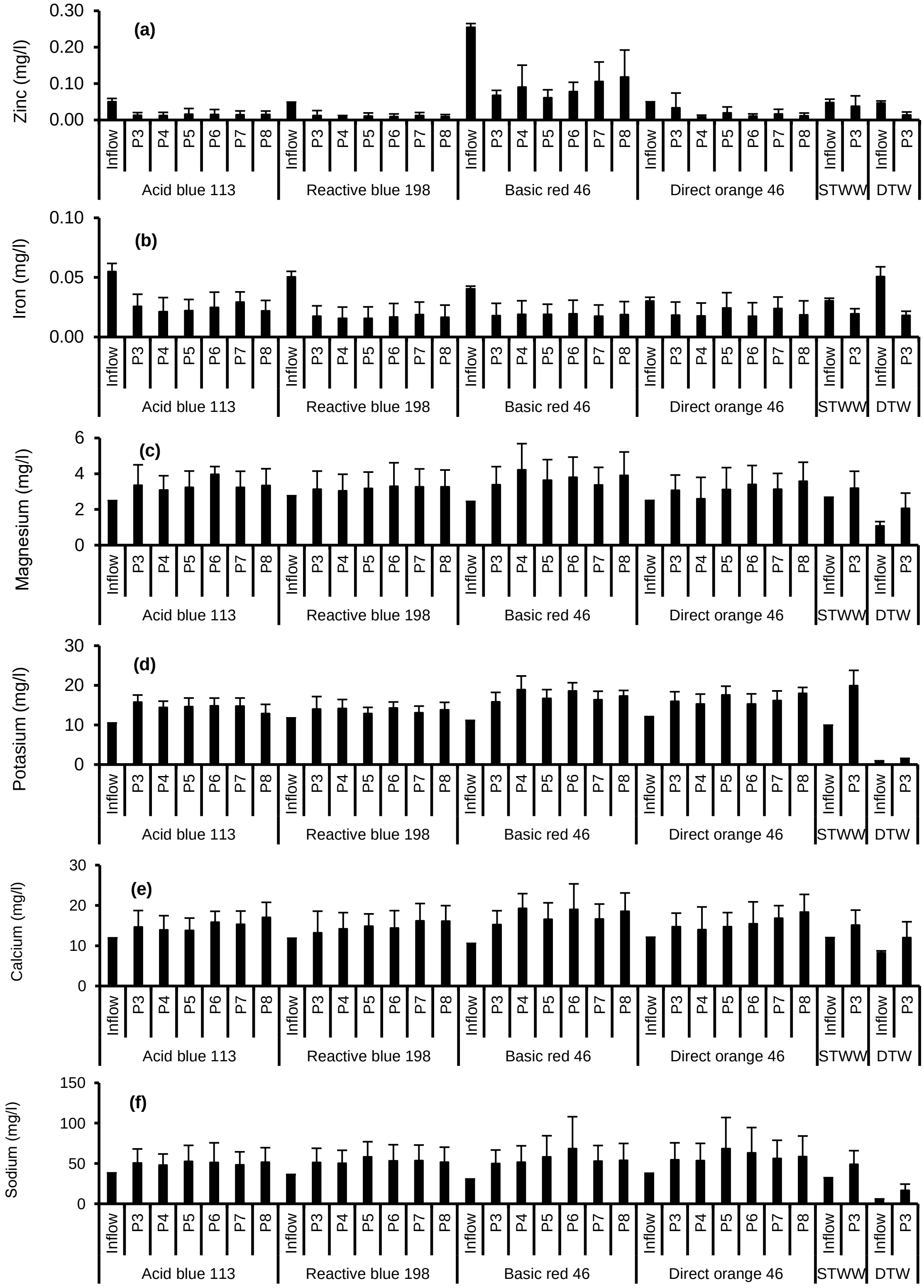


Figure 5.1 Mean inflow and outflow concentrations of the detected trace elements during the experiment between 1 October 2015 and 19 January 2016. Note: (a), zinc; (b), iron; (c), magnesium; (d), potassium; (e), calcium; (f), sodium; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

5.2.2 Treatment performance

5.2.2.1 Dye and apparent colour

The efficiency of dye removal (Figure 5.2) was low for the planted ponds (P3) comprising acid blue 113 (AB113), reactive blue 198 (RB198) and direct orange 46 (DO46) compared to basic red 46 (BR46), which showed mean removals of around 85%. This was mainly due to the absence of sulpho groups and the simple structure of the dye BR46, as mentioned previously (Chapter 4, Sections 4.2.2.1, 4.3.2.1, and 4.4.2.1). The mean removal efficiency of *L. minor* ponds treating RB198 and BR46 (Figure 5.2) was significantly (Table 5.2) higher than the corresponding control ones which reflects the significantly lower outflow concentrations in *L. minor* ponds compared with control ones for these two dyes (Table 5.3). These results indicate that plants can remove RB198. However, both plant and microbial activities affected the dye BR46 molecule and increased dye elimination, although the impact of the plants was higher. The effect of *L. minor* in terms of treatment resembled outcomes reported by Keskinkan and Lugal Goksu (2007), who used submerged plants for treating basic blue 41. The dye AB113 was removed due to the impact of microbes and dye attachment on the walls. This was because no significant differences were found between *L. minor* and control ponds in terms of dye reductions. However, regarding DO46, it seems that biological treatment with shallow ponds planted with *L. minor*, which is associated with elevated DO concentrations, inhibits dye molecule removal. The longitudinal profile of the dye removal is shown in Figure 5.3.

Regarding the impact of pH, the results showed that the mean removal efficiency of the *L. minor* ponds comprising the dyes AB113, RB198 and DO46 was higher at acidic conditions followed by the removal at normal pH and then at alkaline conditions. However, the mean removal of *L. minor* ponds treating the dye BR46 was similar for all different pH treatments (Figure 5.2). Salleh et al. (2011) indicated that at acidic conditions, the positive charge in the solution increased and the adsorbent surface appeared positively charged. Therefore, for cationic dyes (BR46), the dye concentration will increase leading to a reduction of the adsorbent and, consequently, the removal efficiency. However, in the case of anionic dyes (AB113, RB198 and DO46), the concentration will decrease leading to an increase of the adsorbent and, subsequently, the removal efficiency, and vice versa in the case of alkaline conditions. No significant differences (Table 5.4) were found in the mean dye removal among the pH cases for both control and *L. minor* ponds. The results showed that the impact of pH in this study was very low in terms of AB113, RB198 and DO46, and negligible for

BR46. This may be because the pH values of 6 and 9 were not suitable to considerably impact on the removal efficiency within the system and a stronger acid or base is required. In addition, the period of the experiment, of around three months, and the death of the plants resulted in insufficient valuable data being available to discuss.

Based on the ultraviolet visible (UV-visible) scans data (Figure 5.4), the peaks of the inflow samples in the visible region were between 400 nm and 800 nm, at 556, 625, 530 and 421 nm, and are linked to the chromophore groups for AB113, RB198, BR46 and DO46 in this order. However, for the outflow samples, the intensity of the dye adsorption peak at 566 nm reduced for both *L. minor* and control ponds, which confirmed the (low) reduction of AB113, because the colourless outflows were not achieved, with appearance of a new peak at 610 nm (Figures 5.4a, b and c). This result indicates that the dye AB113 removal was due to the bio-sorption by microbes (Somasiri et al., 2006) and/or the dye accumulating on the sides of the pond as dry dye. The outflow samples of planted ponds treating RB198 showed appreciable decreases of the absorbance at 625 nm compared with the control ones. This reduction is not due to the dye molecule degradation or transformation, because the main peak at 625 nm does not completely disappear and the outflow samples were not colourless. Therefore, dye accumulation and/or adsorption by plants was the expected mechanism for the removal (Figures 5.4d, e and f). Regarding the dye BR46, the intensity of the absorption peak of the visible region at 530 nm completely disappeared for planted ponds with the appearance of a colourless product indicating significant decolourisation of this dye (Figures 5.4g, h and i). This complete reduction was due to transformation and adsorption by plants and microbes. However, the mechanism of BR46 removal in the control ponds was due to microbial bio-sorption, because the colour band at 530 nm did not completely disappear, and may be biotransformation. The bands at 421 nm for the dye DO46 (Figures 5.4j, k and l) showed small absorbance reductions for both *L. minor* and control ponds, which may indicate that the removal was due to dye attachment on the sides of the ponds. The figures for each dye do not show appreciable differences in terms of pH variation.

The HPLC chromatogram showed a peak at a retention time of 1.711 min for the AB113 inflow (Appendix E, Figure E.1a). However, the outflow samples showed a peak at 1.687 min for *L. minor* ponds and at 1.66 min for control ponds at normal pH, a peak at 1.895 and 2.083 min for *L. minor* ponds and one at 1.677 min for control ponds at alkaline conditions, and at 1.619 and 1.655 min for *L. minor* ponds and at 1.745 min for control ones at acidic conditions. Peaks shifting or changing for both *L. minor* and control ponds, in addition to the low dye removal for all ponds containing AB113, linked with the not colourless outflow samples indicating that the expected removal of this dye was due to adsorption processes (Appendix E, Figure E.10). The inflow RB198 sample displayed a distinct peak at 1.505 min (Appendix E, Figure E.1b). The peak for the inflow RB198 sample shifted for *L. minor* ponds after treatment, which reflects the low removal of this dye (Figure 5.2). However, small changes were found for peaks related to the control ponds, which implies that RB198 was not treated in the control ponds (Appendix E, Figure E.11). The peaks appeared at 1.613 and 1.566 min for planted and unplanted ponds, respectively, at normal pH. The recordings of peaks were at 1.623 min for planted and 1.593 min for unplanted ponds at alkaline conditions, and at 1.616 and 1.557 min for planted and unplanted ponds, respectively, at acidic conditions. The BR46 inflow showed peaks at retention times of 1.488, 1.693, 2.569 and 2.405 min (Appendix E, Figure E.1c). At normal pH, for *L. minor* ponds, new peaks were noted in HPLC chromatogram analysis for metabolites at 1.61, 1.82, 1.99 and 2.39 min. In addition, all inflow peaks disappeared, which confirms the formation of new products. However, the control ponds showed new peaks at 1.62, 1.81, 1.925 and 2.193 min. For the case of pH 9, the chromatogram indicated new peaks at 1.61, 1.822, 1.93 and 2.41 min for *L. minor* ponds, and at 1.62 and 2.257 min for control ponds. For pH 6, the parent dye BR46 displayed a prominent peak at 1.588 min and other peaks at 1.72, 1.83 and 2.27 min for *L. minor* ponds, and at 1.62 and 2.082 min for the control ones (Appendix E, Figure E.12). As indicated by Khandare et al. (2011) and Kabra et al. (2012), there are peak variations between the inflow and outflow samples, and new peaks are noted as new products are formed by dye phytotransformation. Therefore, due to the presence of new products and the disappearance of the inflow peaks of BR46 linked with high removal of this dye (Figure 5.2) and the colourless outflow samples, the removal mechanisms were expected to be bio-sorption, complete phytotransformation (Khandare et al., 2011; Kabra et al., 2012) and may be biotransformation (Tahir et al., 2016) in planted ponds, and microbial adsorption and may be biotransformation in control ponds (Tahir et al., 2016). Finally, the dye DO46 showed a peak at 1.497 min (Appendix E, Figure E.1d). However, the outflow samples (Appendix E, Figure E.13) showed peaks at 1.711 and 1.68 min for the cases of normal pH, at 1.508 and 1.597 min for pH of 9, and at 1.717 and 1.882 min for pH of 6, linked to planted and unplanted ponds, respectively. These changes may have been due to the interaction between the dye and the synthetic wastewater because the outflow colour was not changed.

Based on colour monitoring, the mean outflow values were lower than the inflow values for all ponds except the *L. minor* and control ponds containing the dye DO46, and the planted ponds treating the dye RB198. The mean outflow (Table 5.3) colour values for all planted ponds were significantly lower (Table 5.2) than the corresponding unplanted ones for the dye BR46 only. These results can be explained by the factors that affect colour (apparent colour) measurements which include the dye concentration and the suspended particles in the system. Therefore, the planted ponds for the dyes AB113, RB198 and DO46 showed higher colour due to the presence of dead plants in the system in addition to the high dye concentration due to the low removal. However, BR46 was treated better and the colour measurements were due to the impact of the dead plants. Inflow and outflow sample pictures are shown in Appendix F, Figure F.2.

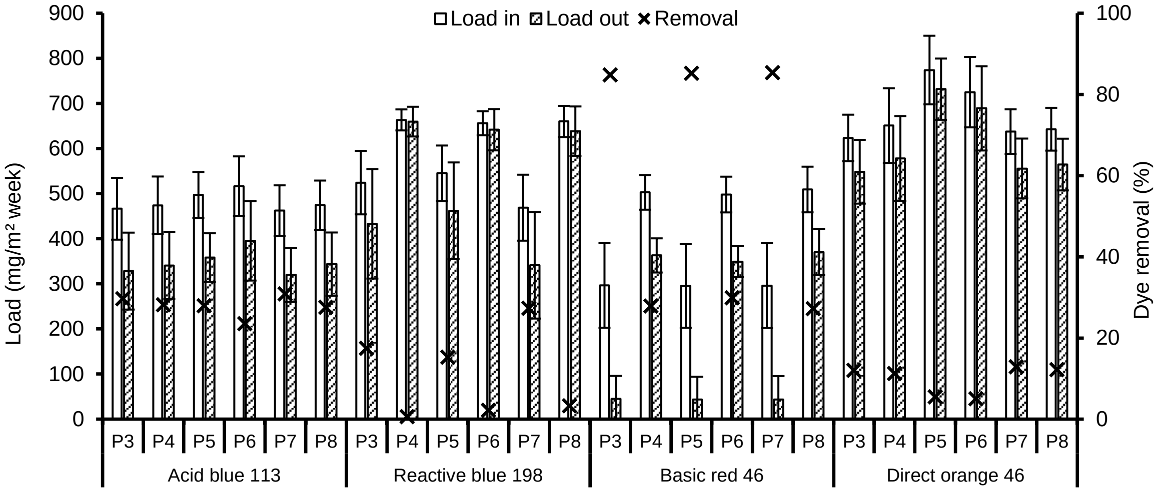


Figure 5.2 Mean dye removal efficiency during the experiment between 1 October 2015 and 19 January 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6**.**

Table 5.2 Overview of the statistical analysis between *Lemna minor* L. and control ponds for outflow water quality parameters and corresponding removal efficiencies for the period between 1 October 2015 and 19 January 2016

Parameter	Shapiro-Wilk test (*p* value[1])	*p* value[1]	Shapiro-Wilk test (*p* value[1])	*p* value[1]	Shapiro-Wilk test (*p* value[1])	*p* value[1]
	Normal pH		pH of 9		pH of 6	
Dye (mg/l)						
AB113	0.001	0.613	0.013	0.408	0.003	0.291
RB198	0.001	0.000	0.022	0.000	0.002	0.000
BR46	0.000	0.000	0.000	0.000	0.001	0.000
DO46	0.001	0.312	0.472	0.29	0.791	0.497
Dye removal (%)						
AB113	0.226	0.686	0.449	0.281	0.829	0.409
RB198	0.000	0.017	0.004	0.000	0.000	0.000
BR46	0.000	0.000	0.000	0.000	0.000	0.000
DO46	0.001	0.963	0.246	0.982	0.654	0.816
Colour (Pt Co)						
AB113	0.096	0.081	0.056	0.808	0.026	0.748
RB198	0.163	0.000	0.463	0.068	0.074	0.229
BR46	0.001	0.000	0.000	0.000	0.000	0.000
DO46	0.287	0.012	0.354	0.003	0.159	0.008
Chemical oxygen demand (mg/l)						
AB113	0.266	0.485	0.554	0.773	0.658	0.633
RB198	0.502	0.795	0.328	0.752	0.843	0.666
BR46	0.511	0.911	0.812	0.653	0.716	0.585
DO46	0.875	0.665	0.875	0.089	0.164	0.364
Chemical oxygen demand removal (%)						
AB113	0.383	0.842	0.787	0.548	0.65	0.797
RB198	0.521	0.983	0.408	0.743	0.37	0.893
BR46	0.139	0.753	0.61	0.741	0.556	0.872
DO46	0.63	0.477	0.751	0.008	0.817	0.807
Ammonium-nitrogen (mg/l)						
AB113	0.316	0.698	0.378	0.749	0.09	0.8

Table 5.2 (Continued)

RB198	0.626	0.322	0.684	0.417	0.948	0.446
BR46	0.696	0.536	0.416	0.867	0.751	0.584
DO46	0.17	0.72	0.563	0.632	0.121	0.737
Ammonium-nitrogen removal (%)						
AB113	0.865	0.533	0.898	0.628	0.657	0.77
RB198	0.147	0.677	0.504	0.787	0.001	0.386
BR46	0.693	0.655	0.170	0.650	0.067	0.745
DO46	0.138	0.690	0.121	0.796	0.530	0.993
Nitrate-nitrogen (mg/l)						
AB113	0.474	0.816	0.695	0.056	0.447	0.062
RB198	0.471	0.601	0.181	0.525	0.647	0.229
BR46	0.721	0.346	0.693	0.302	0.584	0.013
DO46	0.426	0.467	0.666	0.475	0.214	0.843
Nitrate-nitrogen removal (%)						
AB113	0.075	0.841	0.003	0.773	0.009	1.000
RB198	0.318	0.944	0.72	0.929	0.056	0.967
BR46	0.055	0.919	0.468	0.847	0.049	0.564
DO46	0.191	0.913	0.142	0.621	0.084	0.869
Ortho-phosphate-phosphorus (mg/l)						
AB113	0.146	0.958	0.152	0.663	0.84	0.623
RB198	0.038	0.139	0.8	0.005	0.511	0.098
BR46	0.862	0.056	0.479	0.072	0.47	0.05
DO46	0.752	0.072	0.277	0.124	0.781	0.094
Ortho-phosphate-phosphorus removal (%)						
AB113	0.958	0.07	0.192	0.137	0.889	0.026
RB198	0.636	0.038	0.371	0.008	0.85	0.012
BR46	0.628	0.023	0.287	0.019	0.967	0.009
DO46	0.081	0.059	0.822	0.078	0.135	0.221
Suspended solids (mg/l)						
AB113	0.167	0.077	0.069	0.156	0.059	0.093
RB198	0.000	0.47	0.008	0.854	0.390	0.125
BR46	0.008	0.089	0.108	0.805	0.04	0.89

Table 5.2 (Continued)

DO46	0.297	0.69	0.008	0.853	0.078	0.055
Turbidity (NTU)						
AB113	0.254	0.469	0.000	0.646	0.073	0.304
RB198	0.000	0.408	0.029	0.679	0.002	0.597
BR46	0.000	0.215	0.000	0.334	0.066	0.907
DO46	0.463	0.466	0.571	0.705	0.000	0.646
Dissolved oxygen (mg/l)						
AB113	0.524	0.612	0.523	0.767	0.173	0.298
RB198	0.397	0.691	0.863	0.419	0.359	0.347
BR46	0.391	0.438	0.654	0.996	0.063	0.524
DO46	0.143	0.511	0.100	0.963	0.125	0.386
pH (-)						
AB113	0.468	0.214	0.003	0.29	0.051	0.204
RB198	0.142	0.118	0.000	0.215	0.001	0.147
BR46	0.184	0.859	0.000	0.89	0.016	0.160
DO46	0.218	0.173	0.000	0.27	0.110	0.210
Redox potential (mV)						
AB113	0.000	0.358	0.098	0.025	0.068	0.208
RB198	0.180	0.133	0.000	0.300	0.002	0.182
BR46	0.293	0.991	0.000	0.782	0.024	0.182
DO46	0.018	0.231	0.000	0.223	0.129	0.028
Electrical conductivity (µS/cm)						
AB113	0.001	0.000	0.058	0.001	0.001	0.000
RB198	0.008	0.000	0.006	0.002	0.004	0.000
BR46	0.033	0.001	0.208	0.000	0.000	0.000
DO46	0.206	0.000	0.002	0.003	0.000	0.005

Note: ^{1}Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); ^2p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$, the variables are statistically significantly different); NTU, nephelometric turbidity unit; Mann-Whitney U test used when the data are not normally distributed and independent sample t-test used when the data are normally distributed; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46.

Table 5.3 Outflow water quality parameters for each system between 1 October 2015 and 19 January 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Acid blue113 + STWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	6.1	1.67	4.2	10.1	14
pH	-	7.5	0.20	7.8	7.2	14
Redox	mV	-62.6	31.57	-121.0	-31.0	14
Dissolved oxygen	mg/l	7.6	0.38	7.1	8.2	14
Electrical conductivity	µS/cm	715.3	27.92	668.0	766.0	14
Total dissolved solids	mg/l	357.6	13.96	334.0	383.0	14
Suspended solids	mg/l	19.1	4.39	12.0	25.5	14
Turbidity	NTU	6.5	2.36	2.9	12.0	14
Colour	Pt Co	817.0	51.65	736.5	894.0	14
Chemical oxygen demand	mg/l	112.9	29.01	83.7	161.0	4
Ammonium -nitrogen	mg/l	12.4	3.97	7.9	18.8	4
Nitrate-nitrogen	mg/l	12.2	2.37	9.6	15.0	4
Ortho-phosphate-phosphorus	mg/l	13.6	2.32	9.9	16.0	4
Acid blue113 + STWW at normal pH for control ponds						
Dye concentration	mg/l	6.4	1.53	4.8	10.0	14
pH	-	7.7	0.25	8.0	7.1	14
Redox	mV	-66.9	28.07	-114.0	-32.0	14
Dissolved oxygen	mg/l	7.7	0.36	7.1	8.5	14
Electrical conductivity	µS/cm	763.9	5.62	752.5	774.0	14
Total dissolved solids	mg/l	382.0	2.81	376.3	387.0	14
Suspended solids	mg/l	15.8	4.81	8.0	24.0	14
Turbidity	NTU	5.9	1.46	3.5	8.7	14
Colour	Pt Co	846.2	26.49	793.5	886.5	14
Chemical oxygen demand	mg/l	98.5	16.77	75.1	122.0	4
Ammonium -nitrogen	mg/l	13.9	4.51	9.1	21.0	4
Nitrate-nitrogen	mg/l	13.0	5.53	7.1	21.1	4
Ortho-phosphate-phosphorus	mg/l	13.7	2.09	10.5	15.6	4
Acid blue113 + STWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	6.9	1.13	5.4	9.2	14
pH	-	7.9	0.46	8.8	7.5	14
Redox	mV	-56.6	9.82	-82.0	-47.0	14
Dissolved oxygen	mg/l	7.7	0.32	7.2	8.3	14
Electrical conductivity	µS/cm	730.1	36.18	666.0	786.5	14
Total dissolved solids	mg/l	365.1	18.09	333.0	393.3	14
Suspended solids	mg/l	16.8	5.07	9.5	26.0	14
Turbidity	NTU	6.7	4.57	2.3	20.4	14
Colour	Pt Co	857.9	24.30	811.0	888.5	14
Chemical oxygen demand	mg/l	98.6	19.11	72.3	126.0	4
Ammonium -nitrogen	mg/l	10.7	3.33	6.1	15.5	4
Nitrate-nitrogen	mg/l	12.9	2.88	9.2	15.9	4
Ortho-phosphate-phosphorus	mg/l	12.5	2.69	8.1	15.5	4
Acid blue113 + STWW at pH 9 for control ponds						
Dye concentration	mg/l	7.6	1.75	5.6	11.3	14

Table 5.3 (Continued)

pH	-	8.0	0.47	8.7	7.1	14
Redox	mV	-66.3	10.95	-87.0	-47.0	14
Dissolved oxygen	mg/l	7.8	0.33	7.3	8.5	14
Electrical conductivity	µS/cm	777.7	26.87	721.0	810.5	14
Total dissolved solids	mg/l	388.9	13.44	360.5	405.3	14
Suspended solids	mg/l	14.1	4.24	9.0	23.0	14
Turbidity	NTU	6.0	1.15	3.8	7.9	14
Colour	Pt Co	853.6	56.89	738.5	956.5	14
Chemical oxygen demand	mg/l	103.2	18.61	81.9	133.0	4
Ammonium -nitrogen	mg/l	11.8	4.49	6.7	19.0	4
Nitrate-nitrogen	mg/l	21.1	5.23	14.5	28.8	4
Ortho-phosphate-phosphorus	mg/l	13.4	2.20	9.9	15.5	4
Acid blue113 + STWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	6.0	1.20	4.7	9.3	14
pH	-	7.2	0.42	7.8	6.5	14
Redox	mV	-30.4	22.68	-61.0	12.0	14
Dissolved oxygen	mg/l	7.5	0.29	7.1	8.1	14
Electrical conductivity	µS/cm	751.9	24.25	698.0	779.0	14
Total dissolved solids	mg/l	376.0	12.12	349.0	389.5	14
Suspended solids	mg/l	20.7	5.67	9.0	35.0	14
Turbidity	NTU	8.1	3.70	3.5	15.0	14
Colour	Pt Co	918.7	38.82	866.0	978.0	14
Chemical oxygen demand	mg/l	120.2	16.79	97.8	145.0	4
Ammonium -nitrogen	mg/l	12.5	5.77	6.6	22.0	4
Nitrate-nitrogen	mg/l	11.4	2.62	8.0	15.1	4
Ortho-phosphate-phosphorus	mg/l	15.4	3.27	10.2	19.0	4
Acid blue113 + STWW at pH 6 for control ponds						
Dye concentration	mg/l	6.5	1.31	4.7	9.1	14
pH	-	7.4	0.30	7.8	6.9	14
Redox	mV	-40.6	17.31	-66.0	-10.0	14
Dissolved oxygen	mg/l	7.6	0.34	7.2	8.3	14
Electrical conductivity	µS/cm	783.2	6.42	771.5	798.5	14
Total dissolved solids	mg/l	391.6	3.21	385.8	399.3	14
Suspended solids	mg/l	16.5	6.44	6.5	25.0	14
Turbidity	NTU	7.0	0.68	5.7	8.6	14
Colour	Pt Co	914.2	37.20	861.0	980.0	14
Chemical oxygen demand	mg/l	112.4	21.15	85.4	144.0	4
Ammonium -nitrogen	mg/l	13.9	6.45	7.2	24.5	4
Nitrate-nitrogen	mg/l	19.9	5.87	13.1	28.8	4
Ortho-phosphate-phosphorus	mg/l	16.6	2.74	12.8	20.2	4
Reactive blue 198 + STWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	8.0	2.13	4.6	11.4	14
pH	-	7.5	0.26	7.9	6.9	14
Redox	mV	-48.6	14.51	-67.0	-14.0	14
Dissolved oxygen	mg/l	7.6	0.30	7.0	8.2	14
Electrical conductivity	µS/cm	726.7	14.30	705.0	759.0	14
Total dissolved solids	mg/l	363.4	7.15	352.5	379.5	14

Table 5.3 (Continued)

Suspended solids	mg/l	15.8	5.08	10.0	25.0	14
Turbidity	NTU	6.6	3.10	4.6	17.2	14
Colour	Pt Co	246.5	23.59	194.5	287.5	14
Chemical oxygen demand	mg/l	80.8	27.05	47.9	123.0	4
Ammonium -nitrogen	mg/l	9.3	3.82	5.1	15.5	4
Nitrate-nitrogen	mg/l	18.0	3.81	14.1	24.3	4
Ortho-phosphate-phosphorus	mg/l	7.6	1.58	6.2	10.1	4
Reactive blue 198 + STWW at normal pH for control ponds						
Dye concentration	mg/l	12.1	0.41	11.3	12.8	14
pH	-	7.7	0.20	8.0	7.3	14
Redox	mV	-56.2	10.28	-74.0	-36.0	14
Dissolved oxygen	mg/l	7.6	0.32	7.2	8.2	14
Electrical conductivity	µS/cm	769.0	7.34	757.5	784.0	14
Total dissolved solids	mg/l	384.5	3.67	378.8	392.0	14
Suspended solids	mg/l	13.4	1.07	11.5	15.0	14
Turbidity	NTU	6.0	0.55	5.1	6.8	14
Colour	Pt Co	217.3	10.71	203.5	242.5	14
Chemical oxygen demand	mg/l	74.5	29.36	32.0	115.0	4
Ammonium -nitrogen	mg/l	12.4	3.15	8.0	16.9	4
Nitrate-nitrogen	mg/l	16.2	3.98	12.0	22.2	4
Ortho-phosphate-phosphorus	mg/l	9.7	0.42	9.1	10.1	4
Reactive blue 198 + STWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	8.7	1.90	5.6	11.7	14
pH	-	7.9	0.47	8.7	7.4	14
Redox	mV	-67.8	26.59	-115.0	-39.0	14
Dissolved oxygen	mg/l	7.7	0.28	7.2	8.3	14
Electrical conductivity	µS/cm	741.7	34.55	664.0	794.0	14
Total dissolved solids	mg/l	370.8	17.27	332.0	397.0	14
Suspended solids	mg/l	14.4	2.51	9.5	20.0	14
Turbidity	NTU	5.8	0.83	4.9	7.5	14
Colour	Pt Co	263.0	16.91	236.5	290.0	14
Chemical oxygen demand	mg/l	77.6	30.74	35.4	122.0	4
Ammonium -nitrogen	mg/l	8.6	3.53	4.9	14.4	4
Nitrate-nitrogen	mg/l	15.2	5.39	10.5	24.0	4
Ortho-phosphate-phosphorus	mg/l	6.4	1.16	5.0	7.8	4
Reactive blue 198 + STWW at pH 9 for control ponds						
Dye concentration	mg/l	12.1	0.88	10.8	14.7	14
pH	-	8.0	0.47	8.8	7.5	14
Redox	mV	-73.0	25.29	-117.0	-45.0	14
Dissolved oxygen	mg/l	7.8	0.29	7.3	8.3	14
Electrical conductivity	µS/cm	781.3	30.94	748.0	880.5	14
Total dissolved solids	mg/l	390.6	15.47	374.0	440.3	14
Suspended solids	mg/l	13.2	4.00	4.0	17.0	14
Turbidity	NTU	5.7	2.02	2.7	10.7	14
Colour	Pt Co	248.4	21.78	215.0	285.0	14
Chemical oxygen demand	mg/l	86.1	31.75	35.2	121.0	4
Ammonium -nitrogen	mg/l	11.3	3.85	6.4	17.1	4

Table 5.3 (Continued)

Nitrate-nitrogen	mg/l	18.1	5.29	13.8	27.0	4
Ortho-phosphate-phosphorus	mg/l	10.1	0.87	9.2	11.5	4
Reactive blue 198 + STWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	6.4	2.06	3.6	10.5	14
pH	-	6.8	0.91	7.8	5.2	14
Redox	mV	-17.8	39.21	-61.0	36.0	14
Dissolved oxygen	mg/l	7.5	0.26	7.2	8.2	14
Electrical conductivity	µS/cm	744.6	18.55	711.5	768.0	14
Total dissolved solids	mg/l	372.3	9.27	355.8	384.0	14
Suspended solids	mg/l	14.8	2.08	11.0	18.0	14
Turbidity	NTU	6.1	0.64	5.3	7.5	14
Colour	Pt Co	215.9	18.16	169.0	250.5	14
Chemical oxygen demand	mg/l	83.6	24.31	50.5	118.0	4
Ammonium -nitrogen	mg/l	10.8	3.78	6.0	16.6	4
Nitrate-nitrogen	mg/l	21.1	4.10	16.4	27.7	4
Ortho-phosphate-phosphorus	mg/l	8.6	1.30	6.9	10.5	4
Reactive blue 198+ STWW at pH 6 for control ponds						
Dye concentration	mg/l	12.0	0.85	9.8	13.0	14
pH	-	7.3	0.47	7.8	6.1	14
Redox	mV	-37.5	27.93	-74.0	34.0	14
Dissolved oxygen	mg/l	7.6	0.30	7.1	8.1	14
Electrical conductivity	µS/cm	780.3	5.14	771.5	788.0	14
Total dissolved solids	mg/l	390.1	2.57	385.8	394.0	14
Suspended solids	mg/l	13.3	2.70	7.0	18.0	14
Turbidity	NTU	6.0	1.14	4.8	7.1	14
Colour	Pt Co	208.5	11.78	187.0	235.5	14
Chemical oxygen demand	mg/l	75.1	21.76	41.3	102.0	4
Ammonium -nitrogen	mg/l	13.5	4.16	7.7	19.3	4
Nitrate-nitrogen	mg/l	26.6	5.69	18.4	34.0	4
Ortho-phosphate-phosphorus	mg/l	10.3	0.76	9.1	11.0	4
Basic red 46 + STWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	1.1	1.39	0.3	5.0	14
pH	-	7.6	0.30	8.3	7.2	14
Redox	mV	-55.2	16.20	-90.0	-30.0	14
Dissolved oxygen	mg/l	7.6	0.31	7.2	8.2	14
Electrical conductivity	µS/cm	702.2	48.84	622.0	764.0	14
Total dissolved solids	mg/l	351.1	24.42	311.0	382.0	14
Suspended solids	mg/l	17.8	7.14	8.0	30.5	14
Turbidity	NTU	7.1	1.31	4.8	9.8	14
Colour	Pt Co	198.0	48.48	155.5	313.0	14
Chemical oxygen demand	mg/l	89.4	22.44	58.8	121.0	4
Ammonium -nitrogen	mg/l	11.8	4.55	6.3	18.8	4
Nitrate-nitrogen	mg/l	15.2	4.65	10.0	22.0	4
Ortho-phosphate-phosphorus	mg/l	8.6	1.07	7.5	10.3	4
Basic red 46 + STWW at normal pH for control ponds						
Dye concentration	mg/l	7.2	0.80	6.3	9.1	14
pH	-	7.7	0.21	8.0	7.3	14

Table 5.3 (Continued)

Redox	mV	-56.2	12.91	-78.0	-34.0	14
Dissolved oxygen	mg/l	7.7	0.34	7.1	8.2	14
Electrical conductivity	µS/cm	763.0	32.82	692.5	799.0	14
Total dissolved solids	mg/l	381.5	16.41	346.3	399.5	14
Suspended solids	mg/l	14.4	10.70	4.0	33.0	14
Turbidity	NTU	6.9	2.46	3.9	15.3	14
Colour	Pt Co	456.9	52.27	326.0	527.5	14
Chemical oxygen demand	mg/l	87.2	23.04	53.0	118.0	4
Ammonium -nitrogen	mg/l	14.5	5.47	8.0	23.0	4
Nitrate-nitrogen	mg/l	19.5	5.42	14.2	27.4	4
Ortho-phosphate-phosphorus	mg/l	10.9	1.34	9.4	13.0	4
Basic red 46 + STWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	0.9	1.16	0.2	4.5	14
pH	-	7.9	0.41	8.7	7.5	14
Redox	mV	-71.0	22.61	-109.0	-43.0	14
Dissolved oxygen	mg/l	7.7	0.34	7.2	8.4	14
Electrical conductivity	µS/cm	731.7	25.07	682.0	759.5	14
Total dissolved solids	mg/l	365.8	12.54	341.0	379.8	14
Suspended solids	mg/l	12.1	3.31	7.0	17.5	14
Turbidity	NTU	6.7	1.53	5.2	11.0	14
Colour	Pt Co	205.0	9.69	188.0	221.5	14
Chemical oxygen demand	mg/l	83.8	27.75	45.3	122.0	4
Ammonium -nitrogen	mg/l	10.5	3.71	6.1	16.3	4
Nitrate-nitrogen	mg/l	12.7	3.63	9.0	17.8	4
Ortho-phosphate-phosphorus	mg/l	9.8	1.23	8.6	11.8	4
Basic red 46 + STWW at pH 9 for control ponds						
Dye concentration	mg/l	7.0	0.84	5.8	8.9	14
pH	-	8.0	0.42	8.8	7.5	14
Redox	mV	-71.3	21.99	-107.0	-44.0	14
Dissolved oxygen	mg/l	7.7	0.33	7.1	8.3	14
Electrical conductivity	µS/cm	781.2	28.65	712.0	809.0	14
Total dissolved solids	mg/l	390.6	14.33	356.0	404.5	14
Suspended solids	mg/l	11.8	3.26	7.5	18.0	14
Turbidity	NTU	6.5	0.58	5.9	7.2	14
Colour	Pt Co	562.9	48.65	481.5	639.0	14
Chemical oxygen demand	mg/l	93.1	19.90	60.9	115.0	4
Ammonium -nitrogen	mg/l	11.0	3.18	6.5	15.5	4
Nitrate-nitrogen	mg/l	16.3	4.09	12.2	22.1	4
Ortho-phosphate-phosphorus	mg/l	12.3	1.57	10.1	14.0	4
Basic red 46 + STWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	0.9	1.26	0.2	5.0	14
pH	-	7.3	0.26	7.8	6.9	14
Redox	mV	-34.4	15.04	-63.0	-13.0	14
Dissolved oxygen	mg/l	7.5	0.27	7.2	8.0	14
Electrical conductivity	µS/cm	724.1	40.90	668.0	778.0	14
Total dissolved solids	mg/l	362.1	20.45	334.0	389.0	14
Suspended solids	mg/l	15.2	6.35	7.0	31.0	14

Table 5.3 (Continued)

Turbidity	NTU	6.6	0.98	5.0	7.6	14
Colour	Pt Co	212.9	14.36	192.5	240.5	14
Chemical oxygen demand	mg/l	99.3	16.02	79.0	122.0	4
Ammonium -nitrogen	mg/l	11.1	4.15	6.0	17.5	4
Nitrate-nitrogen	mg/l	11.4	5.60	5.4	20.2	4
Ortho-phosphate-phosphorus	mg/l	9.8	1.42	8.7	12.2	4
Basic red 46 + STWW at pH 6 for control ponds						
Dye concentration	mg/l	7.3	1.36	5.3	9.9	14
pH	-	7.4	0.30	7.9	7.1	14
Redox	mV	-42.7	16.55	-66.0	-25.0	14
Dissolved oxygen	mg/l	7.6	0.36	7.1	8.2	14
Electrical conductivity	µS/cm	780.0	5.60	770.1	792.0	14
Total dissolved solids	mg/l	390.0	2.80	385.0	396.0	14
Suspended solids	mg/l	14.8	6.49	7.0	25.5	14
Turbidity	NTU	6.5	3.04	2.7	12.6	14
Colour	Pt Co	538.8	58.73	471.0	648.0	14
Chemical oxygen demand	mg/l	90.2	22.25	58.3	121.0	4
Ammonium -nitrogen	mg/l	13.1	4.36	7.7	19.8	4
Nitrate-nitrogen	mg/l	25.0	3.69	22.0	31.2	4
Ortho-phosphate-phosphorus	mg/l	12.8	1.49	11.1	14.5	4
Direct orange 46 + STWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	10.8	1.48	8.6	13.3	14
pH	-	7.5	0.31	8.2	7.1	14
Redox	mV	-52.0	17.36	-85.0	-34.0	14
Dissolved oxygen	mg/l	7.8	0.24	7.5	8.2	14
Electrical conductivity	µS/cm	733.7	13.42	714.5	758.0	14
Total dissolved solids	mg/l	380.3	51.13	357.3	563.3	14
Suspended solids	mg/l	12.4	3.21	8.0	19.0	14
Turbidity	NTU	5.8	0.82	4.0	6.8	14
Colour	Pt Co	1477.4	119.52	1265.0	1621.5	14
Chemical oxygen demand	mg/l	76.8	20.56	50.7	108.0	4
Ammonium -nitrogen	mg/l	10.0	4.05	5.5	16.6	4
Nitrate-nitrogen	mg/l	18.9	1.87	16.8	21.0	4
Ortho-phosphate-phosphorus	mg/l	15.5	2.77	11.0	18.6	4
Direct orange 46 + STWW at normal pH for control ponds						
Dye concentration	mg/l	11.6	2.33	9.4	18.3	14
pH	-	7.7	0.21	8.0	7.3	14
Redox	mV	-56.4	11.97	-74.0	-38.0	14
Dissolved oxygen	mg/l	7.9	0.42	7.1	8.8	14
Electrical conductivity	µS/cm	764.2	8.91	746.0	780.0	14
Total dissolved solids	mg/l	382.1	4.45	373.0	390.0	14
Suspended solids	mg/l	11.9	2.10	7.0	15.5	14
Turbidity	NTU	5.6	1.01	3.7	7.5	14
Colour	Pt Co	1351.6	119.40	1168.0	1681.5	14
Chemical oxygen demand	mg/l	85.7	26.88	44.6	120.0	4
Ammonium -nitrogen	mg/l	11.4	4.55	6.8	18.8	4
Nitrate-nitrogen	mg/l	17.7	1.77	15.6	20.0	4

Table 5.3 (Continued)

Ortho-phosphate-phosphorus	mg/l	20.8	3.18	15.5	24.0	4
Direct orange 46 + STWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	15.4	1.55	13.0	18.5	14
pH	-	8.0	0.60	9.3	7.5	14
Redox	mV	-74.1	33.46	-148.0	-45.0	14
Dissolved oxygen	mg/l	8.1	0.41	7.6	9.2	14
Electrical conductivity	µS/cm	737.8	22.35	711.0	779.0	14
Total dissolved solids	mg/l	368.9	11.17	355.5	389.5	14
Suspended solids	mg/l	12.1	3.46	7.5	20.0	14
Turbidity	NTU	5.3	0.68	4.5	6.5	14
Colour	Pt Co	1632.9	111.90	1337.5	1765.5	14
Chemical oxygen demand	mg/l	106.4	13.88	84.4	122.0	4
Ammonium -nitrogen	mg/l	9.5	3.83	4.9	15.5	4
Nitrate-nitrogen	mg/l	23.1	2.78	19.6	26.6	4
Ortho-phosphate-phosphorus	mg/l	18.4	2.84	13.7	21.0	4
Direct orange 46 + STWW at pH 9 for control ponds						
Dye concentration	mg/l	15.9	2.11	11.6	18.9	14
pH	-	8.1	0.63	9.2	7.2	14
Redox	mV	-79.3	34.14	-144.0	-47.0	14
Dissolved oxygen	mg/l	8.1	0.50	7.5	9.4	14
Electrical conductivity	µS/cm	766.1	8.02	743.0	778.0	14
Total dissolved solids	mg/l	383.0	4.01	371.5	389.0	14
Suspended solids	mg/l	11.8	3.29	8.0	21.0	14
Turbidity	NTU	5.2	0.97	3.3	7.2	14
Colour	Pt Co	1496.3	99.39	1344.0	1717.0	14
Chemical oxygen demand	mg/l	126.9	10.70	116.0	143.0	4
Ammonium -nitrogen	mg/l	11.0	3.61	6.2	16.4	4
Nitrate-nitrogen	mg/l	25.1	3.48	22.5	31.0	4
Ortho-phosphate-phosphorus	mg/l	21.9	1.75	19.0	23.5	4
Direct orange 46 + STWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	11.1	1.44	8.1	13.8	14
pH	-	7.1	0.42	7.8	6.5	14
Redox	mV	-25.2	22.37	-62.0	7.0	14
Dissolved oxygen	mg/l	7.7	0.27	7.1	8.1	14
Electrical conductivity	µS/cm	749.3	27.92	672.0	784.0	14
Total dissolved solids	mg/l	374.6	13.96	336.0	392.0	14
Suspended solids	mg/l	13.1	1.15	11.5	15.0	14
Turbidity	NTU	6.0	0.44	5.1	6.5	14
Colour	Pt Co	1405.4	73.12	1216.5	1489.5	14
Chemical oxygen demand	mg/l	106.9	7.43	100.0	118.2	4
Ammonium -nitrogen	mg/l	10.9	5.56	5.0	20.0	4
Nitrate-nitrogen	mg/l	18.7	2.73	15.5	22.2	4
Ortho-phosphate-phosphorus	mg/l	16.3	2.93	11.5	19.1	4
Direct orange 46 + STWW at pH 6 for control ponds						
Dye concentration	mg/l	11.5	1.29	9.5	13.8	14
pH	-	7.5	0.30	7.9	7.1	14
Redox	mV	-43.3	16.79	-66.0	-24.0	14

Table 5.3 (Continued)

Dissolved oxygen	mg/l	7.8	0.34	7.1	8.3	14
Electrical conductivity	µS/cm	773.0	7.99	759.9	789.8	14
Total dissolved solids	mg/l	386.5	3.99	380.0	394.9	14
Suspended solids	mg/l	12.1	1.06	10.5	14.0	14
Turbidity	NTU	5.5	1.31	2.4	7.0	14
Colour	Pt Co	1332.5	55.57	1244.0	1457.5	14
Chemical oxygen demand	mg/l	98.5	12.80	76.9	110.0	4
Ammonium -nitrogen	mg/l	12.4	5.37	6.6	21.2	4
Nitrate-nitrogen	mg/l	18.2	2.92	14.2	21.0	4
Ortho-phosphate-phosphorus	mg/l	20.7	2.40	16.9	23.3	4
STWW at normal pH for *Lemna minor* L. ponds						
pH	-	7.7	0.26	8.0	7.3	14
Redox	mV	-55.2	14.85	-79.0	-38.0	14
Dissolved oxygen	mg/l	8.2	0.49	7.4	9.5	14
Electrical conductivity	µS/cm	726.5	8.15	716.5	741.0	14
Total dissolved solids	mg/l	363.2	4.08	358.3	370.5	14
Suspended solids	mg/l	8.7	2.31	6.0	13.0	14
Turbidity	NTU	3.4	0.86	2.1	5.1	14
Colour	Pt Co	42.8	8.48	30.5	58.0	14
Chemical oxygen demand	mg/l	104.2	14.76	83.5	122.3	4
Ammonium -nitrogen	mg/l	7.6	2.93	5.0	12.2	4
Nitrate-nitrogen	mg/l	21.3	3.22	16.5	25.0	4
Ortho-phosphate-phosphorus	mg/l	9.1	3.17	5.6	13.8	4
DTW at normal pH for *Lemna minor* L. ponds						
pH	-	7.3	0.14	7.5	7.1	14
Redox	mV	-34.6	7.99	-46.0	-23.0	14
Dissolved oxygen	mg/l	8.1	0.36	7.6	8.8	14
Electrical conductivity	µS/cm	71.6	5.68	61.5	78.5	14
Total dissolved solids	mg/l	35.8	2.84	30.8	39.3	14
Suspended solids	mg/l	4.8	0.86	3.0	6.0	14
Turbidity	NTU	2.4	0.96	1.0	4.1	14
Colour	Pt Co	21.1	7.37	8.0	30.5	14
Chemical oxygen demand	mg/l	20.1	12.24	7.0	36.0	4
Ammonium-nitrogen	mg/l	1.2	0.63	0.6	2.1	4
Nitrate-nitrogen	mg/l	1.0	0.27	0.6	1.3	4
Ortho-phosphate-phosphorus	mg/l	1.2	0.28	0.9	1.6	4

Note: NTU, nephelometric turbidity unit; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

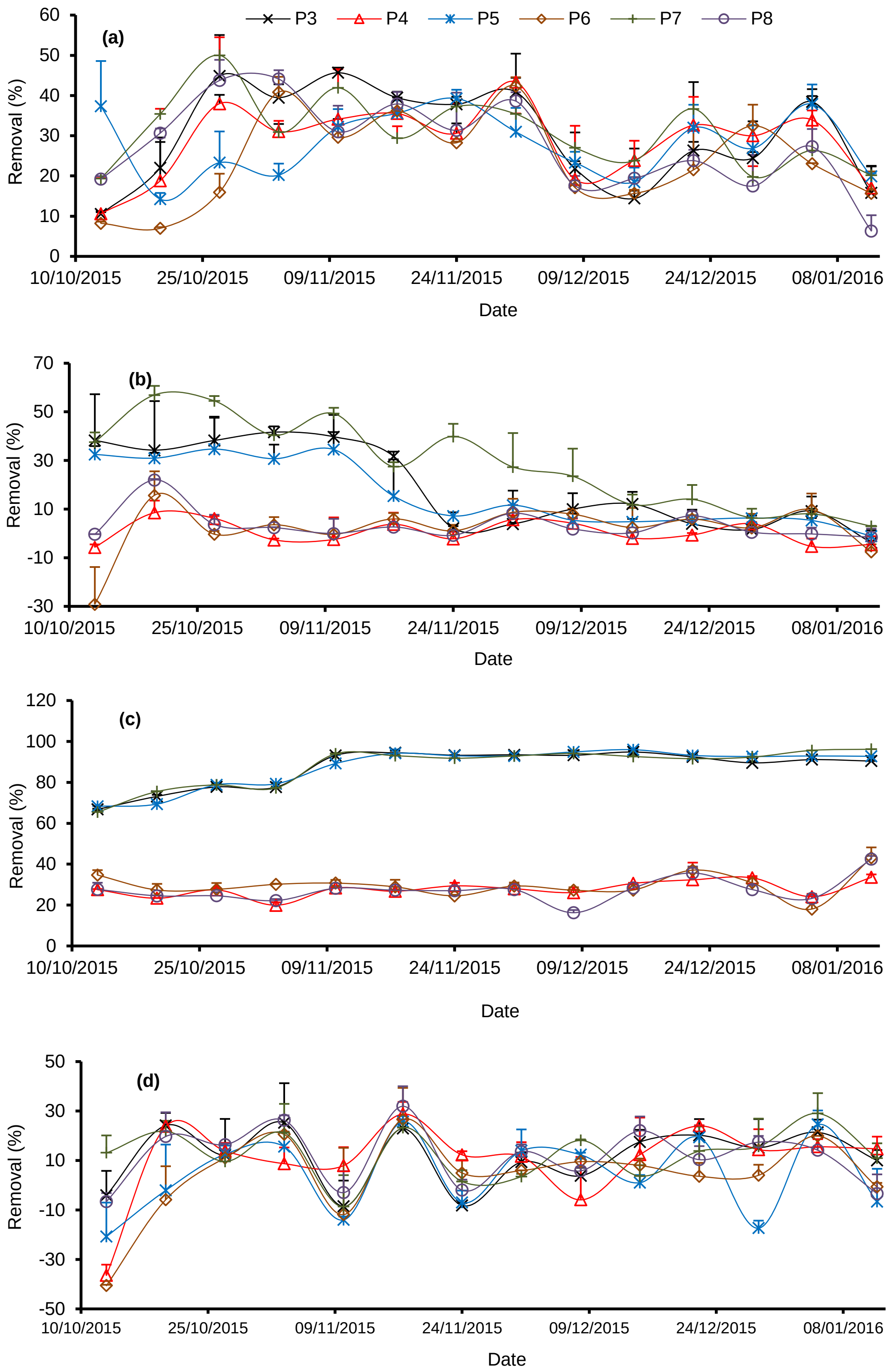


Figure 5.3 Mean values of dye removal profile between 1 October 2015 and 19 January 2016. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6**.**

Table 5.4 Overview of the statistical analysis in terms of the impact of pH factor for outflow water quality parameters and corresponding removal efficiencies for the period between 1 October 2015 and 19 January 2016

Parameter	Shapiro-Wilk test (*p* value[1])	*p* value[1]				Shapiro-Wilk test (*p* value[1])	*p* value[1]			
		Normal pH & pH 9 & pH 6	Normal pH & pH 9	Normal pH & pH 6	pH 9 & pH 6		Normal pH & pH 9 & pH 6	Normal pH & pH 9	Normal pH & pH 6	pH 9 & pH 6
		Lemna minor L. ponds					Control ponds			
Dye (mg/l)										
AB113	0.018	0.102	N/A	N/A	N/A	0.001	0.041	0.013	0.713	0.073
RB198	0.092	0.022	0.720	0.117	0.020	0.006	0.307	N/A	N/A	N/A
BR46	0.000	0.883	N/A	N/A	N/A	0.003	0.827	N/A	N/A	N/A
DO46	0.284	0.000	0.000	0.802	0.000	0.004	0.002	0.003	0.696	0.002
Dye removal (%)										
AB113	0.368	0.713	N/A	N/A	N/A	0.170	0.47	N/A	N/A	N/A
RB198	0.004	0.125	N/A	N/A	N/A	0.000	0.289	N/A	N/A	N/A
BR46	0.000	0.949	N/A	N/A	N/A	0.027	0.268	N/A	N/A	N/A
DO46	0.028	0.366	N/A	N/A	N/A	0.001	0.229	N/A	N/A	N/A
Colour (Pt Co)										
AB113	0.171	0.000	0.033	0.000	0.001	0.396	0.000	0.894	0.001	0.002
RB198	0.554	0.000	0.099	0.001	0.000	0.003	0.000	0.001	0.081	0.000
BR46	0.000	0.000	0.024	0.007	0.140	0.290	0.000	0.000	0.001	0.491
DO46	0.742	0.000	0.001	0.000	0.192	0.036	0.000	0.001	0.818	0.000
Chemical oxygen demand (mg/l)										
AB113	0.823	0.508	N/A	N/A	N/A	0.831	0.673	N/A	N/A	N/A
RB198	0.297	0.965	N/A	N/A	N/A	0.321	0.852	N/A	N/A	N/A
BR46	0.541	0.705	N/A	N/A	N/A	0.247	0.947	N/A	N/A	N/A
DO46	0.221	0.057	N/A	N/A	N/A	0.716	0.057	N/A	N/A	N/A
Chemical oxygen demand removal (%)										
AB113	0.145	0.842	N/A	N/A	N/A	0.858	0.792	N/A	N/A	N/A
RB198	0.537	0.980	N/A	N/A	N/A	0.261	0.947	N/A	N/A	N/A
BR46	0.495	0.991	N/A	N/A	N/A	0.048	0.925	N/A	N/A	N/A
DO46	0.298	0.175	N/A	N/A	N/A	0.376	0.078	N/A	N/A	N/A

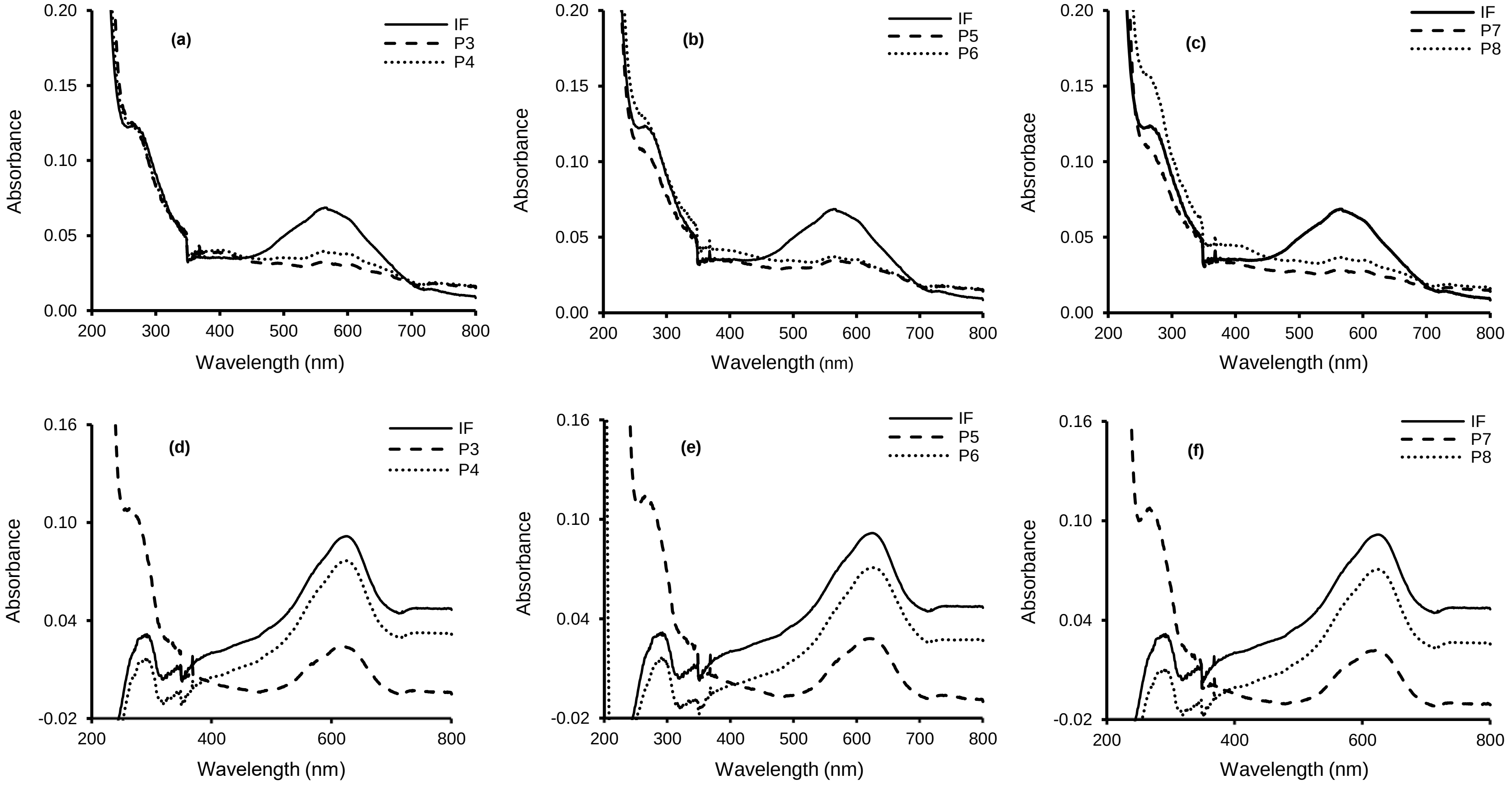
Table 5.4 (Continued)

Ammonium-nitrogen (mg/l)										
AB113	0.203	0.857	N/A	N/A	N/A	0.125	0.858	N/A	N/A	N/A
RB198	0.179	0.768	N/A	N/A	N/A	0.775	0.777	N/A	N/A	N/A
BR46	0.159	0.932	N/A	N/A	N/A	0.441	0.64	N/A	N/A	N/A
DO46	0.036	0.591	N/A	N/A	N/A	0.071	0.928	N/A	N/A	N/A
Ammonium-nitrogen removal (%)										
AB113	0.931	0.722	N/A	N/A	N/A	0.785	0.759	N/A	N/A	N/A
RB198	0.159	0.555	N/A	N/A	N/A	0.131	0.653	N/A	N/A	N/A
BR46	0.521	0.708	N/A	N/A	N/A	0.187	0.762	N/A	N/A	N/A
DO46	0.123	0.770	N/A	N/A	N/A	0.468	0.796	N/A	N/A	N/A
Nitrate-nitrogen (mg/l)										
AB113	0.17	0.783	N/A	N/A	N/A	0.751	0.214	N/A	N/A	N/A
RB198	0.761	0.321	N/A	N/A	N/A	0.31	0.072	N/A	N/A	N/A
BR46	0.614	0.407	N/A	N/A	N/A	0.526	0.108	N/A	N/A	N/A
DO46	0.606	0.099	N/A	N/A	N/A	0.395	0.018	0.026	0.974	0.037
Nitrate-nitrogen removal (%)										
AB113	0.051	0.976	N/A	N/A	N/A	0.842	0.87	N/A	N/A	N/A
RB198	0.235	0.663	N/A	N/A	N/A	0.246	0.887	N/A	N/A	N/A
BR46	0.971	0.351	N/A	N/A	N/A	0.384	0.201	N/A	N/A	N/A
DO46	0.091	0.177	N/A	N/A	N/A	0.503	0.181	N/A	N/A	N/A
Ortho-phosphate-phosphorus (mg/l)										
AB113	0.851	0.464	N/A	N/A	N/A	0.702	0.226	N/A	N/A	N/A
RB198	0.838	0.194	N/A	N/A	N/A	0.277	0.641	N/A	N/A	N/A
BR46	0.233	0.411	N/A	N/A	N/A	0.311	0.322	N/A	N/A	N/A
DO46	0.311	0.458	N/A	N/A	N/A	0.064	0.814	N/A	N/A	N/A
Ortho-phosphate-phosphorus removal (%)										
AB113	0.365	0.587	N/A	N/A	N/A	0.454	0.151	N/A	N/A	N/A
RB198	0.378	0.085	N/A	N/A	N/A	0.183	0.821	N/A	N/A	N/A
BR46	0.007	0.098	N/A	N/A	N/A	0.447	0.584	N/A	N/A	N/A
DO46	0.393	0.313	N/A	N/A	N/A	0.017	0.118	N/A	N/A	N/A
Suspended solids (mg/l)										
AB113	0.014	0.276	N/A	N/A	N/A	0.043	0.56	N/A	N/A	N/A

Table 5.4 (Continued)

RB198	0.001	0.913	N/A	N/A	N/A	0.000	0.479	N/A	N/A	N/A
BR46	0.001	0.102	N/A	N/A	N/A	0.000	0.504	N/A	N/A	N/A
DO46	0.028	0.209	N/A	N/A	N/A	0.001	0.087	N/A	N/A	N/A
Turbidity (NTU)										
AB113	0.001	0.388	N/A	N/A	N/A	0.499	0.029	0.991	0.046	0.061
RB198	0.000	0.284	N/A	N/A	N/A	0.000	0.531	N/A	N/A	N/A
BR46	0.011	0.34	N/A	N/A	N/A	0.000	0.926	N/A	N/A	N/A
DO46	0.047	0.038	0.06	0.854	0.013	0.253	0.641	N/A	N/A	N/A
Dissolved oxygen (mg/l)										
AB113	0.058	0.927	0.92	0.98	0.978	0.839	0.823	0.808	0.929	0.964
RB198	0.237	0.665	0.983	0.668	0.774	0.867	0.611	0.995	0.698	0.637
BR46	0.534	0.803	0.997	0.855	0.816	0.416	0.775	0.999	0.784	0.802
DO46	0.108	0.927	0.921	0.988	0.97	0.153	0.983	0.996	0.995	0.982
pH (-)										
AB113	0.015	0.004	0.031	0.034	0.005	0.03	0.004	0.073	0.054	0.002
RB198	0.000	0.011	0.093	0.048	0.008	0.003	0.008	0.129	0.029	0.006
BR46	0.025	0.000	0.113	0.005	0.000	0.004	0.017	0.154	0.039	0.014
DO46	0.007	0.000	0.007	0.026	0.000	0.000	0.016	0.147	0.035	0.014
Redox potential (mV)										
AB113	0.026	0.010	0.231	0.026	0.006	0.109	0.002	0.997	0.005	0.006
RB198	0.000	0.010	0.129	0.041	0.007	0.003	0.017	0.118	0.077	0.010
BR46	0.080	0.000	0.117	0.013	0.000	0.018	0.010	0.089	0.034	0.009
DO46	0.008	0.000	0.019	0.011	0.000	0.000	0.011	0.089	0.034	0.011
Electrical conductivity (μS/cm)										
AB113	0.103	0.012	0.423	0.009	0.163	0.141	0.013	0.091	0.012	0.0672
RB198	0.484	0.141	0.265	0.154	0.949	0.000	0.011	0.27	0.001	0.301
BR46	0.053	0.186	0.363	0.909	0.879	0.000	0.111	0.066	0.323	0.129
DO46	0.294	0.189	0.883	0.183	0.39	0.749	0.0260	0.827	0.027	0.100

Note: [1]Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); [2]*p* value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$, the variables are statistically significantly different); NTU, nephelometric turbidity unit; Kruskal-Wallis test used when the data are not normally distributed and one-way ANOVA used when the data are normally distributed; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46.


(a)
IF
P3
P4
Absorbance
0.20
0.15
0.10
0.05
0.00
200
300
400
500
600
700
800
Wavelength (nm)
(b)
IF
P5
P6
Absorbance
Wavelength (nm)
(c)
IF
P7
P8
Absrorbace
Wavelength (nm)
(d)
IF
P3
P4
Absorbance
0.16
0.10
0.04
-0.02
200
400
600
800
Wavelength (nm)
(e)
IF
P5
P6
Absorbance
Wavelength (nm)
(f)
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P7
P8
Absorbance
Wavelength (nm)

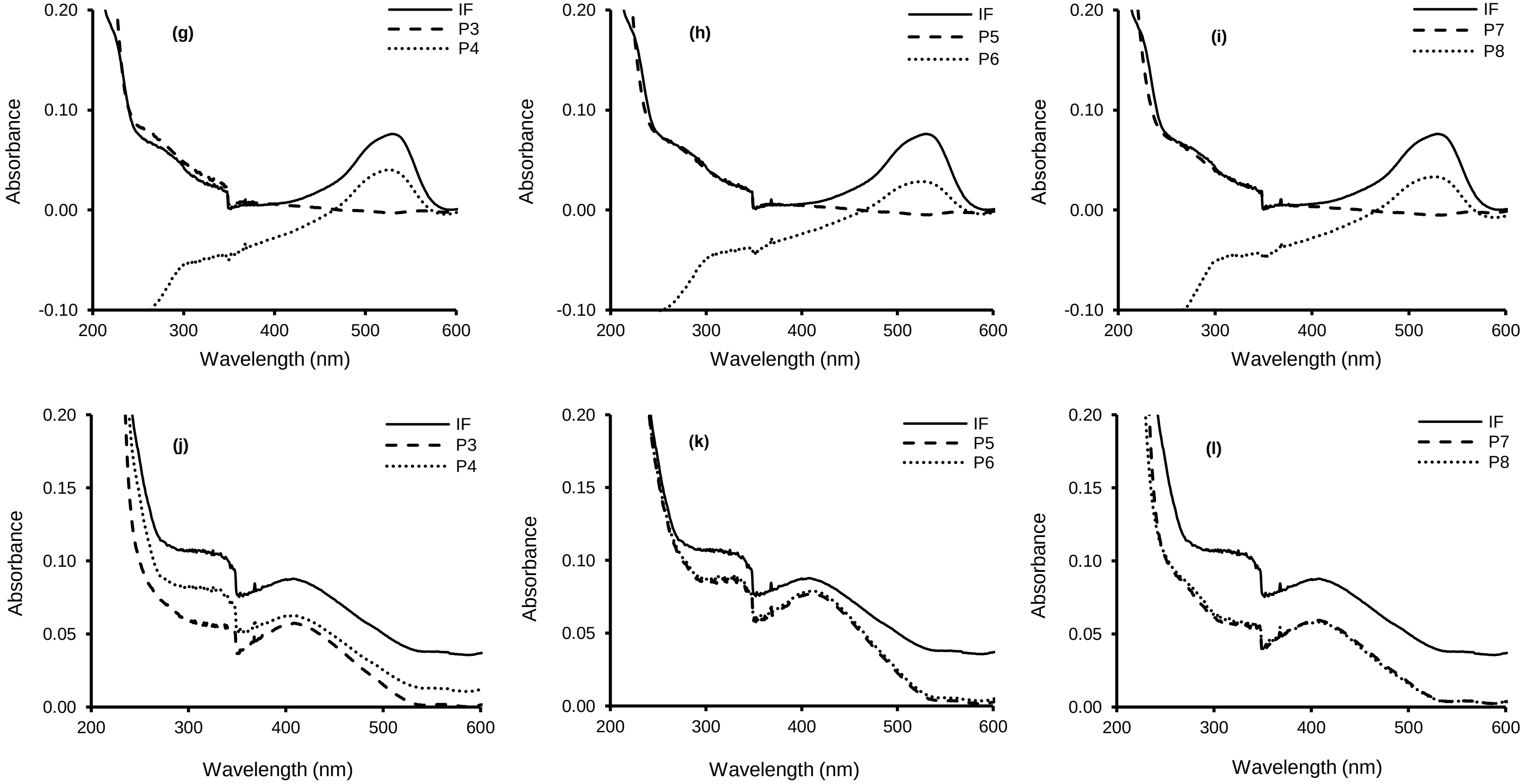


Figure 5.4 Ultraviolet visible analysis for the outflow samples at the end of the experimental rig, which was operated between 1 October 2015 and 19 January 2016. Note: (a), (b) and (c) ponds treating the dye acid blue 113; (d), (e) and (f) ponds treating the dye reactive blue 198; (g), (h) and (i), ponds treating the dye basic red 46; and (j), (k) and (l), ponds treating the dye direct orange 46; IF, inflow; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

5.2.2.2 Chemical oxygen demand and dissolved oxygen

Most of the mean outflow COD concentrations were slightly higher in *L. minor* ponds than the corresponding control ones as shown in Table 5.3, although no significant differences were found (Table 5.2). This was due to the impact of plant decay which increased the organic load in the system (Dalu & Ndamba, 2003).

The average of COD removal efficiencies for all ponds were in the range between 50% and 68% as shown in Figure 5.5, these results are clearly noticed from the high inflow COD concentrations compared with the lower outflow values (Tables 5.1 and 5.3). In this experiment, anoxic conditions were the dominant outflow conditions, although aerobic and anaerobic conditions also occur in the top layer and the bed of the duckweed ponds, respectively (Ozengin & Elmaci, 2007). It seems that these conditions linked with high levels of dissolved oxygen are able to degrade the organic matter in the system due to the impact of the heterotrophic bacteria rather than the uptake mechanism by the plants, which may be negligible as indicated by Ong et al. (2009). They mentioned that the main mechanism of COD removal in wetland systems is the biodegradation by heterotrophic micro-organisms under both aerobic and anaerobic conditions, although the aeration boosted the removal efficiency. In addition, the impact of the plants is normally neglected compared with the biodegradation by microbes. The results of COD removal in this experiment don't match with the very low removal observed in the first experiment, Chapter 4 (Section 4.2.2.2) using the same macrophytes. This may be due to the operation of this experiment by removing the water to a level equivalent to 3 l, which reduced the accumulated load of COD. Statistically, the pH factor didn't significantly affect (Table 5.4) the COD outflow concentrations and COD removal in all the ponds.

Correlation analysis results indicate that the COD removal was significantly ($r = 0.175$, $p = 0.096$) positively correlated with dye removal. The European and international standards set a limit for COD of 125 mg/l in the case of discharge of effluent directly into water courses. The results have shown that the COD outflow concentrations for *L. minor* ponds at normal pH, *L. minor* and control ponds at acidic conditions, *L. minor* and control ponds at basic conditions containing the dye AB113, and control ponds at basic conditions treating the dye DO46 were 1,1,1,1,1, and 2 times non-compliant, respectively.

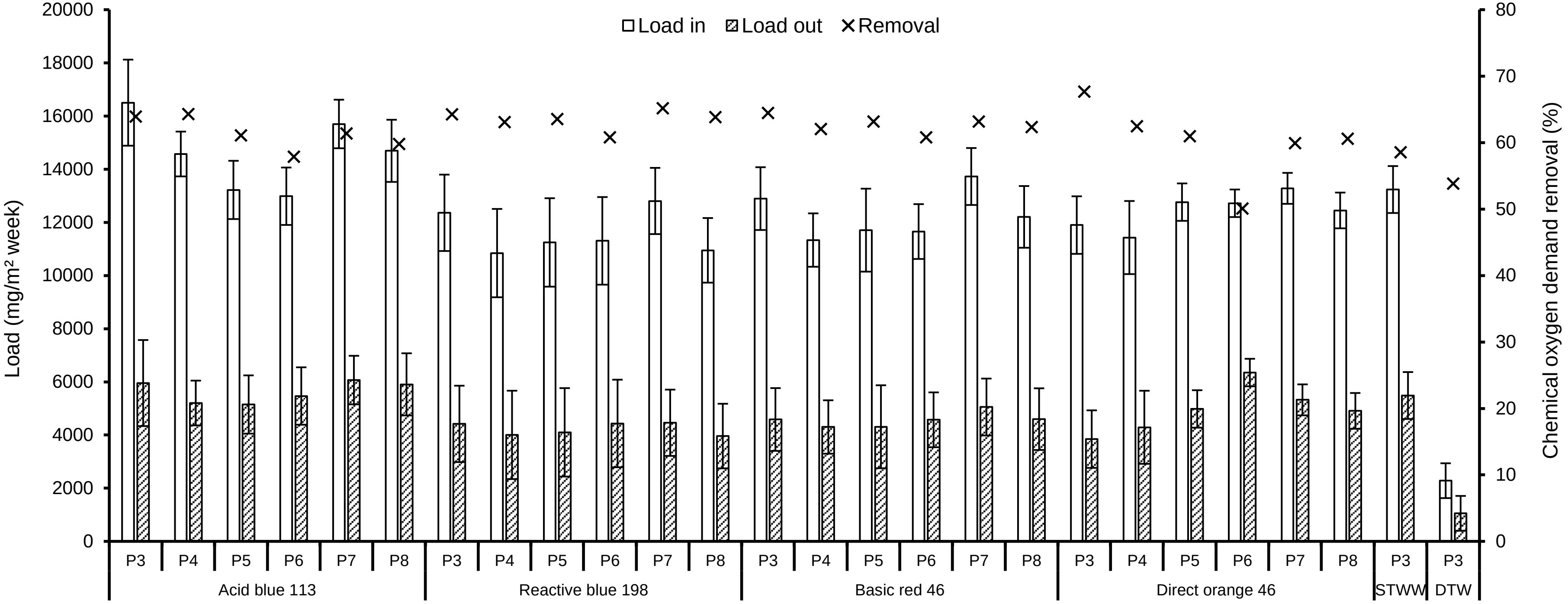


Figure 5.5 Mean chemical oxygen demand removal efficiency during the experiment between 1 October 2015 and 19 January 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

Based on DO, the outflow values ranged between 7.0 and 9.4 mg/l for the ponds containing dyes (Table 5.3), which were lower or equal to the inflow values, which varied between 7.4 and 9.6 mg/l. Statistically, no significant differences were observed between the designs variables (Table 5.2) for all dyes. These results indicate that the oxygen diffusion by the atmosphere affects the DO level in the system rather than the impact of the plants. Regarding the pH level in the system, the mean DO outflow values ranged as follows: at basic conditions > at normal pH > acidic conditions. Note that there is no direct relationship between pH and DO but at basic conditions carbon dioxide level decreases leading to an increase in the DO content, and vice versa for acidic conditions

5.2.2.3 pH and redox potential

The mean inflow pH values at initial conditions were between 7.4 and 7.6 for all dyes, however after adjustment the mean inflow pH values were 9± 0.08 and 6± 0.04 for the basic and acidic conditions, respectively. The mean pH values obtained from the outflow (Table 5.3) were equal to or higher than the inflow pH values at normal conditions (Table 5.1). However, the mean outflow values dropped to be between 7.9 and 8.1 at basic conditions, and increased to be between 6.8 and 7.5 at acidic conditions. These results indicate that the chemicals in the system made the pH approximately neutral during the contact time. Furthermore, the mean outflow values for all dyes were slightly lower (not significant differences, Table 5.2) in *L. minor* ponds than in the corresponding control ponds (Table 5.3). This small difference indicates that the plants also have ability to modify the pH values to balance the chemical contents in the cells by proton and ion exchanges from the coloured synthetic wastewater (Noonpui & Thiravetyan, 2011). The mean pH outflow values for all dyes ranged as follows: at basic conditions > at normal conditions > at acidic conditions for both, *L. minor* and control ponds. European and international standard thresholds for pH are 6.5–8.5, and the results indicate that the planted and unplanted ponds at pH 9 containing AB113, planted ponds at pH 6 containing AB113, planted and unplanted ponds at pH 9 treating RB198, planted and unplanted ponds treating RB198 at pH 6, planted and unplanted ponds fed by BR46 at pH 9, and planted and unplanted ponds at pH 9 containing DO46 were 3, 4, 1, 3, 4, 6, 1, 3, 3, 4 and 4 times non-compliant.

Redox potential is used as an indicator for the aerobic and anaerobic conditions in the aquatic system (Ong et al., 2009a). The maximum and minimum outflow values of redox potential indicate the presence of anaerobic and/or anoxic conditions for ponds at normal pH and basic conditions, and aerobic and/or anoxic conditions at acidic conditions (Table 5.3).

5.2.2.4 Suspended solids and turbidity

The mean outflow SS concentrations (Table 5.3) for each dye were higher than the mean corresponding inflow values, which may be due to the organic matter biodegradation (Dalu & Ndamba, 2003). Regarding the design variables, the mean outflow SS values in *L. minor* ponds were slightly higher (Table 5.2) than the corresponding values of control ponds. This is because in addition to the impact of organic matter degradation, the die-off of *L. minor* and decomposition affected the concentration of SS in the planted ponds. Regarding the impact of pH level on the SS outflow values, no significant dissimilarity was found between the values of each dye (Table 5.4). According to the European and international standards, the results showed that all outflow SS concentrations were equal to or less than the standard limits of 35 mg/l (Carmen & Daniela, 2012).

Based on the turbidity (Table 5.3), the mean outflow values were higher than the corresponding mean inflow values, except for RB198 and DO46 at basic conditions, which were slightly lower than the inflow. All mean outflow turbidity values were slightly higher (no significant dissimilarity, Table 5.2) in *L. minor* ponds compared with the corresponding values in control ponds. A correlation analysis indicated that SS was significantly ($p < 0.01$) positively correlated ($r = 0.27$, $p = 0.000$) with turbidity and significantly negatively ($r = -0.212$, $p = 0.000$) correlated with DO. Therefore, higher COD removal and high outflow SS concentrations are results of low DO content in the system, this is because the DO is consumed by microbial activities for organic matter degradation (Ong et al., 2011). A correlation analysis indicated that COD removal was significantly ($p < 0.01$) negatively correlated with DO ($r = -0.339$, $p = 0.001$) and significantly ($p < 0.01$) positively correlated with SS ($r = 0.333$, $p = 0.001$).

5.2.2.5 Electrical conductivity and total dissolved solids

The mean outflow EC values were lower than the corresponding inflow ones in *L. minor* ponds for each dye. However, control ponds showed mean outflow values higher than the inflow ones (Tables 5.1 and 5.3). In addition, the mean outflow EC values in *L. minor* ponds were significantly (Table 5.2) lower than the corresponding control ones. These results indicate that the plants reduced the EC content in the system. This is because large numbers of dye molecules might be caught in barriers within the vascular plant system (Nilratnisakorn et al., 2009). The optimum growth rate of *L. minor* is linked with EC values ranging between 600 and 1400 µS/cm (Wendeou et al., 2013), all the outflow values of EC in this experiment did not affect the optimum growth rate of *L. minor*.

The mean outflow TDS values were half the EC values, and all the inflow and outflow TDS values showed the same trend as the inflow and outflow EC values (Table 5.3). This is because the EC is a function of TDS (Amankwaah et al., 2014). According to the European and international standards, the results showed that all outflow TDS concentrations were less than the standard limits of 3000 mg/l and 500 mg/l (Carmen & Daniela, 2012).

5.2.2.6 Nutrients

Based on nitrogen removal, the eutrophication that was caused by adding nitrogen to the surface water and the negative impacts on some aquatic organisms, which was caused by the presence of NH_4–N in receiving watercourses, even in low concentrations, makes the removal of nitrogen a very important parameter for wastewater treatment systems. The main processes for nitrogen reduction in pond systems are nitrification and denitrification (which are affected by temperature, pH level, and sedimentation) as well as nutrient uptake by plants.

Regarding ammonium-nitrogen (NH_4–N), the removal efficiencies (Figure 5.6) ranged between 40% and 58% for all treatment systems which was clearly observed from the low outflow concentrations compared with the inflow ones (Tables 5.1 and 5.3). The removal values were slightly higher in *L. minor* ponds (no significant differences, Table 5.2) than the corresponding values for control ones, which reflects the small decrease (no significant differences, Table 5.2) in the mean outflow NH_4–N concentrations in *L. minor* ponds compared with the corresponding control ones for each dye (Tale 5.3). These results indicate that the uptake of nitrogen by the plants was very low or absent. This is because the plants were not harvested during the experiment period due to their death and decomposition which indicates that the mechanism of nitrogen reduction by plant harvesting also did not occur. However, nitrogen accumulation in sediment is possible by decaying duckweed after sedimentation. Ong et al. (2009a) mentioned that the impact of the plant in biological treatment for ammonium-nitrogen removal varies, as some researchers found higher removal in planted than unplanted reactors, whereas others didn’t find any differences. Note that the impact of the plants doesn’t exceed 10% of the total removal in the treatment system as clarified by Kadlec et al. (2000). The results observed were similar to Vanitha et al.’s (2013) findings that duckweed is not very effective in removing ammonia-nitrogen. In this experiment, the role of NH_4–N removal was the biological oxidation of the ammonia to nitrate with nitrite as an intermediate by nitrifying bacteria. During nitrification processes, this micro-organism requires oxygen which is supplied by diffusion from the atmosphere and from the plants, which transfer the oxygen by their roots. Note that the temperature conditions and the pH values were within the range for nitrification occurrence (Kadlec et al., 2000; Ozengin & Elamic, 2007). The removal efficiency of the ponds containing only synthetic wastewater without dyes was higher than the removal in all ponds containing dyes. This is due to the impact of the dye, and the aromatic amine intermediate if it is generated which inhibits the activities of nitrifying microbes in the system leading to a low NH_4–N removal efficiency, as discussed by Ong et al. (2009a). The regulations (UK Government, 1994) set no threshold for ammonia–nitrogen that would relate to the treatment system considered in this article. A realistic guideline threshold value for ammonium-nitrogen, proposed by Al-Isawi et al. (2017), is 20 mg/l. The values for control ponds at normal pH, *L. minor* ponds at acidic pH, control ponds at acidic conditions containing the dye AB113, control ponds at normal pH treating BR46, and *L. minor* and control ponds at acidic pH comprising DO46 were 1, 1, 1, 1, 1, and 1 times non-compliant.

Although the outflow nitrate-nitrogen (NO_3–N) concentrations were less than the inflow ones (Table 5.3), the results showed low NO_3–N removal efficiencies for all ponds that ranged (Figure 5.7) between 31% and 46%. This could be due to the very low anaerobic conditions or the limited denitrification process, which is attributed to the fact that most carbon sources, required for denitrification to occur, have been utilised by the nitrification process (Mohammed, 2017). Statistically, no significant differences (Table 5.2) were found between the removal efficiency of *L. minor* and control ponds which indicated that the removal mechanism was the denitrification processes only and *L. minor* was not effective for NO_3–N removal. This result is in agreement with Vanitha et al.'s (2013) outcomes that the potential of ponds with and without duckweed was similar in removing NO_3–N. Although they reported around 60% of NO_3–N removal which was higher than the results found in this experiment which may attributed to the very high loading rate of the nutrients, the toxic effect of the ammonia level, and the limited amount of plants used in this study. No threshold regulations set for nitrate-nitrogen have relevance for the treatment system examined in this study (UK Government, 1994). Nonetheless, a realistic guideline threshold value for nitrate-nitrogen is proposed by Al-Isawi et al. (2017) to be 50 mg/l, and all values were less than this range. Regarding the impact of the pH factor, no significant differences (Table 5.4) were found among the removal efficiencies of NH_4–N and NO_3–N corresponding to each pH case. This is because the pH variation affected plant growth which consequently impacted on the potential of nitrogen uptake or accumulation. Therefore, the death of the plants in all systems made the impact of pH negligible.

Mean values of ortho-phosphate-phosphorus (PO_4–P) removal efficiency were low in all ponds containing dyes, ranging between 7% and 28%. However, higher removal was found in ponds treating only synthetic wastewater without dyes(Figure 5.8). The *L. minor* ponds showed PO_4–P removal higher than the control ones for all dyes. This reflected the outflow concentrations of PO_4–P which were lower in planted ponds compared with the corresponding unplanted ones for all dyes (Table 5.3). The mechanisms of phosphorus removal in planted ponds are the uptake and assimilation into *L. minor* protein, adsorption on the leaves of the plant, chemical precipitation and microbial uptake. However, the uptake by microbes and biological activities only are responsible for phosphorus reduction in controlled ponds (Vanitha et al., 2013). Regarding the impact of pH inflow values on the PO_4–P outflow concentrations and removal efficiencies, no significant (Table 5.4) difference was found among the pH cases for both planted and unplanted ponds. The UK Government (1994) doesn’t set regulation for ortho-phosphate-phosphorus that relate to the treatment system in this study. However, a realistic guideline threshold value, as mentioned by Al-Isawi et al. (2017), is 1 mg/l and all outflow values were non-compliant.

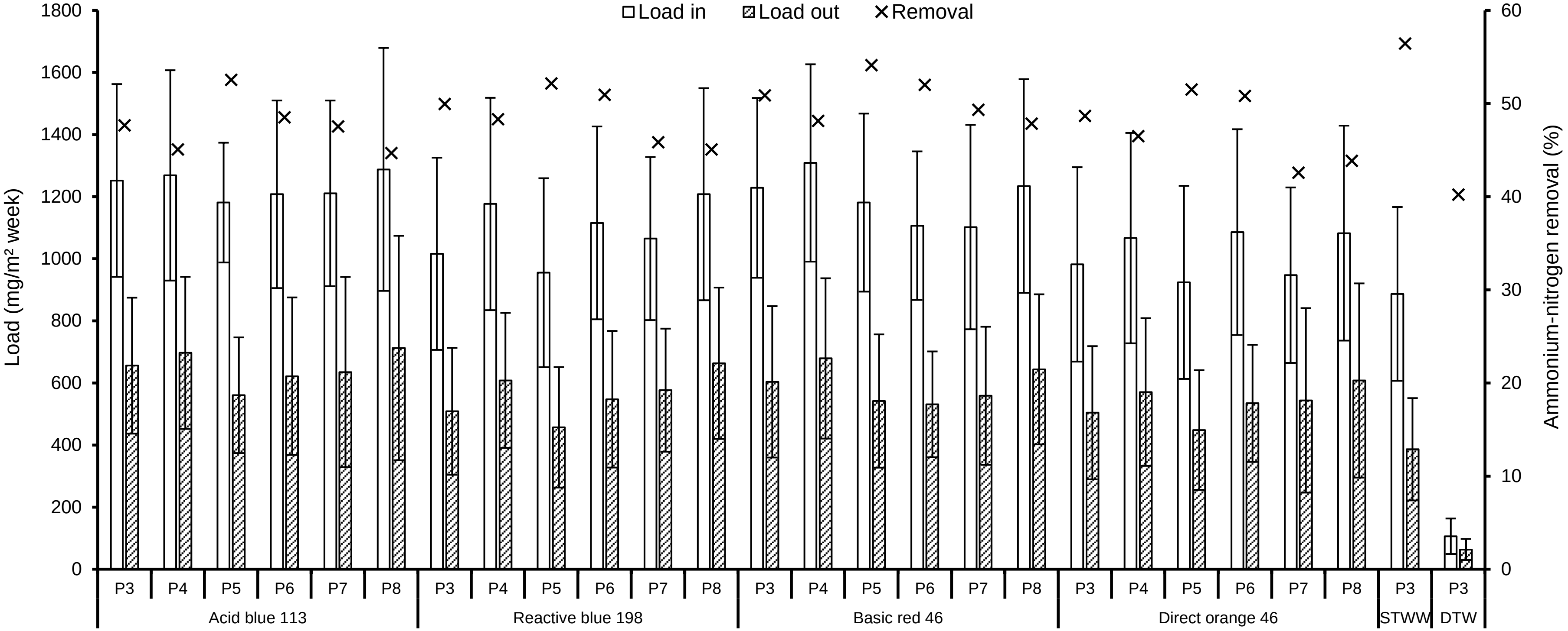


Figure 5.6 Mean ammonium-nitrogen removal efficiency during the experiment between 1 October 2015 and 19 January 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

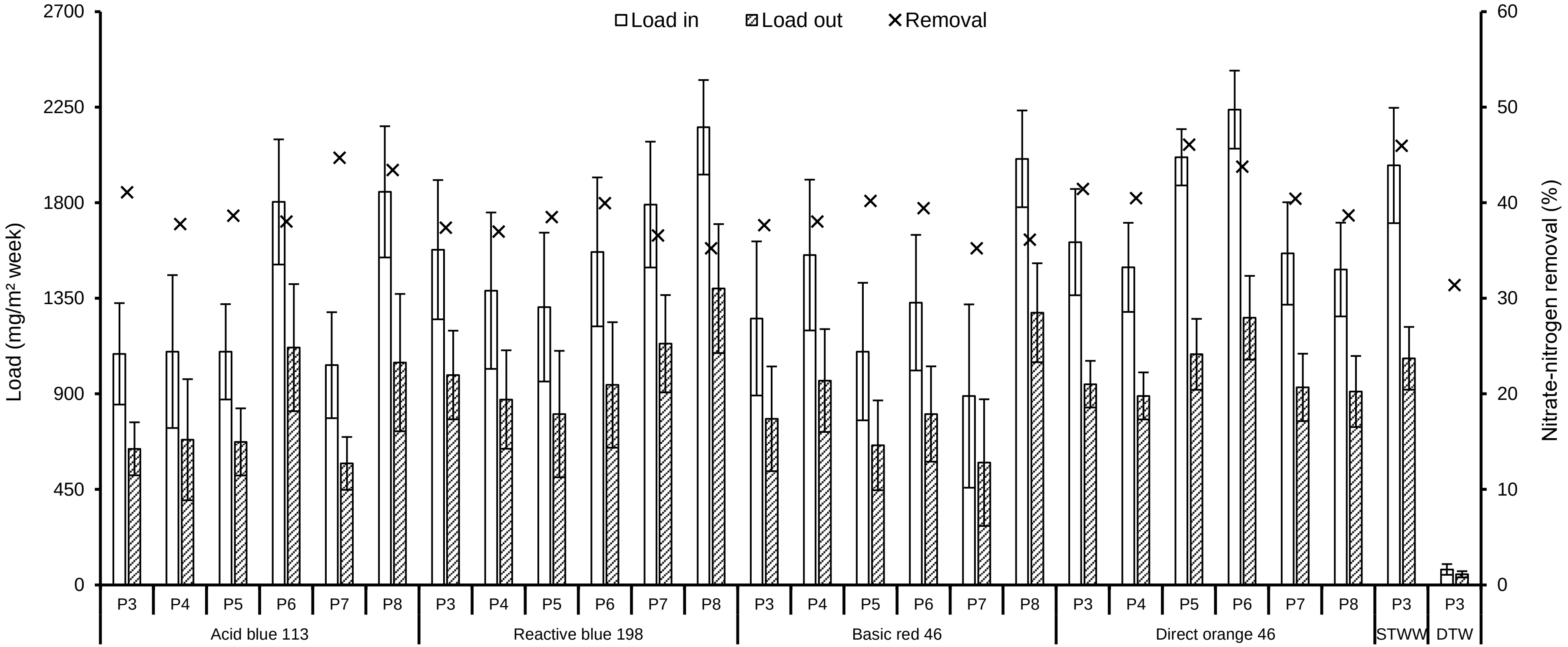


Figure 5.7 Mean nitrate-nitrogen removal efficiency during the experiment between 1 October 2015 and 19 January 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

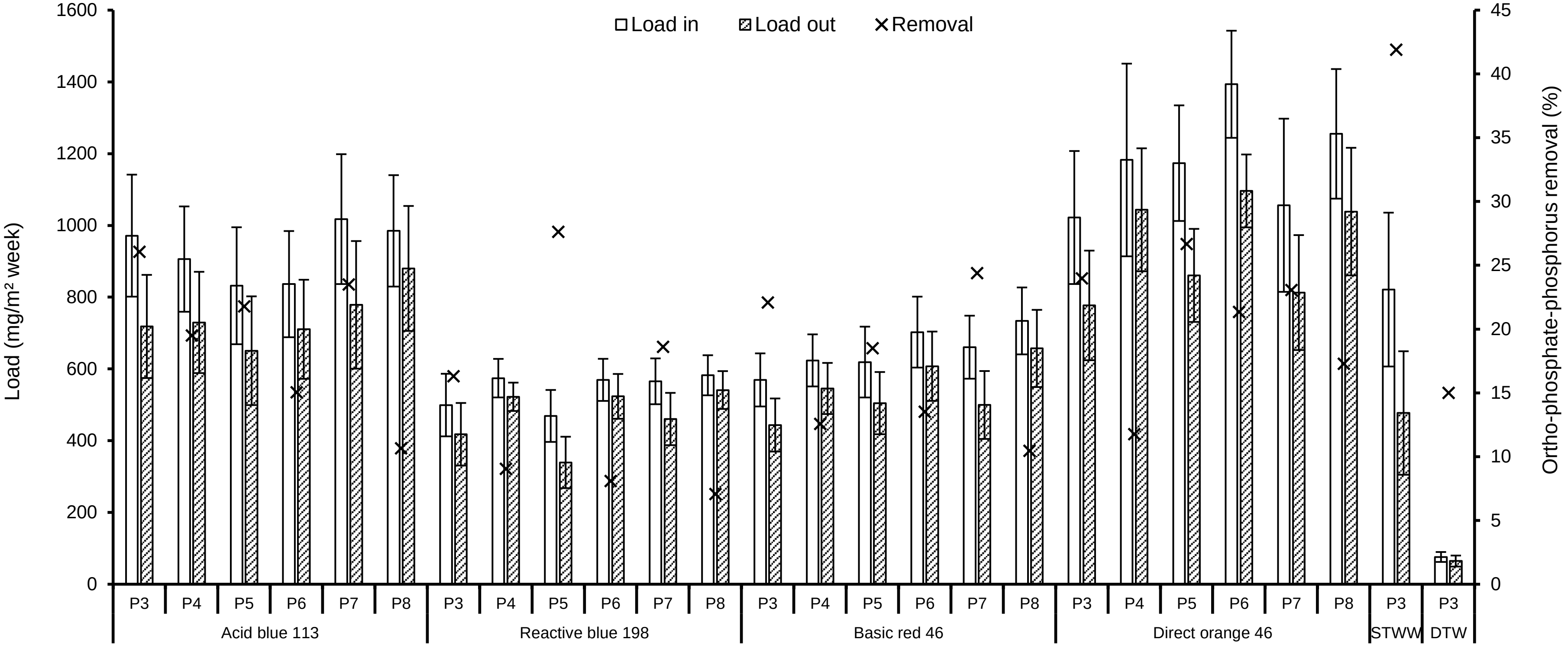


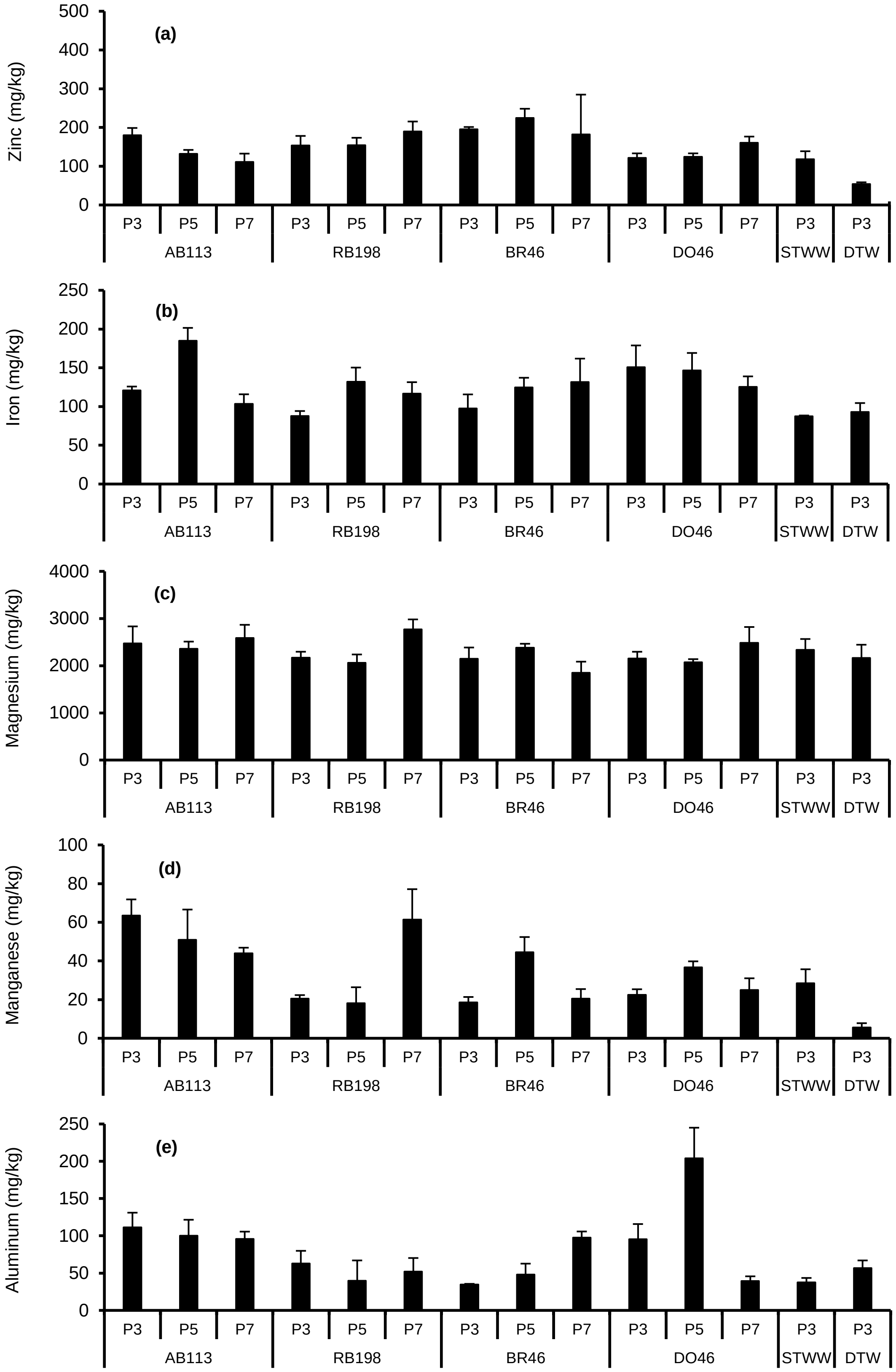
Figure 5.8 Mean ortho-phosphate-phosphorus removal efficiency during the experiment between 1 October 2015 and 19 January 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

5.2.2.7 Trace elements

The mean inflow and outflow concentrations of the elements detected through the ICP-OES analyses are presented in Figure 5.1. The main source of the elements was the synthetic textile wastewater, in addition to the presence of zinc ion in the dye BR46. Mean outflow zinc and iron values (Figures 5.1a and b) were lower than the mean inflow concentrations for both *L. minor* and control ponds. In addition, no significant differences were found between *L. minor* and control ponds ($p > 0.05$, Mann-Whitney U test), although most of the zinc outflow values were lower in ponds containing plants, confirming the uptake of zinc by plants. Regarding the impact of the pH factor, no significant differences ($p > 0.05$) were found. Mean zinc outflows were within the acceptable range for *L. minor* survival (Khellaf & Zerdaoui, 2009). In addition, the mean outflow zinc and iron concentrations were lower than the threshold set of 2 mg/l and 5 mg/l for irrigation, respectively (Metcalf & Eddy, 2003). Higher mean outflow values of magnesium, potassium, calcium and sodium (Figures 5.1c, d, e and f, respectively) than the inflow ones were found for all ponds containing dyes.

Figure 5.9 provides an overview of the concentration of elements accumulated in plant tissues for all ponds with and without dyes. Lower zinc accumulated in plant tissues in ponds containing only dechlorinated tap water. The levels of zinc and iron in plants (Figures 5.9a and b) were higher than the permissible limits of 50 mg/kg and 20 mg/kg, respectively, as mentioned by Nazir et al. (2015). Manganese, aluminium and boron were accumulated and detected in the plants (Figures 5.9d, e and f, respectively), although these metals were not detected in the inflow and outflow aqueous samples.

The BCF of higher than 1000 indicated that the plant as a positive accumulator for heavy metals as mentioned by Sukumaran (2013). The results in Figures 5.10a and b, indicated that the plant was positive for phytoremediation of zinc and iron, respectively, for all samples except the plants treating BR46 in the case of zinc metal which were considered as moderate accumulators.


(a)
Zinc (mg/kg)
0
100
200
300
400
500
(b)
Iron (mg/kg)
0
50
100
150
200
250
(c)
Magnesium (mg/kg)
0
1000
2000
3000
4000
(d)
Manganese (mg/kg)
0
20
40
60
80
100
(e)
Aluminum (mg/kg)
0
50
100
150
200
250
P3
P5
P7
AB113
RB198
BR46
DO46
STWW
DTW

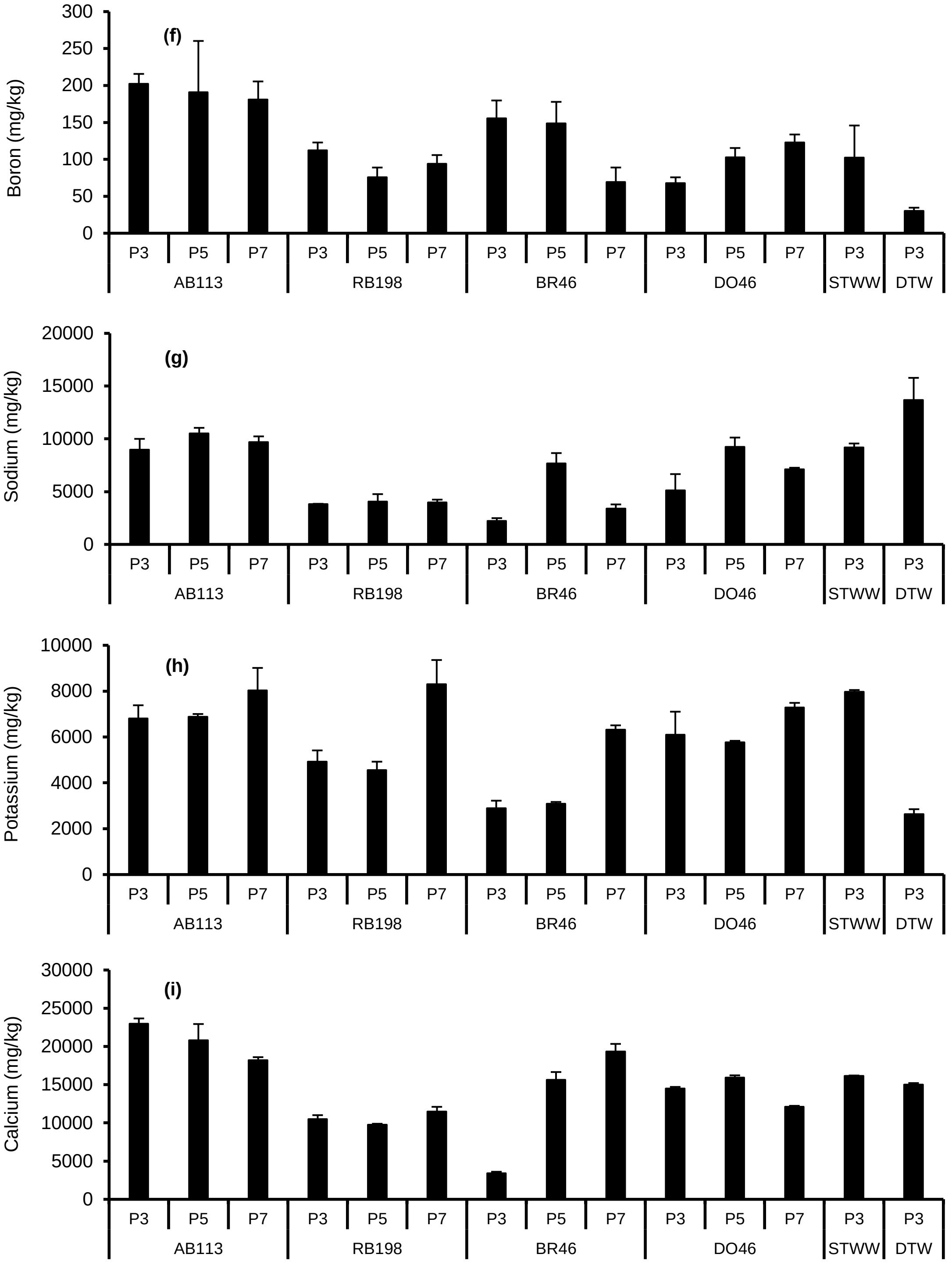


Figure 5.9 Mean concentrations of the detected elements in plant tissue harvested at the end of the experiment that operated between 1 October 2015 and 19 January 2016. Note: (a), zinc; (b), iron; (c), magnesium; (d), manganese; (e), aluminium; (f), boron; (g), sodium; (h), potassium; (i), calcium; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

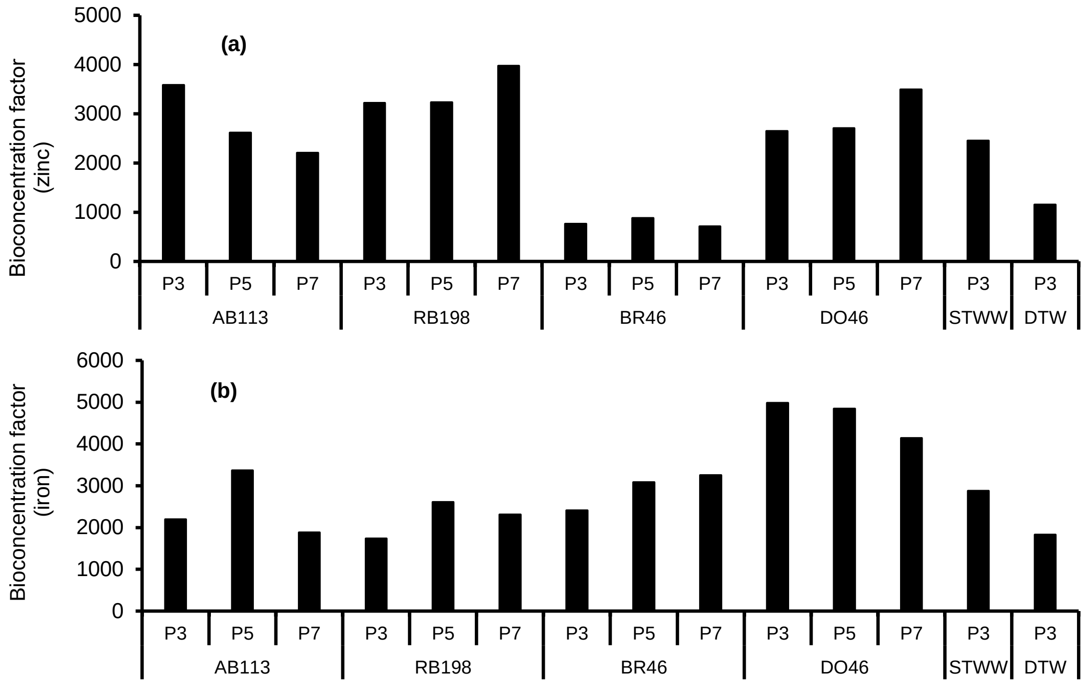


Figure 5.10 Bioconcentration factor during the experiment between 1 October 2015 and 19 January 2016. Note: (a), zinc; (b), iron; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; STWW, synthetic textile wastewater; DTW, dechlorinated tap water.

5.2.3 Plant monitoring

The plants within the system were monitored regularly, and during the first two weeks of adding synthetic dye wastewater, they were still green and their number started to increase, but they did not cover the entire surface area. Thereafter, the green colour faded (reduced). In week 4, the colour of the fronds of *L. minor* turned to yellow and subsequently to brown for all ponds with and without dyes except for ponds containing only dechlorinated tap water. This indicated that the high concentrations of chemicals in the synthetic textile wastewater negatively impacted on plant growth. At the end of the experiment, some of the fronds remained brown, but most of them turned white. The dead plants settled in the pond or stuck on the sides of the ponds. The death of the plants was an indication of the toxicity, such as the high ammonium concentration, of the synthetic wastewater. However, the dead plant biomass contributed to the treatment performance during the operation period. Therefore, it was advisable to re-examine the wastewater constituent for different dilution ratios. The dilution of one part synthetic wastewater to 24 parts raw water was seen as appropriate for the plants to survive.

5.2.4 Environmental conditions

The mean temperature of 28°C, and maximum and minimum values of 29°C and 27°C, respectively, didn’t affect the growth of the plants in the system (Ozengin & Elmaci, 2007). Regarding light intensity, Yin et al. (2015) found that the plant production increases with increasing the light intensity between 1480 lux and 8140 lux. The mean value of 6996 lux was within this range. Table 5.5 provides an overview of environmental boundary conditions in the laboratory during the experimental period.

Table 5.5 Overview of environmental boundary conditions in the laboratory between 1 October 2015 and 19 January 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	N
Temperature	°C	28.0	0.58	26.7	28.8	
Temperature (minimum within 24 h)	°C	24.8	1.22	22.8	28.5	74
Temperature (maximum within 24 h)	°C	28.6	0.24	28.0	29.1	74
Relative humidity	%	50.5	2.46	46.0	60.0	74
Relative humidity (minimum within 24 h)	%	47.8	3.34	42.0	58.0	74
Relative humidity (maximum within 24 h)	%	57.3	3.76	46.0	66.0	74
Illuminance (one-off records)	lux	6995.7	345.8	6430.0	7721.0	58

Note: N, number of records; h, hours.

5.3 Long-term impact of pH on pond performance treating diluted STWW containing dyes

5.3.1 Inflow water quality parameters

Table 5.6, and Figures 5.11 and 5.12 summarise the values of the inflow water quality parameters including trace elements content regarding the diluted synthetic textile wastewater with and without dyes for both the first and second phase of the experiment. The mean inflow values of pH, colour, NO_3–N and NH_4–N, zinc and iron were within the typical characteristics of textile wastewater (Ghaly et al., 2014). The initial dye concentration was 10 mg/l and was regarded to be within the lower range of dye effluents (10–250 mg/l). Regarding COD, SS and TDS, the mean inflow values were lower than the typical ones (Ghaly et al., 2014). The wastewater used in this experiment was suitable for operating pond systems as a polishing stage, which deals with wastewater in relatively low concentrations.

Table 5.6 Inflow water quality parameters for the first phase (20 January 2016 to 18 October 2016) and second phase (18 October 2016 to 30 June 2017) of the experiment

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
First phase						
Reactive blue 198 + diluted synthetic textile wastewater						
Dye concentration	mg/l	10	0.11	9.8	10.2	37
pH	-	7.4	0.08	7.1	7.5	37
Redox	mv	-39.2	3.32	-45	-30	37
Dissolved oxygen	mg/l	9.3	0.45	8.6	10	37
Electrical conductivity	µS/cm	130.4	1.36	126.4	132.6	37
Total dissolved solids	mg/l	65.2	0.68	63.2	66.3	37
Suspended solids	mg/l	3.1	0.77	2	4	37
Turbidity	NTU	2.3	0.54	1.1	3.1	37
Colour	Pt Co	225.4	3.95	222	233	37
Chemical oxygen demand	mg/l	29.2	0.24	28.8	29.5	10
Ammonium-nitrogen	mg/l	0.17	0.02	0.16	0.17	10
Nitrate-nitrogen	mg/l	0.43	0.011	0.41	0.44	10
Ortho-phosphate-phosphorus	mg/l	1.55	0.027	1.53	1.6	10
Basic red 46 + diluted synthetic textile wastewater						
Dye concentration	mg/l	10	0.09	9.8	10.2	37
pH	-	7.3	0.07	7.1	7.4	37
Redox	mv	-37.3	2.77	-41	-30	37
Dissolved oxygen	mg/l	9.1	0.43	8.5	9.9	37
Electrical conductivity	µS/cm	126	1.15	124	128.1	37
Total dissolved solids	mg/l	63	0.57	62	64.1	37
Suspended solids	mg/l	2.1	0.51	1	3	37
Turbidity	NTU	2.2	0.5	1.1	3.3	37
Colour	Pt Co	741.4	3.07	737	746	37
Chemical oxygen demand	mg/l	34.3	0.27	34	34.7	10
Ammonium -nitrogen	mg/l	0.26	0.005	0.26	0.27	10
Nitrate-nitrogen	mg/l	0.6	0.012	0.58	0.61	10
Ortho-phosphate-phosphorus	mg/l	1.6	0.006	1.58	1.6	10
Diluted synthetic textile wastewater						
pH	-	7.2	0.13	7	7.4	37
Redox	mv	-26.6	6.88	-37	-18	37
Dissolved oxygen	mg/l	9.3	0.6	8.5	10	37
Electrical conductivity	µS/cm	90	1.61	88.5	96	37
Total dissolved solids	mg/l	45	0.8	44.3	48	37
Suspended solids	mg/l	1.5	0.5	1	2	37
Turbidity	NTU	1.5	0.73	1	2.1	37
Colour	Pt Co	7.8	1.64	6	12	37
Chemical oxygen demand	mg/l	20.5	0.34	19.8	20.8	10
Ammonium -nitrogen	mg/l	0.16	0.005	0.16	0.168	10
Nitrate-nitrogen	mg/l	0.41	0.005	0.4	0.415	10
Ortho-phosphate-phosphorus	mg/l	1.51	0.011	1.5	1.53	10
Second phase						
Reactive blue 198 + diluted synthetic textile wastewater						
Dye concentration	mg/l	10.1	0.10	10.0	10.3	36
pH	-	7.4	0.07	7.2	7.5	36
Redox	mv	-39.3	3.12	-46.0	-29.0	36

Table 5.6 (Continued)

Dissolved oxygen	mg/l	9.4	0.45	8.7	10.2	36
Electrical conductivity	µS/cm	130.6	1.45	127.0	136.0	36
Total dissolved solids	mg/l	65.3	0.73	63.5	68.0	36
Suspended solids	mg/l	3.0	0.76	1.0	4.0	36
Turbidity	NTU	2.5	0.51	1.4	3.3	36
Colour	Pt Co	226.3	4.49	221.0	235.0	36
Chemical oxygen demand	mg/l	29.2	0.24	28.8	29.5	15
Ammonium -nitrogen	mg/l	0.17	0.003	0.16	0.17	15
Nitrate-nitrogen	mg/l	0.43	0.011	0.41	0.44	15
Ortho-phosphate-phosphorus	mg/l	1.55	0.027	1.53	1.60	15
Basic red 46 + diluted synthetic textile wastewater						
Dye concentration	mg/l	10.0	0.10	9.8	10.2	36
pH	-	7.3	0.08	7.1	7.5	36
Redox	mv	-37.5	2.87	-42.0	-28.0	36
Dissolved oxygen	mg/l	9.2	0.45	8.4	9.8	36
Electrical conductivity	µS/cm	126.0	1.41	124.0	130.0	36
Total dissolved solids	mg/l	63.0	0.70	62.0	65.0	36
Suspended solids	mg/l	2.1	0.54	1.0	3.0	36
Turbidity	NTU	2.2	0.52	1.1	3.2	36
Colour	Pt Co	741.7	3.22	735.0	746.0	36
Chemical oxygen demand	mg/l	34.3	0.27	34.0	34.7	10
Ammonium -nitrogen	mg/l	0.26	0.005	0.26	0.27	10
Nitrate-nitrogen	mg/l	0.60	0.012	0.58	0.61	10
Ortho-phosphate-phosphorus	mg/l	1.60	0.006	1.58	1.60	10
Diluted synthetic textile wastewater						
pH	-	7.2	0.13	7.0	7.4	36
Redox	mv	-23.0	15.51	-37.0	35.0	36
Dissolved oxygen	mg/l	9.3	0.59	8.5	10.0	36
Electrical conductivity	µS/cm	89.9	1.61	88.5	96.0	36
Total dissolved solids	mg/l	45.0	0.80	44.3	48.0	36
Suspended solids	mg/l	1.6	0.50	1.0	2.0	36
Turbidity	NTU	1.5	0.73	1.0	2.1	36
Colour	Pt Co	7.8	1.62	6.0	12.0	36
Chemical oxygen demand	mg/l	20.5	0.34	19.8	20.8	10
Ammonium -nitrogen	mg/l	0.16	0.005	0.16	0.17	10
Nitrate-nitrogen	mg/l	0.41	0.005	0.40	0.42	10
Ortho-phosphate-phosphorus	mg/l	1.51	0.011	1.50	1.53	10
Dechlorinated tap water for both phases						
pH	-	7.1	0.16	6.9	7.4	30
Redox	mv	-26.3	9.19	-40	-12	30
Dissolved oxygen	mg/l	8.8	0.49	8.4	9.8	30
Electrical conductivity	µS/cm	66.4	1.53	64.4	68.8	30
Total dissolved solids	mg/l	33.2	0.8	32.2	34.4	30
Suspended solids	mg/l	1.2	0.69	0.0	2	30
Turbidity	NTU	1.2	0.38	0.6	1.7	30
Colour	Pt Co	0.0	0.00	0.0	0.0	30
Chemical oxygen demand	mg/l	2.4	0.93	0.9	3.4	9
Ammonium -nitrogen	mg/l	0.04	0.003	0.04	0.05	9
Nitrate-nitrogen	mg/l	0.38	0.139	0.31	0.40	9
Ortho-phosphate-phosphorus	mg/l	1.03	0.221	0.8	1.44	9

Note: NTU, nephelometric turbidity unit.

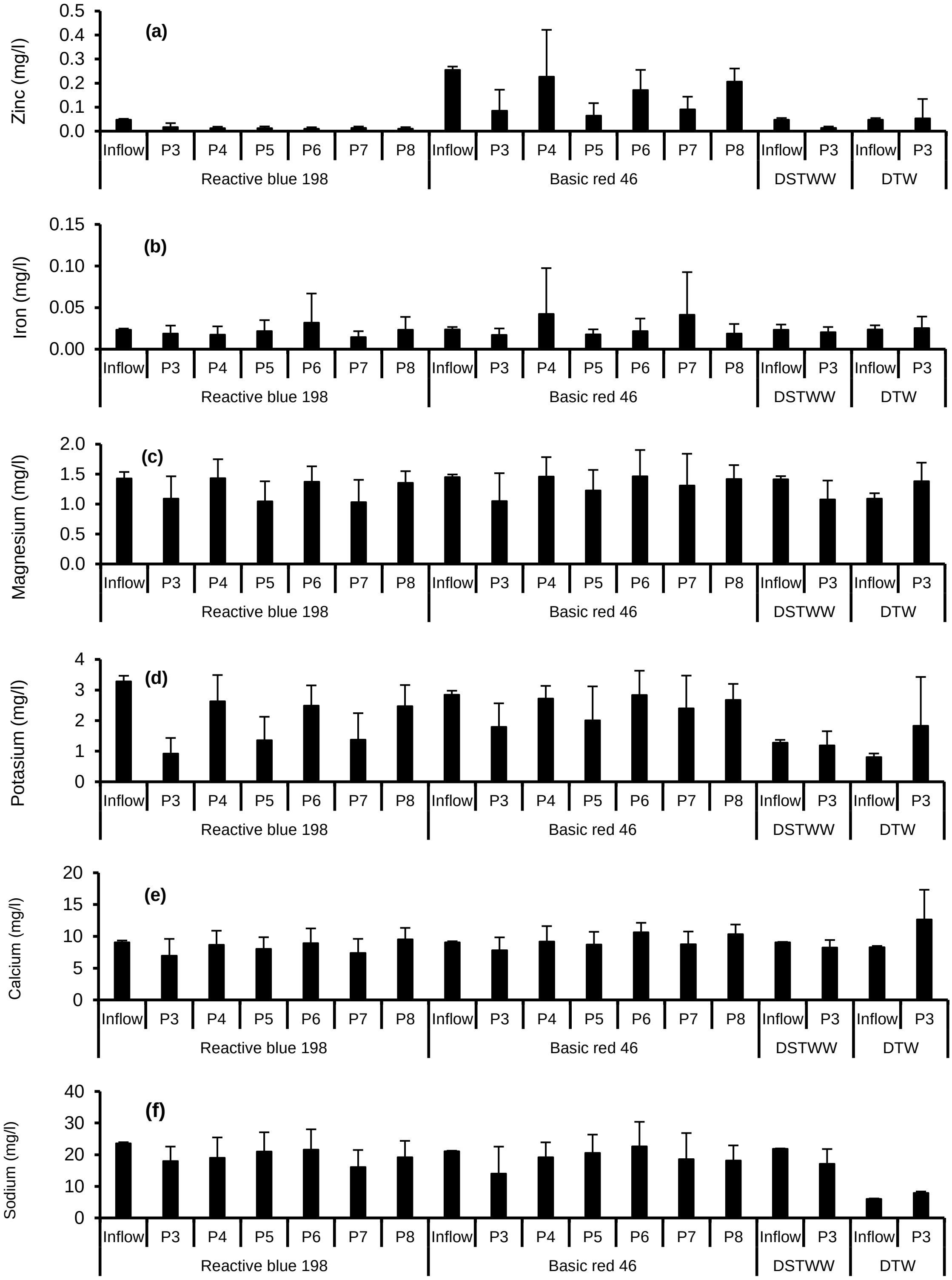


Figure 5.11 Mean inflow and outflow concentrations of the detected elements during the first experimental phase between 20 January 2016 and 18 October 2016. Note: (a), zinc; (b), iron; (c), magnesium; (d), potassium; (e), calcium; (f), sodium; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

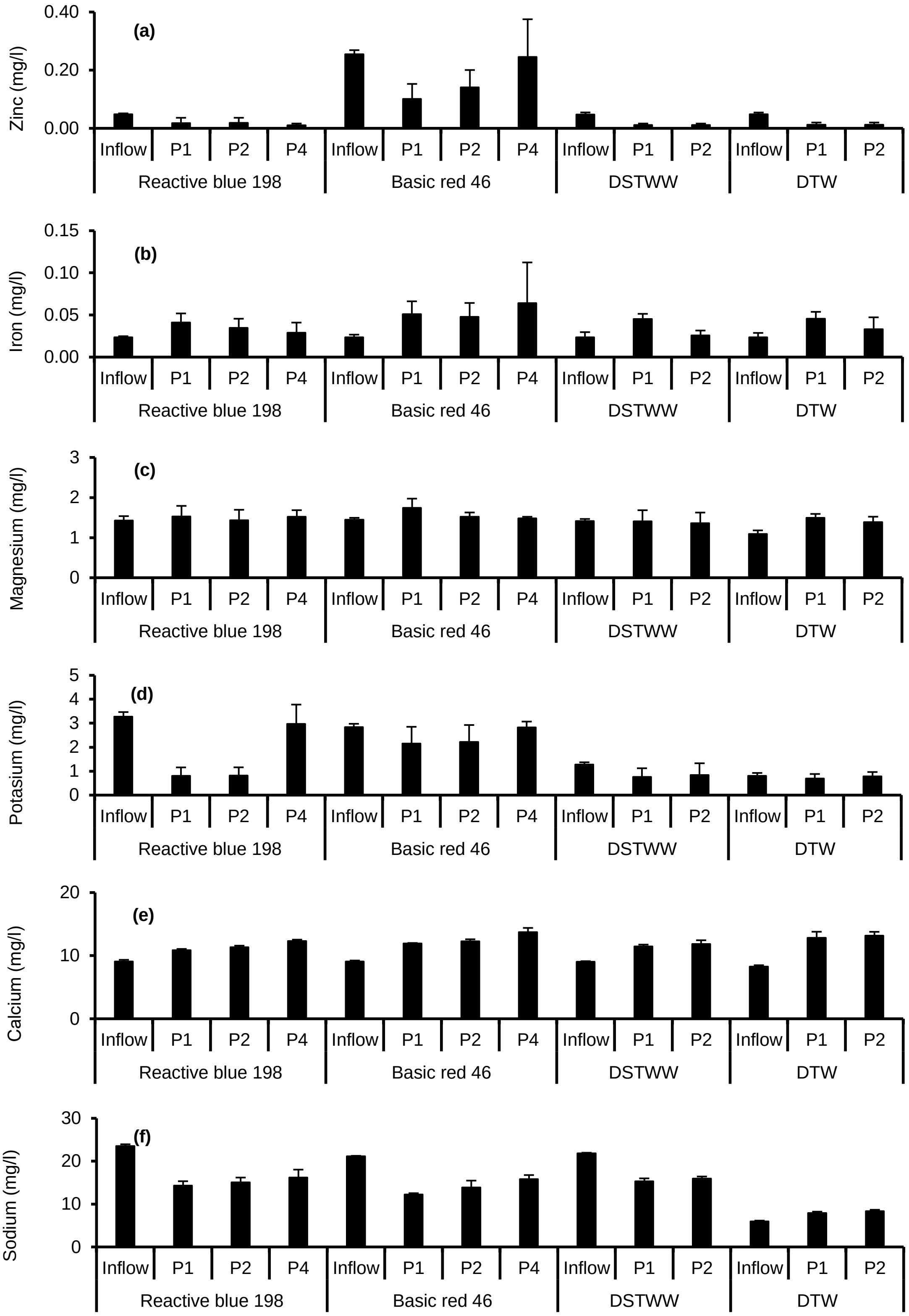


Figure 5.12 Mean inflow and outflow concentrations of the detected trace elements during the second experimental phase between 18 October 2016 and 30 June 2017. Note: (a), zinc; (b), iron; (c), magnesium; (d), potassium; (e), calcium; (f), sodium; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.2 Treatment performance (phase 1)

5.3.2.1 Dye and apparent colour

Figure 5.13 illustrates the mean removal efficiency of the dyes for each system related to the first experimental phase that ran between 20 January 2016 and 18 October 2016. Based on the dye RB198 removal (Figure 5.13), findings showed that although the mean removal of RB198 was very low for all ponds, significant differences were found (Table 5.7) between *L. minor* and control ponds in the case of high (P5 and P6) and low (P7 and P8) pH, attributed to the impact of the plants in the system. This low removal can be explained by the presence of two sulpho groups, high molecular weight and the complex structure of RB198 (Chapter 3, Table 3.1) making this dye challenging to degrade. The mean removal at acidic conditions was significantly (Table 5.8) higher than that at normal conditions, indicating that the adsorption capacity of RB198 increased at low pH as interpreted by Salleh et al. (2011). At acidic conditions, the positive charge in the solution interface increases and the adsorbent surface appears positively charged, which results in an increase in anionic dye (e.g., RB198) adsorption and a decrease in cationic dye (e.g., BR46) adsorption. Findings indicate that the mean RB198 removal for the experimental period lasting three months (Section 5.2.2.1, Figure 5.2) was higher than for the operation of nine months during this experiment (Figure 5.13), which highlights that time has a significant impact on RB198 removal. This is because the adsorbent property of the plant has limited capacity for RB198 molecule adsorption, which can be noted by the longitudinal profile of RB198 removal efficiency, which was higher during the first few weeks of the operation period for the three months experimental period (Figure 5.3b) and the nine months experimental period (Figure 5.14). In contrast, a high mean removal efficiency was noticed for the dye BR46 (Figure 5.13). The treatment performance for *L. minor* ponds was significantly (Table 5.7) higher than the corresponding one for control ponds, although the control ponds had removals of more than 24%. This result was seen as evidence that the plant has a significant contribution to BR46 removal. This high removal is attributed to the BR46 characteristics of low molecular weight, simple structure, and the absence of sulpho groups, which all improve dye elimination (Pearce et al., 2003). These results matched those presented in Chapter 4 (Section 4.2.2.1) for the treatment of BR46 at inflow concentrations of 5 mg/l. The impact of inflow at acidic and alkaline pH values on the treatment of BR46 showed no significant dissimilarity (Table 5.8) compared with the normal pH for both the *L. minor* and control systems. Although the reduction of cationic dye increased at alkaline conditions and vice versa (Salleh et al., 2011), it seems that the selected pH values of 6 and 9 in this study had no considerable impact on the treatment of this dye. Similar outcomes by Movafeghi et al. (2013) were confirmed. It is important to mention that in June 2016, algae started to appear in the ponds planted with *L. minor*. These algae belonged to *Oedogonium*, which was identified by the Culture Collection of Algae and Protozoa (SAMS UK). *Oedogonium* are green and sexual species (Appendix D, Figure D 3), which live in calm and fresh water either free-floating or attached to macrophytes. Therefore, it was necessary to find the impact of this kind of algae on the system performance as will be explained later on in the second phase.

The treatment performance in terms of the outflow dye concentrations (Table 5.9) showed that the average outflow concentrations of RB198 were higher than those of the corresponding inflow values, reflecting very low dye removal except for the *L. minor* ponds at acidic conditions, which were similar. In addition, the mean outflow RB198 concentrations at acidic conditions were significantly lower (Table 5.8) than those at normal conditions for *L. minor* ponds due to enhanced RB198 removal at low pH, as discussed previously, and consequently reduced outflow concentrations (Salleh et al., 2011). However, lower mean outflow concentrations compared to the inflow for all ponds comprising BR46 was noticed. *L. minor* ponds displayed mean outflow concentrations that were significantly (Table 5.7) reduced compared to those of the control ponds without any impact on pH. These results confirm the removal efficiencies discussed above. A correlation analysis showed that the dye concentration significantly (Spearman's r = -0.910, p = 0.000) correlated negatively with the removal efficiency of dyes.

The spectral changes between the treated and untreated samples indicate modifications to the dye molecule structure after treatment. The spectrum results (Figure 5.15) in the visible region (400–800 nm) show peaks of the inflow samples at 625 and 530 nm of RB198 and BR46, respectively, which are linked to the chromophore groups of the dye molecules. However, for the outflow samples, the intensity of the dye adsorption peak at 625 nm (Figures 5.15a, b, and c) does not change for both *L. minor* and control ponds confirming the un-decolourisation of RB198 and the increase in outflow concentrations. The dye BR46 (Figures 5.15d, e and f) showed appreciable disappearances of the peak at 530 nm for all planted ponds, confirming the complete decolourisation and decreases in the outflow concentrations. However, control ponds showed a reduction in the peak at 530 nm indicating a decrease in the outflow concentration due to microbial bio-sorption processes and may be another microbial activity.

The HPLC chromatogram showed a peak at a retention time of 1.505 min for RB198 influent (Appendix E, Figure E.1b). However, the peak for the outflow samples (Appendix E, Figure E.14) shifted to 1.53 and 1.566 min at normal pH, 1.552 and 1.593 min at alkaline, and 1.541 and 1.557 min at acidic conditions for *L. minor* and control ponds, respectively. These changes are due to the interaction between the synthetic wastewater and the dye, because the main peak of RB198 at 625 nm does not change (Figures 5.15a, b and c) and the removal of this dye, as shown in Figure 5.13, was not achieved (outflow samples were coloured). The inflow BR46 showed peaks at retention times of 1.488, 1.693, 2.569 and 2.405 min (Appendix E, Figure E.1c). Based on the outflow samples (Appendix E, Figure E.15), *L. minor* ponds indicated the appearance of a new peak in the HPLC chromatogram results of the metabolites at 1.938, 1.919 and 1.908 min for normal, high and low pH values, respectively. In addition, all inflow peaks disappeared, which confirms the formation of new products. However, the outflow samples of control ponds showed the appearance of a new peak at 1.736, 1.732 and 1.768 min for normal, high and low pH values, respectively. The variation between the inflow and outflow samples with presence of new peaks (in the outflow samples), as a result of new products, were evidence to confirm that the dye removal mechanism was due to phytotransformation by plants (Khandare et al., 2011; Kabra et al., 2012) and biotransformation by microbes (Tahir et al., 2016). Therefore, according to HPLC data linked with dye BR46 removal and UV spectra results, it seems that the dye BR46 is removed in *L. minor* ponds due to phytotransformation (Kabra et al., 2012), adsorption and/or accumulation processes, and may be by biotransformation processes as well (Kagalkar, 2011). However, the mechanism of BR46 removal in the control ponds was mainly due to microbial bio-sorption (Pearce et al., 2003) and may be biotransformation (Tahir et al., 2016) processes.

Based on colour monitoring (Table 5.9) the mean RB198 outflow levels were higher or only slightly lower, compared to the corresponding inflow ones due to low dye removal. Also, the outflow colour values were higher within *L. minor* ponds than the control ones for all ponds. This can be explained by the impact of plant death and the increase of algae growth in the planted wetlands. However, the mean colour outflow values for the dye BR46 mirrored the outflow dye concentrations, as the correlation analysis showed that the colour values had a significantly positive (Spearman's r = 0.151, p = 0.001) correlation with the dye concentrations. Panswad et al. (2001) mentioned that a carbon source in the synthetic wastewater boosted the colour reduction and consequently the dye removal, however, in this experiment, the carbon source from the synthetic textile wastewater may have affected BR46 only. Inflow and outflow sample pictures are shown in Appendix F, Figure F.3.

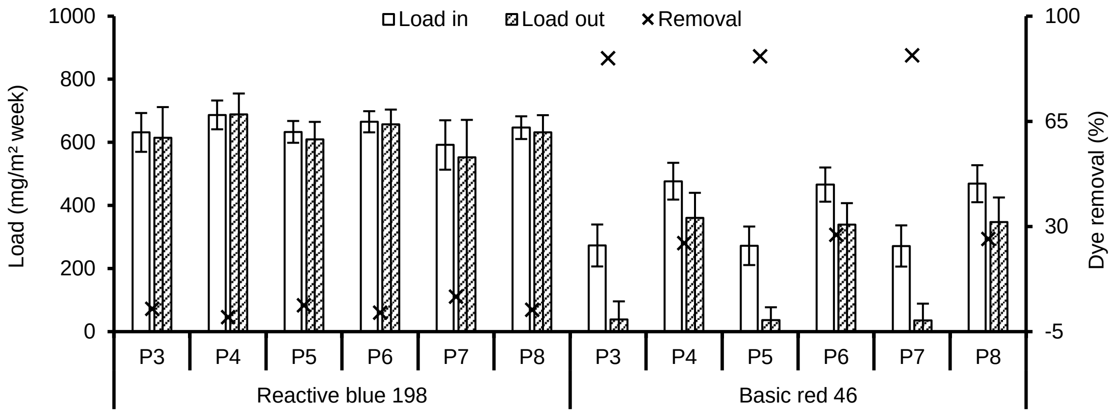


Figure 5.13 Mean dye removal efficiency during the experiment between 20 January 2016 and 18 October 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds obtaining inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

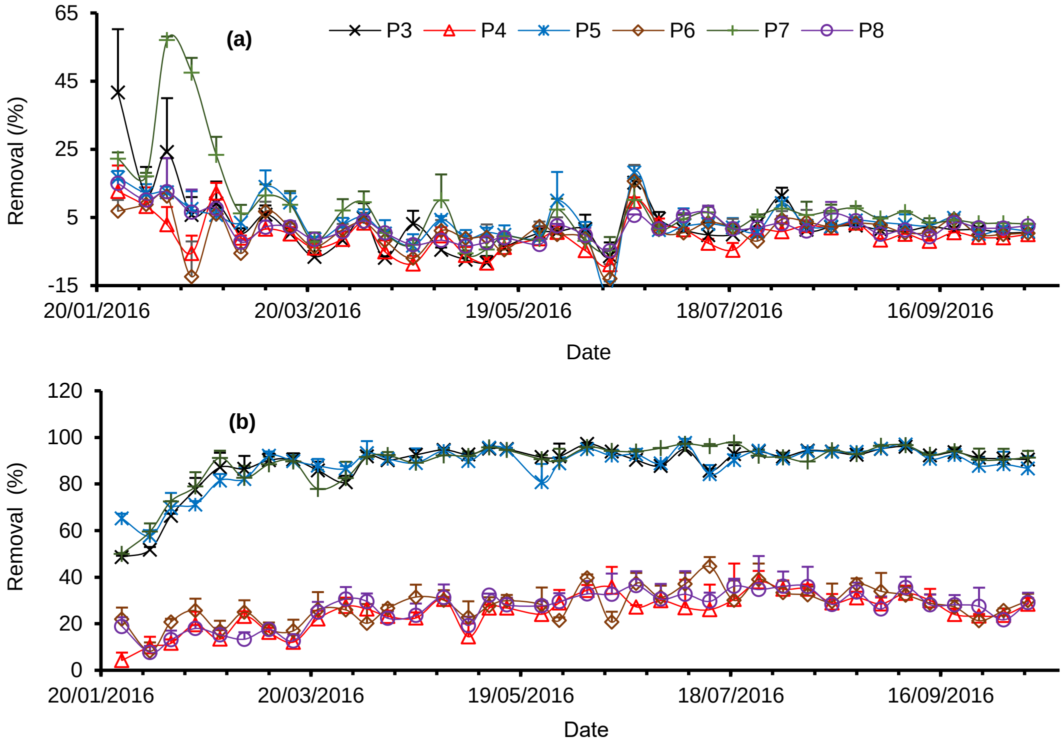


Figure 5.14 Mean values of dye removal profile between 20 January 2016 and 18 October 2016. Note: (a), reactive blue 198; (b), basic red 46; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

Table 5.7 Overview of the statistical analysis between *Lemna minor* L. and control ponds for outflow water quality parameters and corresponding removal efficiencies for the period from 20 January 2016 to 18 October 2016

Parameter	Shapiro-Wilk test (*p* value[1])	*p* value[1]	Shapiro-Wilk test (*p* value[1])	p value[1]	Shapiro-Wilk test (*p* value[1])	*p* value[1]
	Normal pH		pH of 9		pH of 6	
Dye mg/l						
RB198	0.004	0.000	0.000	0.000	0.000	0.000
BR46	0.000	0.000	0.000	0.000	0.000	0.000
Dye removal (%)						
RB198	0.001	0.055	0.000	0.033	0.000	0.011
BR46	0.000	0.000	0.000	0.000	0.000	0.000
Colour (Pt Co)						
RB198	0.000	0.006	0.003	0.147	0.000	0.081
BR46	0.000	0.000	0.000	0.000	0.000	0.000
Chemical oxygen demand (mg/l)						
RB198	0.201	0.176	0.096	0.564	0.303	0.149
BR46	0.000	0.001	0.003	0.031	0.191	0.000
Chemical oxygen demand removal (%)						
RB198	0.144	0.742	0.919	0.211	0.124	0.763
BR46	0.526	0.175	0.427	0.596	0.774	0.187
Ammonium-nitrogen (mg/l)						
RB198	0.000	0.489	0.000	0.666	0.000	0.290
BR46	0.000	0.666	0.000	1.000	0.000	0.931
Ammonium-nitrogen removal (%)						
RB198	0.172	0.014	0.470	0.003	0.599	0.007
BR46	0.073	0.04	0.427	0.01	0.573	0.049
Nitrate-nitrogen (mg/l)						
RB198	0.000	0.931	0.000	0.546	0.000	0.489
BR46	0.002	0.489	0.942	0.233	0.000	0.546
Nitrate-nitrogen removal (%)						
RB198	0.121	0.003	0.241	0.002	0.313	0.000
BR46	0.648	0.031	0.495	0.255	0.848	0.609

Table 5.7 (Continued)

Ortho-phosphate-phosphorus (mg/l)						
RB198	0.654	0.033	0.063	0.027	0.372	0.001
BR46	0.318	0.077	0.876	0.004	0.65	0.002
Ortho-phosphate-phosphorus						
RB198	0.534	0.021	0.555	0.307	0.034	0.356
BR46	0.019	0.000	0.578	0.003	0.003	0.000
Suspended solids (mg/l)						
RB198	0.000	0.001	0.000	0.001	0.000	0.000
BR46	0.0080	0.000	0.000	0.000	0.000	0.000
Turbidity (NTU)						
RB198	0.000	0.001	0.000	0.035	0.000	0.006
BR46	0.000	0.000	0.000	0.000	0.000	0.000
Dissolved oxygen (mg/l)						
RB198	0.002	0.681	0.028	0.367	0.130	0.996
BR46	0.003	0.892	0.032	0.940	0.031	0.320
pH (-)						
RB198	0.001	0.058	0.000	0.204	0.000	0.039
BR46	0.02	0.787	0.165	0.595	0.000	0.001
Redox potential (mV)						
RB198	0.001	0.075	0.000	0.375	0.000	0.020
BR46	0.001	0.681	0.039	0.449	0.000	0.000
Electrical conductivity (μS/cm)						
RB198	0.029	0.000	0.000	0.000	0.000	0.000
BR46	0.000	0.000	0.000	0.000	0.000	0.000

Note: ^{1}Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); ^{2}p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; Mann-Whitney U test used when the data are not normally distributed and independent sample t-test used when the data are normally distributed; RB198, reactive blue 198; BR46, basic red 46.

Table 5.8 Overview of the statistical analysis in terms of the impact of pH factor for outflow water quality parameters and corresponding removal efficiencies for the period from 20 January 2016 to 18 October 2016

Parameter	Shapiro-Wilk test (*p* value[1])	*p* value[1]				Shapiro-Wilk test (*p* value[1])	*p* value[1]			
Type of the dye		Normal pH & pH 9 & pH 6	Normal pH & pH 9	Normal pH & pH 6	pH 9 & pH 6		Normal pH & pH 9 & pH 6	Normal pH & pH 9	Normal pH & pH 6	pH 9 & pH 6
	Lemna. minor L. ponds					Control ponds				
Dye (mg/l)										
RB198	0.000	0.003	0.581	0.004	0.004	0.000	0.017	0.282	0.083	0.005
BR46	0.000	0.653	NA	NA	NA	0.000	0.571	NA	NA	NA
Dye removal (%)										
RB198	0.000	0.023	0.111	0.009	0.159	0.011	0.044	0.104	0.013	0.473
BR46	0.000	0.478	NA	NA	NA	0.025	0.434	NA	NA	NA
Colour (Pt Co)										
RB198	0.000	0.806	NA	NA	NA	0.005	0.705	NA	NA	NA
BR46	0.000	0.356	NA	NA	NA	0.003	0.997	NA	NA	NA
Chemical oxygen demand (mg/l)										
RB198	0.753	0.94	NA	NA	NA	0.555	0.37	NA	NA	NA
BR46	0.15	0.547	NA	NA	NA	0.404	0.826	NA	NA	NA
Chemical oxygen demand removal (%)										
RB198	0.714	0.972	NA	NA	NA	0.946	0.108	NA	NA	NA
BR46	0.144	0.218	NA	NA	NA	0.346	0.584	NA	NA	NA
Ammonium-nitrogen (mg/l)										
RB198	0.011	0.937	NA	NA	NA	0.000	0.979	NA	NA	NA
BR46	0.000	0.062	NA	NA	NA	0.000	0.741	NA	NA	NA
Ammonium-nitrogen removal (%)										
RB198	0.505	0.419	NA	NA	NA	0.431	0.822	NA	NA	NA
BR46	0.205	0.227	NA	NA	NA	0.773	0.867	NA	NA	NA
Nitrate-nitrogen (mg/l)										
RB198	0.03	0.988	NA	NA	NA	0.000	0.436	NA	NA	NA

Table 5.8 (Continued)

BR46	0.024	0.968	NA	NA	NA	0.000	0.904	NA	NA	NA
Nitrate-nitrogen removal (%)										
RB198	0.021	0.37	NA	NA	NA	0.768	0.628	NA	NA	NA
BR46	0.505	0.88	NA	NA	NA	0.520	0.04	0.253	0.033	0.543
Ortho-phosphate-phosphorus (mg/l)										
RB198	0.444	0.498	NA	NA	NA	0.012	0.922	NA	NA	NA
BR46	0.388	0.534	NA	NA	NA	0.125	0.478	NA	NA	NA
Ortho-phosphate-phosphorus removal (%)										
RB198	0.353	0.08	NA	NA	NA	0.013	0.983	NA	NA	NA
BR46	0.692	0.792	NA	NA	NA	0.000	0.007	0.014	0.989	0.019
Suspended solids (mg/l)										
RB198	0.000	0.066	NA	NA	NA	0.000	0.223	NA	NA	NA
BR46	0.000	0.765	NA	NA	NA	0.000	0.644	NA	NA	NA
Turbidity (NTU)										
RB198	0.000	0.8	NA	NA	NA	0.000	0.775	NA	NA	NA
BR46	0.000	0.683	NA	NA	NA	0.000	0.294	NA	NA	NA
Dissolved oxygen (mg/l)										
RB198	0.008	0.722	NA	NA	NA	0.001	0.888	NA	NA	NA
BR46	0.003	0.64	NA	NA	NA	0.002	0.917	NA	NA	NA
pH (-)										
RB198	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.011	0.000	0.000
BR46	0.000	0.000	0.204	0.000	0.000	0.000	0.000	0.023	0.001	0.000
Redox potential (mV)										
RB198	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.043	0.002	0.000
BR46	0.003	0.000	0.034	0.000	0.000	0.000	0.000	0.091	0.001	0.000
Electrical conductivity (µS/cm)										
RB198	0.000	0.000	0.001	0.000	0.155	0.000	0.011	0.104	0.004	0.101
BR46	0.892	0.002	0.081	0.002	0.358	0.000	0.000	0.028	0.1	0.000

Note: [1]Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); [2]p value, probability of the statistic test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; Kruskal-Wallis test used when the data not normally distributed and one-way ANOVA used when the data normally distributed; AB113, acid blue 113; RB198, reactive blue 198; BR46, basic red 46; DO46, direct orange 46; NA, not applicable.

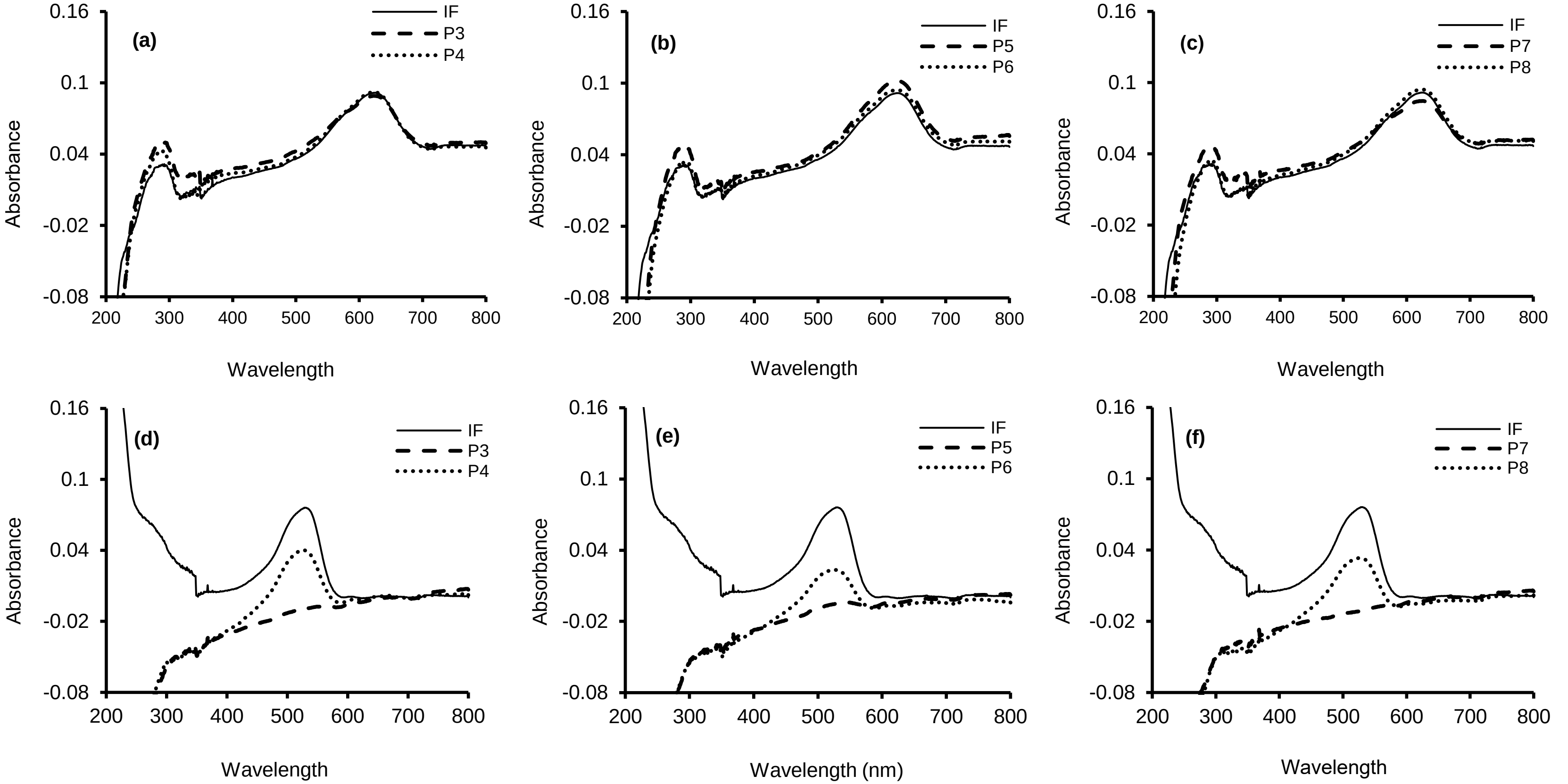


Figure 5.15 Ultraviolet visible analysis for the outflow samples at the end of the experimental rig, which was operated between 20 January 2016 and 18 October 2016. Note: (a), (b) and (c) ponds treating the dye reactive blue 198; (d), (e) and (f) ponds treating the dye basic red 46; IF, inflow; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

Table 5.9 Outflow water quality parameters for each system between 20 January 2016 and 18 October 2016

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Reactive blue 198 + DSTWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	11.1	1.73	6.7	14.4	37
pH	-	7.6	0.25	7.0	8.0	37
Redox	mv	-54.2	14.12	-76.0	-19.5	37
Dissolved oxygen	mg/l	8.2	0.27	7.8	9.0	37
Electrical conductivity	µS/cm	108.9	10.89	85.5	129.0	37
Total dissolved solids	mg/l	54.4	5.44	42.8	64.5	37
Suspended solids	mg/l	8.5	5.30	1.7	29.0	37
Turbidity	NTU	5.0	1.80	1.4	8.7	37
Colour	Pt Co	240.5	50.36	17.0	321.7	37
Chemical oxygen demand	mg/l	21.2	7.22	7.4	30.3	10
Ammonia-nitrogen	mg/l	0.1	0.06	0.0	0.2	10
Nitrate-nitrogen	mg/l	0.2	0.10	0.1	0.3	10
Ortho-phosphate-phosphorus	mg/l	2.1	0.52	1.2	2.8	10
Reactive blue 198 + DSTWW at normal pH for control ponds						
Dye concentration	mg/l	12.6	1.22	10.1	15.2	37
pH	-	7.7	0.21	7.2	8.0	37
Redox	mv	-59.5	11.06	-74.5	-34.0	37
Dissolved oxygen	mg/l	8.2	0.29	7.8	8.9	37
Electrical conductivity	µS/cm	135.6	14.64	91.4	166.3	37
Total dissolved solids	mg/l	67.8	7.32	45.7	83.1	37
Suspended solids	mg/l	5.2	3.36	0.7	15.7	37
Turbidity	NTU	3.6	0.95	1.1	6.1	37
Colour	Pt Co	222.2	34.47	104.5	292.7	37
Chemical oxygen demand	mg/l	16.2	7.09	6.9	28.3	10
Ammonia-nitrogen	mg/l	0.3	0.40	0.0	1.4	10
Nitrate-nitrogen	mg/l	0.3	0.27	0.1	1.0	10
Ortho-phosphate-phosphorus	mg/l	2.8	0.63	2.0	3.8	10
Reactive blue 198 + DSTWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	11.1	0.96	9.2	13.8	37
pH	-	7.8	0.20	7.5	8.2	37
Redox	mv	-63.4	10.99	-79.5	-43.5	37
Dissolved oxygen	mg/l	8.3	0.31	7.7	9.1	37
Electrical conductivity	µS/cm	120.2	23.43	74.5	227.1	37
Total dissolved solids	mg/l	60.1	11.72	37.3	113.6	37
Suspended solids	mg/l	10.6	7.91	1.0	36.3	37
Turbidity	NTU	5.4	2.70	1.1	11.9	37
Colour	Pt Co	241.9	43.25	132.5	411.0	37
Chemical oxygen demand	mg/l	22.4	9.24	11.4	41.6	10
Ammonia-nitrogen	mg/l	0.1	0.08	0.0	0.2	10
Nitrate-nitrogen	mg/l	0.2	0.12	0.0	0.4	10
Ortho-phosphate-phosphorus	mg/l	1.9	0.49	1.3	3.0	10
Reactive blue 198 + DSTWW at pH 9 for control ponds						
Dye concentration	mg/l	12.0	0.89	9.7	14.2	37
pH	-	7.9	0.18	7.5	8.1	37
Redox	mv	-64.5	11.41	-78.7	-38.5	37
Dissolved oxygen	mg/l	8.2	0.29	7.8	9.0	37

Table 5.9 (Continued)

Electrical conductivity	µS/cm	139.4	14.90	101.5	171.4	37
Total dissolved solids	mg/l	69.7	7.45	50.8	85.7	37
Suspended solids	mg/l	5.0	3.73	0.3	20.3	37
Turbidity	NTU	4.0	1.40	2.2	9.0	37
Colour	Pt Co	232.1	42.41	159.0	348.7	37
Chemical oxygen demand	mg/l	20.1	6.16	11.0	33.5	10
Ammonia-nitrogen	mg/l	0.3	0.32	0.0	1.1	10
Nitrate-nitrogen	mg/l	0.2	0.28	0.0	1.0	10
Ortho-phosphate-phosphorus	mg/l	2.7	0.79	1.8	4.0	10
Reactive blue 198 + DSTWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	10.0	2.13	3.6	13.0	37
pH	-	7.1	0.73	5.3	7.8	37
Redox	mv	-22.8	41.88	-61.3	76.0	37
Dissolved oxygen	mg/l	8.3	0.30	7.7	9.1	37
Electrical conductivity	µS/cm	124.2	12.03	97.9	150.3	37
Total dissolved solids	mg/l	62.1	6.01	48.9	75.1	37
Suspended solids	mg/l	12.1	7.55	0.7	37.0	37
Turbidity	NTU	5.7	2.87	1.8	13.8	37
Colour	Pt Co	235.6	40.95	116.7	299.7	37
Chemical oxygen demand	mg/l	21.1	7.51	7.7	31.0	10
Ammonia-nitrogen	mg/l	0.1	0.08	0.0	0.2	10
Nitrate-nitrogen	mg/l	0.2	0.10	0.1	0.3	10
Ortho-phosphate-phosphorus	mg/l	2.3	0.60	1.3	3.2	10
Reactive blue 198+ DSTWW at pH 6 for control ponds						
Dye concentration	mg/l	11.5	0.98	9.1	13.1	37
pH	-	7.4	0.37	6.5	7.8	37
Redox	mv	-44.2	22.11	-71.0	8.5	37
Dissolved oxygen	mg/l	8.2	0.28	7.8	8.9	37
Electrical conductivity	µS/cm	141.4	29.64	1.1	189.5	37
Total dissolved solids	mg/l	70.7	14.82	0.6	94.8	37
Suspended solids	mg/l	6.7	4.91	1.5	26.0	37
Turbidity	NTU	4.0	1.60	0.4	9.5	37
Colour	Pt Co	226.7	28.78	122.0	270.3	37
Chemical oxygen demand	mg/l	15.9	6.06	7.7	26.4	10
Ammonia-nitrogen	mg/l	0.2	0.29	0.1	1.1	10
Nitrate-nitrogen	mg/l	0.2	0.25	0.0	0.9	10
Ortho-phosphate-phosphorus	mg/l	2.9	0.70	2.0	3.8	10
Basic red 46 + DSTWW at normal pH for *Lemna minor* L. ponds						
Dye concentration	mg/l	0.7	1.11	0.1	5.9	37
pH	-	7.7	0.30	7.2	8.2	37
Redox	mv	-61.4	18.61	-87.7	-32.0	37
Dissolved oxygen	mg/l	8.3	0.28	7.8	9.1	37
Electrical conductivity	µS/cm	110.7	18.16	72.7	154.0	37
Total dissolved solids	mg/l	55.4	9.08	36.4	77.0	37
Suspended solids	mg/l	8.3	6.12	1.3	25.7	37
Turbidity	NTU	4.8	2.24	0.5	11.1	37
Colour	Pt Co	162.4	91.83	16.0	470.7	37
Chemical oxygen demand	mg/l	37.4	18.09	18.5	61.6	10
Ammonia-nitrogen	mg/l	0.2	0.07	0.1	0.3	10
Nitrate-nitrogen	mg/l	0.2	0.14	0.0	0.4	10

Table 5.9 (Continued)

Ortho-phosphate-phosphorus	mg/l	2.0	1.07	0.6	4.2	10
Basic red 46 +DSTWW at normal pH for control ponds						
Dye concentration	mg/l	6.6	1.54	4.9	10.8	37
pH	-	7.7	0.19	7.3	8.0	37
Redox	mv	-60.4	11.27	-77.7	-36.0	37
Dissolved oxygen	mg/l	8.3	0.29	7.8	9.0	37
Electrical conductivity	µS/cm	147.3	97.15	117.0	722.1	37
Total dissolved solids	mg/l	73.6	48.57	58.5	361.0	37
Suspended solids	mg/l	2.6	2.34	0.0	12.0	37
Turbidity	NTU	3.2	0.70	0.7	4.8	37
Colour	Pt Co	551.4	77.42	364.0	709.3	37
Chemical oxygen demand	mg/l	13.1	4.09	8.8	20.4	10
Ammonium -nitrogen	mg/l	0.3	0.35	0.0	1.2	10
Nitrate-nitrogen	mg/l	0.3	0.21	0.2	0.9	10
Ortho-phosphate-phosphorus	mg/l	3.5	0.60	2.8	4.4	10
Basic red 46 + DSTWW at pH 9 for *Lemna minor* L. ponds						
Dye concentration	mg/l	0.7	0.94	0.1	5.3	37
pH	-	7.8	0.24	7.5	8.5	37
Redox	mv	-64.7	15.04	-100.0	-44.0	37
Dissolved oxygen	mg/l	8.3	0.30	7.8	9.1	37
Electrical conductivity	µS/cm	119.2	11.13	95.4	146.7	37
Total dissolved solids	mg/l	59.6	5.57	47.7	73.4	37
Suspended solids	mg/l	7.1	3.51	0.7	15.0	37
Turbidity	NTU	5.0	2.00	2.6	9.9	37
Colour	Pt Co	176.6	83.61	67.0	409.3	37
Chemical oxygen demand	mg/l	32.8	21.00	7.7	69.1	10
Ammonium -nitrogen	mg/l	0.1	0.05	0.1	0.2	10
Nitrate-nitrogen	mg/l	0.2	0.13	0.0	0.4	10
Ortho-phosphate-phosphorus	mg/l	1.8	0.68	0.6	3.1	10
Basic red 46 + DSTWW at pH 9 for control ponds						
Dye concentration	mg/l	6.2	1.33	4.1	9.7	37
pH	-	7.8	0.19	7.3	8.3	37
Redox	mv	-65.1	9.71	-79.0	-45.0	37
Dissolved oxygen	mg/l	8.3	0.32	7.2	8.9	37
Electrical conductivity	µS/cm	136.0	19.87	80.1	176.5	37
Total dissolved solids	mg/l	68.0	9.94	40.1	88.3	37
Suspended solids	mg/l	3.2	2.14	0.0	19.0	37
Turbidity	NTU	3.1	0.53	1.0	4.1	37
Colour	Pt Co	541.4	77.23	365.0	696.3	37
Chemical oxygen demand	mg/l	12.0	5.83	2.8	22.6	10
Ammonium -nitrogen	mg/l	0.3	0.37	0.1	1.3	10
Nitrate-nitrogen	mg/l	0.3	0.09	0.2	0.5	10
Ortho-phosphate-phosphorus	mg/l	3.2	0.75	1.9	4.6	10
Basic red 46 + DSTWW at pH 6 for *Lemna minor* L. ponds						
Dye concentration	mg/l	0.7	1.04	0.1	5.8	37
pH	-	7.2	0.43	6.4	7.8	37
Redox	mv	-30.0	23.37	-64.0	14.0	37
Dissolved oxygen	mg/l	8.3	0.28	7.8	9.0	37
Electrical conductivity	µS/cm	124.6	19.22	88.0	157.3	37
Total dissolved solids	mg/l	62.3	9.61	44.0	78.6	37

Table 5.9 (Continued)

Suspended solids	mg/l	8.5	5.28	1.0	23.0	37
Turbidity	NTU	4.8	2.04	1.2	11.4	37
Colour	Pt Co	182.3	82.25	91.0	427.0	37
Chemical oxygen demand	mg/l	42.5	11.67	17.0	61.5	10
Ammonium -nitrogen	mg/l	0.3	0.19	0.1	0.8	10
Nitrate-nitrogen	mg/l	0.2	0.10	0.0	0.4	10
Ortho-phosphate-phosphorus	mg/l	2.2	0.74	0.9	3.1	10
Basic red 46 +DSTWW at pH 6 for control ponds						
Dye concentration	mg/l	6.4	1.54	4.7	9.7	37
pH	-	7.5	0.29	7.1	7.9	37
Redox	mv	-48.6	15.68	-67.0	-25.0	37
Dissolved oxygen	mg/l	8.3	0.28	7.8	8.9	37
Electrical conductivity	µS/cm	147.6	22.01	129.5	199.0	37
Total dissolved solids	mg/l	73.8	11.01	64.7	99.5	37
Suspended solids	mg/l	3.6	3.19	0.0	11.0	37
Turbidity	NTU	3.4	0.78	0.8	4.7	37
Colour	Pt Co	549.5	82.10	405.0	713.3	37
Chemical oxygen demand	mg/l	13.7	6.16	3.2	23.3	10
Ammonium -nitrogen	mg/l	0.4	0.45	0.1	1.6	10
Nitrate-nitrogen	mg/l	0.4	0.32	0.1	1.2	10
Ortho-phosphate-phosphorus	mg/l	3.6	0.72	2.4	4.5	10
DSTWW at normal pH for *Lemna minor* L. ponds						
pH	-	7.7	0.31	7.2	8.5	37
Redox	mv	-57.4	16.22	-101.5	-36.0	37
Dissolved oxygen	mg/l	8.4	0.25	8.0	9.0	37
Electrical conductivity	µS/cm	107.9	7.45	91.0	117.5	37
Total dissolved solids	mg/l	54.0	3.73	45.5	58.8	37
Suspended solids	mg/l	8.3	0.29	7.9	9.1	37
Turbidity	NTU	5.0	1.52	2.6	8.7	37
Colour	Pt Co	38.8	9.85	13.0	49.5	37
Chemical oxygen demand	mg/l	30.3	9.23	21.6	50.1	10
Ammonium -nitrogen	mg/l	0.1	0.07	0.0	0.2	10
Nitrate-nitrogen	mg/l	0.2	0.19	0.0	0.7	10
Ortho-phosphate-phosphorus	mg/l	1.2	0.48	0.6	2.3	10
DTW at normal pH for *Lemna minor* L. ponds						
pH	-	7.4	0.17	7.0	7.7	37
Redox	mv	-38.6	9.57	-59.0	-20.5	37
Dissolved oxygen	mg/l	7.2	4.63	1.5	17.0	37
Electrical conductivity	µS/cm	80.4	7.85	64.5	97.5	37
Total dissolved solids	mg/l	40.2	3.93	32.3	48.8	37
Suspended solids	mg/l	5.1	3.06	0.5	11.0	37
Turbidity	NTU	3.1	0.39	2.4	3.9	37
Colour	Pt Co	24.4	7.84	5.5	34.5	37
Chemical oxygen demand	mg/l	21.8	12.28	8.8	44.5	9
Ammonium-nitrogen	mg/l	0.1	0.03	0.0	0.1	9
Nitrate-nitrogen	mg/l	0.3	0.27	0.0	0.8	9
Ortho-phosphate-phosphorus	mg/l	0.8	0.43	0.3	1.7	9

Note: NTU, nephelometric turbidity unit; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.2.2 Chemical oxygen demand and dissolved oxygen

Mean outflow COD concentrations (Table 5.9) were not as high as the corresponding inflow concentrations (Table 5.6) for all ponds containing RB198, indicating high microbial activities for COD degradation as shown in Figure 5.16. In addition, no significant differences (Table 5.7) were found between *L. minor* and control ponds in terms of COD outflow concentrations and removal efficiency. These results indicate that COD removal was due to microbial activities and the impact of the plants was negligible. Similar findings were presented, previously (Ong et al., 2009a). The mean COD outflow concentrations (Table 5.9) related to ponds treating BR46 were higher than the mean inflow (Table 5.6) concentrations for *L. minor* ponds, and lower than the average inflow for control ponds. In addition, the outflow concentrations linked to the *L. minor* wetlands were significantly (Table 5.7) higher than those for ponds without plants. This may be ascribed to the high percentage of BR46 decolourisation and degradation in planted ponds, which consequently increased the organic load. The death of some plants during their life cycle also increased the COD concentrations in *L. minor* compared to the control systems (Dalu & Ndamba, 2003). However, COD removal (Figure 5.16) for both ponds with and without plants comprising BR46 showed no significant dissimilarity (Table 5.7), suggesting that there was no contribution by plants in terms of organic matter uptake, and COD degradation was due to micro-organism activities (Ong et al., 2009a). No significant (Table 5.8) differences were found among the outflow concentrations and the removal values concerning COD in terms of pH adjustment. Results indicate that the impact of different pH values used in this study was mostly negligible. The results of COD removal in this experiment do not match with the very low removal observed in the first experiment (Chapter 4, Section 4.2.2.2). This may be due to the mode of operation, and the carbon source in the synthetic textile wastewater. According to common international standards (Carmen & Daniela, 2012), the limits for COD are around 125 mg/l for direct discharge to watercourses. The results show that all outflow COD values for each system were less than the standard threshold.

Concerning DO, the average outflow concentrations were lower compared to the inflow ones as shown in Tables 5.9 and 5.6 for all ponds with and without dyes. No significant differences were observed between the ponds with and without plants regardless of the pH conditions (Table 5.7) for all dyes. These results indicate that oxygen diffusion by the atmosphere impacts on the DO level in the system rather than oxygen release by plants.

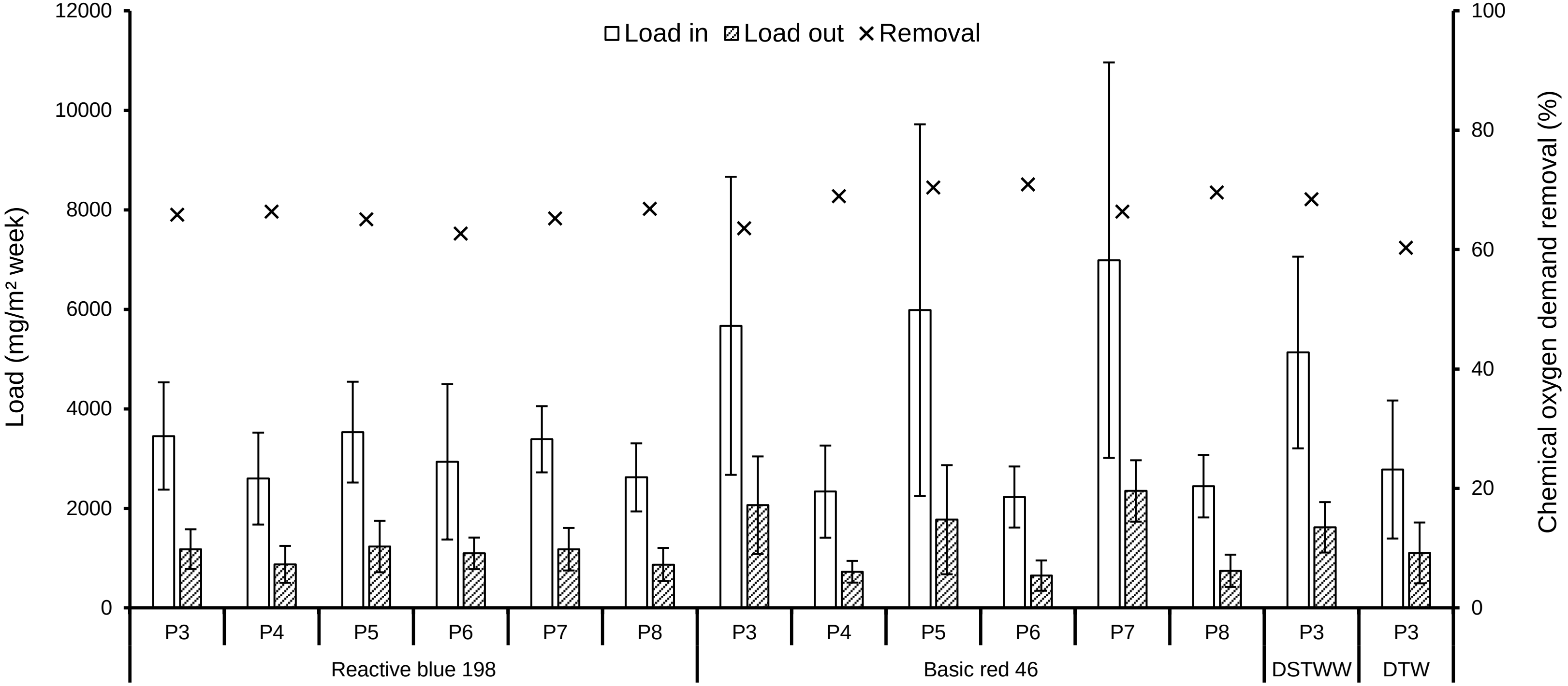


Figure 5.16 Mean chemical oxygen demand removal efficiency during the experiment between 20 January 2016 and 18 October 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.2.3 pH and redox potential

The mean inflow pH (Table 5.6) was 7.4 for the dye RB198 and 7.5 for the dye BR46. However, after pH adjustment, the inflow values were 9.0± 0.08 for alkaline and 6.0± 0.04 for acidic conditions concerning RB198, and 9.0± 0.05 for alkaline and 6± 0.05 for acidic conditions with respect to BR46. As a result, the mean pH outflow values (Table 5.9) followed this ranking order: alkaline > normal > acidic for both *L. minor* and control ponds due to the impact of pH adjustment, although the outflow values at alkaline conditions dropped and at acidic conditions increased. These results indicate the system buffer capacity, which leads to approximately neutral pH values after seven days of contact time. The mean pH values obtained from the outflow for the *L. minor* ponds were equal to or slightly lower than the control ones for both dyes. This indicates that the plants are able to modify the pH by proton and ion exchanges. Similar findings were published, previously (Noonpui & Thiravetyan, 2011). Common international standard thresholds for pH are between 6.5 and 8.5 (Carmen & Daniela, 2012). The results indicate that the planted ponds at acidic conditions containing RB198 and BR46 were 11 and 2 times non-compliant, respectively. The outflow (Table 5.9) redox potential values ranged between 76 mV and -100 mV, indicating the presence of anoxic conditions in all ponds.

5.3.2.4 Suspended solids and turbidity

The average outflow concentrations of SS, (Table 5.9) were higher than the average inflow ones (Table 5.6) for all ponds with and without dye. This was due to organic matter degradation. In addition, the outflow values in *L. minor* ponds comprising RB198 and BR46 were significantly (Table 5.7) higher than the corresponding values for the control ones. This could be due to the die-off of some plants, dry plant on the walls of the ponds and the growth of algae in planted ponds. Algae are one of the main factors effecting SS removal efficiency, and consequently increasing the SS concentration in the pond system (Dalu & Ndamba, 2003). Regarding the impact of pH level on the SS outflow values, no significant dissimilarity was found between the values of each dye (Table 5.8). According to common international standards, the results showed that all outflow SS concentrations were equal to or less than the threshold of 35 mg/l. The outflow values for *L. minor* ponds comprising RB198 at high pH and low pH were 1 and 1 times non-compliant, respectively. In this study, high SS concentrations were linked with lower DO outflows compared to the inflow values, confirming organic matter degradation by consuming DO via micro-organisms.

In terms of turbidity, the mean outflow values (Table 5.9) mirrored those for SS. A related correlation analysis shows that SS was significantly ($p < 0.01$) positively correlated ($r = 0.609$, $p = 0.000$) with turbidity.

5.3.2.5 Electrical conductivity and total dissolved solids

The average outflow EC numbers were lower than the corresponding inflow ones for all *L. minor* ponds containing dyes (Tables 5.6 and 5.9). However, higher mean EC outflows compared well with the inflow values for all control ponds. In addition, the average outflow EC values for the planted ponds were significantly lower (Table 5.7) than the corresponding unplanted ones, indicating that the plant was efficient in reducing the EC level in the ponds. This is because large dye molecules are caught in barriers within the vascular system of plants, as explained by Noonpui and Thiravetyan (2011).

The average inflow and outflow TDS concentrations indicated a similar trend compared to the inflow and outflow EC numbers (Tables 5.6 and 5.9). This is because the EC is a function of TDS (Amankwaah et al., 2014) and in this experiment, is equal to twice the TDS values. According to common international standards, the results showed that all outflow TDS concentrations were less than the threshold of 3000 mg/l and 500 mg/l (Carmen & Daniela, 2012).

5.3.2.6 Nutrients

All *L. minor* ponds showed mean outflow NH_4–N values (Table 5.9) lower than the inflow concentrations (Table 5.6). However, control ponds showed higher values than the corresponding mean inflows. These results are confirmed by NH_4–N removal efficiencies, which were significantly (Table 5.7) higher in *L. minor* ponds than the corresponding control ones for all systems (Figure 5.17). Results indicate that the uptake of nitrogen by plants was one of the main reasons for nitrogen reduction in this study, although Kadlec et al. (2000) clarified that the impact of plants does not exceed 10% of the total removal in treatment systems. However, Ong et al. (2009a) mentioned that some researchers found higher removals of NH_4–N in planted than unplanted reactors, and others did not find any differences at all. Similar outcomes were reported by Selvarani et al. (2015), highlighting that *L. minor* takes-up nitrogen from municipal wastewater within pond systems. The plants removed more NH_4–N in this experiment (Figure 5.17) compared with the negligible removal, by plants, in the second experiment (Section 5.2.2.6; Figure 5.6), which could be attributed to the low loading rate of NH_4–N in diluted synthetic wastewater within this experiment, which increased the uptake level as indicated by Zimmo (2003). The algae grown in this operation and the high plant survival rate contributed to good nitrogen removal as well. High NH_4–N removal was also due to biological oxidation of the ammonia to nitrate, with nitrite as an intermediate, by nitrifying bacteria. This process requires oxygen supplied by the atmosphere, diffusion and/or plant transfer via their roots. Note that the temperature conditions and the pH values were within the range of possible nitrification (Kadlec et al., 2000; Ozengin & Elamic, 2007).

Regarding the impact of pH, removal at low pH conditions was lower than at other conditions although no significant differences (Table 5.8) were found. Normally, in the presence of *L. minor* or algae in pond systems at high pH (8-9) values, nitrogen removal increases, because of boosted sedimentation and ammonia volatilisation processes. This is because the limited density of *L. minor* at higher pH values leads to an increase in nitrogen accumulation within the sediment comprising decaying *L. minor*, and reduced covered surface area consequently allowing for enhanced volatilisation processes to take place. In addition, a greater dispersal of algal biomass taking-up nitrogen is due to high light penetration and favourable growth conditions for algae (Zimmo, 2003). The NH_4–N removal efficiency of ponds containing only synthetic textile wastewater without dyes was higher than those for all ponds containing dyes. This is due to the impact of dyes and aromatic amine intermediates. The latter inhibits the nitrification activities by microbes in the system leading to a low NH_4–N removal efficiency, as discussed by Ong et al. (2009a). No thresholds have been set for NH_4–N that have relevance for the treatment system in this study (UK Government 1994). However, the standard limit relating to secondary wastewater treatment is 20 mg/l (Al-Isawi et al., 2017). All outflow values were lower than this limit.

Concerning NO_3–N, all mean outflow concentrations (Table 5.9) were lower compared to the inflow ones (Table 5.6). In terms of removal efficiency, ponds containing dye exhibited low NO_3–N removal, which ranged between 16 and 47%. In addition, all NO_3–N removal values (Figure 5.18) were significantly higher (Table 5.7) in *L. minor* ponds than in control ones, except for ponds treating BR46 at low and high pH values. These findings suggest that the plants acquire nitrate as a second source after ammonia has been taken-up (Selvarani et al., 2015). This results in a significant potential for NO_3–N reduction by plants in addition to the denitrification mechanism. However, ponds treating BR46 at acidic conditions provide unexpected results of higher removal in control ponds than planted ponds, which indicates that denitrification was the main mechanism for NO_3–N removal and the plant contribution was marginal. This may be attributed to plant detritus, which plays an important role in nutrient recycling within the system (Zhang et al., 2014). The UK Government (1994) has set no threshold for NO_3–N. Nonetheless, the traditional limit for secondary wastewater is 50 mg/l, as discussed by Al-Isawi et al. (2017). All outflow values were less than this limit. It is important to mention that anaerobic micro-niches within the pond system also have a small contribution to the increase in the denitrification rate. In addition, around 4% of the initial nitrogen was reduced by nitrification and denitrification due to the micro sites of the biofilm-attached *L. minor* (Zimmo, 2003).

Ortho-phosphate-phosphorus (PO_4–P) mean outflow concentrations (Table 5.9) were elevated compared to the inflow recordings (Table 5.6) with respect to all dyes, indicating low PO_4–P reduction. Mean outflow values for *L. minor* ponds were significantly lower (Table 5.7) than the respective values for the control ones concerning all dyes. This reflects the PO_4–P removal efficiencies (Figure 5.19), which were significantly (Table 5.7) higher for *L. minor* ponds compared to the control ones, excluding the ponds comprising RB198 at low and high pH, which were not significant. These findings indicate that plants contributed to the removal of PO_4–P, and that the mechanism of phosphorus removal in planted ponds was attributed to the uptake and assimilation of phosphorus into *L. minor* protein, adsorption on the fronds of the plant, chemical precipitation and uptake by micro-organisms. However, only the uptake by microbes and biological activities are responsible for PO_4–P reduction in control ponds. Similar trends were stated by Vanitha et al. (2013). The realistic guideline threshold for PO_4–P is 1 mg/l (Al-Isawi et al., 2017), most outflow values were then non-compliant. Regarding the impact of pH inflow values, PO_4–P removal by control ponds treating BR46 at high pH was significantly (Table 5.8,) higher than those at normal and low pH.

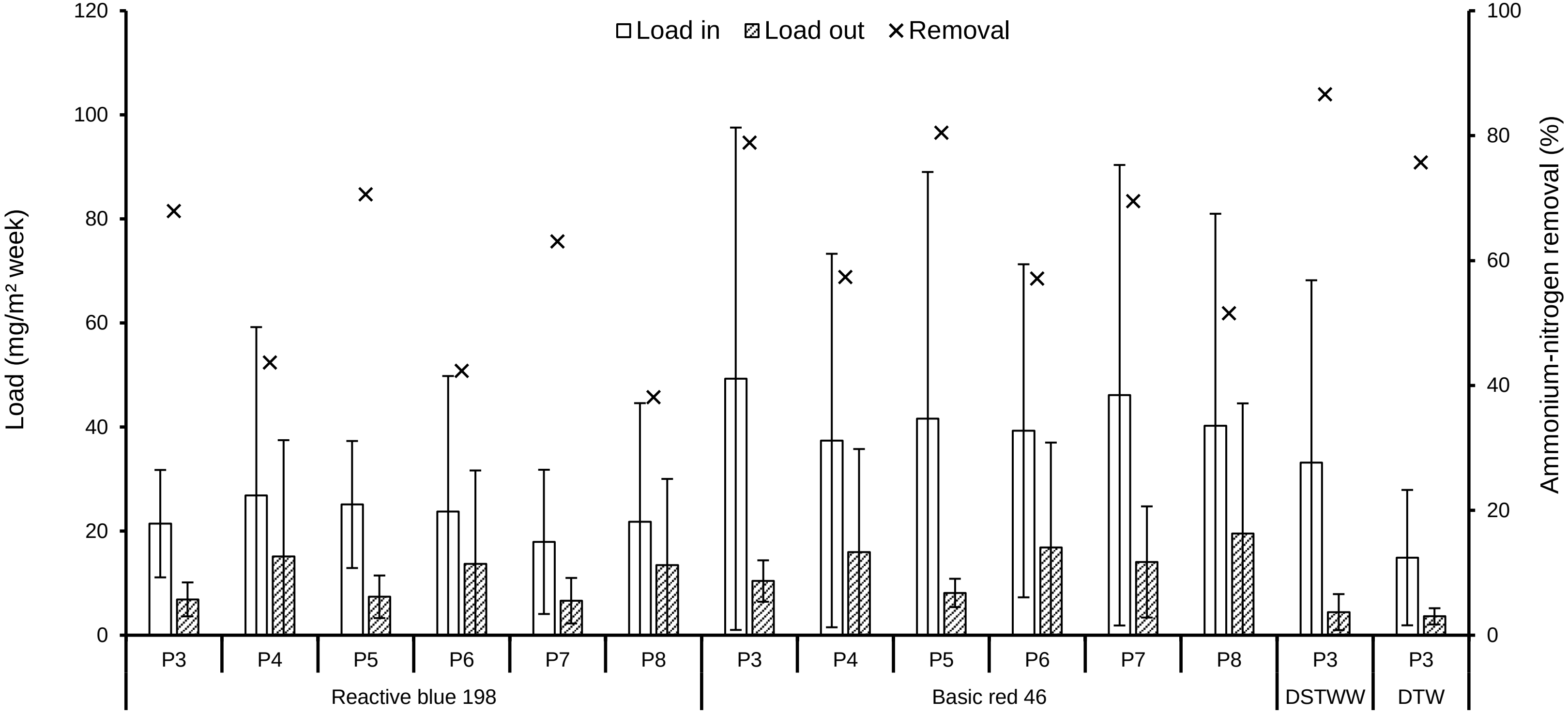


Figure 5.17 Mean ammonium-nitrogen removal efficiency during the experiment between 20 January 2016 and 18 October 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water**.**

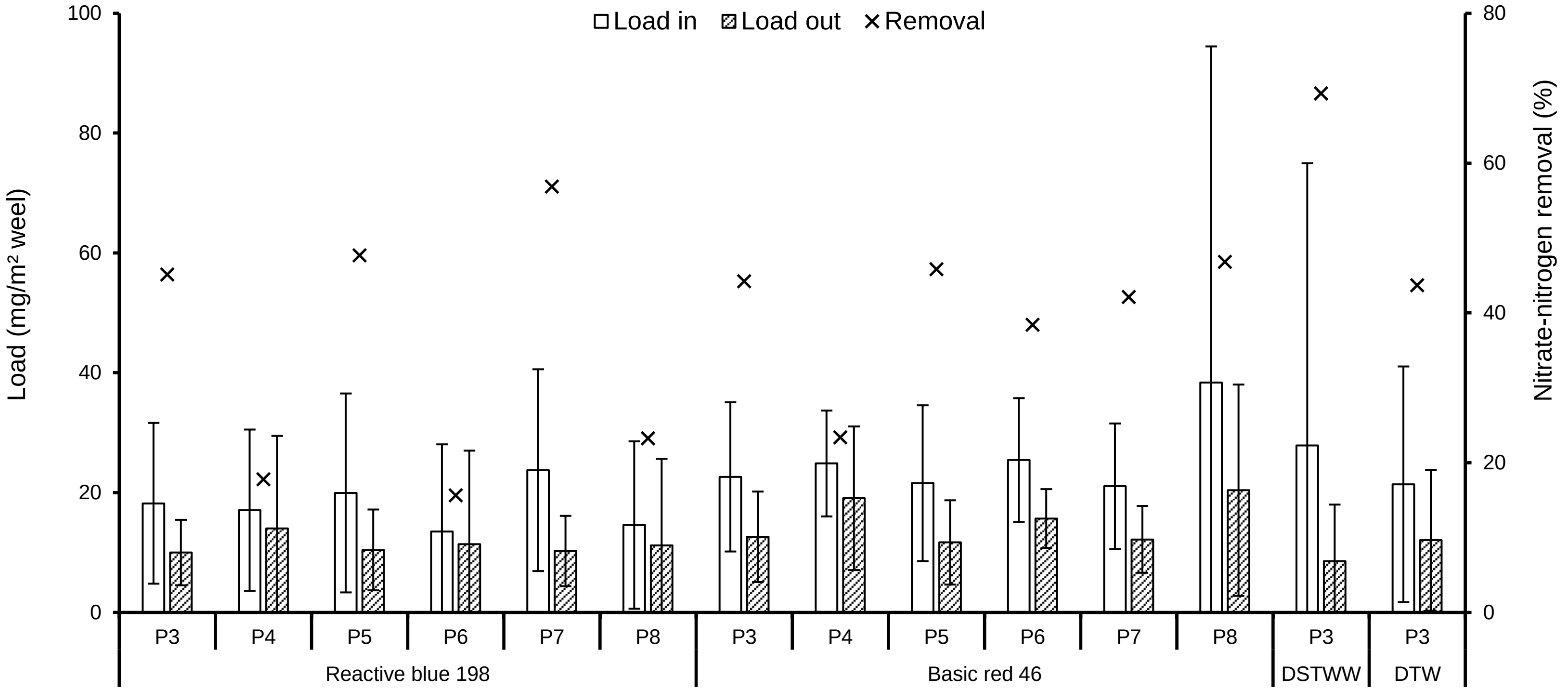


Figure 5.18 Mean nitrate-nitrogen removal efficiency during the experiment between 20 January 2016 and 18 October 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

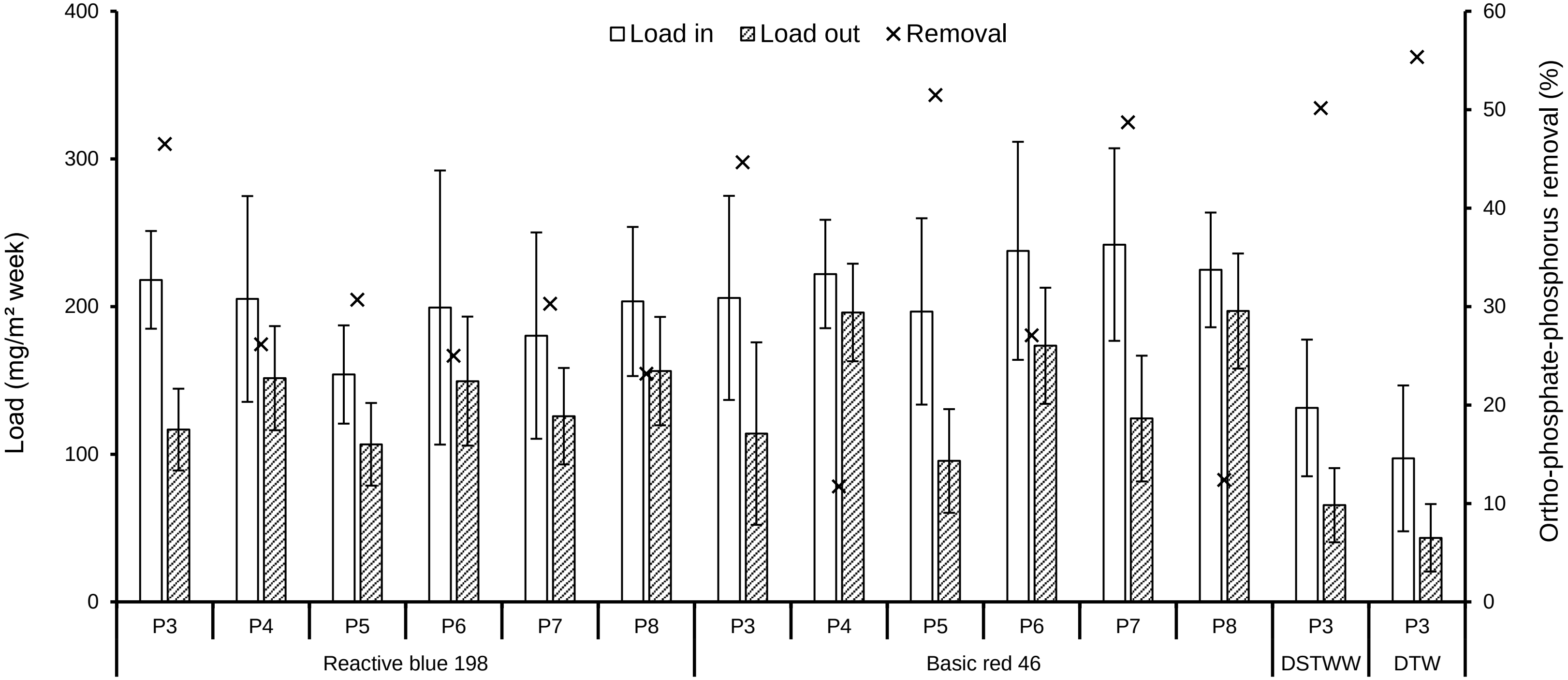


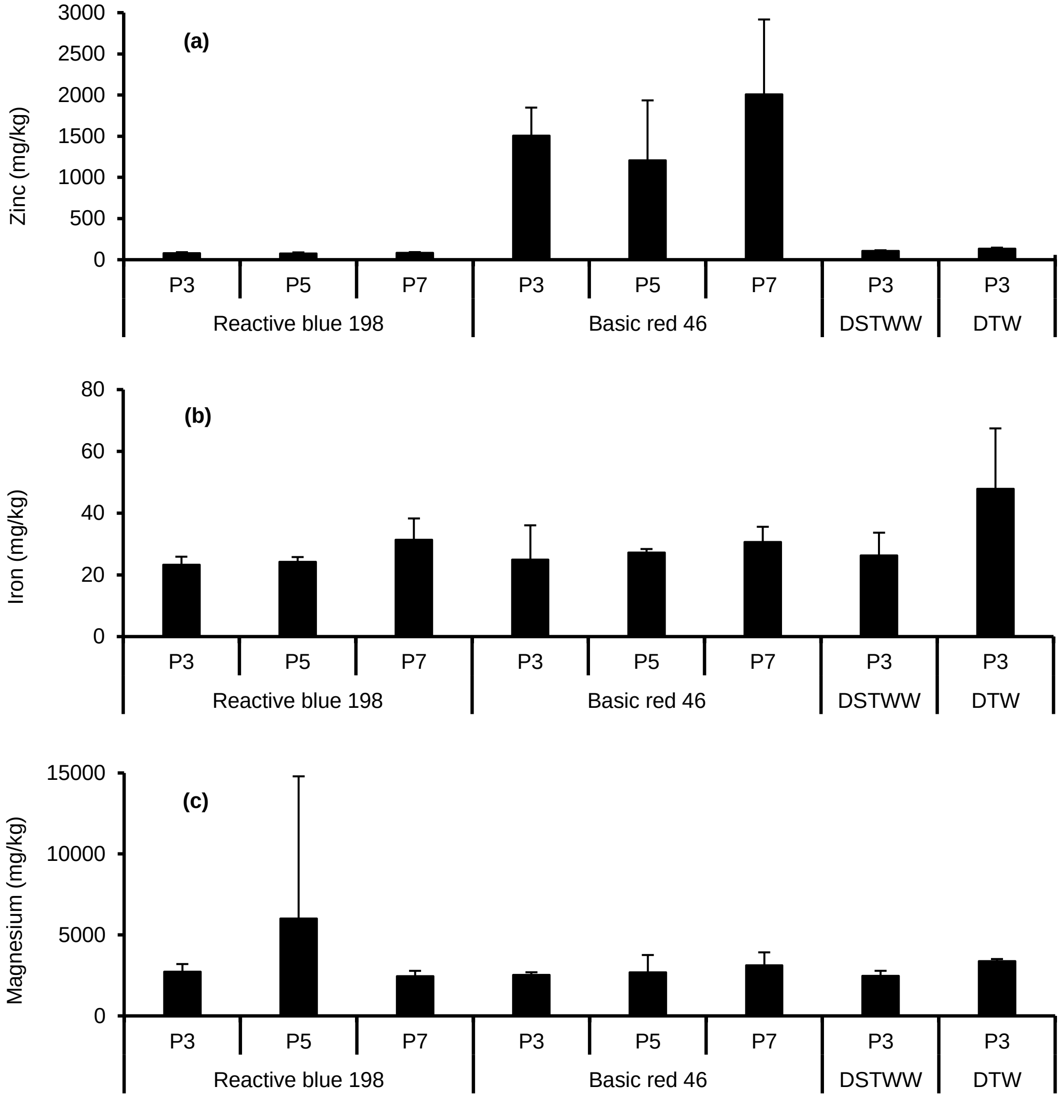
Figure 5.19 Mean ortho-phosphate-phosphorus removal efficiency during the experiment between 20 January 2016 and 18 October 2016. Note: P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.2.7 Trace elements

The mean inflow and outflow concentrations of the elements detected through the ICP-OES analyses are presented in Figure 5.11. Mean outflow zinc values (Figure 5.11a) were lower than the mean inflow concentrations for both *L. minor* and control ponds containing dye, as well as those fed by only synthetic textile wastewater. In addition, all the outflow zinc values were higher for ponds containing plants compared with the corresponding outflow values for control ponds in terms of the dye RB198, although no significant differences were found ($P > 0.05$, t-test). However, for BR46, which is the main source of zinc in the wastewater, the outflow values for *L. minor* ponds were significantly ($p < 0.05$, t-test) lower than those in control ones, which may reflect the BR46 degradation and consequently zinc ion reduction by *L. minor* ponds. Regarding the impact of the pH factor, no significant differences ($P > 0.05$, Kruskal-Wallis test) were found for either dye. Mean zinc outflows were within the acceptable range for *L. minor* survival (Khellaf & Zerdaoui, 2009). In addition, the mean outflow zinc concentrations were lower than the threshold set of 2 mg/l for irrigation (Metcalf & Eddy, 2003). Mean outflow iron values (Figure 5.11b) were lower than the mean inflow concentrations for both *L. minor* and control ponds containing dye as well as those fed by only synthetic textile wastewater, except for the outflow values of control ponds at pH of 9 (P6) treating RB198, and control ponds at normal pH (P4) and *L. minor* pond at pH of 6 (P7) comprising BR46. In addition, although the outflow iron values were lower in *L. minor* ponds compared with those in control ponds for both dyes, which indicates some iron uptake by *L. minor* except the case of normal pH (P3 and P4) for treating RB198 and pH of 6 (P7 and P8) treating BR46, no significant differences were found ($P > 0.05$). These exceptions may be attributed to plant die-off and subsequent decomposition in some ponds being higher than others due to the effect of pH values with the dyes and the synthetic wastewater. The standard limit of iron for irrigation is 5 mg/l (Metcalf & Eddy, 2003), and all outflow values were compliant. Lower mean outflow values of potassium (Figure 5.11d) than the inflow ones were found for all ponds containing dye as well as those comprising only synthetic textile wastewater. Other elements detected in the wastewater including magnesium, calcium and sodium are presented in Figures 5.11c, e and f, respectively

Figure 5.20 provides an overview of the concentration of elements accumulated in plant tissues for all ponds with and without dyes. The higher concentrations of zinc in BR46 were linked with the high outflow zinc values in ponds treating BR46 (Figure 5.20a), which were attributed to the higher inflow zinc in these ponds compared with the systems not comprising BR46. The level of zinc and iron in plants (Figures 5.20a and b) were higher than the permissible limits of 50 mg/kg and 20 mg/kg, respectively, as mentioned by Nazir et al. (2015). Manganese was detected in the plants (Figure 5.20d), although it was not detected in the inflow and outflow aqueous samples.

The BCF, Figures 5.21a and b, indicated that the plant was positive for phytoremediation of zinc and iron, respectively, for all samples.



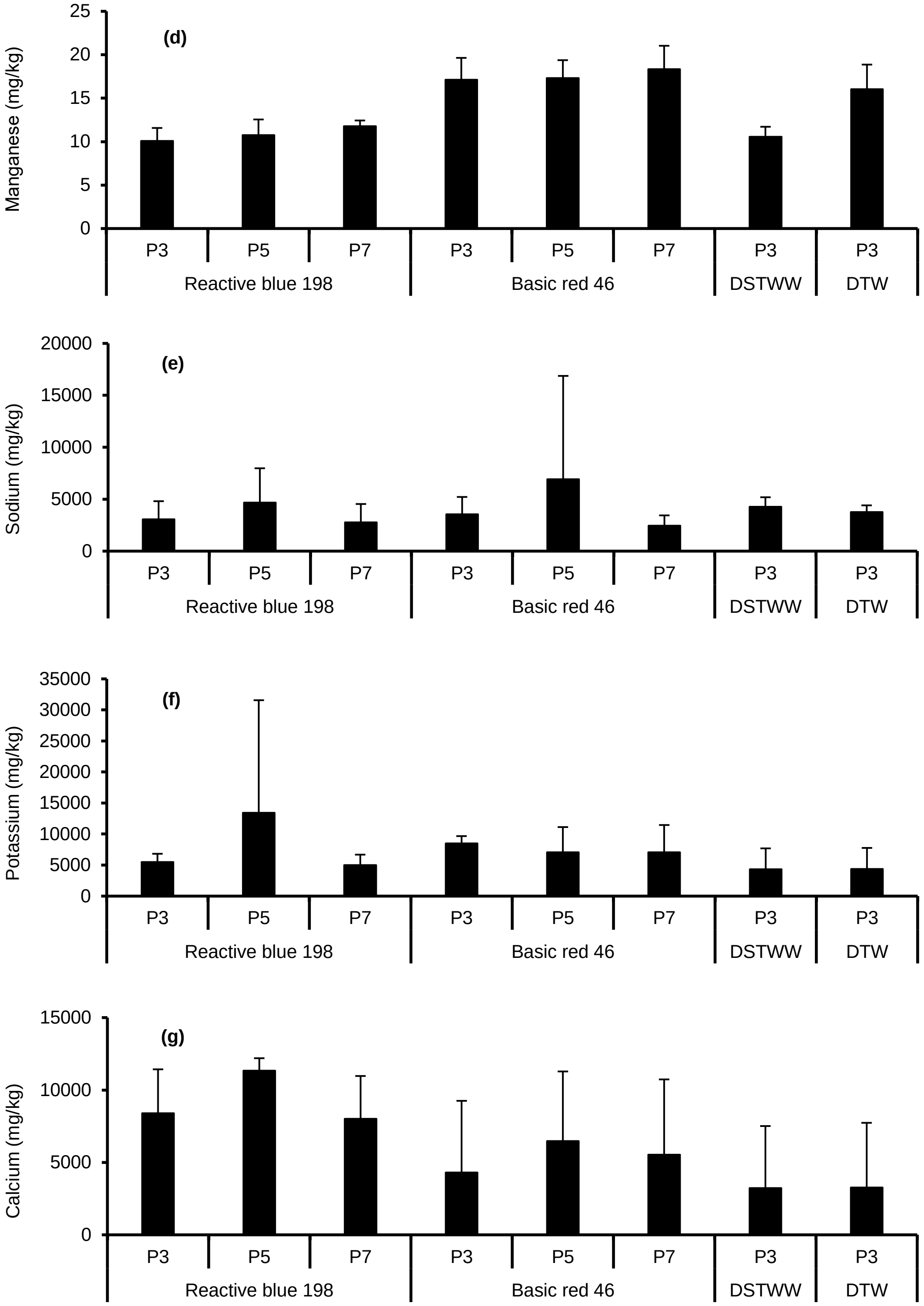


Figure 5.20 Mean concentrations of the detected elements in plant tissue during the experimental between 20 January 2016 and 18 October 2016. Note: (a), zinc; (b), iron; (c), magnesium; (d), manganese; (e), sodium; (f), potassium; (g), calcium; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

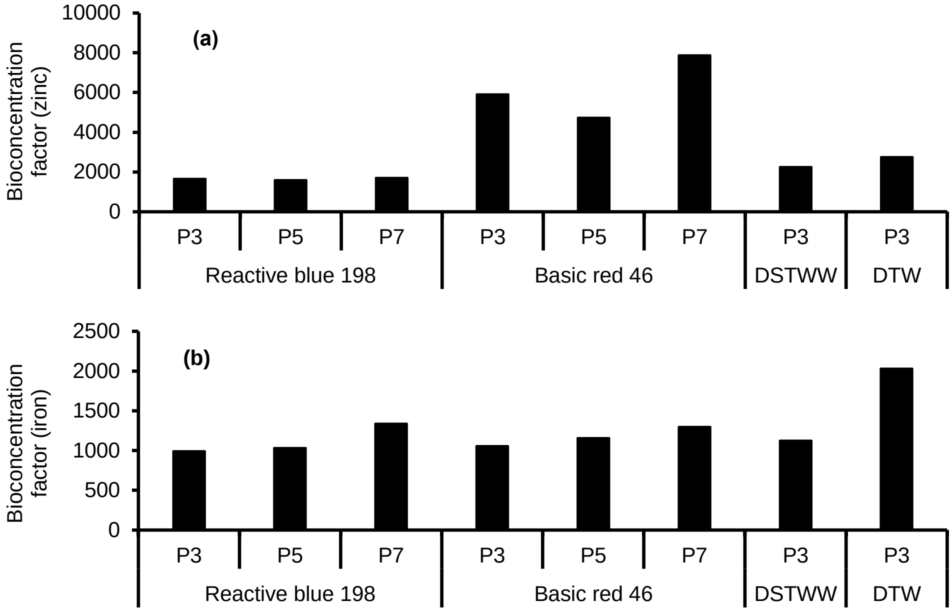


Figure 5.21 Bioconcentration factor during the experiment between 20 January 2016 and 18 October 2016. Note: (a), zinc; (b), iron; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.3 Treatment performance (phase 2)

5.3.3.1 Dye and apparent colour

Figure 5.22 shows the mean removal efficiency of the dyes for each system related to the second phase that ran between 18 October 2016 and 30 June 2017. Tables 5.10 and 5.11 present the overall outflow water quality parameters for each system, and statistical analysis between the treatment systems for the period from 18 October 2016 to 30 June 2017. The pond systems exhibited no removal efficiencies for the dye RB198 and the differences between the design parameters were low (Figure 5.22). In addition, the mean outflow concentrations (Table 5.10) were higher compared to those of the inflow (Table 5.6) and showed no significant differences (Table 5.11) among the design variables. These results indicate that the presence or absence of algae in the system do not affect the pond performance in terms of RB198 treatment. The highest and lowest removals were 6 and -26% for *L. minor* and algae ponds, respectively, and 13 and -30% for algae ponds in that order, and 11% and -23% for control ponds, respectively. The negative removal numbers suggested that the system works as a source rather than a sink, which could be due to surface evaporation phenomena or dried dye sticking on the wall of the pond sides after weekly doses. The removal of dye BR46 was significantly higher than that of RB198 due to the absence of sulpho groups, low molecular weight and the simple structure of BR46 (Chapter 3, Table 3.1), making this dye easily absorbed and degraded. The mean removal of BR46 (Figure 5.22) was highest for *L. minor* and algae ponds (89%) followed by only algae ponds (58%), and the lowest removal (33%) is linked to unplanted ponds. These results reflected the mean outflow BR46 (Table 5.10) concentrations, which were as follows: *L. minor* and algae ponds (P1; 0.5 mg/l) < algae ponds (P2; 2.4 mg/l) < control ponds (P4; 5 mg/l). All dissimilarities between the design parameters in terms of the outflow concentrations and the removal values were statistically significant (Table 5.11). These findings suggest that the influence of *L. minor* and algae on BR46 elimination are 31 and 25% in this order. These findings of BR46 removal efficiency match with those in Chapter 4, Section 4.2.2.1 (Table 4.2), although the potential of *L. minor* was slightly lower in this experiment, which may be due to the impact of increasing the concentration to 10 mg/l in this experiment compared with only 5 mg/l in the previous one.

Figure 5.23 highlights the UV-Vis analysis for the inflow and outflow samples for each dye linked with the second phase of the experiment. The main peak for the RB198 colour at 625 nm showed a clear increase in outflow samples related to *L. minor* and algae, algae and control ponds (Figure 5.23a). This indicates that the outflow concentrations increased and confirms the results of Table 5.10 related to the outflow RB198 concentrations. However, the dye BR46 (Figure 5.23b) showed a complete disappearance of the colour peak at 530 nm for ponds planted with *L. minor* and algae, confirming complete decolourisation. In addition, algae ponds showed considerable elimination of the peak at 530 nm confirming the high reduction of outflow concentrations, although the outflow samples were not fully colourless. However, control ponds showed some reduction in the peak at 530 nm indicating a decrease in the outflow concentration by microbes.

The HPLC chromatogram analysis showed that the RB198 inflow peak at a retention time of 1.505 min (Appendix E, Figure E.1b) shifted to 1.633, 1.793 and 1.523 min in ponds planted with *L. minor* and algae, algae only and controls, respectively (Appendix E, Figure E.16). These bands in planted ponds could considerably shift in contrast to the control ponds due to interactions between plants, algae and the wastewater. However, for the dye BR46, the inflow peak (Appendix E, Figure E.1c) at 1.488 min shifted to 1.485 min with the appearance of a new peak at 2.779 min for *L. minor* and algae ponds, and the inflow peak at 1.488 shifted to 1.52 min with the appearance of a new peak at 2.276 min for algae ponds (Appendix E, Figures E.17a and b, respectively). In addition, all other inflow peaks disappeared, confirming dye adsorption and degradation due to phytotransformation (Khandare et al., 2011; Kabra et al., 2012) and biotransformation by microbes (Tahir et al., 2016) processes. However, the control ponds showed appearances of new peaks at 2.796 min and the band at 2.405 shifted to 2.2, and the elimination of other inflow peaks was observed which may reflect dye bio-sorption and biotransformation (Pearce et al., 2003; Tahir et al., 2016) (Appendix E, Figure E.17c). As mentioned by Somasiri et al. (2006), the cleavage of azo bonds due to dye degradation produced toxic intermediates called aromatic amines, and due to the short life of these intermediates, it was difficult to identify these products by HPLC analysis. Therefore, gas chromatography mass spectrometry (GCMS) analysis was used for aromatic amine identification, which helped to confirm the mechanism of dye removal.

The GCMS results (provided by Concept Life Sciences Analytical and Development Services Limited) for the outflow of *L. minor* and algae ponds treating RB198 showed that there were no aromatic amines. This was because the breakage of azo bonds as a first step for the treatment did not occur and, consequently, no intermediates (aromatic amine) were produced. This result could be generalised for the algae and control ponds due to the same removal processes being expected for all ponds treating RB198. Concerning the dye BR46 treated by *L. minor* and algae ponds, no aromatic amines were detected in the residual dye bath. This finding indicates that the cleavage product is completely mineralised by phytotransformation and biotransformation (Khandare et al., 2011; Kabra et al., 2012; Tahir et al., 2016). However, the outflow samples of algae ponds containing BR46 showed the presence of three aromatics; benzenemethanamine, N-methyl (CAS number 103-67-3), benzenamine, 4methoxy-N-methyl (CAS number 5961-59-1), and 1-Triazene, 1-(4methylphenyl)-3(phenylmethyl) (CAS number 17683-09-9). The concentrations were lower than 10 mg/l, which is the detection limit of the equipment. This suggests that although algae *Oedogonium* were able to remove a considerable amount of colour, they were incapable of completely mineralising the dye within the systems to water and carbon dioxide. Therefore, in this experiment, biotransformation to colourless intermediates (aromatic amines) and bio-sorption processes are likely to be responsible for colour removal by algae. Holkar et al. (2016) mentioned that the main mechanisms for dye removal by algae are bio-sorption (the dye moves from the water phase to the solid phase) and biotransformation (algae break-down of the azo dye to colourless intermediates or carbon dioxide and water). According to the Regulation (EC) No. 1907/2006, the first aromatic (CAS number 103-67-

3) can cause severe skin burns, eye damage, allergic skin reaction, asthma symptoms and breathing difficulties, if inhaled. Therefore, environmental agencies often prevent this product from entering drains. However, the second amine (CAS number 5961-59-1) has been confirmed as a non-hazardous substance according to the Regulation (EC) No. 1272/2008. In addition, it does not contain components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bio-accumulative (vPvB) at levels of 0.1% or higher. Therefore, no special environmental exposure precautions are required. Furthermore, no component of these two aromatics present at levels greater than or equal to 0.1% has been identified as a probable, possible or confirmed human carcinogen. Whereas, there are no data available for the third aromatic (CAS number 17683-09-9). Although as an environmental precaution, it has been recommended not to allow this aromatic material to enter drains or watercourses.

Regarding the sample collected from the control ponds containing BR46, the analysis detected two peaks of concentrations below 10 mg/l. One peak was identified as N-(4-methylphenyl)-benzenemethanamine (CAS number 5405-15-2) and the other one was unidentifiable, but the closest match had an aromatic amine structure. This confirms that the pond system without *L. minor* was unable to mineralise the dye BR46. However, aromatic amine production confirmed the bio-sorption and biotransformation process by microbes within the control ponds (Tahir et al., 2016). N-(4-methylphenyl)-benzenemethanamine is considered as a non-hazardous substance. However, as an environmental precaution the discharge of this compound must be avoided. Sponza and Isik (2005) conducted different toxicity tests after each stage of direct red 28 treatment. They concluded that the outflow samples of the first stage in dye removal (decolourisation step) are toxic to plants and animals. However, continued degradation completed mineralisation, decreased the toxicity of the parent compound and eliminated the toxic impact on micro-organisms. Therefore, in this study, the toxic impacts of the outflow samples in *L. minor* ponds, which completely mineralised the dye, were expected to be eliminated. Whereas within the algae and control ponds, toxic effects are associated with the effluents depending on the toxicity of the aromatics.

Based on apparent colour monitoring, all mean outflow colour values (Table 5.10) were lower than the corresponding inflow (Table 5.6) values. *L. minor* and algae, and only algae ponds treating RB198 showed outflow colour values higher than the control ponds. However, *L. minor* and algae, and only algae ponds comprising BR46 showed lower outflow colour values than the control ponds. This is because the colour parameter (un-true colour) is affected by the colour of the dye and the suspended particles in the system. Therefore, the planted ponds treating RB198 showed higher colour values due to the presence of dead plants in the system in addition to the high dye concentration due to the low removal rate. However, BR46 was treated better and the colour measurements were affected by the impact of dead plants and other suspended particles only. Inflow and outflow sample pictures are shown in Appendix F, Figure F.4.

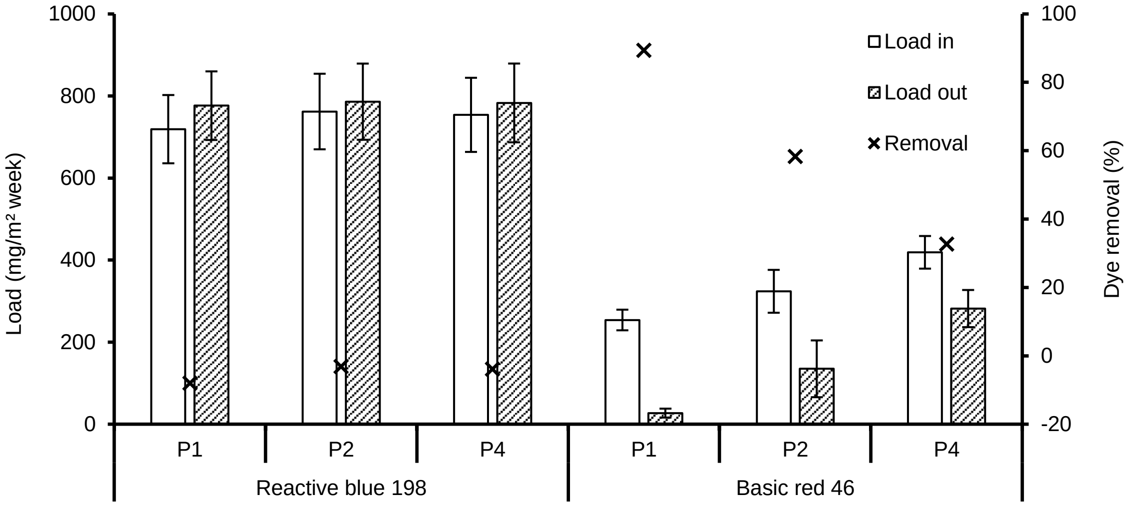


Figure 5.22 Mean dye removal efficiency during the experiment between 18 October 2016 and 30 June 2017. Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds.

Table 5.10 Outflow water quality parameters for each system between 18 October 2016 and 30 June 2017

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Reactive blue 198 + DSTWW for *Lemna minor* L. and algae ponds						
Dye concentration	mg/l	14.4	1.86	11.7	18.4	35
pH	-	7.6	0.32	7.3	9.0	35
Redox	mv	-63.9	17.67	-142.5	-46.5	35
Dissolved oxygen	mg/l	8.5	0.20	8.2	9.0	35
Electrical conductivity	µS/cm	127.2	9.91	111.5	145.9	35
Total dissolved solids	mg/l	54.4	5.44	42.8	64.5	37
Suspended solids	mg/l	6.7	6.46	0.5	26.5	35
Turbidity	NTU	4.4	2.48	2.7	14.3	35
Colour	Pt Co	204.1	67.43	134.5	559.5	35
Chemical oxygen demand	mg/l	19.2	3.52	13.3	26.1	8
Ammonium-nitrogen	mg/l	0.06	0.044	0.01	0.15	8

Table 5.10 (Continued)

Nitrate-nitrogen	mg/l	0.23	0.227	0.01	0.80	8
Ortho-phosphate-phosphorus	mg/l	1.72	0.344	1.12	2.08	8
Reactive blue 198 + DSTWW for algae ponds						
Dye concentration	mg/l	14.1	1.64	11.3	17.1	35
pH	-	7.6	0.16	7.3	8.0	35
Redox	mv	-64.4	9.29	-84.0	-47.5	35
Dissolved oxygen	mg/l	8.5	0.17	8.2	9.0	35
Electrical conductivity	µS/cm	125.9	12.29	109.1	150.9	35
Total dissolved solids	mg/l	67.8	7.32	45.7	83.1	37
Suspended solids	mg/l	3.9	2.49	0.0	10.0	35
Turbidity	NTU	3.5	0.56	2.7	5.3	35
Colour	Pt Co	176.6	24.57	135.0	226.5	35
Chemical oxygen demand	mg/l	17.1	6.36	8.8	32.7	8
Ammonium-nitrogen	mg/l	0.09	0.051	0.04	0.19	8
Nitrate-nitrogen	mg/l	0.23	0.294	0.01	1.00	8
Ortho-phosphate-phosphorus	mg/l	1.96	0.298	1.37	2.34	8
Reactive blue 198 + DSTWW for control ponds						
Dye concentration	mg/l	13.9	1.64	11.0	16.8	35
pH	-	7.5	0.09	7.3	7.7	35
Redox	mv	-58.7	4.87	-69.0	-46.5	35
Dissolved oxygen	mg/l	8.5	0.17	8.2	8.9	35
Electrical conductivity	µS/cm	128.0	12.22	108.3	150.5	35
Total dissolved solids	mg/l	60.1	11.72	37.3	113.6	37
Suspended solids	mg/l	3.8	2.20	0.5	9.0	35
Turbidity	NTU	3.5	0.57	2.5	5.3	35
Colour	Pt Co	175.5	23.80	127.0	224.5	35
Chemical oxygen demand	mg/l	16.9	4.49	9.8	22.0	8
Ammonium-nitrogen	mg/l	0.13	0.107	0.04	0.40	8
Nitrate-nitrogen	mg/l	0.24	0.179	0.11	0.55	8
Ortho-phosphate-phosphorus	mg/l	2.25	0.597	1.25	3.47	8
Basic red 46+ DSTWW for *Lemna minor* L. and algae ponds						
Dye concentration	mg/l	0.5	0.20	0.1	0.8	35
pH	-	7.6	0.31	7.2	8.7	35
Redox	mv	-64.5	17.26	-122.0	-43.0	35
Dissolved oxygen	mg/l	8.5	0.34	7.2	9.4	35
Electrical conductivity	µS/cm	122.6	12.36	103.9	149.4	35
Total dissolved solids	mg/l	55.4	9.08	36.4	77.0	37
Suspended solids	mg/l	3.7	3.51	0.0	17.0	35
Turbidity	NTU	3.7	0.66	2.7	6.3	35
Colour	Pt Co	244.4	51.30	165.0	361.0	35
Chemical oxygen demand	mg/l	21.3	9.33	7.1	33.3	8
Ammonium-nitrogen	mg/l	0.09	0.050	0.02	0.13	8
Nitrate-nitrogen	mg/l	0.22	0.103	0.01	0.35	8

Table 5.10 (Continued)

Ortho-phosphate-phosphorus	mg/l	1.70	1.209	0.60	3.36	8
Basic red 46 + DSTWW for algae ponds						
Dye concentration	mg/l	2.4	1.22	0.5	5.0	35
pH	-	7.6	0.26	6.9	8.3	35
Redox	mv	-60.9	14.98	-104.5	-23.0	35
Dissolved oxygen	mg/l	8.5	0.18	8.2	8.9	35
Electrical conductivity	µS/cm	119.0	11.24	103.3	145.0	35
Total dissolved solids	mg/l	73.6	48.57	58.5	361.0	37
Suspended solids	mg/l	3.4	2.10	0.0	9.0	35
Turbidity	NTU	3.7	0.46	2.9	5.0	35
Colour	Pt Co	351.7	84.70	215.5	589.5	35
Chemical oxygen demand	mg/l	33.1	21.91	5.8	83.1	8
Ammonium-nitrogen	mg/l	0.09	0.056	0.00	0.20	8
Nitrate-nitrogen	mg/l	0.22	0.081	0.02	0.30	8
Ortho-phosphate-phosphorus	mg/l	1.60	0.330	1.22	2.20	8
Basic red 46 + DSTWW for control ponds						
Dye concentration	mg/l	5.0	0.85	2.6	6.0	35
pH	-	7.6	0.16	7.3	8.1	35
Redox	mv	-61.3	8.97	-92.0	-48.0	35
Dissolved oxygen	mg/l	8.5	0.17	8.2	8.9	35
Electrical conductivity	µS/cm	119.0	12.31	99.3	140.2	35
Total dissolved solids	mg/l	59.6	5.57	47.7	73.4	37
Suspended solids	mg/l	2.1	1.61	0.0	6.5	35
Turbidity	NTU	3.4	0.41	2.5	4.6	35
Colour	Pt Co	472.0	87.48	265.0	605.0	35
Chemical oxygen demand	mg/l	15.3	5.25	8.2	26.9	8
Ammonium-nitrogen	mg/l	0.10	0.05	0.02	0.18	8
Nitrate-nitrogen	mg/l	0.23	0.103	0.04	0.33	8
Ortho-phosphate-phosphorus	mg/l	2.25	0.576	1.42	3.14	8
DSTWW for *Lemna minor* L. and algae ponds						
pH	-	7.6	0.19	7.3	8.3	35
Redox	mv	-66.6	10.56	-104.0	-54.0	35
Dissolved oxygen	mg/l	8.5	0.17	8.2	8.9	35
Electrical conductivity	µS/cm	129.8	14.24	103.5	156.0	35
Total dissolved solids	mg/l	54.0	3.73	45.5	58.8	37
Suspended solids	mg/l	3.6	2.80	0.0	12.0	35
Turbidity	NTU	3.6	0.65	2.2	6.0	35
Colour	Pt Co	41.9	18.22	18.0	99.0	35
Chemical oxygen demand	mg/l	19.6	7.90	12.8	35.8	8
Ammonium-nitrogen	mg/l	0.04	0.052	0.01	0.17	8
Nitrate-nitrogen	mg/l	0.17	0.352	0.01	1.10	8
Ortho-phosphate-phosphorus	mg/l	0.58	0.210	0.18	0.94	8
DSTWW for algae ponds						

Table 5.10 (Continued)

pH	-	7.7	0.24	7.4	8.5	35
Redox	mv	-69.0	13.35	-115.0	-50.0	35
Dissolved oxygen	mg/l	8.5	0.17	8.2	9.0	35
Total dissolved solids	mg/l	40.2	3.93	32.3	48.8	37
Suspended solids	mg/l	4.0	4.76	0.0	17.0	35
Turbidity	NTU	4.3	1.30	2.7	8.5	35
Colour	Pt Co	41.7	20.38	12.0	89.0	35
Chemical oxygen demand	mg/l	30.4	9.01	23.5	50.4	8
Ammonium-nitrogen	mg/l	0.05	0.019	0.03	0.10	8
Nitrate-nitrogen	mg/l	0.23	0.479	0.02	1.50	8
Ortho-phosphate-phosphorus	mg/l	0.83	0.224	0.44	1.19	8
DTW for *Lemna minor* L. and algae ponds						
pH	-	7.5	0.14	7.2	7.8	35
Redox	mv	-56.1	7.36	-76.5	-45.0	35
Dissolved oxygen	mg/l	8.5	0.14	8.2	8.8	35
Electrical conductivity	µS/cm	109.4	12.59	87.1	141.9	35
Total dissolved solids	mg/l	54.0	3.73	45.5	58.8	37
Suspended solids	mg/l	3.2	2.59	0.0	10.5	35
Turbidity	NTU	3.6	0.45	2.6	4.5	35
Colour	Pt Co	39.4	15.20	12.0	71.0	35
Chemical oxygen demand	mg/l	24.5	11.97	16.2	51.9	8
Ammonium-nitrogen	mg/l	0.04	0.042	0.02	0.12	8
Nitrate-nitrogen	mg/l	0.05	0.024	0.01	0.08	8
Ortho-phosphate-phosphorus	mg/l	0.58	0.264	0.13	1.12	8
DSTWW for algae ponds						
pH	-	7.5	0.12	7.1	7.7	35
Redox	mv	-57.6	6.68	-69.5	-37.0	35
Dissolved oxygen	mg/l	8.5	0.14	8.2	8.8	35
Electrical conductivity	µS/cm	117.8	5.80	106.0	130.0	35
Total dissolved solids	mg/l	40.2	3.93	32.3	48.8	37
Suspended solids	mg/l	3.1	2.68	0.0	12.5	35
Turbidity	NTU	3.5	0.61	2.5	5.6	35
Colour	Pt Co	34.8	15.69	9.0	66.5	35
Chemical oxygen demand	mg/l	17.9	5.76	12.0	28.6	8
Ammonium-nitrogen	mg/l	0.05	0.055	0.02	0.19	8
Nitrate-nitrogen	mg/l	0.07	0.031	0.02	0.13	8
Ortho-phosphate-phosphorus	mg/l	0.76	0.374	0.13	1.37	8

Note: NTU, nephelometric turbidity unit; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

Table 5.11 Overview of the statistical analysis for outflow water quality parameters and corresponding removal efficiencies for each system between 18 October 2016 and 30 June 2017

Parameter	Shapiro-Wilk test (p value[1])	Statistical test	p values[2] for different system combinations			
			P1 & P2 & P3	P1 & P2	P1 & P3	P2 & P3
Dye (mg/l)						
RB198	0.017	K-W	0.548	N/A	N/A	N/A
BR46	<0.001	K-W	<0.001	<0.001	<0.001	<0.001
Dye removal (%)						
RB198	0.178	ANOVA	0.010	0.016	0.033	0.960
BR46	<0.001	K-W	<0.001	<0.001	<0.001	<0.001
Colour (Pt Co)						
RB198	<0.001	K-W	0.005	0.008	0.003	0.888
BR46	0.001	K-W	<0.001	<0.001	<0.001	<0.001
DSTWW	0.001	M-W		0.874		
DTW	0.014	M-W		0.235		
Chemical oxygen demand (mg/l)						
RB198	0.196	Annova	0.638	N/A	N/A	N/A
BR46	<0.001	K-W	0.071	N/A	N/A	N/A
DSTWW	0.062	t-test		0.033		
DTW	<0.001	M-W		0.140		
Chemical oxygen demand removal (%)						
RB198	0.498	ANOVA	0.202	N/A	N/A	N/A
BR46	0.095	ANOVA	0.639	N/A	N/A	N/A
DSTWW	0.006	M-W		0.009		
DTW	0.005	M-W		0.036		
Ammonium-nitrogen (mg/l)						
RB198	<0.001	K-W	0.188	N/A	N/A	N/A
BR46	0.045	K-W	0.362	N/A	N/A	N/A
DSTWW	0.002	M-W		0.074		

Table 5.11 (Continued)

DTW	<0.001	M-W		0.874		
Ammonium-nitrogen removal (%)						
RB198	0.057	ANOVA	0.039	0.451	0.031	0.300
BR46	0.176	ANOVA	0.001	0.801	0.002	0.009
DSTWW	0.043	M-W		0.462		
DTW	0.098	t-test		0.257		
Nitrate-nitrogen (mg/l)						
RB198	<0.001	K-W	0.758	N/A	N/A	N/A
BR46	0.006	K-W	0.744	N/A	N/A	N/A
DSTWW	<0.001	M-W		0.128		
DTW	0.652	t-test		0.245		
Nitrate-nitrogen removal (%)						
RB198	0.002	K-W	0.001	0.141	0.001	0.002
BR46	0.137	ANOVA	<0.001	0.742	<0.001	<0.001
DSTWW	0.826	t-test		0.014		
DTW	0.683	t-test		0.038		
Ortho-phosphate-phosphorus (mg/l)						
RB198	0.067	ANOVA	0.099	N/A	N/A	N/A
BR46	0.171	ANOVA	0.278	N/A	N/A	N/A
DSTWW	0.358	t-test		0.048		
DTW	0.794	t-test		0.297		
Ortho-phosphate-phosphorus removal (%)						
RB198	0.909	ANOVA	0.008	0.043	0.008	0.740
BR46	0.231	ANOVA	0.124	N/A	N/A	N/A
DSTWW	0.084	t-test		N/A	N/A	N/A
DTW	0.002	M-W		0.005		
Suspended solids (mg/l)						
RB198	<0.001	K-W	0.120	N/A	N/A	N/A
BR46	<0.001	K-W	0.029	N/A	N/A	N/A

Table 5.11 (Continued)

DSTWW	<0.001	M-W		0.422		
DTW	<0.001	M-W		0.962		
Turbidity (NTU)						
RB198	<0.001	K-W	0.611	N/A	N/A	N/A
BR46	<0.001	K-W	0.058	N/A	N/A	N/A
DSTWW	<0.001	M-W		0.052		
DTW	<0.001	M-W		0.055		
Dissolved oxygen (mg/l)						
RB198	0.001	K-W	0.391	N/A	N/A	N/A
BR46	<0.001	K-W	0.805	N/A	N/A	N/A
DSTWW	0.123	t-test		0.326		
DTW	0.027	M-W		0.651		
pH (-)						
RB198	0.000	K-W	0.062	N/A	N/A	N/A
BR46	0.000	K-W	0.580	N/A	N/A	N/A
DSTWW	0.000	M-W		0.414		
DTW	0.275	t-test		0.266		
Electrical conductivity (µS/cm)						
RB198	0.000	K-W	0.672	N/A	N/A	N/A
BR46	0.001	K-W	0.432	N/A	N/A	N/A
DSTWW	0.077	t-test		0.477		
DTW	0.414	t-test		0.001		

Note: [1]Test of normality (if $p > 0.05$, data are normally distributed; if $p < 0.05$, data are not normally distributed); [2]p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; RB198, reactive blue 198; BR46, basic red 46; K-W, Kruskal-Wallis test; ANOVA, one-way analysis of variance test; t-test, independent samples t-test; M-W, Mann-Whitney U test; N/A, not applicable, because the difference among the variables is not significant; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

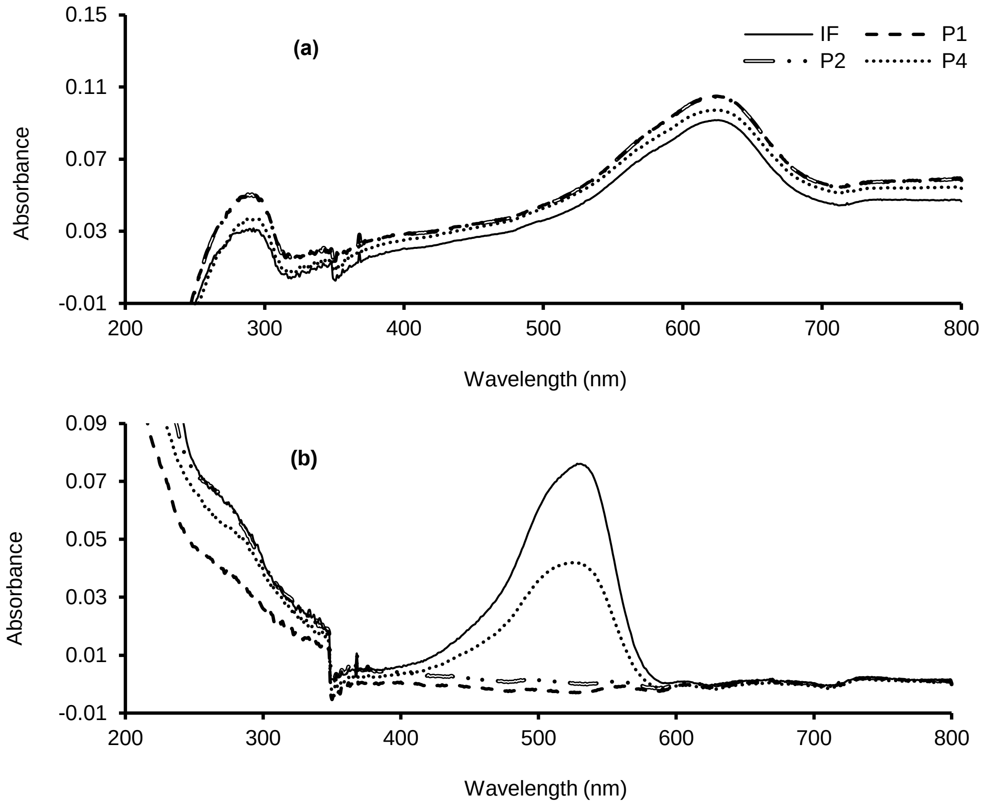


Figure 5.23 Ultraviolet visible analysis for the outflow samples at the end of the second phase of the experimental rig, which was operated between 18 October 2016 and 30 June 2017. Note: (a), ponds treating the dye reactive blue 198; (b), ponds treating the dye basic red 46; IF, inflow; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds.

5.3.3.2 Chemical oxygen demand and dissolved oxygen

Figure 5.24 summarises the overall COD removal efficiency for each system, the results showed no significant differences among the design variables in ponds containing dyes. However, for ponds without dyes, the values were significantly higher (Table 5.11) in the combined *L. minor* and algae ponds than the corresponding algae only ponds. In addition, no significant differences (Table 5.11) were found between the design parameters in terms of average outflow COD concentrations (Table 5.10) for either dye, which were lower compared to the inflow ones (Table 5.6). All these findings indicate that the presence of dyes in the system has an adverse effect on organic matter degradation, which can be attributed to the dye impact on the plants. However, algae seem to be not affected by the dye due to the COD removal similarity in all algae ponds with and without dye. This indicates that the COD degradation was due to microbial activities for all systems except *L. minor* and algae ponds without dyes. In this phase, all outflow COD values were less than the standard limits. For Do, the average outflow concentrations were lower compared to the inflow ones (Tables

5.6 and 5.10) for all ponds. Statistically significant (Tables 5.11) differences were not found among the pond design variables indicating that the oxygen content in all ponds was affected by the atmosphere rather than the plants and/or algae.

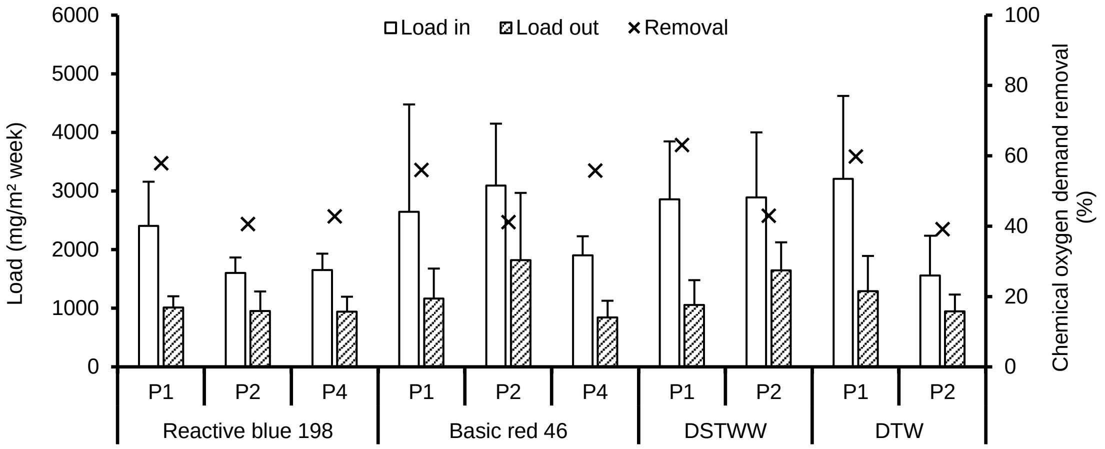


Figure 5.24 Mean chemical oxygen demand removal efficiency during the experiment between 18 October 2016 and 30 June 2017. Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.3.3 pH and redox potential

The mean pH outflow values (Table 5.10) were a little bit higher than the inflow ones (Table 5.6) concerning all systems. In addition, a statistical analysis of the outflow pH values showed no significant (Table 5.11) differences among the pond types. This could be attributed to the long-term nature of this study, as it is continuous for the first phase leading to the absence of the impact of plants on pH modification. According to European and international standards, the pH outflow values for *L. minor* and algae ponds containing RB198, *L. minor* and algae ponds treating BR46, and algae ponds containing synthetic wastewater were 1, 1 and 1 times non-compliant, respectively.

Regarding redox potential, some outflow values of the first few weeks of this phase were lower than -100 mV indicating anaerobic conditions. However, after that, all outflow values ranged between -98 and -23 mV (Table 5.10) indicating the dominant anoxic conditions in the systems (Ong et al., 2009a). The mean outflow redox potential values showed no significant differences between the design parameters for all ponds (Table 5.11).

5.3.3.4 Suspended solids and turbidity

Regarding SS concentrations, the mean outflow concentrations (Table 5.10) were elevated compared to the corresponding inflow ones (Table 5.6) for all ponds which could be due to organic matter degradation. Furthermore, the outflow concentrations for the planted ponds (P1 and P2) comprising dyes were higher than the corresponding values of the control ponds. This was attributed to the die-off of some plants, dried plants on the walls of the artificial ponds and the presence of algae in the planted ponds. These results indicate that both algae and *L. minor* impacted on the SS concentrations in this study. Note that the outflow values followed this order: P1 > P2 > P4. Significant (Table 5.11) differences were found between control ponds and planted ponds (*L. minor* and algae, and algae ponds) containing BR46. This could be due to the high degradation of this dye in planted ponds compared to ponds without *L. minor* and/or algae. Dye degradation also increased the SS content in the system. According to the European and international standards (Carmen & Daniela, 2012), all outflow values were below the standard limits.

Concerning turbidity (Tables 5.6 and 5.10), the average outflow turbidity concentrations mirrored those for SS. Based on a correlation analysis, SS was significantly ($p < 0.01$) positively correlated with turbidity.

5.3.3.5 Electrical conductivity and total dissolved solids

The average outflow EC (Table 5.10) was slightly lower than the corresponding inflow one (Table 5.6) for all ponds containing dyes. In addition, no significant (Table 5.11) differences were found between the design parameters in terms of the outflow EC, whereas there was a significant difference between *L. minor* ponds and control ponds in the first phase. This was attributed to salt saturation and/or exhaustion after two phases of weekly dosages due to using the same plants from the first phase, which were not harvested during the second phase as a result of their low growth rate.

Based on TDS values, both average inflow and outflow TDS concentrations had the same trend with respect to the inflow and outflow EC, as explained previously (Tables 5.6 and 5.10, respectively). According to the European and international standards, the results showed that all outflow TDS concentrations were less than the standard limits of 500 mg/l and 2000 mg/l for discharge of effluents to the receiving watercourses (Carmen & Daniela, 2012).

5.3.3.6 Nutrients

Regarding NH_4–N, higher removal was found in ponds planted with *L. minor* and algae followed by algae ponds and the lowest removal was noticed in control ponds for all treatment systems (Figure 5.25). Significant differences (Table 5.11) were found between ponds planted with *L. minor* and algae, and control ponds for both dyes. In terms of outflow concentrations, the values were lower compared to the corresponding inflow concentrations (Table 5.6 and 5.10), although the outflow numbers were higher in the control ponds than the planted ponds. These results showed that both *L. minor* and algae contributed to nitrogen removal. However, the removal values for algae ponds compared with *L. minor* and algae ponds for all treatment systems (Figure 5.25) suggest that algae have higher contributions to nitrogen removal than *L. minor*. This result confirmed findings previously reported by Kadlec et al. (2000) that the plant potential does not exceed 10% of the total nitrogen removal. In addition, this is also attributed to the impact of mean pH outflows, which were higher than 7 in this study, as clarified by Zimmo (2003). He mentioned that higher nitrogen removal occurs in algae ponds than *L. minor* ponds in the case of pH values between 7 and 9, as a best condition for algae growth. Nitrogen removal is due to biological uptake by dispersed algae, which settle and attach themselves to the wall of the ponds. However, *L. minor* is able to accumulate nitrogen, which depends on the plant state in the treatment system and on other conditions related temperature and pH level. The other impact for NH_4–N removal in this experiment was the nitrification process by nitrifying bacteria that depends on the oxygen supplied by the atmosphere, diffusion and/or plant transfer via their roots. The results shown in Figure 5.25 confirm the outcome of the first phase highlighting that the presence of the dye impacted on the of NH_4–N removal efficiency. All the outflow values of NH_4–N in this phase were lower than the threshold limit.

Based on NO_3–N, a low mean removal efficiency was found in all ponds especially the control ones (Figure 5.26). The removal values follow this order: P1 > P2 > P4. Statistically, significant (Table 5.11) differences were found between *L. minor* and algae ponds, and algae ponds compared to the control wetlands. In terms of NO_3–N concentrations, all average outflow concentrations were lower compared to the inflow ones (Table 5.6 and Table 5.10), although the control ponds showed outflow concentrations higher than the planted ponds. These findings indicate that *L. minor* and algae use nitrate as a source for nutrients (Selvarani et al., 2015) resulting in a significant ($p < 0.05$) potential for NO_3–N reduction by both *L. minor* and algae, in addition to the denitrification mechanism. However, it seems that the potential of algae is higher than that for *L. minor* due to the same removal found in algae ponds compared with ponds containing both algae and *L. minor*. This is because algae reduces in the presence of *L. minor* making the impact of both of them together similar to ponds containing algae or *L. minor* separately. Note that in this phase, anaerobic micro-niches within the container also have some contribution to the denitrification rate, in addition to the impact of micro-sites of the biofilm attached to *L. minor*, supporting both nitrification and denitrification (Zimmo, 2003). For both phases, the synthetic wastewater composition supplied energy to micro-organisms to enhance denitrification by providing a long-term source of carbon and nutrients, which supported the micro-organism population as well. This source of carbon is necessary, as during the nitrification process carbon dioxide is utilised as a source of carbon and energy. The denitrification process under anoxic conditions requires carbon, which is lacking at the end of the nitrification process. High nitrate concentrations present in the system outflow indicate limited denitrification, which is due to the fact that most carbon sources have been utilised by the nitrification process (Mohammed, 2017). Therefore, the carbon source promoted the long-term functioning of the wetlands.

The PO_4–P removal efficiency was very low for all ponds containing dyes compared with ponds without dyes (Figure 5.27). In addition, the removal of ponds planted with *L. minor* and algae was significantly higher than that for algae ponds containing RB198, control wetland systems comprising RB198, and algae wetlands without dyes. It was evident that the state of *L. minor* was better in these ponds due to the potential of *L. minor* for phosphorus uptake compared with systems treating BR46. Results of low removal reflect the high outflow values, which were higher than the inflow values for all ponds containing dyes (Table 5.6 and Table 5.10). These findings indicate that the mechanisms of phosphorus removal were the uptake by plants and microbes, which are the main mechanisms for phosphorus removal in wetlands (Vymazal, 2007). Generally, these low removal efficiencies were expected, due to the relatively low potential of wetland systems for PO_4–P removal. Concerning the guideline threshold for PO_4–P of 1 mg/l, this is indicated by Al-Isawi et al. (2017), as the UK Government (1994) does not set regulations for PO_4–P relating to the treatment system in this study. All outflow values concerning *L. minor* and algae, algae, and control ponds containing RB198 as well as algae and control ponds treating BR46 were non-compliant. However, *L. minor* and algae ponds comprising BR46, algae ponds containing synthetic wastewater, *L. minor* and algae, and algae ponds containing tap water were 3, 1, 1 and 1 times non-compliant.

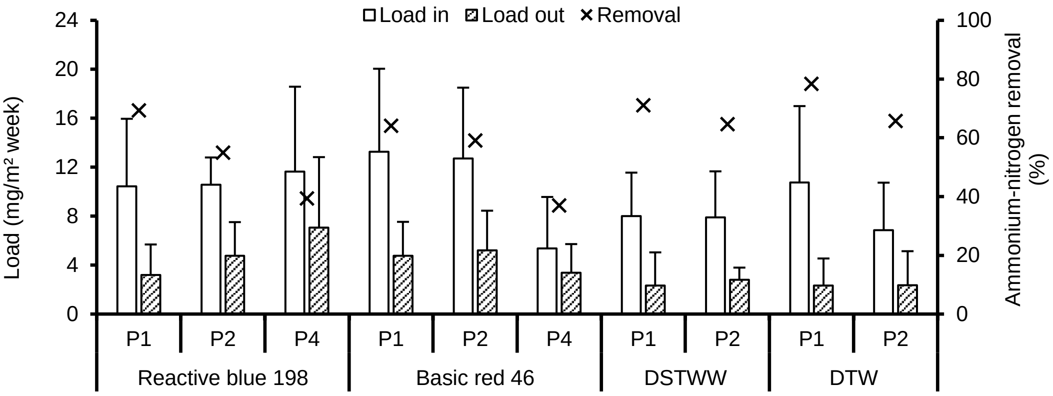


Figure 5.25 Mean ammonium-nitrogen removal efficiency during the experiment between 18 October 2016 and 30 June 2017. Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

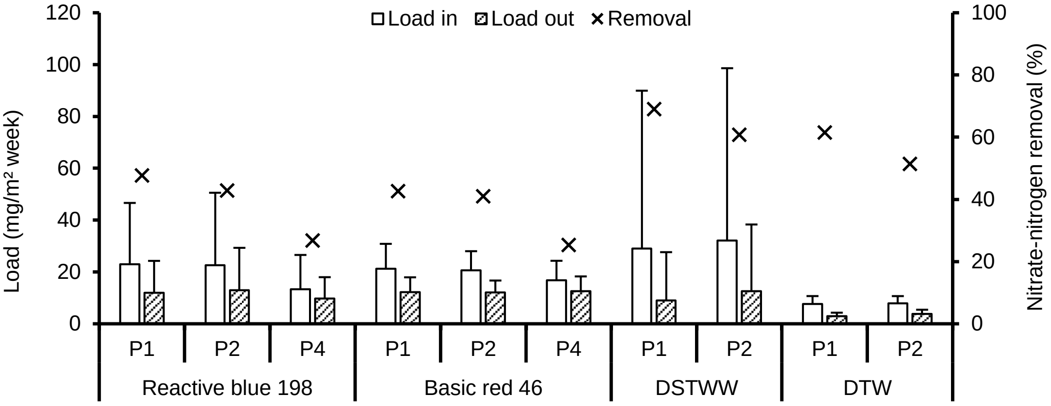


Figure 5.26 Mean nitrate-nitrogen removal efficiency during the experiment between 18 October 2016 and 30 June 2017. Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

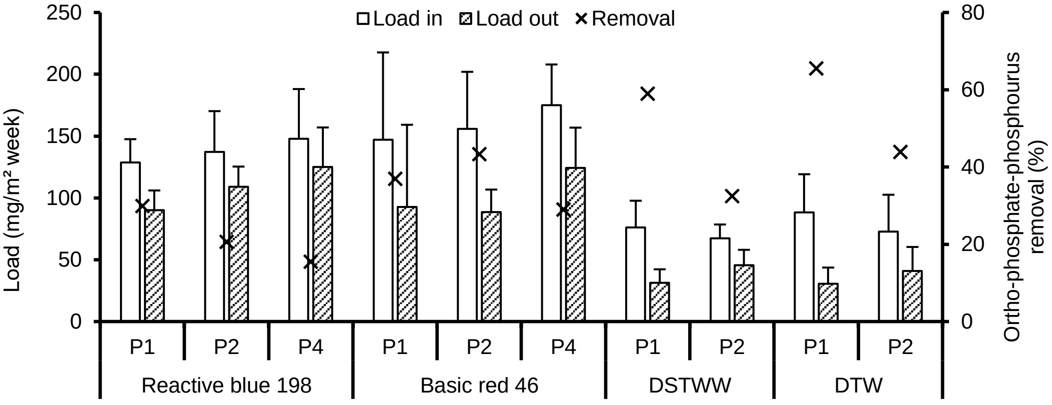


Figure 5.27 Mean ortho-phosphate-phosphorus removal efficiency during the experiment between 18 October 2016 and 30 June 2017. Note: P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.3.7 Trace elements

The mean inflow and outflow concentrations of the elements detected through the ICP-OES analyses are presented in Figure 5.12. Mean outflow zinc values (Figure 5.12a) were lower than the mean inflow concentrations for all systems with and without dye. In addition, all the outflow zinc values were higher for ponds containing plants compared with the corresponding outflow values for control ponds in terms of the dye RB198, although no significant differences were found ($p > 0.05$,) between the outflow values in terms of the design variable for all ponds, except those treating BR46 which were significantly higher in P4 followed by P2 and then P1 ($p < 0.05$). This confirmed zinc reduction by *L. minor* ponds. Mean zinc outflows were within the acceptable range for *L. minor* survival (Khellaf & Zerdaoui, 2009), as well as being lower than the threshold set of 2 mg/l for irrigation (Metcalf & Eddy, 2003). Mean outflow iron values (Figure 5.12b) were slightly higher than the mean inflow ones for all treatment systems, indicating low iron removal efficiency. This result of outflow iron values does not match with the outcome of the first phase (Figure 5.11b), where outflow values were slightly lower than the inflow values for most of the treatment systems. This is because after weekly doses iron increased gradually in the systems, due to the low reduction rate, to be higher during the second phase as it continued on from the first phase. No differences were found between the design variables for all systems ($p > 0.05$). The standard limit of iron for irrigation is 5 mg/l (Metcalf & Eddy, 2003), and all outflow values were compliant. Lower mean outflow values of potassium and sodium (Figures 5.12d and f, respectively) than the inflow ones were found for all ponds, and the outflow values were ranked as follows P4 > P2 > P1. However, the mean outflow values of magnesium and calcium were higher than the corresponding inflow values indicating accumulation of these elements in the system (Figures 5.12c and e, respectively). Figure 5.28 shows an overview of the concentration of elements accumulated in plant tissues for all ponds with and without dyes. The results of zinc in plants (Figure 5.28a) reflect the inflow and outflow zinc concentrations in treatment systems for all ponds, and therefore higher mean zinc is found in plant tissue for ponds comprising BR46, which is linked with high inflow values. The levels of zinc and iron in plants (Figures 5.28a and b) were higher than the permissible limits of 50 mg/kg and 20 mg/kg, respectively (Nazir et al., 2015). The BCF, Figures 5.29a and b, indicates that the plant was positive for phytoremediation of zinc and iron, respectively, for all samples. These results were mirrored for the zinc and iron concentrations in *L. minor* which are presented in Figures 5.28a and b, respectively.

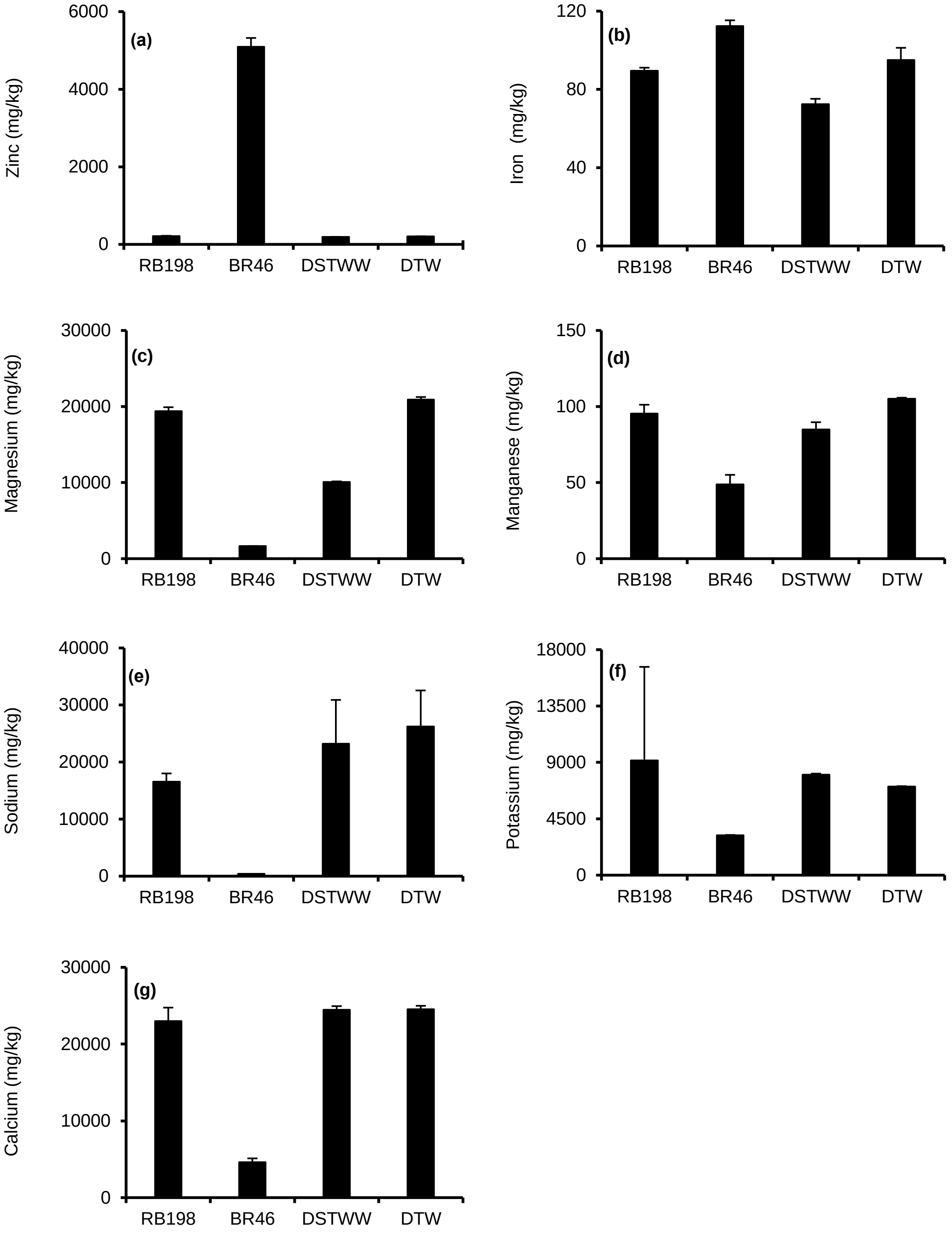


Figure 5.28 Mean concentrations of the detected elements in plant tissue harvested at the end of the experiment between 18 October 2016 and 30 June 2017. Note: (a), zinc; (b), iron; (c), magnesium; (d), manganese; (e), sodium; (f), potassium; (g), calcium; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

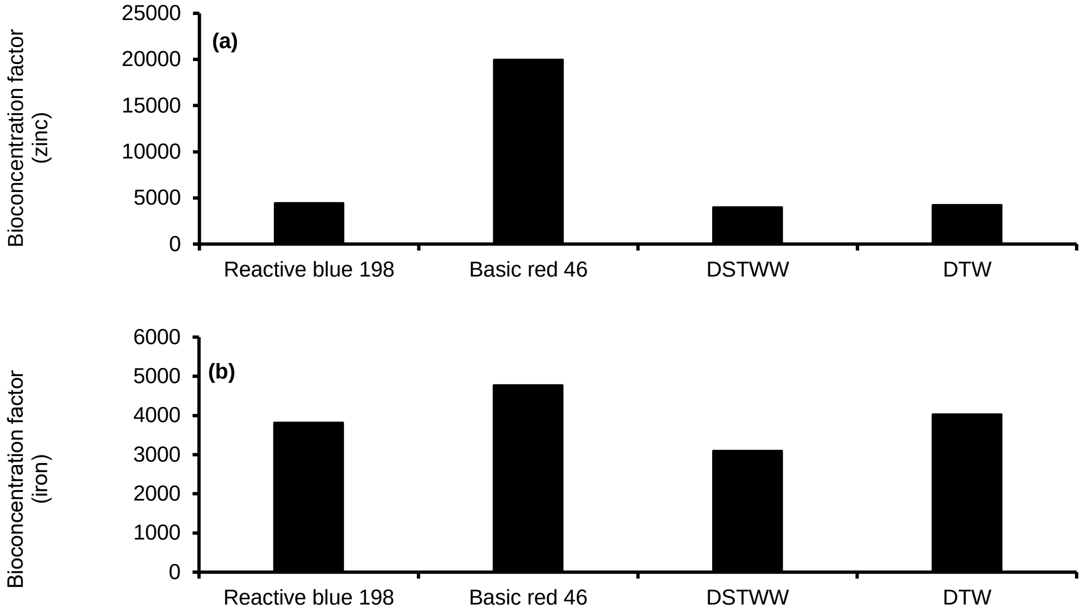


Figure 5.29 Bioconcentration factor during the experiment between 18 October 2016 and 30 June 2017. Note: (a), zinc; (b), iron; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

5.3.4 Plant monitoring

The overall average values of the relative growth rate during the experimental period based on the fresh weights are shown in Table 5.12. This growth factor has been used as a pointer for the toxicity of dyes and the diluted synthetic textile wastewater on *L. minor* growth. For the first phase between 20 January 2016 and 18 October 2017, results showed that the mean values of plant growth rate were higher in ponds comprising only dechlorinated tap water followed by wetlands treating diluted synthetic textile wastewater. Then came the ponds comprising diluted textile synthetic wastewater mixed with RB198, and finally ponds containing diluted synthetic textile wastewater mixed with BR46. Statistically, plant growth rate in wetlands comprising BR46 was significantly (one-way ANOVA, $p < 0.05$) lower than in those containing RB198, as well as in those without dye. These outcomes suggest that the synthetic wastewater used in this study reduced the growth of the plants but not the toxicity level, as in Section 5.2.3. In addition, the presence of dyes negatively impacts on the growth rate, which is especially the case for BR46, which has been successfully treated. The same findings were observed for BR46 at a concentration of 5 mg/l inhibiting *L. minor* growth (Chapter 4, Section 4.2.3). The impact of pH on the plant development was not significant for either dye, although the growth values were slightly higher in ponds receiving inflow of a pH value of 6 followed by the values at normal conditions. A lower growth rate was linked to ponds receiving inflow of a pH value of 9 for both studied dyes. This can be explained by the mean outflow pH values (Table 5.9). The findings matched those by Movafeghi et al. (2013) and Khataee et al. (2012), indicating that the optimum growth conditions for *L. minor* were at pH values between 6 and 7.5. At the end of the second phase, the overall growth rate was calculated according to the total experimental period between 20 January 2016 and 30 June 2017. The growth values were significantly lower than the growth rate within the first period. This could be attributed to the weekly doses accumulated in the system during this long period making the plant exhausted and over-saturated with nutrients. Especially in the second phase, the plants were not harvested monthly, because they suffered and did not spread over the surface area. Overall, the relative growth rates of the ponds containing dechlorinated tap water were higher than for the other ponds followed by ponds containing RB198 and ponds containing only diluted synthetic textile wastewater, which were not significantly (one-way ANOVA, $p > 0.05$) different from each other. Finally, a significantly (one-way ANOVA, $p < 0.05$) lower growth was observed in ponds comprising BR46. Note that, all plants in ponds treating BR46 died (Appendix F, Figure F.5) and decomposed. This was not due to the impact of aromatic amine, because all systems containing *L. minor* completely degraded the dyes, consequently decreasing the possible toxicity of the parent compound as shown by Sponza and Isık (2005). In addition, the plants in the first experiment (Chapter 4, Section 4.2.3) did not die after treatment of the same dye, indicating the aromatic amine was not the main reason for the plant death. Therefore, the expected reason for the plant death in ponds treating BR46 is due to the dye concentration of 10 mg/l or as a result of mixing this dye with the diluted synthetic textile wastewater.

Visual monitoring of the plants at the end of the experiment with the help of the Munsell (1977) chart showed that *L. minor* in ponds containing tap water was associated with light green (2.5GY) and dark green (5GY) fronds. In comparison, ponds comprising diluted synthetic textile wastewater only and RB198 mixed with diluted synthetic textile wastewater showed that most of the fronds were linked to dark green (5GY). This can be ascribed to the lack of nutrients in ponds receiving only tap water, although a lack of nutrients did not affect the growth of *L. minor*. The photosynthetic process and the chlorophyll content are not affected considerably (Khataee et al., 2012), as the fronds remained green in colour with some variation of the degree of this colour among the ponds. Furthermore, the presence of white fronds in all ponds with and without dyes indicated the die-off of *L. minor* during its life cycle. For ponds comprising BR46 mixed with diluted synthetic textile wastewater, most of the plants turned a brown colour and only a low percentage of plants were associated with light green (2.5 GY), dark yellow (5 Y) and white colours.

Table 5.12 Overview of the relative growth rate of *Lemna minor* L. during the periods between 20 January 2016 and 18 October 2017, and 18 October 2016 and 30 June 2017

Treatment system	Relative growth rate (per day)			
	period between 20/1/2016 and 18/10/2017			period between 20/1/2016 and 30/6/2017
	pH of 6	Normal pH	pH of 9	pH of 7
Reactive Blue 198+DSTWW	0.01380	0.01378	0.01371	0.00713
Basic Red 46 +DSTWW	0.01174	0.01161	0.01137	0.00561
DSTWW	N/A	0.01414	N/A	0.00703
DTW	N/A	0.01492	N/A	0.00733

Note: DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water; N/A, not applicable.

5.3.5 Environmental conditions

The mean temperature was 25°C and 23°C for the first and second phase, respectively. The maximum and minimum values were 29°C and 21°C for the first phase, and 27°C and 19°C for the second phase in this order. Temperature records in this experiment did not affect the growth of plants in the system (Ozengin & Elmaci, 2007).

Regarding light intensity, the mean readings of light intensity were 6962 lux and 6853 lux for the first and second phase, respectively, which were within the acceptable range for plant growth (Yin et al., 2015). Table 5.13 provides an overview of laboratory conditions during the experimental period.

Table 5.13 Overview of environmental boundary conditions in the laboratory during the periods between 20 January 2016 and 18 October 2017, and 18 October 2016 and 30 June 2017

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	N
First phase between 20/1/2016 and 18/10/2017						
Temperature	°C	24.5	1.87	21.2	28.5	174
Temperature (minimum within 24 h)	°C	22.0	1.82	18.5	26.9	174
Temperature (maximum within 24 h)	°C	25.4	2.20	20.4	28.9	174

Table 5.13 (Continued)

Relative humidity	%	61.8	7.39	48.0	76.0	174
Relative humidity (minimum within 24 h)	%	58.9	8.99	41.0	74.0	174
Relative humidity (maximum within 24 h)	%	68.3	7.62	50.0	84.0	174
Illuminance (one-off records)	lux	6962.1	354.17	6220	7735	98
Second phase between18/10/2016 and 30/6/2017						
Temperature	°C	23.3	1.85	19.4	26.8	171
Temperature (minimum within 24 h)	°C	21.7	1.42	17.4	26.9	171
Temperature (maximum within 24 h)	°C	23.7	3.01	18.1	26.9	171
Relative humidity	%	64.0	4.05	51.0	73.0	171
Relative humidity (minimum within 24 h)	%	61.9	4.49	44.0	70.0	171
Relative humidity (maximum within 24 h)	%	69.9	5.47	53.0	80.0	171
Illuminance (one-off records)	lux	6853.5	382.90	6335	7722	94

Note: N, number of records; h, hours.

5.4 Chapter summary

- Pond systems effectively removed the dye BR46 at a concentration of 10 mg/l compared with the dyes AB113, RB198 and DO46.
- The potential of *L. minor* and algae for elimination of BR46 was around 31% and 25%, respectively, without any noticeable impacts of different pH values regarding the treatment performance.
- The removal of BR46 within ponds planted with *L. minor* and algae or only algae by phyto-transformation, bio-transformation and adsorption process, was significantly ($p < 0.05$) higher than the corresponding removal by the control ponds, which was due to bio-transformation and bio-sorption processes.
- *L. minor* ponds completely mineralise BR46 by removing the aromatic amines after dye decolourization compared with algae and control ponds, which consequently shows the effectiveness of *L. minor*.
- All outflow COD, NH_4–N, NO_3–N, SS, and TDS concentrations were below the standard discharge thresholds. Therefore, the treated effluents were suitable for safe discharge or reusable for other purposes.
- Synthetic textile wastewater used in this research was not suitable, and dilution of (1 part of artificial wastewater to 24 parts of raw water) was required for the treatment systems using *L. minor*, although the growth was inhibited.
- The outflow values of zinc and iron were below the thresholds set for irrigation purposes, and within the tolerated limits for plants.
- Dyes reduced the growth rate of *L. minor*. This was particularly the case for BR46.

Chapter 6
Assessment of System Performance for Treating Dye Mixtures

6.1 Overview

The overall results and discussions of the fourth experiments are documented in this chapter. The set-up and operational processes of this experiment are explained Chapter 3 (Section 3.4.5). Section 6.1 provides an overview of the chapter. Section 6.2 presents the evaluation results of the system performance for the treatment of diluted synthetic textile wastewater containing three mixtures of two dyes reactive blue 198 (RB198) and basic red 46 (BR46). The chapter summary is presented in Section 6.3.

6.2 Performance of *L. minor* ponds treating wastewater containing dye mixtures

6.2.1 Inflow water quality parameters

The characteristics of the inflow water used in this experiment are summarised in Table 6.1 and Figure 6.1. All mean inflow values, as well as the inflow dye concentrations of each mixture, were within the typical range of textile effluent characteristics (Ghaly et al., 2014), except for chemical oxygen demand (COD), suspended solids (SS) and total dissolved solids (TDS) concentrations, which were lower.

Table 6.1 Inflow water quality parameters for the experiment between 14 October 2016 and 27 June 2017

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Mixture 1: (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198)						
pH	-	7.3	0.11	7.1	7.5	34
Redox	mv	-41.9	5.42	-54.0	-33.0	34
Dissolved oxygen	mg/l	8.9	0.30	8.3	9.4	34
Electrical conductivity	µS/cm	109.9	14.20	88.9	128.0	34
Total dissolved solids	mg/l	55.0	7.10	44.5	64.0	34
Suspended solids	mg/l	2.3	0.76	1.0	3.0	34

Table 6.1 (Continued)

Turbidity	NTU	2.4	0.39	1.8	3.4	34
Colour	Pt Co	571.4	47.06	452.0	615.0	34
Absorbance	-	0.45	0.016	0.43	0.520	34
Chemical oxygen demand	mg/l	33.7	0.30	33.0	34.0	9
Ammonium-nitrogen	mg/l	0.24	0.021	0.22	0.288	9
Nitrate-nitrogen	mg/l	0.58	0.027	0.52	0.610	9
Ortho-phosphate-phosphorus	mg/l	1.71	0.119	1.50	1.940	9
Mixture 2: (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198)						
pH	-	7.2	0.09	7.1	7.4	34
Redox	mv	-41.8	5.70	-50.0	-32.0	34
Dissolved oxygen	mg/l	8.8	0.29	8.2	9.3	34
Electrical conductivity	µS/cm	112.4	15.47	85.5	131.9	34
Total dissolved solids	mg/l	56.2	7.73	42.8	66.0	34
Suspended solids	mg/l	3.0	1.22	2.0	5.0	34
Turbidity	NTU	3.1	0.56	2.1	3.6	34
Colour	Pt Co	254.7	17.76	220.0	288.0	34
Absorbance	-	0.22	0.022	0.12	0.251	34
Chemical oxygen demand	mg/l	29.2	0.35	28.8	30.0	9
Ammonium-nitrogen	mg/l	0.19	0.013	0.17	0.22	9
Nitrate-nitrogen	mg/l	0.48	0.027	0.44	0.53	9
Ortho-phosphate-phosphorus	mg/l	1.59	0.052	1.48	1.65	9
Mixture 3: (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198)						
pH	-	7.2	0.08	7.1	7.3	34
Redox	mv	-39.3	4.77	-46.0	-32.0	34
Dissolved oxygen	mg/l	8.9	0.37	8.2	9.5	34
Electrical conductivity	µS/cm	112.1	15.54	86.6	132.1	34
Total dissolved solids	mg/l	56.1	7.77	43.3	66.1	34
Suspended solids	mg/l	2.8	0.86	2.0	4.0	34
Turbidity	NTU	3.0	0.57	2.1	3.6	34
Colour	Pt Co	411.5	37.33	338.0	469.0	34
Absorbance	-	0.32	0.032	0.21	0.391	34
Chemical oxygen demand	mg/l	31.8	0.35	31.2	32.5	9
Ammonium -nitrogen	mg/l	0.22	0.022	0.17	0.260	9
Nitrate-nitrogen	mg/l	0.53	0.025	0.49	0.580	9
Ortho-phosphate-phosphorus	mg/l	1.64	0.028	1.60	1.700	9
Diluted synthetic textile wastewater						
pH	-	7.1	0.07	7.0	7.3	31
Redox	mv	-26.2	7.16	-37.0	-18.0	34
Dissolved oxygen	mg/l	9.2	0.42	8.5	9.7	34
Electrical conductivity	µS/cm	89.9	1.34	88.5	95.4	34
Total dissolved solids	mg/l	45.0	0.67	44.3	47.7	34
Suspended solids	mg/l	1.5	0.50	1.0	2.0	34
Turbidity	NTU	1.6	0.43	1.0	2.1	34
Colour	Pt Co	8.0	1.71	6.0	12.0	34
Chemical oxygen demand	mg/l	18.5	0.16	18.1	18.7	9
Ammonium -nitrogen	mg/l	0.16	0.004	0.16	0.17	9
Nitrate-nitrogen	mg/l	0.41	0.020	0.38	0.44	9
Ortho-phosphate-phosphorus	mg/l	1.19	0.038	1.11	1.25	9

Note: NTU, nephelometric turbidity unit.

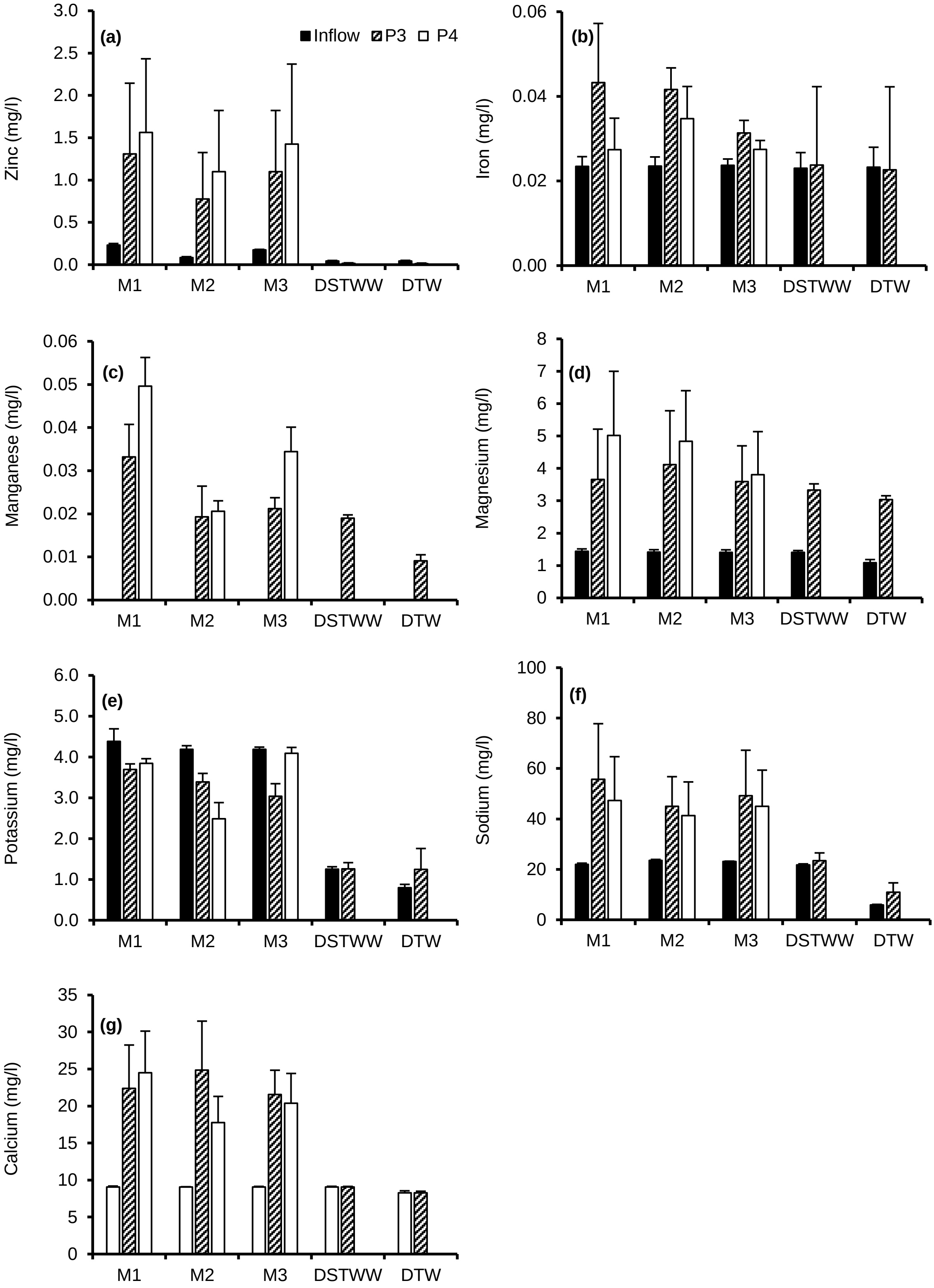


Figure 6.1 Mean inflow and outflow concentrations of the detected trace elements during the experiment between 14 October 2016 and 27 June 2017. Note: (a), zinc; (b), iron; (c), manganese; (d), magnesium; (e), potassium; (f), sodium; (g), calcium; M1, mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); M2, mixture 2, (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); M3, mixture 3, (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198); DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water; P3, *Lemna minor* L. ponds; P4, control ponds.

6.2.2 Treatment performance

6.2.2.1 Dye and apparent colour

Figure 6.2 shows the mean removal efficiency of dye mixtures during the experiment period between 14 October 2016 and 27 June 2017, and Table 6.2 provides an overview of the statistical analysis between the treatment systems. Findings showed that the mean removal efficiency of each mixture in *L. minor* ponds was significantly (Table 6.2) higher than unplanted ponds (Figure 6.2). This indicates the high influence of *L. minor* in enhancing the potential of dye removal within the ponds. The mean removal values of dye mixtures were as follows: mixture one > mixture three > mixture two for both *L. minor* and control ponds. Significant differences were found among all *L. minor* ponds (Kruskal-Wallis, $p < 0.05$). However, control ponds showed that the removal values of mixture two were significantly lower than the corresponding removals of mixtures one and three (Kruskal-Wallis, $p < 0.05$), which may due to the presence of a high percentage of non-biodegradable dye RB198 in this mixture.

The results of mixed dye elimination are attributed to the impact of dye BR46 removal in each solution. This is because BR46 exhibited a high percentage of degradation by *L. minor* ponds of around 85% in previous experiments (Chapter 5, Section 5.2.2.1, Figure 5.2) and 64% (Chapter 5, Section 5.3.2.1, Figure 5.22) at concentrations of 10 mg/l, and 69% (Chapter 4, Section 4.2.2.1, Table 4.2) at concentrations of 5 mg/l compared with very low or negligible removal linked to the dye RB198. Therefore, mixture one, which contained 80% BR46, displayed higher removal than mixture three, which had 50% BR46, whereas lower removal was found in mixture two comprising only 20% BR46. This suggests that the removal levels by pond systems are stable. However, high loads of BR46 led to achieving high percentages of degradation. This observation matched findings by Davies et al. (2006). The authors noticed that although the removal rate of the system was constant, the percentage of dye degradation enhanced when the loading rate of the dyes was increasing. By comparing the mean removal of dye mixtures achieved in this experiment (Figure 6.2) with the individual dye BR46 removal values, as mentioned above, the results clearly showed that RB198, which is recalcitrant to degrade, had an adverse impact on the removal efficiency of the mixture. The inflow and outflow absorbance values (Table 6.1 and 6.3) reflect the mixed dye elimination findings. The longitudinal profiles and trends of the mixed dye removals are shown in Figure 6.3 highlighting that the removal values were low and fluctuated during the first period of the experiment. This may be explained by considering the first period as an acclimatisation stage for the plants and organisms within the inflows, which contained mixtures of two dyes and synthetic wastewater chemicals. Note that since the amount of plants was limited during the first period, according to the set-up design, sufficient time would have been required for them to grow and regenerate due to the shock received from the dye mixture dosages. However, higher removal was achieved in planted ponds between 17 January 2017 and 18 April 2017, which was possible because the biomass of *L. minor* reached a high level. Then, the plants started to become saturated with dyes, except for the plants within mixture two. This consequently led to a noticeable reduction in the removal by *L. minor* ponds, especially ponds treating mixture one. The maximum and minimum mean removal values were as follows: for ponds treating mixture one: 66% and 38% for *L. minor* ponds, and 35% and 13% for control ponds; for ponds comprising mixture two: 33% and 20% for *L. minor* ponds, and 27% and -5% for control ponds, respectively; and for ponds containing mixture three: 47% and 31% for *L. minor* ponds, and 32% and 13% for control ponds, in that order. Negative removal values were found in control ponds treating mixture two, which contained a high percentage of RB198. This was attributed to dried RB198 continuously attaching itself to the walls of the pond sides. The findings of dye mixture removal confirmed the low removal of RB198 and high degradation of BR46. The main causes of high BR46 removal are the absence of sulpho groups, low molecular weight and the simple chemical structure compared to RB198, as mentioned previously.

Ultraviolet visible scans showed that the maximum adsorption wavelength of each separate dye was 625 and 530 nm for RB198 (blue inflow colour) and BR46 (red inflow colour), respectively (Chapter 3, Table 3.1). However, the inflow mixtures, after dilution, showed maximum absorbances at wavelengths of 528, 524 and 524 nm with dark red, purple and red inflow colours for mixtures one, two and three, respectively (Figure 6.4). This was due to the interference between dyes mixed together. The outflow samples illustrated a reduction of the dye intensity for control ponds, which was due to dye adsorption by microbes in the system, as well as biotransformation. However, the intensity dropped in *L. minor* ponds for all mixtures. This was confirmed by the higher decolourisation of each mixture in *L. minor* ponds compared to the control ones (Figure 6.4). The peaks for the outflow samples in this study did not completely disappear, which indicated that particularly the dye RB198 was difficult to biologically degrade. The inflow peaks shifted in the decolourised samples to be at 282, 277 and 277 nm for mixtures one, two and three, in this order. These changes probably resulted from the metabolites or degradation of the dye (BR46). Similar results and explanations have been reported by Chen et al. (2003).

The HPLC chromatogram analysis for dye mixtures before treatment showed two minor peaks besides the main peak at 3.385 min for mixture one (Appendix E, Figure E.18a). However, the outflow samples for *L. minor* ponds showed that the main peak disappeared and new major peaks appeared at 1.88 and 2.58 min besides several minor peaks (Appendix E, Figure E.19a); whereas the control ponds showed only appearances of new major peaks at 1.543 and 2.101 min (Appendix E, Figure E.19b). Regarding the second mixture, the inflow samples presented major peaks at 2.175 min with minor peaks at 1.49 and 1.9 min (Appendix E, Figure E.18b). The outflow samples of *L. minor* ponds showed that the inflow peaks disappeared with appearances of major peaks at 2.155 min in addition to several minor peaks (Appendix E, Figure E.20a). In comparison, the control ponds had major peaks at 1.885 min and minor peaks at 2.714 and 2.37 min (Appendix E, Figure E.20b). Finally, the third mixture showed major peaks for inflow samples at 2.813 min and two other minor peaks (Appendix E, Figure E.18c). However, the outflow samples for *L. minor* ponds were linked to a major peak at 1.7 min and a minor one at 2.216 min (Appendix E, Figure E.21a). The control ponds had a major peak at 2.207 min and other minor ones (Appendix E, Figure E.21b). The variation between the inflow and outflow samples concerning the presence of new peaks can be explained by the formation of different products during molecule transformation and consequently decolourisation (Joshi et al., 2010). These changes occurred for both *L. minor* and control ponds due to dye BR46 degradation, although the mechanism and percentage of elimination was different. As a result, the mechanism of removal was due to phytotransformation with adsorption and/or accumulation by plants (Kabra et al., 2012; Khandare et al., 2011) besides the microbial impact in *L. minor* ponds, such as biotransformation and bio-sorption. However, the control pond potential was due to microbial bio-sorption, as well as by the biotransformation process due to the production of aromatic amine in these control ponds, as indicated below.

The results of GCMS analysis related to mixture one showed that the treated samples of *L. minor* ponds did not contain aromatic amines. This indicates that the cleavage products completely mineralised. Although aromatic amines are toxic to plants and organisms, Sponza and Isik (2005) confirmed that toxicity effects were eliminated during the decolourisation process, being followed by complete mineralisation. However, the control ponds provide outflows containing one trace peak for N-(4-methylphenyl)-benzenemethanamine (CAS: 5405-15-2). The concentration of this amine was below 10 mg/l, which was the detection limit of the machine. This aromatic compound was considered as a non-hazardous material. However, as an environmental precaution, discharge of this compound should be avoided. Toxicological and ecological properties were not evaluated for this type of aromatic amine. This result indicates that the wetland systems without plants were unable to achieve complete mineralisation.

Regarding apparent colour monitoring (Table 6.3), the average outflow colour numbers for both planted and control ponds were lower than the corresponding inflow numbers (Table 6.1) for all mixtures. In addition, all outflow colour values in the *L. minor* ponds were very significantly lower (Table 6.2) than the control ones. This reflects the significant removal achieved for dye mixtures in *L. minor* ponds compared with the control ones, which consequently reduced the apparent colour in the planted system. The colour of inflow samples was dark red, purple and red for mixtures one, two and three, respectively. However, the outflow samples were rather colourless for *L. minor* ponds, and light pink for control ponds containing mixture one. Blue and dark blue outflows were found in *L. minor* and control ponds treating mixture two, and, finally, light blue and blue outflows were recorded for *L. minor* and control ponds treating mixture three. The colour parameter of ponds without dyes showed a significantly (Kruskal-Wallis, $p < 0.05$) reduced outflow colour compared to ponds containing dyes. Inflow and outflow sample pictures are shown in Appendix F, Figure F.6.

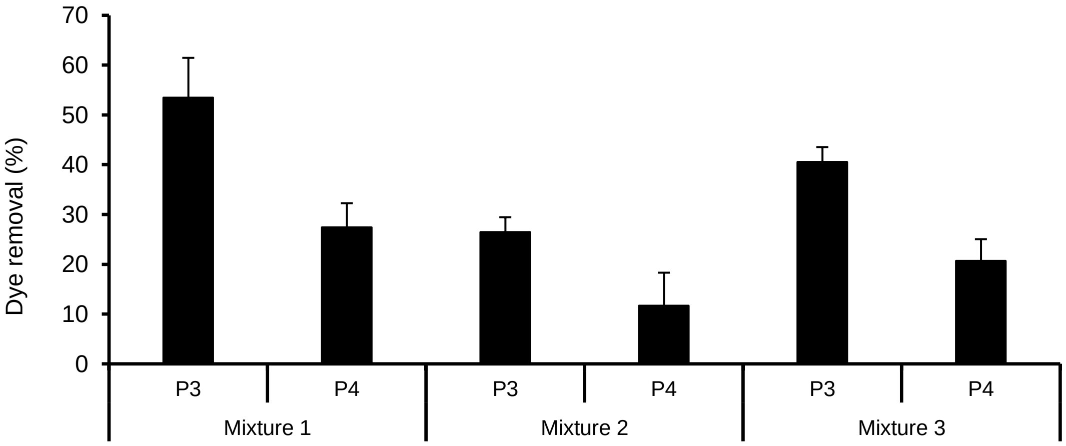


Figure 6.2 Mean removal efficiency of dye mixtures during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198.

Table 6.2 Overview of the statistical analysis for outflow water quality parameters and corresponding removal efficiencies for each system between 14 October 2016 and 27 June 2017

Parameter	Shapiro-Wilk test (p value[1])	p value[2]	Parameter	Shapiro-Wilk test (p value[1])	p value[2]
Dye removal (%)			Colour (Pt Co)		
Mixture 1	0.00	0.00	Mixture 1	0.01	0.00
Mixture 2	0.00	0.00	Mixture 2	0.00	0.00
Mixture 3	0.00	0.00	Mixture 3	0.07	0.00
Chemical oxygen demand (mg/l)			Chemical oxygen demand removal (%)		
Mixture 1	0.05	0.55	Mixture 1	0.15	0.16
Mixture 2	0.28	0.63	Mixture 2	0.07	0.12
Mixture 3	0.24	0.77	Mixture 3	0.39	0.19
Ammonium-nitrogen (mg/l)			Ammonium-nitrogen removal (%)		
Mixture 1	0.38	0.08	Mixture 1	0.08	0.04
Mixture 2	0.00	0.01	Mixture 2	0.55	0.03
Mixture 3	0.00	0.10	Mixture 3	0.32	0.01
Nitrate-nitrogen (mg/l)			Nitrate-nitrogen removal (%)		
Mixture 1	0.00	0.00	Mixture 1	0.00	0.00
Mixture 2	0.04	0.00	Mixture 2	0.06	0.00
Mixture 3	0.03	0.01	Mixture 3	0.03	0.01
Ortho-phosphate-phosphorus (mg/l)			Ortho-phosphate-phosphorus removal (%)		
Mixture 1	0.15	0.09	Mixture 1	0.88	0.09
Mixture 2	0.99	0.00	Mixture 2	0.83	0.04
Mixture 3	0.16	0.01	Mixture 3	0.73	0.00
Suspended solids (mg/l)			Turbidity (NTU)		
Mixture 1	0.01	0.00	Mixture 1	0.00	0.02
Mixture 2	0.00	0.00	Mixture 2	0.00	0.00
Mixture 3	0.00	0.02	Mixture 3	0.00	0.01
Dissolved oxygen (mg/l)			pH (-)		
Mixture 1	0.11	0.89	Mixture 1	0.03	0.10
Mixture 2	0.13	0.83	Mixture 2	0.53	0.45
Mixture 3	0.05	0.71	Mixture 3	0.15	0.06
Electrical conductivity (µS/cm)			Turbidity (NTU)		
Mixture 1	0.00	0.08	Mixture 1	0.06	0.11
Mixture 2	0.00	0.00	Mixture 2	0.27	0.49
Mixture 3	0.00	0.04	Mixture 3	0.01	0.09

Note: [1]Test of normality (if $p > 0.05$, data are normally distributed and the test used is independent samples t-test; if $p < 0.05$, data are not normally distributed and the test used is Mann-Whitney U test); [2]p value, probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different; if $p < 0.05$; the variables are statistically significantly different); NTU, nephelometric turbidity unit; Mixture 1, (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); Mixture 2, (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); Mixture 3, (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198).

Table 6.3 Outflow water quality parameters for the experiment between 14 October 2016 and 27 June 2017

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Lemna minor L. ponds containing mixture 1						
pH	-	7.4	0.12	7.3	7.7	34
Redox	mv	-54.9	7.10	-65.5	-44.0	34
Dissolved oxygen	mg/l	8.5	0.18	8.1	8.8	34
Electrical conductivity	µS/cm	115.8	11.99	101.1	144.4	34
Total dissolved solids	mg/l	57.9	6.00	50.6	72.2	34
Suspended solids	mg/l	5.1	1.38	2.5	8.5	34
Turbidity	NTU	4.3	1.49	2.9	9.2	34
Colour	Pt Co	277.0	81.66	156.0	413.5	34
Absorbance	-	0.14	0.045	0.09	0.30	34
Chemical oxygen demand	mg/l	12.13	2.232	8.91	15.00	9
Ammonium-nitrogen	mg/l	0.06	0.027	0.03	0.10	9
Nitrate-nitrogen	mg/l	0.21	0.023	0.17	0.25	9
Ortho-phosphate-phosphorus	mg/l	2.00	0.373	1.30	2.64	9
Control ponds containing mixture 1						
pH	-	7.5	0.17	7.2	7.9	34
Redox	mv	-58.5	10.61	-80.5	-40.0	34
Dissolved oxygen	mg/l	8.5	0.17	8.0	8.9	34
Electrical conductivity	µS/cm	117.5	17.89	64.6	149.4	34
Total dissolved solids	mg/l	58.8	8.95	32.3	74.7	34
Suspended solids	mg/l	3.2	1.82	1.0	8.0	34
Turbidity	NTU	3.4	0.38	2.6	4.3	34
Colour	Pt Co	447.6	124.00	213.5	644.5	34
Absorbance	-	0.29	0.057	0.22	0.43	34
Chemical oxygen demand	mg/l	13.10	3.506	9.66	21.00	9
Ammonium-nitrogen	mg/l	0.10	0.041	0.03	0.17	9
Nitrate-nitrogen	mg/l	0.32	0.089	0.24	0.49	9
Ortho-phosphate-phosphorus	mg/l	2.41	0.455	2.00	3.49	9
Lemna minor L. ponds containing mixture 2						
pH	-	7.5	0.13	7.1	7.7	34
Redox	mv	-54.9	7.58	-69.0	-36.5	34
Dissolved oxygen	mg/l	8.5	0.14	8.2	8.9	34
Electrical conductivity	µS/cm	114.8	14.57	99.3	146.9	34
Total dissolved solids	mg/l	57.4	7.29	49.6	73.5	34
Suspended solids	mg/l	5.3	1.74	3.0	9.5	34
Turbidity	NTU	4.9	1.75	3.1	8.8	34
Colour	Pt Co	151.0	26.85	70.0	193.0	34
Absorbance	-	0.13	0.020	0.10	0.22	34
Chemical oxygen demand	mg/l	10.80	1.995	8.11	15.10	9
Ammonium-nitrogen	mg/l	0.06	0.014	0.04	0.08	9
Nitrate-nitrogen	mg/l	0.16	0.029	0.11	0.20	9
Ortho-phosphate-phosphorus	mg/l	1.62	0.249	1.15	2.00	9
Control ponds containing mixture 2						
pH	-	7.5	0.11	7.2	7.7	34

Table 6.3 (Continued)

Redox	mv	-56.1	6.26	-66.0	-40.0	34
Dissolved oxygen	mg/l	8.5	0.14	8.2	8.8	34
Electrical conductivity	µS/cm	121.5	13.71	105.4	152.0	34
Total dissolved solids	mg/l	60.8	6.86	52.7	76.0	34
Suspended solids	mg/l	3.4	1.09	2.0	5.5	34
Turbidity	NTU	3.3	0.45	2.4	4.1	34
Colour	Pt Co	210.9	46.68	115.5	362.5	34
Absorbance	-	0.21	0.043	0.13	0.35	34
Chemical oxygen demand	mg/l	11.40	2.458	8.18	16.00	9
Ammonium-nitrogen	mg/l	0.13	0.064	0.04	0.27	9
Nitrate-nitrogen	mg/l	0.24	0.055	0.20	0.38	9
Ortho-phosphate-phosphorus	mg/l	2.16	0.309	1.55	2.61	9
Lemna minor L. ponds containing mixture 3						
pH	-	7.4	0.14	7.2	7.6	34
Redox	mv	-54.6	7.68	-65.5	-42.5	34
Dissolved oxygen	mg/l	8.4	0.14	8.2	8.8	34
Electrical conductivity	µS/cm	115.1	13.12	100.8	144.6	34
Total dissolved solids	mg/l	57.5	6.56	50.4	72.3	34
Suspended solids	mg/l	4.4	1.69	2.0	8.0	34
Turbidity	NTU	4.1	1.22	2.8	6.8	34
Colour	Pt Co	199.7	49.28	92.5	270.0	34
Absorbance	-	0.14	0.023	0.10	0.21	34
Chemical oxygen demand	mg/l	11.66	3.378	6.61	18.00	9
Ammonium-nitrogen	mg/l	0.05	0.014	0.02	0.07	9
Nitrate-nitrogen	mg/l	0.13	0.043	0.10	0.23	9
Ortho-phosphate-phosphorus	mg/l	1.78	0.309	1.23	2.10	9
Control ponds containing mixture 3						
pH	-	7.5	0.12	7.3	7.7	34
Redox	mv	-57.4	7.10	-68.5	-44.0	34
Dissolved oxygen	mg/l	8.5	0.15	8.2	8.8	34
Electrical conductivity	µS/cm	119.4	12.85	103.0	149.0	34
Total dissolved solids	mg/l	59.7	6.43	51.5	74.5	34
Suspended solids	mg/l	3.3	1.16	1.5	5.5	34
Turbidity	NTU	3.3	0.39	2.8	4.2	34
Colour	Pt Co	327.7	80.11	118.0	446.5	34
Absorbance	-	0.23	0.031	0.16	0.28	34
Chemical oxygen demand	mg/l	12.19	3.208	7.73	18.50	9
Ammonium-nitrogen	mg/l	0.09	0.058	0.04	0.23	9
Nitrate-nitrogen	mg/l	0.21	0.062	0.14	0.31	9
Ortho-phosphate-phosphorus	mg/l	2.19	0.388	1.62	3.08	9
Lemna minor L. ponds containing diluted synthetic textile wastewater						
pH	-	7.5	0.14	7.2	7.8	34
Redox	mv	-60.3	8.87	-74.5	-40.0	34
Dissolved oxygen	mg/l	8.3	0.76	4.5	8.9	34
Electrical conductivity	µS/cm	110.6	14.98	79.8	141.5	34
Total dissolved solids	mg/l	55.3	7.49	39.9	70.8	34
Suspended solids	mg/l	3.9	2.50	1.0	9.0	34

Table 6.3 (Continued)

Turbidity	NTU	3.8	0.83	2.6	5.9	34
Colour	Pt Co	32.0	17.86	7.0	88.0	34
Chemical oxygen demand	mg/l	9.60	1.043	8.20	11.70	9
Ammonium-nitrogen	mg/l	0.04	0.018	0.02	0.07	9
Nitrate-nitrogen	mg/l	0.06	0.075	0.02	0.26	9
Ortho-phosphate-phosphorus	mg/l	0.87	0.403	0.24	1.71	9
Lemna minor L. ponds containing dechlorinated tap water						
pH	-	7.4	0.13	7.2	7.7	34
Redox	mv	-54.4	7.14	-66.0	-40.0	34
Dissolved oxygen	mg/l	8.4	0.16	8.2	8.9	34
Electrical conductivity	µS/cm	93.1	8.98	77.0	107.5	34
Total dissolved solids	mg/l	46.6	4.49	38.5	53.8	34
Suspended solids	mg/l	3.0	1.71	1.0	6.5	34
Turbidity	NTU	3.5	0.68	2.6	5.4	34
Colour	Pt Co	27.1	11.27	2.0	45.0	34
Chemical oxygen demand	mg/l	4.74	0.840	3.30	6.20	9
Ammonium-nitrogen	mg/l	0.02	0.004	0.01	0.03	9
Nitrate-nitrogen	mg/l	0.05	0.049	0.03	0.18	9
Ortho-phosphate-phosphorus	mg/l	0.40	0.289	0.11	0.87	9

Note: NTU, nephelometric turbidity unit; mixture 1, (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); mixture 2, (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); mixture 3, (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198).

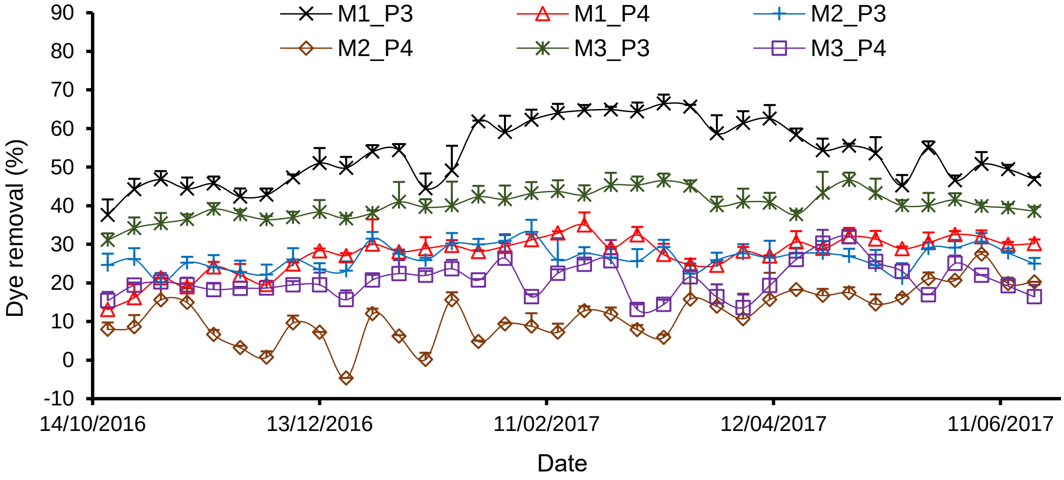


Figure 6.3 Mean values of dye mixture removal profiles during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; M1, mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); M2, Mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); M3, Mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198).

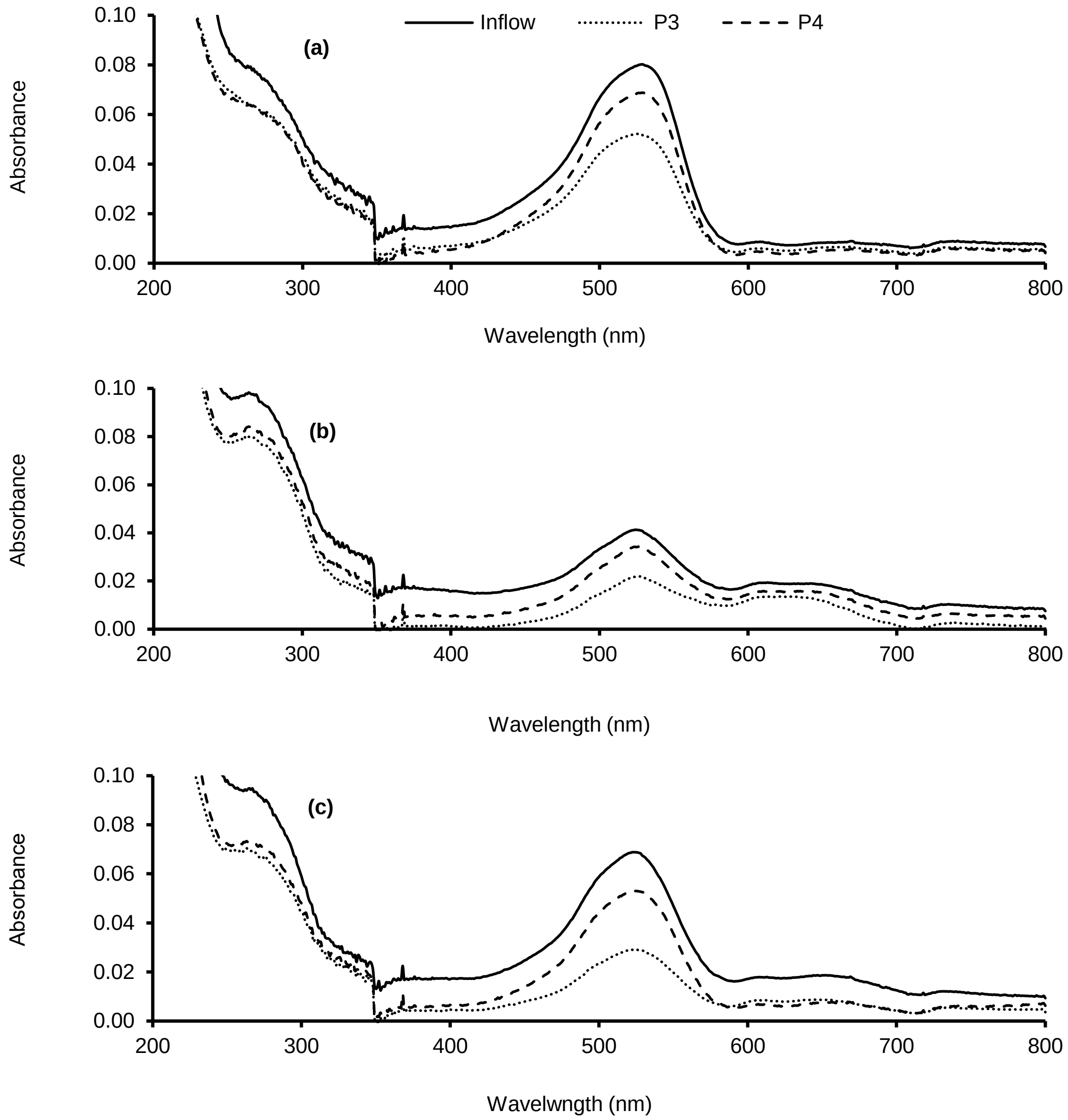


Figure 6.4 Ultraviolet visible analysis for the inflow and outflow samples at the end of the experiment, which was operated between 14 October 2016 and 27 June 2017. Note: (a), mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); (b), mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); (c), mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198); IF, inflow; P3, *Lemna minor* L. ponds; P4, control ponds.

6.2.2.2 Chemical oxygen demand and dissolved oxygen

Tables 6.1 and 6.3 show that the mean COD values after treatment were less than the average inflows. Moreover, all mean outflow COD values in *L. minor* wetlands were lower than the values for the control ponds. This has been confirmed by COD removal values (Figure 6.5), which were higher in *L. minor* ponds than the control ones. However, no significant (Table 6.2) differences were found in the case of COD mean outflow values and COD removal efficiencies. These results confirm the low impact of plants on COD degradation besides microbial activities. Similarly, a negligible impact of COD removal by reeds was concluded by Ong et al. (2009a). The removal of COD for all simulated ponds containing dyes was lower in comparison to those ponds without dyes (Figure 6.5). This may be attributed to the impact of incomplete organic molecule degradation for all dye mixtures. Comparable findings were reported by Sarayu et al. (2007) using ozonation for dye removal. The authors found that the presence of small molecules of untreated dyes has a considerable contribution to incomplete COD reduction. Common international standards state limits for COD of around 125 mg/l in the case of direct discharge. The results highlight that all COD outflow concentrations were below this limit.

Based on DO, all ponds with and without dyes showed lower outflow concentrations compared with the inflow ones (Table 6.1 and 6.3, respectively). The outflow concentrations were high and varied between 8.0 and 8.9 mg/l. No significant differences (Table 6.2) were found between *L. minor* and control ponds in terms of mean outflow DO values. These results indicate that the main source of oxygen in the system was atmospheric diffusion and the plants did not play a major role in enhancing the DO content.

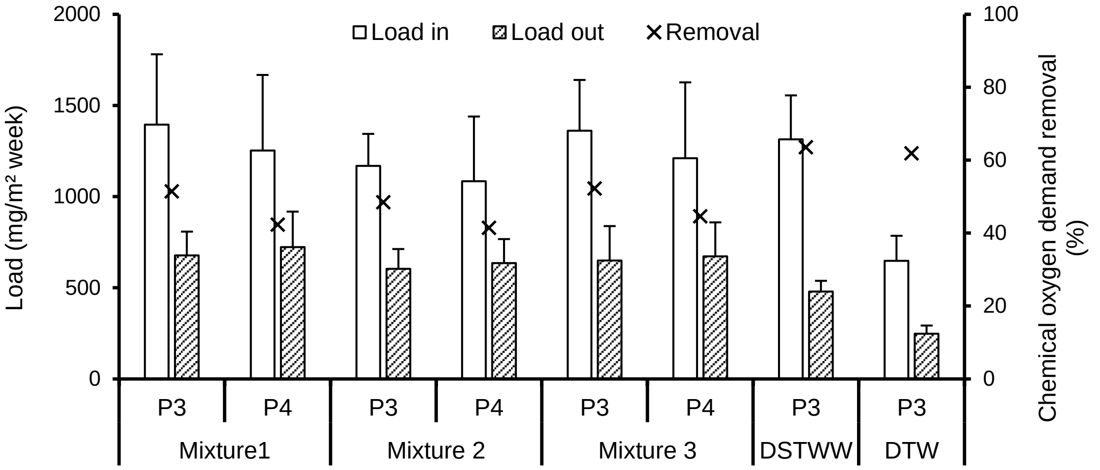


Figure 6.5 Mean chemical oxygen demand removal efficiency during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

6.2.2.3 pH and redox potential

Regarding pH, all inflow and outflow (Table 6.1 and 6.3, respectively) values were within the tolerable range for *L. minor* growth which is between 4.0 and 9.0, as mentioned by

Movafeghi et al. (2013), and for bacteria survival between 4.0 and 9.5 (Kadlec & Wallace, 2009). The mean pH outflow was slightly more than the mean inflow for all ponds, and all values were within the neutral range. These results confirmed that values of pH were within the optimum range, between 6 and 10, for high colour elimination (Saratale et al., 2011), although the exact amount of pH for higher removal is dependent on the dye itself (Yaseen & Scholz, 2016). No significant (Table 6.2) differences were found between vegetated and control ponds. The international lower and upper thresholds for pH are 6.5 and 8.5 for safe discharge to receiving watercourses. All outflow values were compliant.

The redox potential is used as an indicator for the presence of aerobic or anaerobic environments in wetlands (Ong et al., 2009a). Generally, minimum and maximum redox potential outflow values for all ponds were between -80.5 and -36.5 mV, respectively, which indicates that anoxic conditions dominate.

6.2.2.4 Suspended solids and turbidity

Concerning SS, all ponds with and without dyes showed high mean SS outflow values (Table 6.3), compared with the inflow concentrations (Table 6.1). This reflects that the COD and other organic substances degrade in addition to organic dye molecule degradation in the case of ponds containing dyes. Also, *L. minor* ponds showed that outflows were significantly (Table 6.2) higher than the corresponding concentrations for control ones. This can probably be attributed to the impact of *L. minor* and their die-off, which enhanced the organic load (Dalu & Ndamba, 2003), as well as the effect of higher COD removal, molecule degradation and aromatic amine mineralisation in planted ponds compared with unplanted ones. A typical international standard limit for SS is 35 mg/l; all outflow values of SS were lower than this limit.

The mean inflow and outflow turbidity values (Table 6.1 and 6.3, respectively) had the same trend as SS, and the planted ponds also showed an outflow turbidity significantly higher (Table 6.2) than the unplanted ones. Note that correlation analysis showed that SS was significant ($p < 0.01$) and correlated positively ($r = 0.281$, $p = 0.000$) with turbidity.

6.2.2.5 Electrical conductivity and total dissolved solids

Based on EC (Tables 6.1 and 6.3), all mean outflow numbers were a little higher than the corresponding influent values. *L. minor* ponds showed lower EC values than the control ones, which confirms that *L. minor* has a good ability in reducing the EC. These results are similar to the findings in previous sections for treating the dyes individually. However, the differences were significant (Table 6.2) in terms of *L. minor* compared with control ponds regarding EC outflows found in the case of mixtures two and three. This was perhaps due to the state of *L. minor* in the system, with plants being healthier in ponds treating mixture two followed by mixture three, compared with the plants within mixture one.

Regarding TDS values (Tables 6.1 and 6.3), the inflow and outflow concentrations of TDS in each mixture mirrored the corresponding inflow and outflow EC values. This is because TDS concentrations were equal to half of the EC values, as mentioned in earlier sections. According to common international standards, the results showed that all outflow TDS concentrations were less than the thresholds of 3000 mg/l and 500 mg/l (Carmen & Daniela, 2012).

6.2.2.6 Nutrients

Regarding ammonium-nitrogen (NH_4–N) and nitrate-nitrogen (NO_3–N), all mean outflow values (Table 6.3) were lower, compared with the corresponding inflow concentrations (Table 6.1), indicating a reduction of these two compounds in the system. Also, control ponds showed higher outflow concentrations than the corresponding values associated with *L. minor* ponds. This was attributed to the significant (Table 6.2) removal of NH_4–N and NO_3–N in *L. minor* ponds compared to the corresponding removal related to control wetlands (Figures 6.6 and 6.7, respectively). Ong et al. (2009a) highlighted that the effect of vegetation on nitrogen reduction in wetland systems is not evident in all applications and depends on the type of plants and operational period as well as wastewater characteristics. However, the results of this study confirmed a considerable impact of the plants for take-up of nitrogen, which may due to the low loading rate of nitrogen in the system. These results are confirmed by Bragato et al. (2006) who found that wetland plants potentially sequester nutrients from wastewater to their roots and/or shoots, and, as a result, they remediate these pollutants due to the rapid growth and biomass production of these macrophytes. Similarly, Selvarani et al. (2015) elucidated that *L. minor* has a vital role for nitrogen reduction in pond systems. In addition to the plants, nitrification and denitrification processes are also responsible for NH_4–N and NO_3–N reductions, respectively. Temperature and pH values in addition to the high concentrations of oxygen in all ponds were suitable for enhancing the nitrification level by nitrifying bacteria (Kadlec et al., 2000; Ozengin & Elamic, 2007). Nitrogen reduction of around 4% can be attributed to nitrification and denitrification occurring on micro-sites of the biofilm attached to *L.* minor (Zimmo, 2003). The mean reduction values of NO_3–N were not as high as those for NH_4–N, which may either be because of the plants using NO_3–N after assimilation of ammonia as a second source for nutrients or because the environmental conditions within the ponds were not suitable for high denitrification to occur, for example due to low carbon source, which is normally lacking at the end of nitrification process (Mohammed, 2017). In addition, the high level of nitrification linked with oxygen availability reflects the increase of NO_3–N concentration in the pond systems (Vymazal, 2007). The typical international limits for NH_4–N and NO_3–N regarding secondary treatment of effluent are 20 mg/l and 50 mg/l, in this order, as discussed by Al-Isawi et al. (2017). All outflow values of NH_4–N and NO_3–N were less than the corresponding standard thresholds.

Concerning PO_4–P, higher mean outflow values (Table 6.3) were found, compared to the corresponding inflow records (Table 6.1). Also, the mean outflow PO_4–P concentrations were significantly lower (Table 6.2) in *L. minor* ponds compared to the ponds without plants, reflecting better removal of PO_4–P in ponds containing *L. minor* than the control ones, although the system showed low overall reductions for all ponds (Figure 6.8). This outcome is similar to that of other authors: wetland systems are relatively ineffective in phosphorus removal (Vymazal, 2007; Al-Isawi et al., 2017). Significant removal (Table 6.2) of PO_4–P between *L. minor* and control ponds was noticed in ponds treating mixtures two and three. This may be due to the state of *L. minor* plants in ponds containing mixture one, which were not well compared to the other ponds, making their ability to assimilate phosphorus not effective. The expected mechanisms for PO_4–P removal, except for chemical precipitation, were uptake by plants, especially mixtures two and three, and via microbes in planted ponds (Vanitha et al., 2013). However, only biological processes associated with microbial uptake were possible in control ponds. A common international limit for PO_4–P outflow is 1 mg/l, as indicated by Sani et al. (2013). In this study, all outflow values for ponds containing dyes were higher than this standard value.

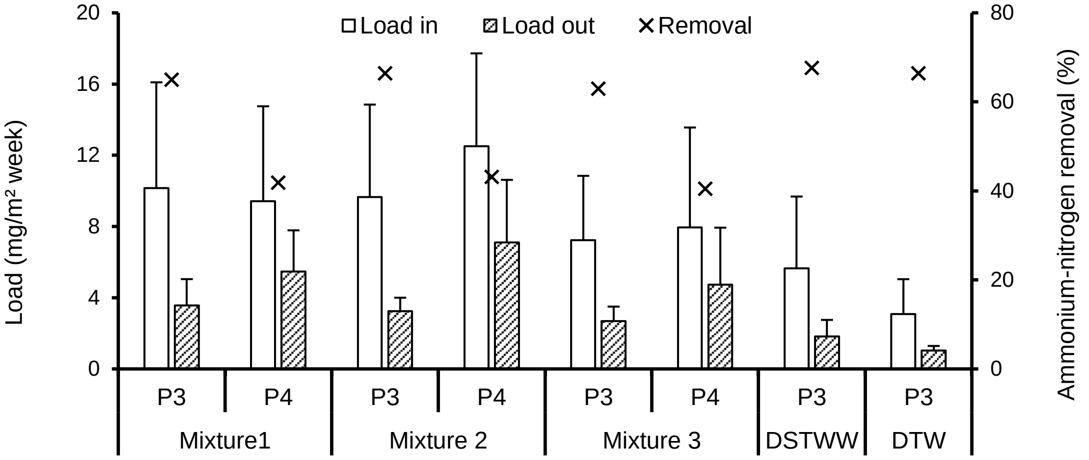


Figure 6.6 Mean ammonium-nitrogen removal efficiency during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

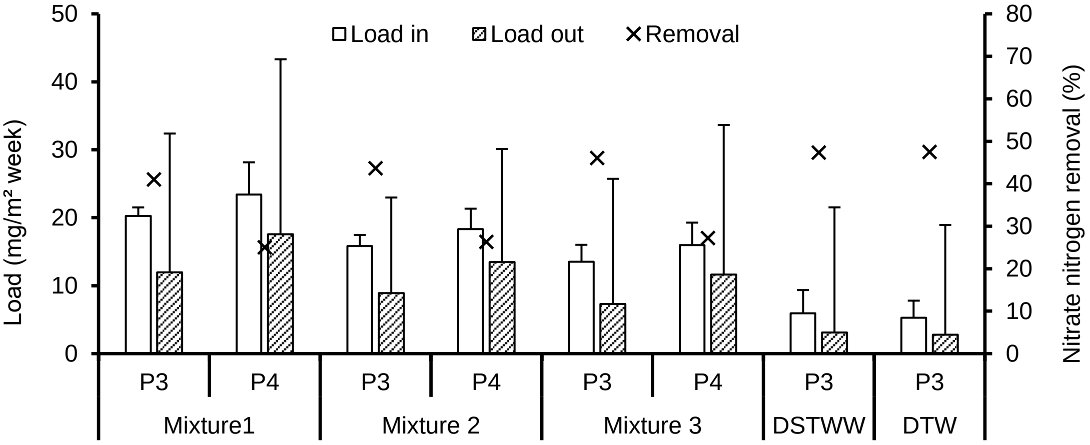


Figure 6.7 Mean nitrate-nitrogen removal efficiency during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

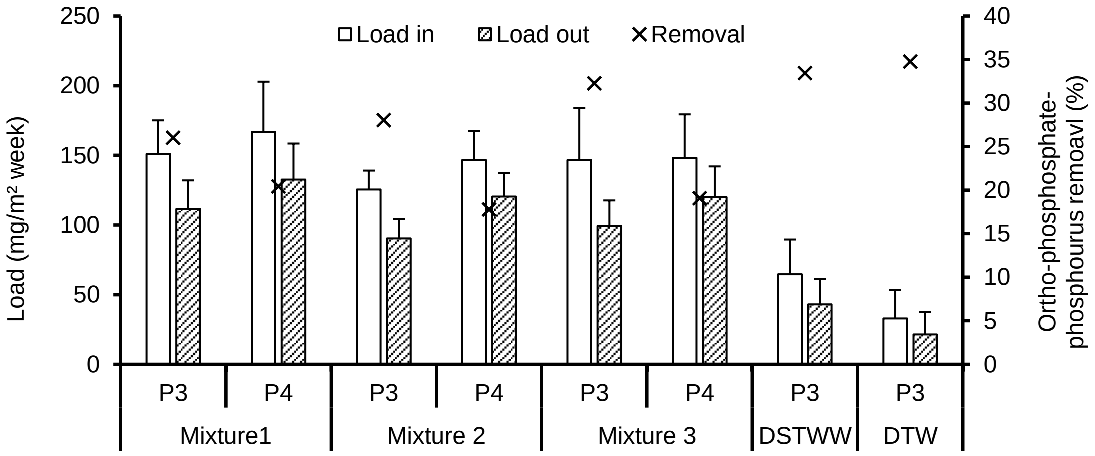


Figure 6.8 Mean ortho-phosphate-phosphorus removal efficiency during the experiment between 14 October 2016 and 27 June 2017. Note: P3, *Lemna minor* L. ponds; P4, control ponds; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

6.2.2.7 Trace elements

Mean outflow zinc and iron values (Figures 6.1a and b, respectively) were higher than the corresponding inflow concentrations for both *L. minor* and control ponds. Ponds containing *L. minor* showed lower zinc outflows than the control ones reflecting zinc uptake by plants, although no significant (t-test, $p > 0.05$) differences were found for any mixtures. All zinc outflows were lower than the values that cause growth reductions in *L. minor*, between 0.5 and 15 mg/l (Khellaf & Zerdaoui, 2009). All zinc outflow values were compliant with the standard threshold for irrigation which is 2 mg/l (Metcalf & Eddy, 2003). Regarding iron, the *L. minor* ponds showed high outflow concentrations compared to the control ones for all dye mixtures, which can be attributed to plant die-off and subsequent decomposition. *Lemna minor* is able to take-up iron from the water and accumulate this metal in its tissue (see also Figure 6.9b). However, when this plant dies during its natural life cycle or due to the toxic impacts of water contaminants, these dead plants consequently will be a source of iron and other elements in the liquid phase. Significant differences between *L. minor* and control ponds were found for mixtures one and two (t-test, $p < 0.05$), which reflect high growth limitations and decompositions related to these ponds. The standard limit of iron for irrigation is 5 mg/l (Metcalf & Eddy, 2003). Concerning manganese (Figure 6.1c), inflow concentrations were not detectable. This is because the only source of manganese in the system was tap water. However, outflow values were detectable during the last few months of operation. This is attributed to low manganese reduction and weekly accumulation within the ponds. Outflow concentrations were higher in the control ponds than in the *L. minor*-planted ponds, which was ascribed to manganese being a nutrient for plants. All outflow values were less than the maximum allowable concentration for irrigation of 0.2 mg/l (Metcalf & Eddy, 2003). Higher mean outflow concentrations than inflow ones were found for magnesium, sodium and calcium (Figures 6.1d, f and g, respectively) due to the weekly dosages and low reductions except for potassium (Figure 6.1e) in all mixtures. The ponds not exposed to the dyes showed lower outflows related to these elements than ponds containing dye, because the plants were healthier and their ability for growth and acquirement of micro-nutrients was higher.

Figure 6.9 provides an overview of the concentrations of elements accumulated in plant tissues for all ponds with and without dyes. The plants linked to ponds without dye showed higher capacity for element accumulation due to their growth state, except for zinc which could be due to the low inflow zinc concentration in ponds without dyes. The levels of zinc and iron in plants (Figures 6.9a and b) were more than the allowable boundaries of 50 mg/kg and 20 mg/kg, respectively, as mentioned by Nazir et al. (2015).

The BCF illustrated in Figure 6.10, is an indicator of the potential of the plants for bio-accumulating heavy metals. A BCF of higher than 1000 indicates that the plant is a positive accumulator for heavy metals, as mentioned by Sukumaran (2013). The results in Figures 6.10a and b, indicate that the plant was positive for phytoremediation of zinc and iron, respectively.

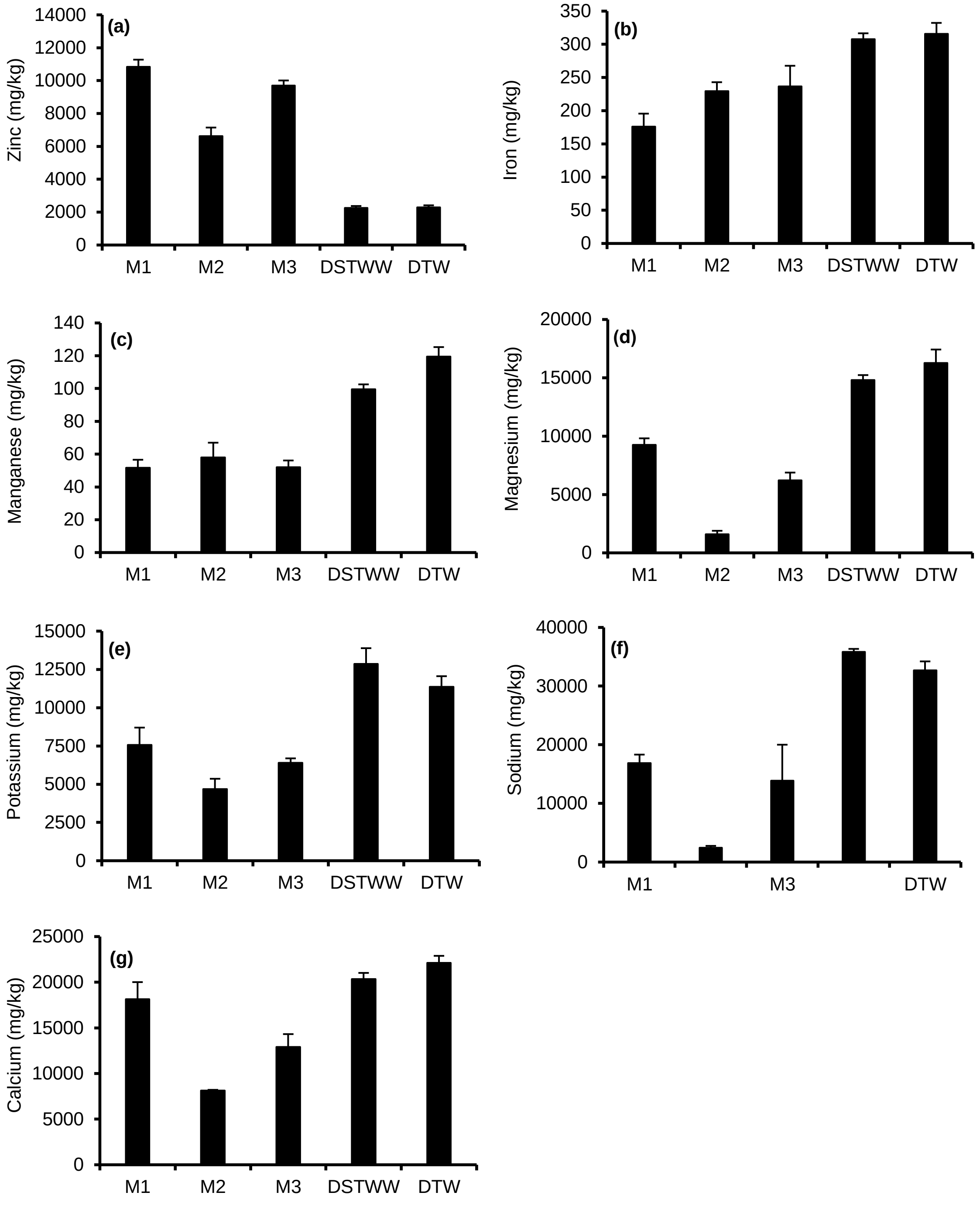


Figure 6.9 Mean concentrations of the detected elements in plant tissue during the experiment between 14 October 2016 and 27 June 2017. Note: (a), zinc; (b), iron; (c), manganese; (d), magnesium; (e), potassium; (f), sodium; (g), calcium; M1, mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); M2, mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); M3, mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198).; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

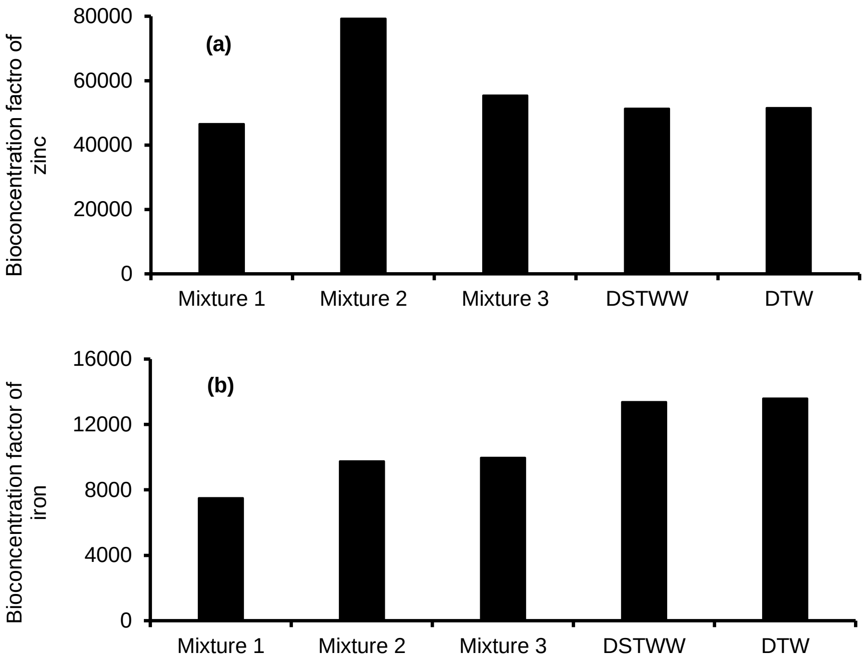


Figure 6.10 Bioconcentration factor during the experiment between 14 October 2016 and 27 June 2017. Note: (a), zinc; (b), iron; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water.

6.2.3 Plant monitoring

The growth of *L. minor* was monitored during the experimental period between 14 October 2016 and 27 June 2017 as an indicator for the toxic impact of each dye mixture as well as the prepared wastewater. During the first two months of the experimental operation, the growth of *L. minor* in all mixtures was limited. This period can be seen as an acclimatisation stage for plants with the inflow dye mixtures, which contained two dyes at a total concertation of 10 mg/l mixed with the synthetic textile wastewater chemicals. After the set-up phase, the plants started to increase in numbers and cover some of the surface area of the ponds, although a full coverage of the simulated pond surface was not achieved. After March 2017, the toxic signs of the dye mixtures were obvious in most systems; green fronds of *L. minor* turned light green and yellow. Although the plants associated with mixture three looked healthier and better than those in mixture one during the experiment, most *L. minor* plants in both mixtures were dead (partly dry at the sides of the pond walls) when the experiment was finished. In addition, the remaining plants in mixture one and three turned dark brown, and no obvious growth was noticed. Regarding mixture two, most plants were light green 2.5 GY (Munsell, 1977) and the remaining plants were white. The growth rate was also inhibited in ponds comprising mixture two in comparison to ponds without dyes. Ponds comprising diluted synthetic textile wastewater and tap water showed that most of the leaves of *L. minor* were light green (2.5 GY) and dark green (7GY) according to Munsell (1977). Plants within diluted synthetic textile wastewater were mostly green and healthier than those in tap water due to the presence of sufficient nutrients in the synthetic wastewater. The growth rate of *L. minor* (Table 6.4) was ranked as follows: ponds containing tap water > ponds containing diluted synthetic textile wastewater > ponds containing mixture two. Significantly (Kruskal-Wallis, $p < 0.05$) lower growth was noted for ponds treating mixture two compared to ponds containing tap water, and ponds containing diluted synthetic textile wastewater. These results suggest that a mixture of dyes restrains the photosynthesis process, impacts on the chlorophyll pigments in the fronds and consequently inhibits the growth of the plants, particularly when the concentration of the dye BR46 in the mixture was high and the dye removal was at a maximum.

Findings regarding chlorophyll pigments do not agree with Khataee et al. (2012) and the findings in Chapter 4, Section 4.2.3, where the dyes do not adversely impact on the chlorophyll content, although the observed growth rate of *L. minor* was reduced. This can be attributed to the separate dye preparation process in aqueous solution (Khataee et al., 2012) and the benefits of fertiliser application in the first experiment under controlled conditions (Chapter 4, Section 4.2) compared to the mixture of dyes added to synthetic textile wastewater chemicals in this experiment. However, the results of the plant growth rate in each mixture matched findings published by Movafeghi et al. (2013). Authors found that an increase in BR46 concentration to 10 mg/l was linked to a reduction in *L. minor* growth rate, although the inhibition in this study was higher in the presence of this dye at concentrations less than10 mg/l, which were 8 mg/l in the first mixture followed by the third and second mixture of 5 and 2 mg/l, respectively. Therefore, a growth reduction of *L. minor* was due to the presence of three adverse factors working together in combination: dye mixture, concentration of BR46 and synthetic textile wastewater. Furthermore, zinc was also present within BR46, although it was within the tolerable range for plant growth within the outflow samples. However, the concentration of zinc accumulated within plant tissue was higher than the allowable limit, as clarified earlier. This could be the main reason for plant damage in ponds containing BR46. The impact of zinc treated by *L. minor* was investigated by Radic et al. (2010). The authors concluded that zinc caused a reduction in the plant growth and chlorophyll pigments. This experiment also showed that the synthetic textile wastewater had a slightly negative influence on plant growth, which could be negligible, if compared with plants in tap water. Significantly (Kruskal-Wallis, $p > 0.05$) no differences were observed between ponds containing diluted synthetic textile wastewater and tap water regarding *L. minor* growth. However, the growth of *L. minor* within tap water ponds (Table 6.4) was significantly lower in comparison with the same species of plants subjected to TNC complete fertiliser for optimum growth, which led to a growth rate of around 0.011 per day (Chapter 4, Section 4.2.3; Appendix F, Figure F.7). This indicates that the growth of *L. minor* was inhibited in ponds containing tap water, and ponds containing synthetic textile wastewater. Note that within the last period of the experiment, an algal biofilm appeared in the bed of each pond (including those without dyes), which was due to the low growth rate of *L. minor* providing an uncovered surface area. However, the impact of algae was neglected during their appearance within the last weeks of the experimental operation, because the results were stable.

Table 6.4 Overview of *Lemna minor* L. growth rate (four replicates) during the experiment between 14 October 2016 and 27 June 2017

Treatment system	Fresh weight (gram)	Dry weight (gram)	Relative growth rate per day
Mixture 1	2.150±0.1872	0.102±0.0165	N/A
Mixture 2	5.793±1.1150	0.309±0.0703	0.00425±0.001517
Mixture 3	2.250±0.1118	0.103±0.0077	N/A
DSTWW	10.241±0.4101	0.609±0.0238	0.00793±0.000209
DTW	10.437±0.8689	0.622±0.04344	0.00801±0.000433

Note: N/A, not applicable; DSTWW, diluted synthetic textile wastewater; DTW, dechlorinated tap water; Mixture 1, 8 mg/l of basic red 46 + 2 mg/l of reactive blue 198; Mixture 2, 2 mg/l of basic red 46 + 8 mg/l of reactive blue 198; Mixture 3, 5 mg/l of basic red 46 + 5 mg/l of reactive blue 198.

6.2.4 Environmental conditions

The optimum temperature required for ideal growth of *L. minor* has been reported by Bekcan (2009) as 26°C. *L. minor* growth reduction occurs at temperature values below 17°C and higher than 35°C (Ozengin & Elmaci, 2007). In this experiment laboratory conditions were controlled and the mean temperature value was 23°C. The maximum and minimum values were 27°C and 19°C, respectively (Table 6.5). These results indicate that temperature records in this experiment do not have any adverse impact on the growth of *L. minor* in the system, although the growth will not be at an optimum rate.

The mean record of light intensity was 6853 lux. The corresponding maximum and minimum readings were 7722 lux and 6335 lux in that order (Table 6.5). These values were within the suitable range, between 1480 lux and 8140 lux, for a high rate of *L. minor* production, as mentioned by Yin et al. (2015).

Table 6.5 Overview of environmental boundary conditions in the laboratory between 14 October 2016 and 27 June 2017

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum	Number
Temperature	°C	23.3	1.83	19.4	26.8	172
Temperature (minimum within 24 h)	°C	21.8	1.42	17.4	26.9	172
Temperature (maximum within 24 h)	°C	23.6	3.00	18.1	26.9	172
Relative humidity	%	64.0	4.05	51.0	73.0	172
Relative humidity (minimum within 24 h)	%	61.9	4.49	44.0	70.0	172
Relative humidity (maximum within 24 h)	%	69.9	5.39	53.0	80.0	172
Illuminance (one-off records)	lux	6853.5	382.9	6335	7722	96

Note: h, hours.

6.3 Chapter summary

- Pond systems effectively improved the main parameters of water quality including COD, NH4-N and NO3-N, but not PO4-P with a considerable impact of *L. minor* ponds compared with the control ones.
- The outflow values of pH, COD, NH4-N, NO3-N, SS and TDS were within the acceptable limits for direct discharge.
- The planted pond efficiency in terms of removing dye mixtures was significantly ($p < 0.05$) higher than that for unplanted ones. High removals were associated with mixtures containing higher percentages of biodegradable dyes.
- The HPLC and UV–Vis analyses confirmed phyto-transformation and adsorption of BR46 in planted ponds. In addition, GC-MS data confirmed the complete aromatic amine mineralisation for the treated dye BR46 within the mixtures to water and carbon dioxide in planted ponds, whereas the control ponds showed the presence of N-(4-methylphenyl)-benzenemethanamine in the outflow samples.
- *L. minor* ponds are more effective to operate in case of wastewater treatment, containing separate (or individual) textile dyes, although the pond systems are able to treat mixtures of dyes.
- The treatment of BR46 when it forms only part of a dye mixture is lower, compared

to its corresponding removal as an individual dye.

- The outflow values of zinc and iron were below the thresholds set for irrigation purposes, and within the tolerated limits for plants.
- The artificial wastewater reduced the growth of *L. minor*. However, dye mixtures have a toxic impact on *L. minor*, particularly when the concentration of BR46 in the mixture was 5 mg/l or more.

Chapter 7
Conclusions and Recommendations

7.1 Conclusions

Four small-scale experimental wetlands (shallow ponds) were operated between July 2014 and June 2017 to fill the knowledge gaps found in previous research for the treatment of synthetic wastewater containing textile dye contamination by assessing the internal processes and the system efficiency using different design variables (presence of *L. minor* and/or algae), operational parameters (pH variation and dye concentration), and environmental conditions (controlled and semi-natural conditions).

The aim of the submitted thesis was to assess the potential of an economic, effective and sustainable approach for treating the coloured effluents from the textile industry to reduce the problems associated with these effluents in developing countries. The examined treatment system was successfully operated and the overall results indicate that the treatment systems vegetated by *Lemna minor* L. were highly efficient as a polishing stage for decolourisation and complete mineralisation the dye contaminant, basic red 46 (BR46) at low concentrations, and for the improvement of most other water quality parameters, especially in warmer regions. Additionally, findings confirmed that phytotransformation, biotransformation and adsorption are likely to be the main mechanisms for BR46 removal. Five objectives were proposed and achieved successfully to fulfil the aim of this research (Section 1.4). The key conclusions emanating from this research, which are linked to each objective consequently, are summarised as follows:

1. Experimental shallow ponds under controlled conditions, which were operated between 15 December 2014 and 15 September 2015, were able to remove the dye BR46 at concentration of 5 mg/l significantly better than other dyes (acid blue 113 (AB113), reactive blue 198 (RB198), and direct orange 46 (DO46)), although all dyes showed higher outflow concentration in control ponds than other ponds. In addition, ponds containing *L. minor* (P1 and P3) significantly ($p < 0.05$) outperformed algae-dominated ponds (P2) and control ponds (P4) in terms of BR46 removal efficiency. Mean BR46 elimination was 67%, 53%, 69% and 31% for *L.*

minor and algae ponds (P1), algae ponds (P2), *L. minor* ponds (P3) and control ponds (P4), respectively. Furthermore, low chemical oxygen demand (COD) removal was achieved in all ponds, with and without dyes, due to the accumulation of weekly doses in the systems and low microbial activities. However, higher COD removal values were found in planted ponds (P1 and P3) treating BR46. Ponds treating BR46, except for the control ones, showed that all outflow values of pH, suspended solids (SS), COD and total dissolved solid (TDS) were within the threshold set for discharge to the aquatic environment. Based on outdoor experimental shallow ponds in Salford, which were operated between 15 December 2014 and 2 February 2016, the results indicated better treatment of BR46 than all other dyes. In addition, significantly higher removal was linked to *L. minor* ponds (P3; 51% and 19%) than control ones (P4; 38% and 11%) regarding the dyes basic red 46 and reactive blue 198, in that order. Furthermore, mean COD removals were higher in *L. minor* ponds than control ponds for all ponds. The treated wastewater values of pH, TDS and COD for *L. minor* ponds treating BR46 were within the allowable ranges for discharge to watercourses.

2. Regarding comparison assessment between the performances of shallow ponds operated under controlled (laboratory) and uncontrolled (semi-natural) conditions during the period between 15 December 2014 and 15 September 2015, findings showed a significant ($p < 0.05$) removal for the dye BR46 at concentration of 5 mg/l by *L. minor* ponds (P3) under both studied environmental conditions. However, the potential of *L. minor* ponds for the treatment of BR46 was considerably better under controlled conditions (mean temperature records of 27.3°C) than those under semi-natural conditions (mean temperature records of 12.6°C), and the potential of *L. minor*-plant only in removing BR46 was 38% and 13%, respectively. The outflow values of zinc, iron, and copper were below the thresholds set and within the tolerated limits for plant survival and for irrigation purposes in both experiments. This result suggests that *L. minor*-based treatment systems would be suitable for operation in tropical and subtropical regions, as the bio-sorption of BR46 by *L. minor* is an endothermic process, and therefore, in this research other experiments designed to evaluate the capability of *L. minor* systems were conducted under controlled conditions only.
3. The impact of pH variation regarding short-term experimental ponds, which were operated between 1 October 2015 and 19 January 2016, was very low for the dyes

AB113, RB198 and DO46, and negligible for BR46. In addition, pH variation did not impact significantly ($p > 0.05$) on the treatment performance of other water quality parameters. Findings also showed that higher mean removal, 85%, was noticed in *L. minor* ponds treating BR46, and only RB198 and BR46 removal was significantly ($p < 0.05$) higher in *L. minor* ponds compared with the control ones. However, the long-term operation, between 20 January 2016 and 18 October 2016, showed that the removal efficiency of RB198 at acidic conditions (pH of 6) was significantly higher ($p < 0.05$) than at normal conditions, indicating that the adsorption capacity of RB198 increased at low pH, although the overall removal was very low in all ponds. Regarding the treatment of BR46, the removal within ponds containing *L. minor* was more than 85%, without any noticeable impact of different pH values. Based on the impact of *Oedogonium* algae within the second phase of the experiment between 18 October 2016 and 30 June 2017, findings showed that the presence or absence of algae in the system do not considerably affect the pond performance when treating RB198. However, BR46 removal was significantly higher ($p < 0.05$) within *L. minor* and algae ponds (P1, 85%) followed by algae ponds (P2, 58%) and control ponds (P4, 33%). Findings also showed that only *L. minor* was able to completely mineralise BR46 by removing the aromatic amines after dye decolourisation, and the very high removal within *L. minor* and algae ponds was due to the phytotransformation, biotransformation and adsorption processes. Whereas, the elimination mechanism was by biotransformation and bio-sorption processes within algae and control ponds, as confirmed by ultraviolet (UV)-scan, high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GCMS) data. All outflow COD, ammonium-nitrogen (NH_4–N), nitrate-nitrogen (NO_3–N), SS, and TDS concentrations were below the standard limits for safe discharge and therefore reusable for other purposes.

4. The assessment of pond systems for treating diluted synthetic textile wastewater containing a mixture of dyes showed that the treatment system effectively improved the main parameters of water quality, including COD, NH_4–N and NO_3–N, but not ortho-phosphate-phosphorus (PO_4–P), with a considerable impact of *L. minor* ponds compared with the control ones. The outflow values of pH, COD, NH_4–N, NO_3–N, SS and TDS were within the acceptable limits for direct discharge. In addition, the outflow concentrations of zinc and copper were below the standard limits for plant survival and for irrigation purposes. The *L. minor* pond (P3) efficiency in terms of

removing dye mixtures was significantly ($p < 0.05$) higher than that for control ones (P4). High removals were associated with mixtures containing higher percentages of biodegradable dyes (Mixture 1; 20% RB198 + 80% BR46). In addition, outcomes confirmed the phytotransformation, biotransformation and adsorption of BR46 in *L. minor* ponds with complete dye mineralisation in each mixture, and biotransformation and bio-sorption of the same dye in control ponds with production of aromatic amine in the outflow samples. Overall findings showed that the system potential for BR46 degradation when it forms only part of a dye mixture is lower, compared to its corresponding removal as an individual dye, suggesting that *L. minor* ponds are more effective in operation for the treatment of wastewater containing separate textile dyes, although the pond systems are able to treat mixtures of dyes.

5. Plant monitoring showed that the impact of environmental conditions on the growth rates of *L. minor* under controlled conditions (higher temperature records) were clearly higher than under semi-natural conditions in Salford, UK (lower temperature records), as the environment in the laboratory was suitable for optimum growth of *L. minor*. In terms of the impact of synthetic wastewater (dechlorinated tap water and TNC complete fertiliser), the plants showed growth rates that were significantly higher compared to other ponds fed by dyes or those fed by only tap water or diluted synthetic textile wastewater. Regarding the impact of pH on plant development, results showed that the pH variation did not significantly affect plant growth rate, although the growth values were slightly higher in ponds receiving inflow of a pH value of 6 followed by the values at normal conditions and lower growth rate was linked to ponds receiving inflow of a pH value of 9. The synthetic textile wastewater (chemicals composition) used at 100% without dilution was not suitable (toxic) for *L. minor*. However, diluted synthetic textile wastewater (1 part wastewater to 24 parts raw water) had a slightly negative influence on plant growth, which was less but not significant ($p > 0.05$), if compared with plants in tap water. Regarding the assessment of dye impact on *L. minor* growth rate, as a toxicity indicator, findings showed that all dyes inhibited the growth rates of *L. minor* compared with ponds without dyes, with the following growth rate ranking: wastewater without dye > AB113 > RB198 > DO46 > BR46. This indicates that BR46, which was successfully treated, had a significantly ($p < 0.05$) higher negative impact on the plant growth rate compared to ponds fed with wastewater without dye. In addition, an increase in BR46 concentration to 10 mg/l, as a separate dye, was linked to a reduction in *L. minor*

growth rate. However, a dye mixture containing diluted synthetic textile wastewater mixed with BR46 at concentrations of 2 mg/l impacted on the chlorophyll pigments in the fronds and consequently inhibited the development of *L. minor*, and at concentrations of 5 or 8 mg/l was toxic to the plant. Overall, findings suggest that the growth reduction of *L. minor* was due to the presence of three adverse factors working together in combination: dye mixture, concentration of BR46 and synthetic textile wastewater after dilution.

7.2 Recommendations for further research

The main recommendations for further research work are listed below:

1. Any future experimental work carried out on field-scale CWs for treating coloured textile effluents would benefit from finding out more about the potential of plants and microbes responsible for dyes, chemical oxygen demand, elements, nutrients and SS reduction.
2. Future research on specifying the microbial population (i.e. characteristics and composition) and examination of their exact contribution to the treatment of dyes and other pollutants in wetland systems is needed.
3. Future study on assessing the system performance using unstable (high and low) inflow dye concentrations is proposed, in addition to the importance of examination of other commercial azo dyes with the same or other plant.
4. Further research on assessing the system efficiency using real textile wastewater could be also applied to give better insight into the overall system performance instead of using synthetic wastewater chemicals that may affect the plant potential and survival.
5. Recycling of the treated wastewater from constructed wetland systems for irrigation purposes under both lab and field conditions to understand the impact of pollutants accumulation on the chemical and biological properties of the soil and crop production would be an additional advantage.

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Appendices

Appendix A Textile wastewater characteristics

Table A.1 Typical characteristics of textile effluents

Reference	Avlonitis et al. (2008)a	Eswaramoorthi et al. (2008)	Al-Kdasi et al. (2004), Turhan and Turgut (2009)	Kalra et al. (2011)	Upadhye and Joshi (2012)	Hussein (2013)	Ghaly et al. (2014), Suresh et al. (2014)	Kehinde and Aziz (2014)
Temp. (°C)		35–45		35–45	35–45	33–45	35–45	21–62
pH (-)		6–10	7–9	6–10	6–10	5.5–10.5	6–10	6.95–11.8
Colour (Pt Co)			50–2500	50–2500	50–2500		50–2500	50–2500
COD (mg/l)	100	1000–1500	150–12000	150–10000	150–10000	150–10000	150–12000	150–30000
BOD (mg/l)		300–500	80–6000	100–4000	100–4000	100–4000	80–6000	80–6000
EC (µS/cm)	1000							
TS (mg/l)								6000–7000
TSS (mg/l)		200–400	15–8000	100–5000	100–5000	100–5000	15–8000	15–8000
TDS (mg/l)		8000–12000	2900–3100	1800–6000	1800–6000	1500–6000	2900–3100	2900–3100
Chlorine (mg/l)			1000–1600				1000–6000	
Chlorides (mg/l)		3000–6000		1000–6000	1000–6000	200–6000		
Free chlorine (mg/l)		<10					<10	
TA (mg/l) as CaCo3				500–800	500–800	500–800		17–22
TH (mg/l) as CaCo3								
TKN (mg/l)			70–80	70–80	70–80	70–80	70–80	70–80

Table A.1 (Continued)

TNK(mg/l)		10–30					10–30	
NO_3–N (mg/l)		<5					<5	
Free ammonia (mg/l)		<10					<10	
Na_2CO_3 (mg/l)	20							
NaOH (mg/l)	10							
NaCl (mg/l)	300							
Phosphate (mg/l)							<10	
Sulphates (mg/l)		600–1000				500–700	600–1000	
Sulphides (mg/l)						5–20		
Sulphur trioxide (mg/l)								
Oil and grease (mg/l)		10–30				10–50	10–30	5–5.5
Dye (mg/l)	70							
Zink (mg/l)		<10				3–6	<10	
Nickel (mg/l)		<10					<10	
Manganese (mg/l)		<10					<10	
Iron (mg/l)		<10					<10	
Copper (mg/l)		<10				2–6	<10	
Boron (mg/l)		<10					<10	
Arsenic (mg/l)		<10					<10	
Silica (mg/l)		<15					<15	
Mercury (mg/l)		<10					<10	
Fluorine (mg/l)		<10					<10	
Chromium (mg/l)						2–5		
Potassium (mg/l)						30–50		
Sodium (mg/l)		7000		610–2175	610–2175	400–2175	7000	

Note: COD, chemical oxygen demand; BOD, five-day biochemical oxygen demand; TOC, total organic carbon; EC, electrical conductivity; TS, total solids; TSS, total suspended solids; TDS, total dissolved solids; TVS, total volatile solids; TA, total alkalinity; TH, total hardness; TKN, total kjeldahl nitrogen; TN, total nitrogen; NO_3–N, nitrate-nitrogen; Na_2CO_3, sodium carbonate; NaOH, sodium hydroxide; NaCl, sodium chloride; a, typical cotton dye industry effluent.

Table A.2 Characteristics of textile wastewater according to process

Reference	Seif and Malak (2001)				
Process	Dyeing				
pH (-)	6–10				
COD (mg/l)	475–1835				
BOD (mg/l)	295–1280				
TS	710–960				
TDS	650–1940				
Reference	Ahmed et al. (2007), Lau and Ismail (2009), Ibrahim et al. (2009)				
Process	Bleaching and Dyeing				
pH (-)	2–10				
Colour (ADMI)	280–2000				
COD (mg/l)	200–5500				
BOD (mg/l)	2000–30000				
TS	100–5000				
Reference	Dos Santos et al. (2006), Carmen and Daniela (2012)				
Process	Desizing	Scouring	Bleaching	Mercerising	Dyeing
pH (-)		10–13	8.5–9.6	5.5–9.5	5–10
Colour (ADMI)	16000–32000	694	153		1450–4750
TS (mg/l)		7600–17400	2300–14400	600–1900	500–14100
TDS (mg/l)			4800–19500	4300–4600	50
COD (mg/l)	4600–5900	8000	6700–13500	1600	1100–4600
BOD (mg/l)	1700–5200	100–2900	100–1700	50–100	10–1800
Reference	Savin and Butnaru (2008)				
Process	Burning	Bleaching	Dyeing	Dyeing Gauge	Dressing
pH (-)	5–6.5	7–12.4	6.3–10.7	6.5–12.1	7–7.11
COD (mg/l)	1512–7802	1060–6556	258–1970.6	458–7561	825–1905
TSS (mg/l)	105–936	56–147	72–956	175–325	135–544
BOD (mg/l)	675–925	80–520	70–300	230–410	60–180
Cl- (mg/l)	64–169	40–175	48–601	70–230	40–80
NH_4 (mg/l)	3–7.9	2.15–18.6	0.48–33.3	18.4–18.8	5.06–14.8
NO_2 (mg/l)	0.08–2.5	0.025–12.8	0.08–1.32	0.19–0.21	1–2.2
NO_3–N (mg/l)	0.025–5.3	3.3–9.4	3.7–8.3	4.7–4.8	1.6–3.6
Reference	Zhang et al. (2012)				
Process	Bleaching	Fibre scouring	Rinsing	Soaping	
pH (-)	9.4	7.3	8.6	12	
Colour (ADMI)	17	21	7	38	
COD (mg/l)	528 ± 7.9		311 ± 2.1	578 ± 23.5	
Salinity (mg/l)		2000	2000	5000	

Note: ADMI, American Dye Manufactures Institute unit; TSS, total suspended solids; TDS, total dissolved solids; TS, total solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand; Cl-, chloride; NH_4, ammonium; NO_2, nitrite; NO_3–N, nitrate-nitrogen.

Table A.3 Reported real textile effluent characteristics from different sources and countries

Reference	Lin and Lin (1993)	Georgiou and Melidis (2002)	Hussain et al. (2004)	Brik et al. (2006)	Chatzisymeon et al. (2006)	Bulc and Ojstrsek (2008)	Bes-Pia et al. (2010)
Source	Typical characteristics of textile wastewater	Cotton textile wastewater characteristics	Range of 6 major textile industries effluents	Mixed textile wastewater from a polyester finishing factory	Actual textile effluent from Epilektos SA manufacturing industry	Real textile wastewater	Textile mill wastewater from secondary treatment plant in the rinsing process
Country	India	Greece	India	Austria	Greece		Spain
Temp. (°C)	289-1030					30–38	
pH (-)	99-1010	8.2	7–9	6.36–9.67	9.5	8–9	7.6–7.8
Colour (Pt Co)							0.39–0.54a
COD (mg/l)	460–1500	150	1600–3200	1380–6033	404	276–1379	200–315
BOD (mg/l)	100–500	80	500–1010	177–720		99–350	
TOC (mg/l)						74–530	
EC (µS/cm)	2100–2900		44309–108710	0.661–4.95		2050–6430	2600–2800
TS (mg/l)					75		
TSS (mg/l)	91–250		8309–101580	75–220		27–408	15–46
TDS (mg/l)			40409–107500				1456–1568
DS (mg/l)			32109–105920				
Turbidity (NTU)							8.2–12.6
DO (mg/l)						1.2–1.7	
Chlorides as Cl- (mg/l)			980–2185				200–365
TH (mg/l) as CaCo$_3$			120–150				133–171
TN (mg/l)				7.53–75.2			
NO$_3$–N (mg/l)			120–627	0.26–11.1			
NH$_4$–N (mg/l)				0.76–23.7		0.2–4.5	

Table A.3 (Continued)

Na_2SO_4 (mg/l)					5500		
Na_2CO_3 (mg/l)					440		
NaOH (mg/l)					110		
Carbonate (mg/l)			110–120				96–96
Bicarbonate (mg/l)			555–1464				800–1000
Total phosphor (mg/l)				0.58–17.9			
Phosphate (mg/l)				0.25–5.38			
Sulphates (mg/l)			307–620	60–294		76–2200	124–176
Sulphur trioxide (mg/l)				0.1–73			
AOX (mg/l)				0.11–1.05			

Reference	Lim et al. (2010)	Abid et al. (2012)	Aouni et al. (2012)	Imtiazuddin et al. (2012)	Joshi and Santani (2012)	Nopkhuntod et al. (2012)	Nopkhuntod et al. (2012)
Source	Textile wastewater from a garment factory	Raw textile wastewater from14-Ramadhan textile mill	Raw textile wastewater from the rinsing baths of Colortex textile industry	Range of raw textile wastewater from 7 textile mills from different steps	Raw textile wastewater from Sumukh textile mill collected from 6 sites	Actual textile wastewaters from dyebath wastewater	Raw textile wastewater from equalising tank
Country	Malaysia	Iraq	Spain	Pakistan	India	Thailand	Thailand
Temp. (°C)	35–58			36–49.2	31.53–50.67		
pH (-)	3.85–11.4	5–8	7.11	7.5–11.5	9.17–12.7		
Colour (Pt Co)	76–1777.33					2502000±500	2105±13
COD (mg/l)	231.67–990		708	115.66–705.25	449.50–2078.55	45500±182	2600±3.78
BOD (mg/l)				125.55–653.75	71.75–852.63	5±0.00	520±0.47
EC (µS/cm)	690–1381	700–1300	3840	175.7–345.0	1340–6550		
TS (mg/l)	39.33–11689.33						
TSS (mg/l)	22.67–150			934–1619	419.87–1549	45±0.73	130±0.85

Table A.3 (Continued)

TDS (mg/l)	14.00–11564	400–1000	3137	2469–7295	1235.58–4975.2	45000±123	5600±6.54
TVS (mg/l)	54.46–531						
Turbidity (NTU)			4.02				
DO (mg/l)	1.5–7.5						
Chlorides as Cl- (mg/l)			>125				
NO_3–N (mg/l)	1.23–5.60						
NH_4–N (mg/l)	0.47–50.83						
Phosphate (mg/l)	0.07–4.01						
Sulphates (mg/l)			28				

Reference	Nopkhuntod et al. (2012)	Paul et al. (2012)	Syafalni et al. (2012)	Al-Shuwaik et al. (2013)	Al-Shuwaik et al. (2013)	Hussein (2013)	Qian et al. (2013)
Source	Actual textile wastewaters from textile industry from activated sludge treatment unit	Range of 6 textile industries effluent	Dye wastewater taken from Penfabric Mill	Textile wastewater from Al-Hilla factory	Textile wastewater from Al-Khadimia factory	Real textile wastewater	Textile wastewater passed from activated sludge unit
Country	Thailand	India	Malaysia	Iraq	Iraq	Iraq	China
Temp. (°C)							
pH (-)		7.54–9.59	9–10.18	7.9–8.5	7–9.5	12.9	8.0–8.3
Colour (Pt Co)	420±3		680–750	85	50–65		310–325b
COD (mg/l)	750±1.67	381–1548	298–360	80–90	120–140	225.084	61–75
BOD (mg/l)	25±0.04	130–500		50–60	15–20	149.3	6–10
TSS (mg/l)	90±0.33		0.0076	312–400	200–300		
TDS (mg/l)	4800±15	2264–7072		1340–1350	600–730		
Turbidity (NTU)			63–74c		26–30		40–61
DO (mg/l)							

Table A.3 (Continued)

Chlorides as Cl- (mg/l)		950–2750		542–550	120–140	63.91	
TA (mg/l) as $CaCo_3$		280–500				73.68	102.5–109.2
TH (mg/l) as $CaCo_3$		470–1050				237	
Free ammonia (mg/l)			2.1–3.8				
Phosphate (mg/l)		1.6–10.45			0.64		
Sulphates (mg/l)		440–912		410-580	140–200		
Sulphides (mg/l)		12–79					

Reference	Sivakumar et al. (2013)	Un and Aytac (2013)	Manekar et al. (2014)	Manekar et al. (2014)	Shehzadi et al. (2014)	Sun et al. (2014)	Uysal et al. (2014)
Source	Textile wastewater from the final clarifier of textile industrial effluent treatment plant	Wastewater from textile factory in Eskisehir	Raw textile wastewater	Raw textile wastewater passing from an equalisation tank	Range of 4 textile industries effluent	Textile dye wastewater from Jinyang textile industry	Real wastewater from textile factory in Bursa city
Country	India	Turkey	India	India	Pakistan	China	Turkey
Temp. (°C)			43–46	28–30	38–42		
pH (-)		9.01	7.8–9	7.7–8.0	7.24–12.93	7.4–7.9	8.22±0.5
Colour (Pt Co)			25–260d	240–290d	42–61		2200±800
COD (mg/l)	3458	1953	752–1120	678–932	320–925	1114–1350	300±100
BOD (mg/l)	2895		368–458e	272–310	172–450		
TOC (mg/l)					124–324		
EC (µS/cm)	4856	739			3.2–8.07		
TS (mg/l)					3112–5125		
TSS (mg/l)			120–170	120–180	200–391		
TDS (mg/l)	3108		1670–2040	1632–1902	2912–4834		
MLSS (mg/l)							165±80

Table A.3 (Continued)

Turbidity (NTU)						125–137
Chlorides as Cl- (mg/l)	942		384–452	384–412	90–800	
TA (mg/l) as $CaCo_3$			480–510	460–500		
TH (mg/l) as $CaCo_3$					380–520	
NO_3–N (mg/l)					20–24	
NH_3–N (mg/l)			5–10	4–8		
Carbonate (mg/l)						
Bicarbonate (mg/l)						
Phosphate (mg/l)			BDL–1.8	BDL–3	9.32–19.02	
Sulphates (mg/l)	758		320–380	300–320	215.6–672.8	
Acidity (mg/l)						
Phenol (mg/l)	155					

Reference	Buscio et al. (2015)	Kaur and Sharma (2015), Singh et al. (2013)	Punzi et al. (2015)	Almazan-Sanchez et al. (2016)	Bhuvaneswari et al. (2016)	Tomei et al. (2016)
Source	Textile effluents from dyeing process	Range of seven woven and knit textile mills of finishing industry	Real textile wastewater from a textile factory in Tirupur	Textile effluents from rinsing step of a denim textile industry	Textile industry effluents limits of three samples	Textile effluents from dyeing bath
Country	Spain	India	India	Mexico	India	Italy
Temp. (°C)					28–29	
pH (-)	6.9	4.3–11.9	10	6.84	8.6–9.2	9±0.5
Colour (Pt Co)	300			330		0.66±0.06a
COD (mg/l)	806	195–3050	1714	344	3880–4400	1017±58
BOD (mg/l)		108–790		91.91	1206–1750	9.8±1.3
TOC (mg/l)		101–7784		84.92		158.0±9.8

Table A.3 (Continued)

EC (µS/cm)	1825		12500	471.3		
TS (mg/l)		450–6510			2670–2850	
TSS (mg/l)	112	0.535±0.1			550–650	
TDS (mg/l)		430–49440			2070–2200	
Turbidity (NTU)				104.66	12.5–16.6	
Chlorides as Cl- (mg/l)	270			338.19	4005–4320	38.6±3.1
TA (mg/l) as $CaCo_3$	150			83.25		
TH (mg/l) as $CaCo_3$	50			119.17	1750–1900	
NO_3–N (mg/l)			3.6	1.9		3.8±0.1
NH_4–N (mg/l)			1.7			40.01±4.5
NH_3–N (mg/l)					73–85.6	
Carbonate (mg/l)				0.035		
Bicarbonate (mg/l)				101.5		
Phosphate (mg/l)			16.8	287.08	72.8-86.8	3.2±0.1
Sulphates (mg/l)	387			227.06	2050–2250	4.5±0.1
Acidity (mg/l)				20.75		

Note: Temp, temperature; COD, chemical oxygen demand; BOD, five-day biochemical oxygen demand; TOC, total organic carbon; EC, electrical conductivity; TS, total solids; TSS, total suspended solids; TDS, total dissolved solids; TVS, total volatile solids; DS, dissolved solids; MLSS, mixed liquor suspended solids; NTU, nephelometric turbidity unit; DO, dissolved oxygen; TA, total alkalinity; TH, total hardness; TKN, total kjeldahl nitrogen; TN, total nitrogen; NO_3–N, nitrate-nitrogen; NO_2–N, nitrite-nitrogen; NH_4–N, ammonium-nitrogen; NH_3–N, ammonia-nitrogen; Na_2SO_4, sodium sulphate; Na_2CO_3, sodium carbonate; NaOH, sodium hydroxide; NaCl, sodium chloride; AOX, adsorbable organic halogens; EC50, half maximal effective concentration; BDL, below detected limits; a, Absorbance unit; b, hazen units; c, BOD_3 days; d, American dye manufacturer's institute unit; e, formazin attenuation units.

Table A.4 Heavy metal and trace element concentrations (mg/l) of real textile wastewater

Reference	Hussain et al. (2004)	Bes-Pia et al. (2010)	Lim et al. (2010)	Aouni et al. (20120	Imtiazuddin et al. (2012)	Joshi and Santani (2012)	Paul et al. (2012)	Syafalni et al. (2012)
Source	Range of six major textile industry effluents	Textile mill wastewater from the rinsing process of a secondary treatment plant	Textile wastewater from a garment factory	Raw textile wastewater from the rinsing baths of a Colortex textile factory	Range of raw textile wastewater from seven textile mills from different steps	Real effluents from Sumukh textile mill	Range of six textile industry effluents	Dye wastewater taken from Penfabric Mill
Country	India	Spain	Malaysia	Spain	Pakistan	India	India	Malaysia
Zinc			0.11–2.93		2.36–6.03	0.48–7.32		<0.2
Lead	0.011–0.061		0.08–0.09		0.16–0.35			
Cadmium			0.01–0.05	42		0.02–0.74		
Nickel			0.02–0.04		0.66–1.53	0.06–1.16		
Magnesium	13–29			18.6			88–210	
Iron	0.017–0.163		0.11–0.16		1.08–3.11	0.3–111.38		0.13–0.15
Chromium	0.015–7.854				1.05–1.86	1.16–2.2		0.5–0.6
Copper	0.006–0.311		<0.001–0.1		0.07–5.14	0.17–9.26		0.03
Fluorine	0.7–2.2							
Calcium	12–28						128–404	
Manganese	0.001–0.022		0.01–0.04		0.88–1.85	0.07–7.74		
Potassium	11–19	54–67				40.50–113.64		
Sodium	975–2185	179–190		1008		86.32–259.06		
Phosphorus						0.17–2.35		
Arsenic			<0.001					

Table A.4 (Continued)

Reference	Al-Shuwaik et al. (2013)	Hussein (2013)	Manekar et al. (2014)	Manekar et al. (2014)	Shehzadi et al. (2014)	Almazan-Sanchez, et al. (2016)	Bhuvaneswari et al. (2016)
Source	Textile wastewater from Al-Khadimia factory	Real textile wastewater	Raw textile wastewater passing from an equalisation tank	Raw textile industry wastewater	Range of 4 textile industries effluent	Textile effluents from the rinsing step of a Denim textile factory	Textile industry wastewater (range for three samples)
Country	Iraq	Iraq	India	India	Pakistan	Mexico	India
Zinc		0.256	0.01–0.3	0.06–0.27			
Lead	1–2		0.1–0.13	0.11–0.12			
Cadmium			0.01	0.01–0.02	0.1–0.27		
Nickel		0.104	0.07–0.26	0.08–0.3	1.1–2.7		
Cobalt			0.03–0.06	0.02–0.03			
Magnesium						4.39	
Iron	0.1–1.0		1.42–8.25	0.7–1.41	1.6–3.3	0.343	
Chromium	0.1–1.0		0.04–0.07	0.03–0.05	0.08–0.25		
Copper		0.27	0.07–0.11	0.03–0.04			
Lithium							68–83.6
Silica						5.6	
Calcium					80.16–104.2	5.65	2185–2378.5
Manganese			BDL–0.26	BDL–0.16	48.6–68.04	1.99	
Potassium		44.831			624–1092	3.08	2490–2685
Sodium		697.6			3242–1656	44.99	2710–2900

Note: BDL; below detection limits.

Table A.5 Level of dye concentrations (mg/l) of real textile wastewater

References	Concentration
Laing (1991)	10–50
Pierce (1994), Shelley (1994)	60
Gahr et al. (1994)	100–200
Koprivanac et al. (1992)	7000
Vandevivere et al. (1998), Jadhav et al. (2007), Saratale et al. (2011)	600–800
Sivakumar (2014)	45
Abid et al. (2012)	20–50
Ghaly et al. (2014)	10–250

Table A.6 Reported chemical constituents used for preparation of synthetic textile wastewater containing dyes

Reference	Marquez and Costa (1996)	Basibuyuk and Forster (1997)		Panswad and Luangdilok (2000)	
Country	Spain	UK			
Method	Adsorption by powdered activated carbon and activated sludge	Sequential biofilter using activated sludge		Anaerobic/aerobic sequential batch reactor	
Meat extract	110 mg/l	Starch	1.28 g/l	Glucose	860 mg/l
Urea	30 mg/l	Lab Lemco	400 mg/l	Acetic acid	0.150 ml/l
Dipotassium hydrogen phosphate	28 mg/l	Ammonium sulphate	353 mg/l	Urea	108 mg/l
Sodium chloride	7 mg/l	Magnesium sulphate heptahydrate	108 mg/l	Potassium dihydrogen phosphate	67 mg/l
Calcium chloride dihydrate	4 mg/l	Calcium chloride	40 mg/l	Sodium bicarbonate	840 mg/l
Magnesium sulphate heptahydrate	2 mg/l	Iron (II) sulphate heptahydrat	750 µg/l	Magnesium sulphate heptahydrat	38 mg/l
Acid Orange 7	20 mg/l	Nickel (II) sulphate heptahydrate	500 µg/ l	Calcium chloride	21 mg/l
		Manganese (II) chloride tetrahydrate	500 µg/ l	Iron (III) chloride hexahydrate	7 mg/l
		Zinc sulphate heptahydrate	500 µg/ l	Reactive Black 5, Reactive Blue 19, Reactive Blue 5, Reactive Blue 198	20 mg/l
		Boric acid	100 µg/ l		
		Cobalt(II) chloride hexahydrate	50 µg/ l		
		Copper(II) sulphate pentahydrate	5 µg/ l		
		Maxilon Red	25-50 mg/l		
Reference	**Panswad et al. (2001)**	**Alaton et al. (2002)**		**Kang et al. (2002)**	
Country	Thailand			Taiwan	
Method	Anaerobic/aerobic sequential batch reactor	Advanced oxidation		Fenton process	
Nutrient broth	500, 350, 250 mg/l (a)	Acetic acid	0.79 g/l	Polyvinyl alcohol	125 mg/l
Sodium acetate	150, 250, 500 mg/l (a)	Sodium chloride	41 g/l	Reactive Blue dye R94H	20 mg/l
Glucose	500 mg/l (a)	Sodium carbonate	13 g/l		

Table A.6 (Continued)

Urea	50, 25 mg/l (as N)	Sodium hydroxide	0.51 g/l		
		Polyether based co-polymer micro-dispersion	1.2 g/l		
Potassium dihydrogen phosphate	15, 5 mg/l (as P)				
Sodium bicarbonate	500 mg/l (as CaCO3)				
Calcium chloride	7.5, 2.5 mg/l (as Ca)	Acryl co-polymer-phosphor mixture	0.85 g/l		
Iron (III) Chloride Hexahydrate	2.5 mg/l (as Fe)	Alcyl phenol polyglycol ether	0.5 g/l		
Magnesium sulphate heptahydrate	3.75, 1.25 mg/l (as Mg)	Procion blue HERD	6.83 mg/l		
Remazol black B	10 mg/l	Procion crimson HEXL	40.6 mg/l		
		Procion Yellow HE4R	15 mg/l		
		Procion navy HEXL	86.3 mg/l		
		Procion yellow HEXL	33.3 mg/l		
Reference	Mohan et al. (2002)	Mbuligwe (2005)		Sakkayawong et al. (2005), Noonpui and Thiravetyan (2011) (c)	
Country	India	Tanzania		Thailand	
Method	Biological treatment by algae	Engineering wetland with emergent plants		Adsorption by chitosan; constructed wetland	
D-glucose	0.1 g/l	Caustic soda	0.2 g/l	Sodium carbonate	90 g/l
Sodium chloride	0.05 g/l	Sodium hydrosulphate	0.3 g/l	Sodium sulfate	20 g/l
$FeCl_3.H_2O$	7100 mg/l (b)	Dye	0.2 g/l	Reactive red 141	4 g/l
Zinc sulphate heptahydrate	1 mg/l (b)				
Magnesium sulphate heptahydrate	5000 mg/l (b)				
Boric acid	1 mg/l (b)				
Copper (II) sulphate pentahydrate	1 mg/l (b)				
Ammonium Molybdate	1.1 mg/l (b)				
Manganese(II) Chloride Dihydrate	80 mg/l (b)				
Aluminium sulphate hexadecahydrate	550 mg/l (b)				

Table A.6 (Continued)

Calcium chloride dihydrate	10000 mg/l (b)
Cobalt(II) chloride hexahydrate	2000 mg/l (b)
Thiamine– HCl hydrogen chloride	2000 mg/l (b)
Reactive Yellow 22	0.25, 0.5, 1.0 g/l

Reference	Khehra et al. (2006)	Bali and Karagozoglu (2007)		Keskinkan and Lugal Goksu (2007) (d)	
Country	India	Turkey		Turkey	
Method	Sequential anoxic/aerobic bioreactor	Fenton process		Constructed wetland with submerged plants	
Disodium phosphate	3.6 g/l	polyvinyl alcohol	100 mg/l	Calcium nitrate	492 mg/l
Ammonium sulphate	1 g/l	Remazol Turquoise Blue G-133	50 mg/l	Ammonium dihydrogen phosphate	230 mg/l
Potassium dihydrogen phosphate	1 g/l			Magnesium sulphate heptahydrate	420 mg/l
Magnesium sulphate heptahydrate	1 g/l			Boric acid	2.86 mg/l
Fe (NH$_4$) citrate	0.01 g/l			Manganese(II) chloride tetrahydrate	1.81 mg/l
Calcium chloride dihydrate	0.1 g/l			Molybdenum(VI) acid monohydrate	0.09 mg/l
Yeast extract	0.05% (w/v)			Iron(II) sulphate heptahydrate	0.07 mg/l
Glucose	2.8 mM			(CHOH)$_2$(COOH)$_2$	0.02 mg/l
Zinc sulphate heptahydrate	10 mg/l (b)			Potassium nitrate	1020 mg/l
Manganese(II) chloride tetrahydrate	3 mg/l (b)			Basic Blue 41	11 mg/ l
Cobalt(II) chloride hexahydrate	1 mg/l (b)				
Nickel(II) chloride hexahydrate	2 mg/l (b)				
Sodium molybdate dihydrate	3 mg/l (b)				
Boric acid	30 mg/l (b)				
Copper(II) chloride dihydrate	1 mg/l (b)				
Acid Red 88	100 mg/l				

Table A.6 (Continued)

Reference	Ojstrsek et al. (2007)			Avlonitis et al. (2008)				Hassani et al. (2008) (e)	
Country	Austria			Austria				Iran	
Method	Constructed wetland with emergent plants			Nanofiltration processes					
Alvirol AGK	0.46 g/l	0.3 g/l	0.3 g/l	Sodium carbonate	20 mg/l	80 mg/l	100 mg/l	Sodium chloride	1000, 2000, 3000 mg/l
Alviron VKSB	0.67 g/l	0.4 g/l		Sodium hydroxide	600 mg/l	40 mg/l	50 mg/l		
Sodium chloride	2 g/l	2 g/l		Sodium chloride	600 mg/l	1200 mg/l	1500 mg/l	Cyanine 5R, Red E3B, Direct red 105, Carmozin 206	5, 50, 100 mg/l
Sodium hydroxide	2 ml/l	2 ml/l		Reactive Black 5	130 mg/l	260 mg/l	230 mg/l		
Irgapadol MP			1.67 g/l						
Cibaflow PAD			0.3 g/l						
Reactive Red 22	0.03 g/l								
Reactive Black 5		0.03 g/l							
Vat Red 13			0.03 g/l						
Reference	Bulc and Ojstrsek (2008)			Ong et al. (2009a, 2010)				Muda et al. (2010)	
Country	Austria			Japan					
Method	Constructed wetland with emergent plants			Up-flow constructed wetland with emergent plants				Sequential batch reactors using granular sludge	
Alvirol AGK	0.3 g/l	0.3 g/l	0.3 g/l	Sodium acetate		204.9 mg/l		Glucose	0.5 g/l
Cibaflow PAD	0.3 g/l	0.3 g/l	0.3 g/l	Ammonium nitrate		176.1 mg/l		Ethanol	0.125 g/l
Sodium chloride	2 g/l			Sodium chloride		7 mg/		Sodium acetate	0.5 g/l
Sodium hydroxide	2 ml/l			Magnesium chloride hexahydrate		3.4 mg/l		Ammonium chloride	0.16 g/l
Irgapadol MP		2 g/l	2 g/l	Calcium chloride dihydrate		4 mg/l		Potassium dihydrogen phosphate	0.23 g/l
Reactive Black 5	0.03 g/l			Potassium Hydrogen Phosphate Trihydrate		36.7 mg/l		Dipotassium phosphate	0.58 g/l
Disperse Yellow 211		0.03 g/l						Calcium chloride dihydrate	0.07 g/l
Vat Yellow 46			0.03 g/l	Sodium benzoate		107.1 mg/l		Magnesium sulphate heptahydrate	0.09 g/l
				Acid Orange 7		50, 100 mg/ l		Ethylenediaminetetraacetic acid	0.02 g/l
								Boric acid	0.15 g/l (b)
								Ferrous chloride	1.5 g/l (b)

Table A.6 (Continued)

						Zinc chloride	0.12 g/l (b)
						Manganese(II) chloride tetrahydrate	0.12 g/l (b)
						Copper(II) chloride dihydrate	0.03 g/l (b)
						Sodium molybdate	0.06 g/l (b)
						Cobalt(II) chloride hexahydrate	0.15 g/l (b)
						Potassium iodide	0.03 g/l (b)
						Sumifix Black EXA, Sumifix Navy Blue EXF and Synozol Red K-4B	50 mg/l
Reference	Ozdemir et al. (2011)	Aouni et al. (2012)				Cumnan and Yimrattanabovorn (2012)	
Country	Turkey	Spain				Thailand	
Method	Fenton process	Ultrafiltration and nanofiltration processes				Constructed wetlands with emergent plants	
Polyvinyl alcohol	100 mg/l	Sodium chloride	500 mg/l	500 mg/l	500 mg/l	Acetic acid sodium salt	204.9 mg/l
Reactive yellow 145	50–250 mg/l	Everzol Black	600 mg/l			Ammonium nitrate	176.1 mg/l
		Everzol Blue		600 mg/l		Sodium chloride	7 mg/l
		Everzol Red			600 mg/l	Magnesium chloride hexahydrate	3.4 mg/l
						Calcium chloride dihydrate	4 mg/l
						Potassium hydrogen phosphate Trihydrate	36.7 mg/l
						Benzoic acid sodium salt	53.55 mg/l
						Dye	11.5 mg/l
Reference	Nopkhuntod et al. (2012)	Verma et al. (2012, 2015)				Al-Amrani et al. (2014)	
Country	Thailand	India					
Method	Adsorption by shale column	Coagulation method				Anoxic/aerobic REACT operated sequential batch reactor	
Sodium chloride	40 g/l	Acetic acid		200 mg/l		Sucrose	563 mg/l
Sodium hydroxide	1.5 g/l	Sucrose		600 mg/l		Bacto-peptone	188 mg/l

Table A.6 (Continued)

Sodium carbonate	2 g/l	Sodium hydroxide	500 mg/l	Iron (III) Chloride Hexahydrate	11.3 mg/l
Reactive dye	0.67 g/l	Sulphuric acid	300 mg/l	Calcium chloride	40 mg/l
		Sodium carbonate	500 mg/l	Magnesium sulphate	49 mg/l
		Sodium chloride	3000 mg/l	Sodium bicarbonate	100 mg/l
		Sodium lauryl sulphate	100 mg/l	Ammonium chloride	172 mg/l
		Starch	1000 mg/l	Potassium dihydrogen phosphate	513 mg/l
		Reactive Black 5, Congo Red, Disperse Blue 3	200 mg/l	Acid Orange 7, Acid Orange 10, Acid Yellow 9 and Acid Red 14	30, 75, 150 mg/l
Reference	Aldoury et al. (2014)	Mountassir et al. (2015)		Punzi et al. (2015)	
Country		Morocco		India	
Method	Sequential anaerobic/aerobic reactor	Electrocoagulation		Anaerobic biofilm reactors	
Calcium chloride	20 mg/l	Starch	2.78 mg/l	Starch	0.465 g/l
Magnesium sulfate heptahydrate	20 mg/l	Ammonium sulphate	5.56 mg/l	Sodium chloride	10 g/l
Zinc sulfate	1 mg/l	Disodium phosphate	5.56 mg/l	Remazol Red	10 g/l
Iron(III) chloride	2 mg/l	Reactive Violet 4	0.8 absorbance unit		
Sodium bicarbonate	50 mg/l				
Ammonium chloride	10 mg/l				
Peptone	50 mg/l				
Dipotassium hydrogen phosphate	15 mg/l				
Acid Orange 12, Disperse Red 17	100, 150, 200 mg/l				
Reference	Dhaouefi et al. (2018)				
Country	Spain				
Method	anoxic-aerobic photobioreactor				
COTOBLANC KRS	330 mg/l				
BIAVIN BPA	330 mg/l				

Table A.6 (Continued)

MEROPAN DA	170 mg/l
Trisodium phosphate	330 mg/l
Sodium hydroxide	1000 mg/l
Sodium hydrosulphate	900 mg/l
Acetic acid	170 mg/l
Ammonium sulphate	600 mg/l
Disperse blue 1	12 mg/l
Disperse orange 3	20 mg/l

Note: a, the chemicals mixed with either NB and SA or with glucose providing 500 mg/l as COD; b, composition of trace element solution using (1 ml/l) except Khehra et al. (2006) used (10 ml/l); c, this ingredient is of stock solution and further dilution applied for making 20 mg/l dye concentration; d, 5% of the chemical solution mixed with the dye; e, the authors mixed each dye separately at three concentrations with sodium chloride which provide three concentrations of TDS (total dissolved solid).

Table A.7 Characteristics of the reported chemical constituents in Table A.6

Parameter	Marquez and Costa (1996)	Panswad et al. (2001)	Kang et al. (2002)	Mbuligwe (2005)	Bali and Karag-ozoglu (2007)	Avlonitis et al. (2008)		
						Bath1	Bath2	Bath3
pH (-)	7.3	7.4–7.5		10.68±0.78				
COD (mg/l)	250±30	492–552	250	85.28±6.5	213	180	360	360
COD/BOD (-)	1.44							
EC (µS/cm)						1500	2120	3070
N (mg/l)		25,50,70						
P (mg/l)		5.3–15.5						
Alkalinity (mg/l)		444–762						
TKN (mg/l)		24.8–72.3						
Sulphate (mg/l)				48.2±7.7				
Colour (Pt Co)			1050a	100.2± 21.87				
Dye (mg/l)						130	260	230

Parameter	Ong et al. (2010)		Aouni et al. (2012)			Verma et al. (2012, 2015)	Aldoury et al. (2014)	Mountassir et al. (2015)	Punzi et al. (2015)
	b	c	d	e	f				
pH (-)			9.89	10.46	10.27	7.5 ± 0.1	6,7,8	6.8	
COD (mg/l)	383.8±13.7	450±8	566	450	470	770 –790	650–900	736	590
COD/BOD (-)								5.37	
EC (µS/cm)			2490	1407	2240			1500	
TDS (mg/l)							370–550		
Chloride (mg/l)								320	
N (mg/l)	55.4±3.4	59±2							
P (mg/l)	6.7±0.3	6.5±0.3							
NH_4–N (mg/l)	34.8±2.8	36±4							
NO_3-N (mg/l)	29.5±2.2	28±2							
Alkalinity (mg/l)						1150–1130			
Colour (Pt Co)			2.05g	1.92g	1.76g			0.8g	
Dye (mg/l)	51±2	103±3	600	600	600				

Note: a, colour based on American dye manufacturer’s institute unit; b, at dye concentration of 50 mg/l; c, at dye concentration of 100 mg/l; d, using the dye everzol black; e, using the dye everzol blue; f, using the dye everzol red; g, colour based on the spectral absorption coefficient; COD, chemical oxygen demand; BOD, biochemical oxygen demand; P, phosphorus; N, nitrogen; NH_4–N, ammonium-nitrogen; NO_4–N, nitrate-nitrogen; TDS, total dissolved solids; EC, electrical conductivity.

Appendix B Pictures of the ponds experiments set-up

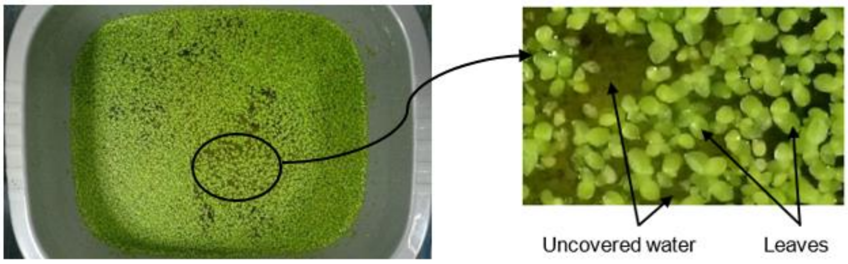
Figure B.1 First phase of the first experiment (plant collection)

Figure B.2 Second phase of the first experiment set-up of pond systems (acclimatisation and monitoring)

Appendix C Aletheia Lemna edition software

1. Goals of Aletheia Software:

- Measure how much of the total water surface area is covered by *L. minor* fronds (relative value %).
- Estimate how many fronds the measured area contains.



2. Using Software Tools

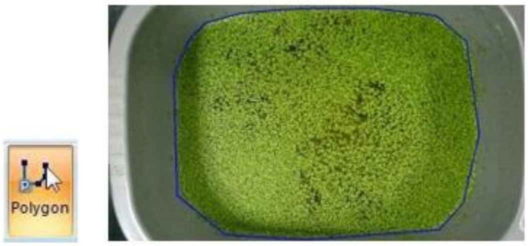
2.1 Open New Image:

Start new document, or drag and drop image file from Windows Explorer into the tool.

2.2 Mark the Border of the Water Surface:

To be able to calculate the relative frond coverage, the border of the water surface has to be specified as follow:

- Activate the ‘Polygon’ tool.
- Click on the image to add a point, and keep adding points.
- Finish with double click (adds a last point) or with right click.

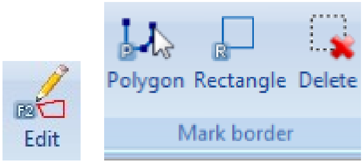


To Edit or Delete the Current Border:

- Activate the ‘Edit’ tool.
- Hover with the mouse over a corner point of the border and click and hold to move it or press the ‘Delete’ key to remove it, or click and drag on a line segment to add a new point and move it.

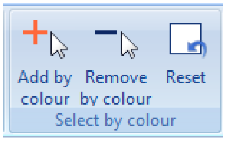
To delete the current border:

- Click on ‘Delete’ to remove the current border and start again.



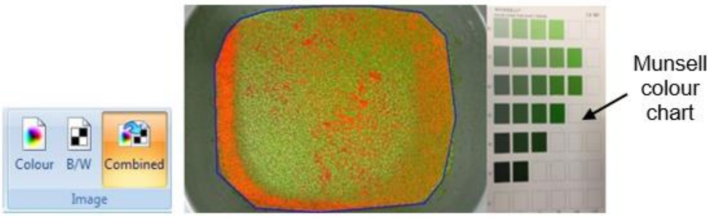
2.3 Highlighting/Selecting the *L. minor* Fronds:

After activating the ‘Add by colour’ tool and selecting a specific colour in the Munsell colour chart, all *L. minor* fronds in the image that are similar to the colour selected in Munsell chart (as found at the click position) are highlighted and added to the current selection.

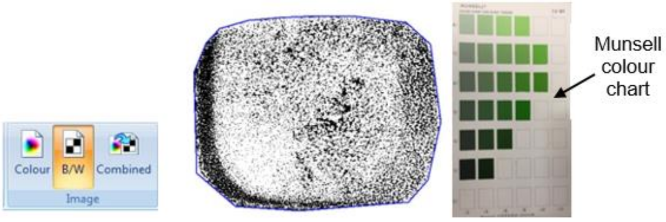


The current selection can be viewed in three ways:

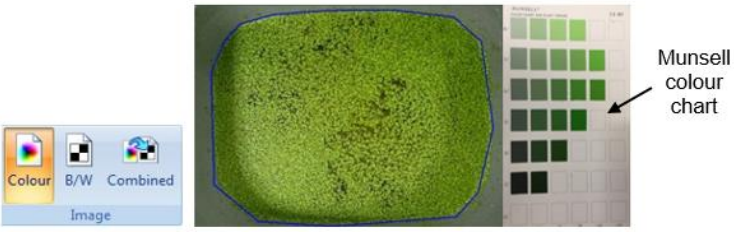
- Colourised overlay on the original image (combined)



- Black-and-white image (black; selected area; white; background)

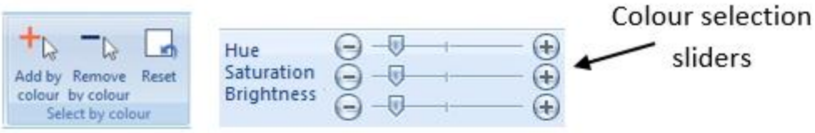


- Hidden (only the original image is visible)



2.4 Modifying the Selection of Fronds:

- Activate ‘Add by colour’ or ‘Remove by colour’.
- Adjust the colour selection sliders.
- Click on the Munsell colour chart, which refers to a specific *L. minor* frond, and repeat until all *L. minor* fronds have the same clicked colour highlighted.
- Use ‘Reset’ to delete the current selection and start again.



2.5 Viewing and Zoom Functions:

To move the image:

- Activate the ‘Hand’ tool (ESC key) and click and drag the mouse.
- Use the mouse wheel to move the image up or down or use the scroll bars at the side of the window.

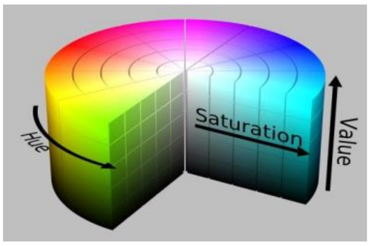
To zoom in or out:

- Use the toolbar buttons or use keyboard shortcuts on number pad.

3. Understanding the selection by colour:

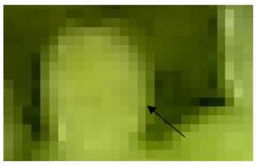
3.1 Hue, Saturation, and Brightness

Colour selection is done via hue (colour tone), saturation (greyness), and brightness (value). Each point (pixel) of the image has a specific colour, defined by those three values. When the user clicks on the Munsell colour chart image, the tool automatically finds all areas of the *L. minor* image that have a 'similar' colour and adds to the current selection (or removes them, if the 'Remove' tool is used). The sliders for hue, saturation and brightness can be used to change 'how similar' the colours of the other areas can be. Low values (slider towards left side) mean very similar. High values (slider towards right side) mean rather different colours are added to the selection.

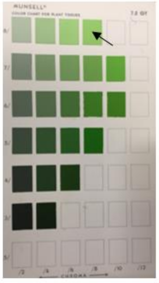


Colour selection sliders

Example for using 'Add by colour' in presence of calibration card after clicking on a specific colour in the Munsell colour chart:



L. minor



Munsell colour chart example

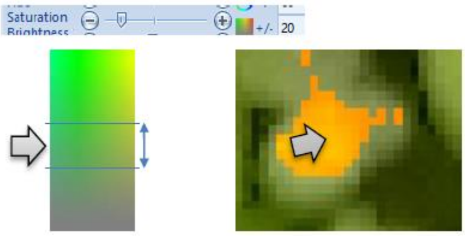
3.1.1 Allowed hue deviation (hue; colour tone)

- Low value

- Higher value

3.1.2 Allowed saturation deviation (saturation; greyness)

- Low value
- Higher value

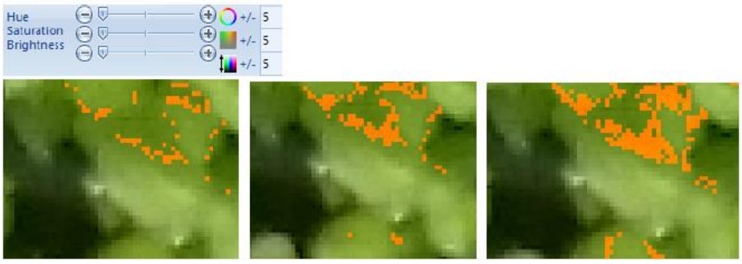


3.1.3 Allowed brightness deviation (dark colours; low brightness)

- Low value
- Higher value

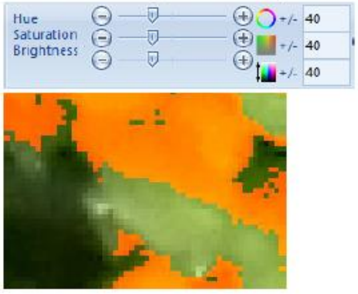
3.2 Strategies to Select all Fronds:

3.2.1 Small slider settings, many clicks (set the sliders to a low value and click on many different colours).



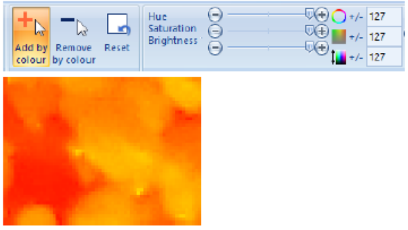
Hue +/- 5
Saturation +/- 5
Brightness +/- 5

3.2.2 High slider settings, few clicks (if the sliders are set to a high value, less clicks are necessary; risks selecting too much).

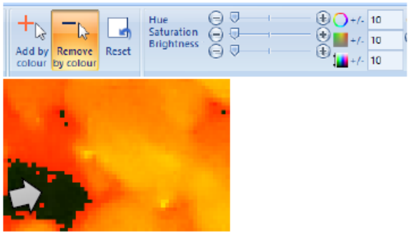


Hue +/- 40
Saturation +/- 40
Brightness +/- 40

3.2.3 Reverse: select everything (all sliders to highest setting) and remove the non-leaf area by using the ‘Remove by colour’ tool.



Hue +/- 127
Saturation +/- 127
Brightness +/- 127



Hue +/- 10
Saturation +/- 10
Brightness +/- 10

4. Calculating the Coverage Area and Fronds Number

The main steps of using Aletheia software, are mentioned previously in Chapter 3 Section 3.5. These steps lead to the appearance of numbers in the ribbon including:

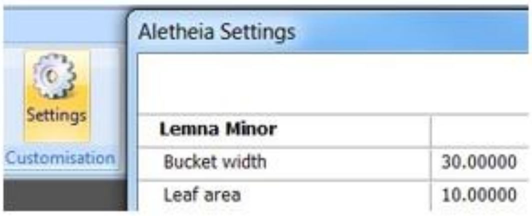
- Pixel count of current selection (black area in B/W image).
- Relative area of selection in relation to inside area of marked border (or full image area if no border defined).
- Number of *L. minor* leaves (based on predefined bucket and leaf size).

Note: use ‘Recalculate’ if the border has been changed manually.

Pixel count (absolute) 2172293

Leafs count 12306

Relative area (%) 40.4%



Lemna Minor	
Bucket width	30.00000
Leaf area	10.00000

5. Saving and Loading:

- Click ‘Save’ (or Ctrl+S) when finished or any time before (saves XML file and black-and-white image).
- To open saved data, use the XML file (‘Open document’ or drag and drop from Windows Explorer).

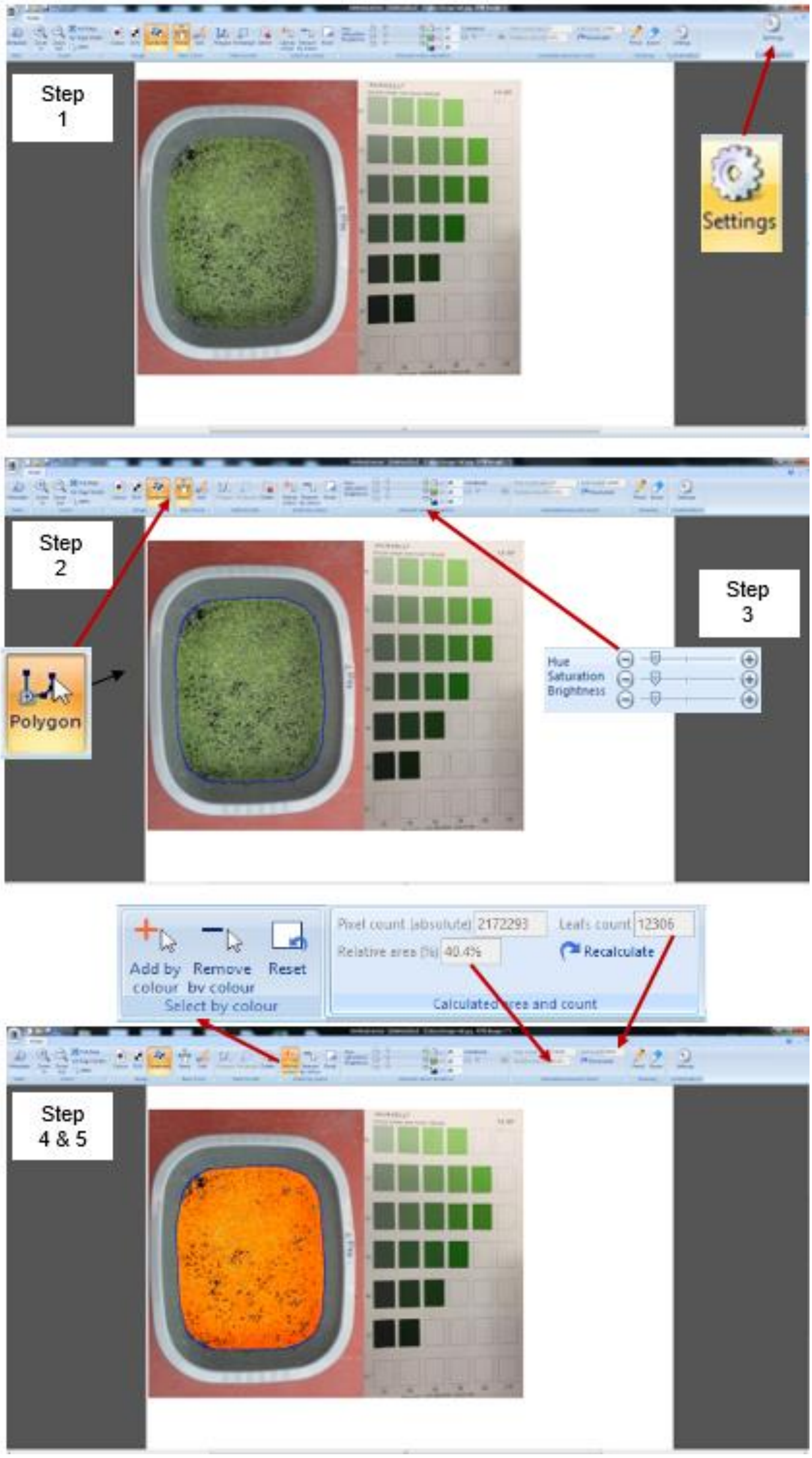
Note: The specified water border is saved to the XML file. When loading an image that has been previously worked on, it is important to open the XML file and not the image. Otherwise the border will not be there and will have to be redone.

Aletheia Lemna Edition Software

Version 3.0

Date: October 2014

www.primaresearch.org



Pixel count (absolute) 2172293

Leafs count 12306

Relative area (%) 40.4%

Figure C.1 Steps of counting *Lemna minor* L. fronds number and coverage area by using Aletheia Lemna Edition software

Appendix D Pictures of algae, and samples sent for external analysis

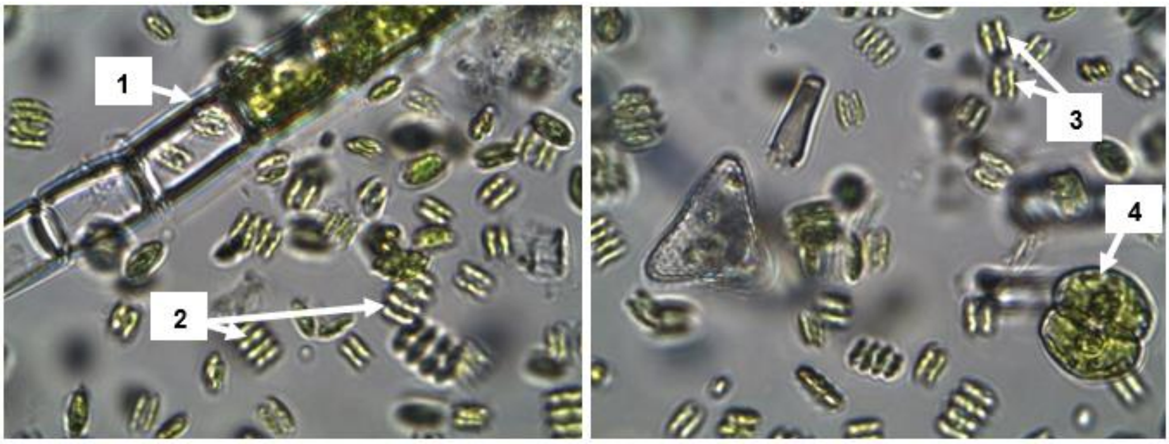


Figure D.1 Algae identification by microscope in the pond systems of the first experiment. Note: (1), Oedogonium; (2), four-celled Scenedesmus; (3), two-celled Scenedesmus; (4), Cosmarium species.

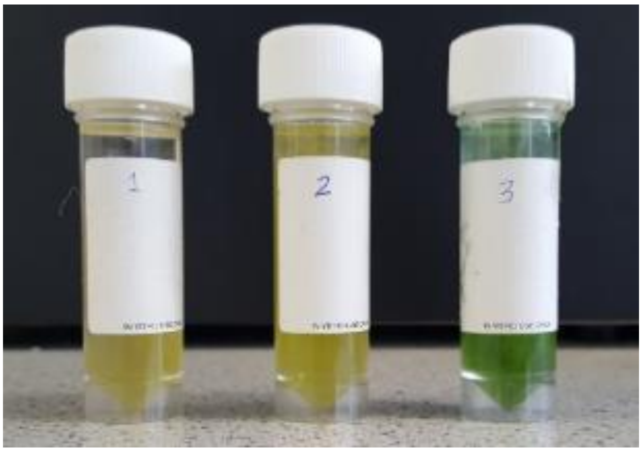


Figure D.2 Aqueous samples sent to Culture Collection of Algae and Protozoa Research Services Limited for algae identification. Note: (1), pond comprising RB198; (2), pond comprising BR46; (3), pond comprising synthetic wastewater without dye.

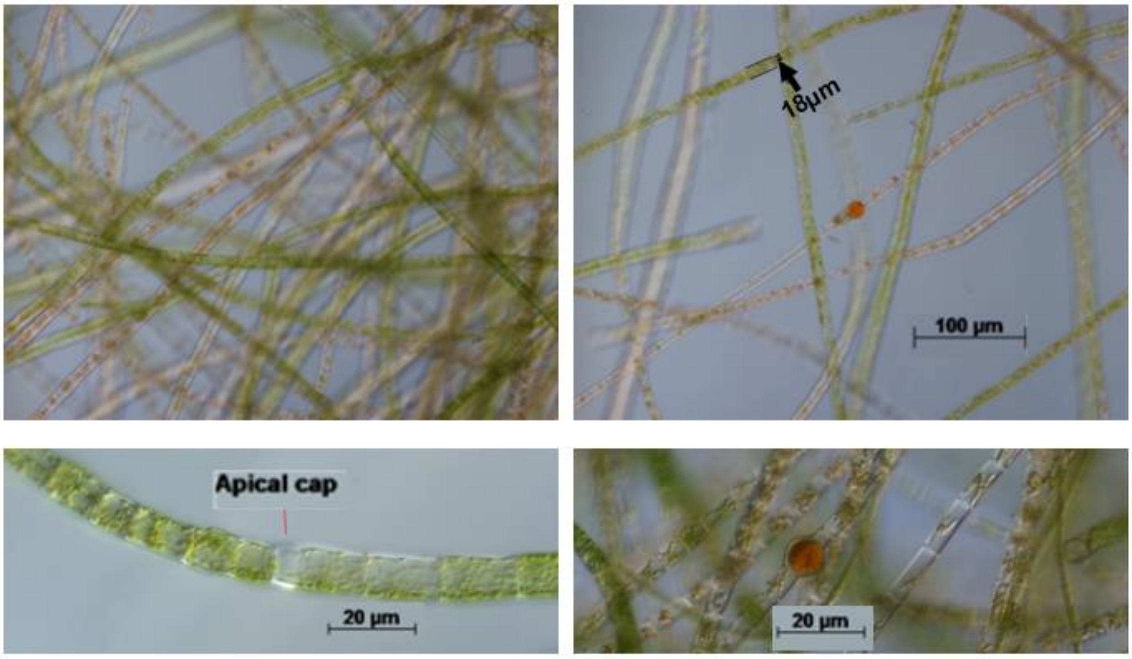
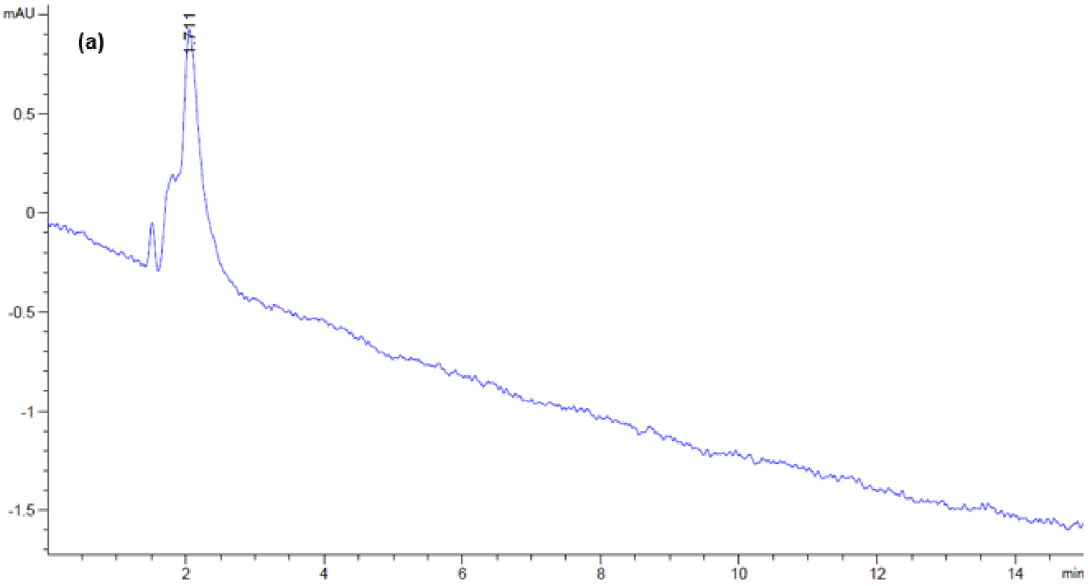
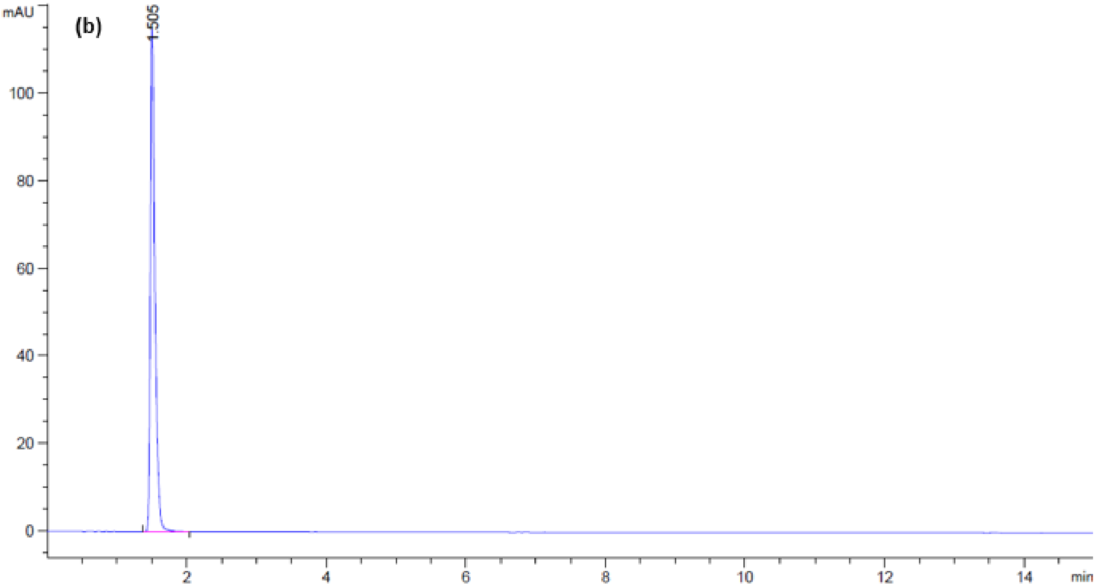


Figure D.3 Filamentous algae of Oedogonium species identified in pond systems of the third experiment by The Culture Collection of Algae and Protozoa Research Services Limited

Figure D.4 Outflow samples sent to Concept Life Sciences Analytical & Development Services Limited for GC-MS analysis

Appendix E High performance liquid chromatograph (HPLC) data





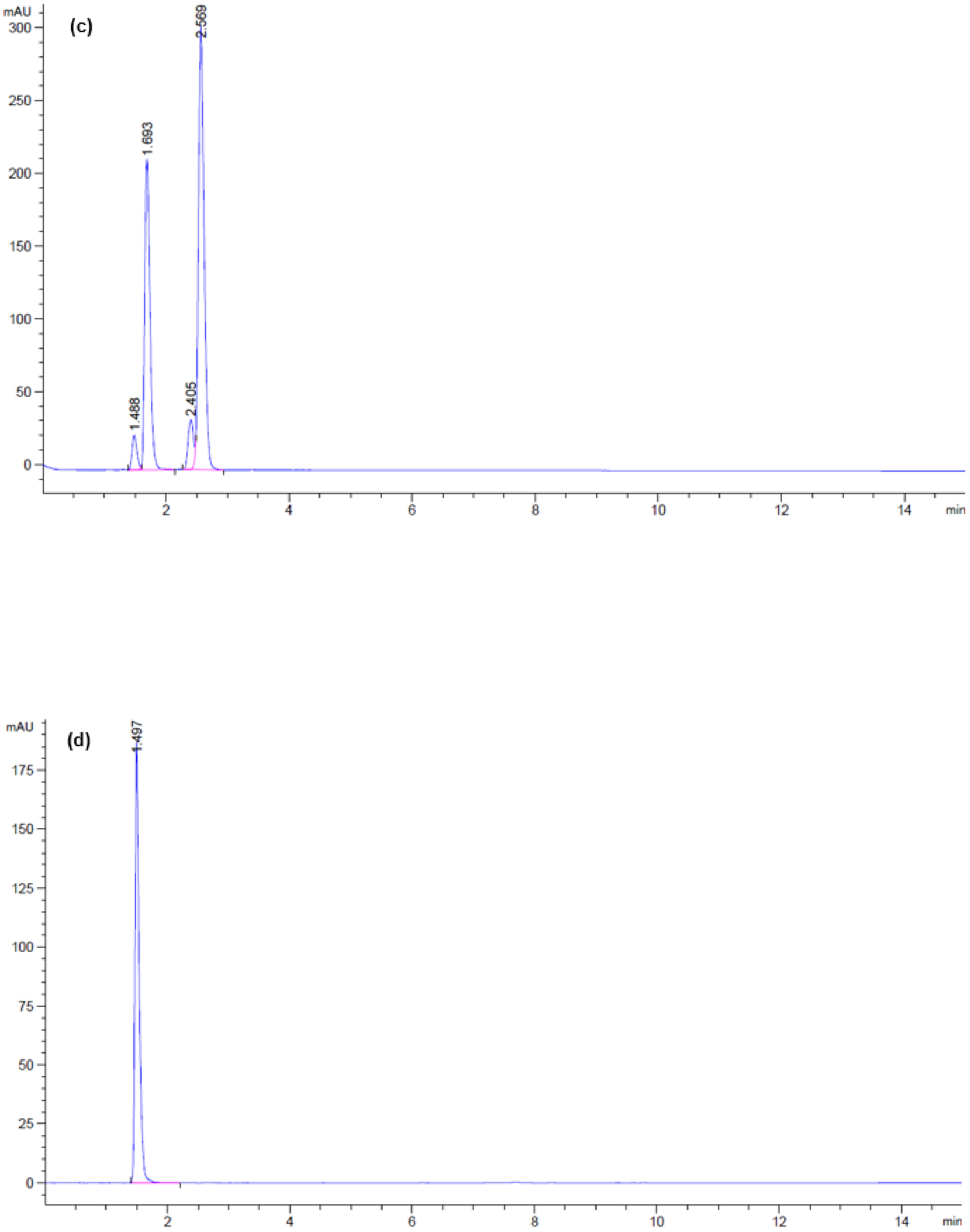
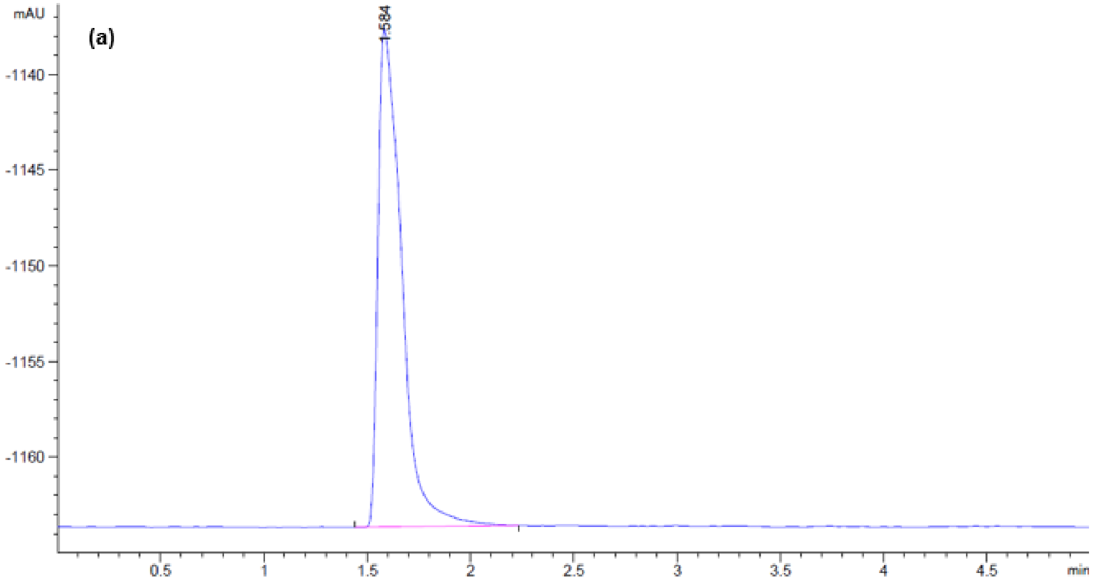
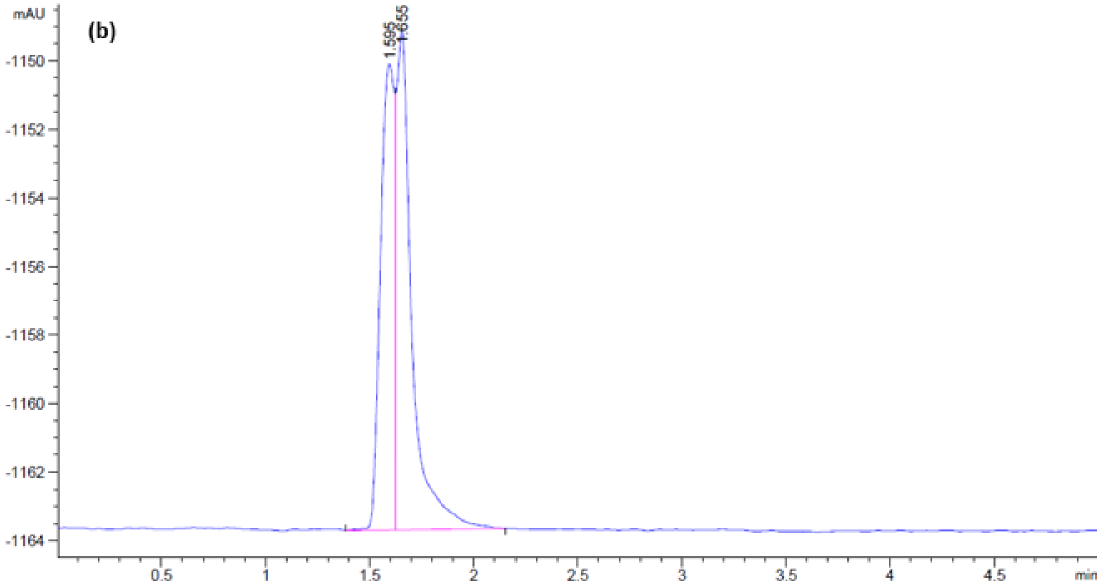


Figure E.1 HPLC analysis of dye solutions before treatment. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46.


mAU
(a)
1.584
-1140
-1145
-1150
-1155
-1160
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min


mAU
(b)
1.595
1.655
-1150
-1152
-1154
-1156
-1158
-1160
-1162
-1164
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

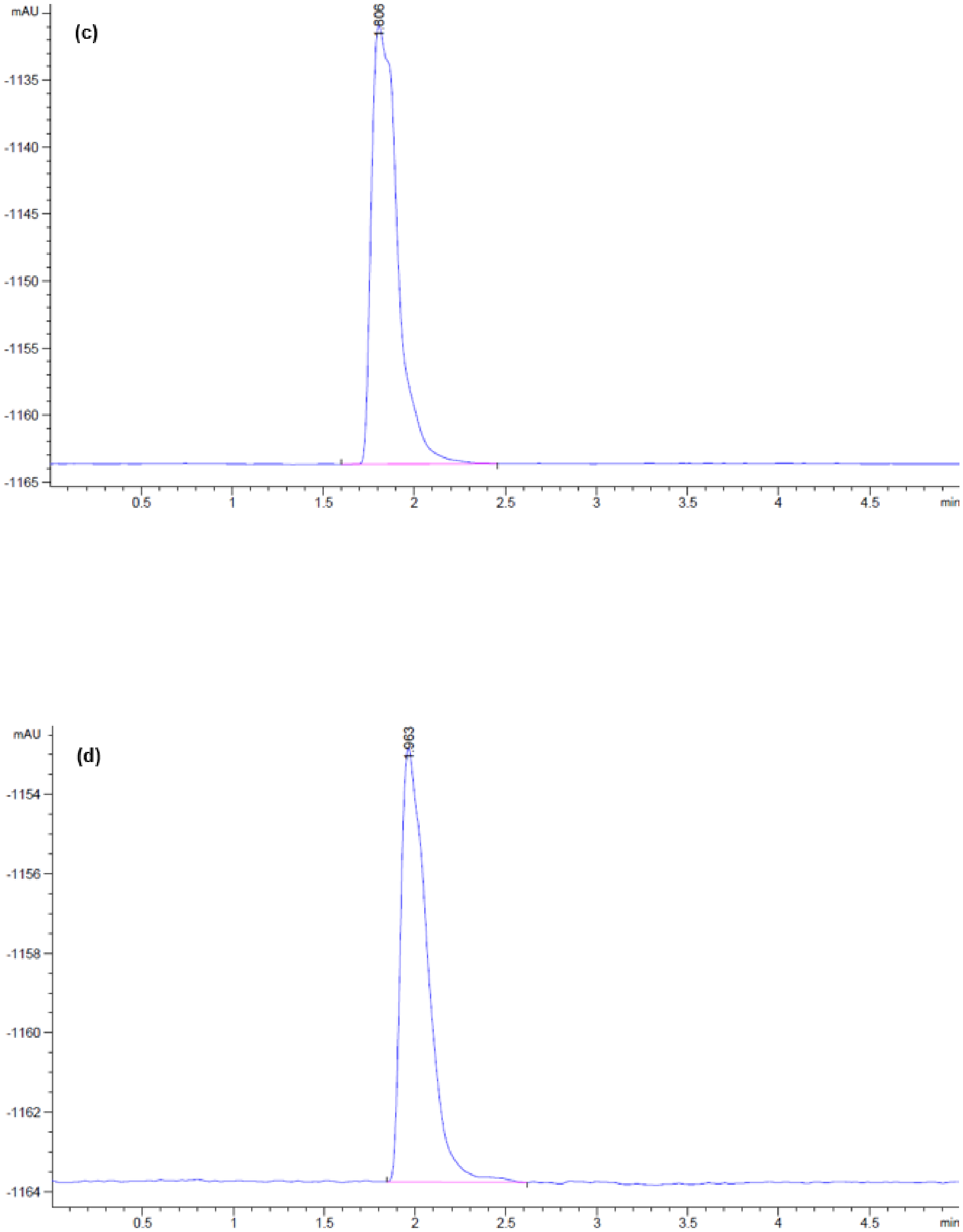
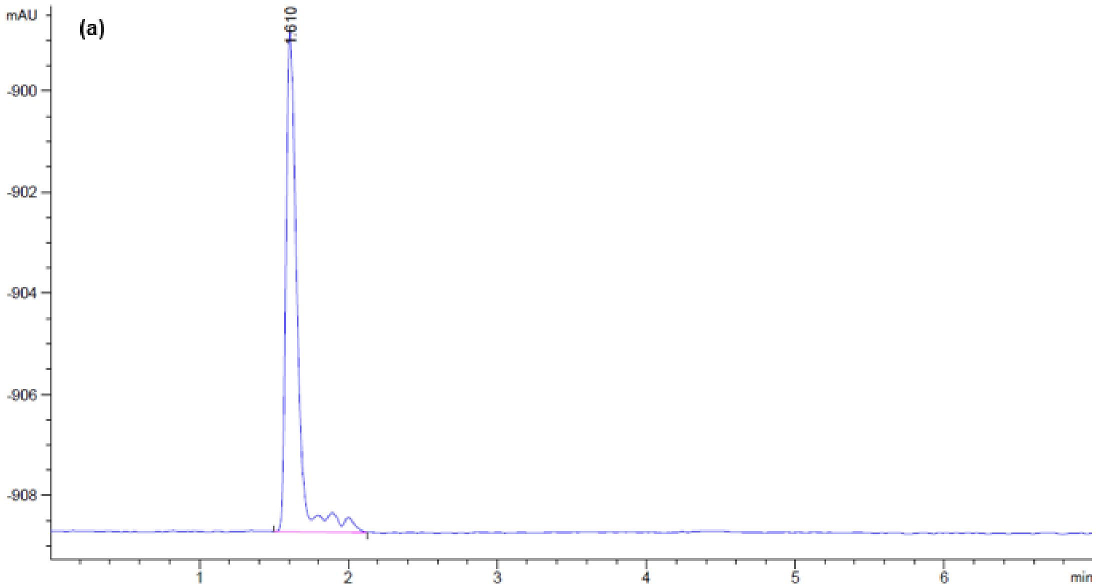
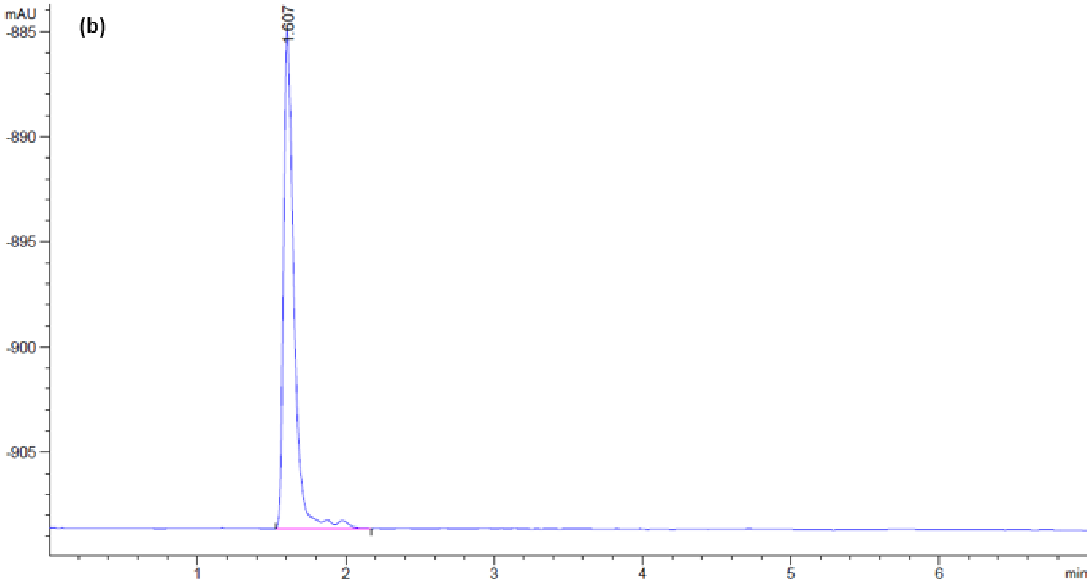


Figure E.2 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 15 September 2015 for acid blue 113 after treatment. Note: (a), *Lemna minor* L. and algae ponds; (b), algae ponds; (c), *Lemna minor* L. ponds; (d), control ponds.


mAU
(a)
1.610
-900
-902
-904
-906
-908
1
2
3
4
5
6
min


mAU
-885
(b)
1.607
-890
-895
-900
-905
1
2
3
4
5
6
min

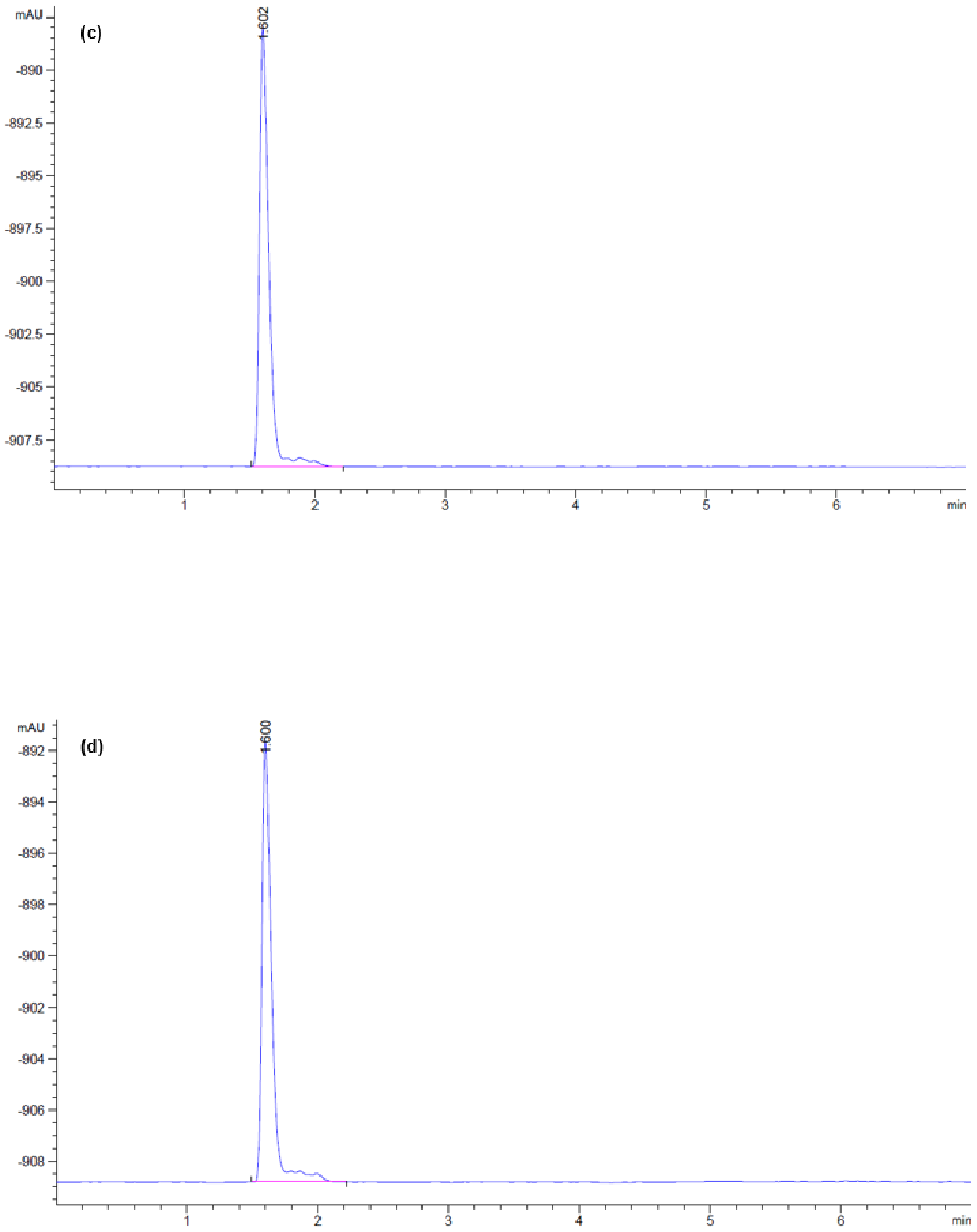
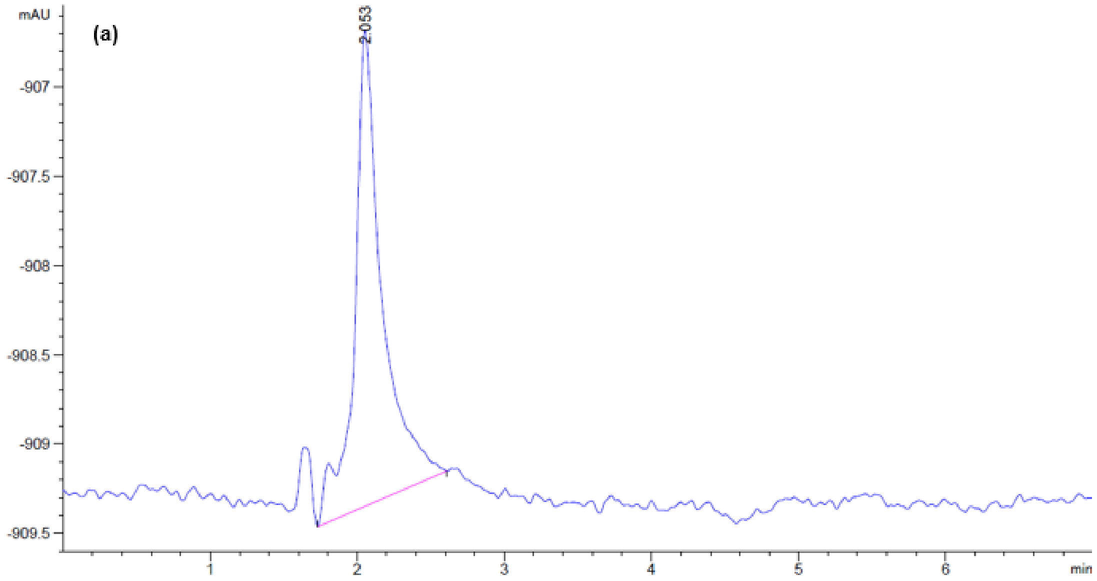
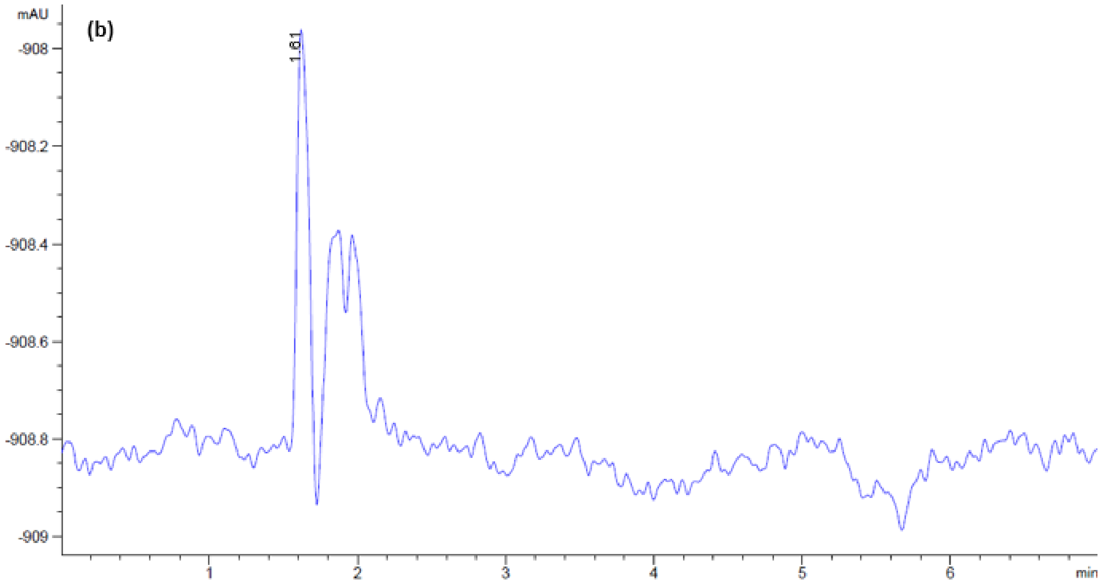
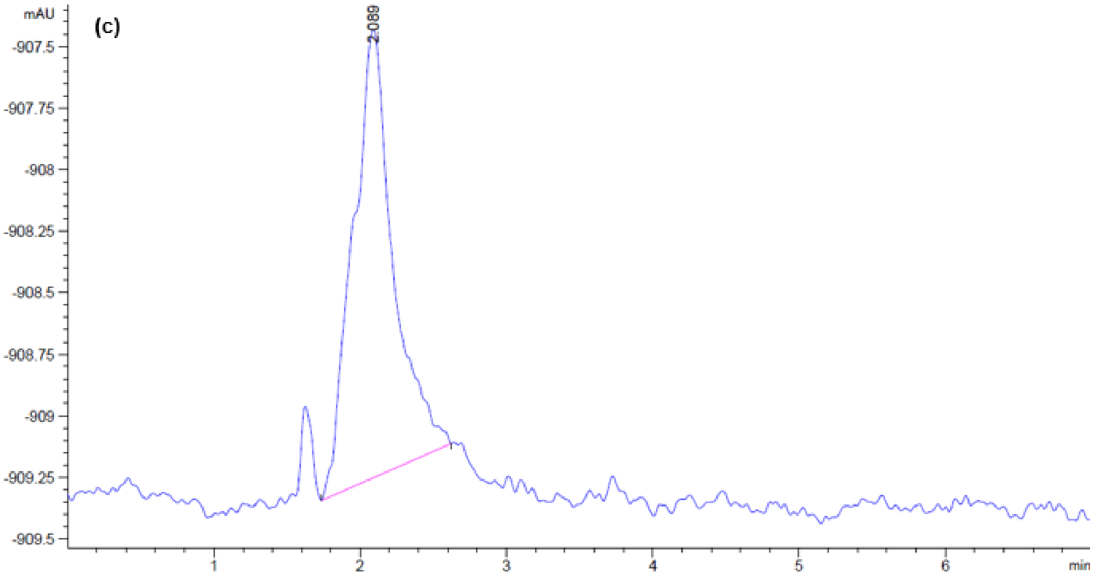


Figure E.3 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 15 September 2015 for reactive blue 198 after treatment. Note: (a), *Lemna minor* L. and algae ponds; (b), algae ponds; (c), *Lemna minor* L. ponds; (d), control ponds.


mAU
(a)
2.053
-907
-907.5
-908
-908.5
-909
-909.5
1
2
3
4
5
6
min


mAU
(b)
1.61
-908
-908.2
-908.4
-908.6
-908.8
-909
1
2
3
4
5
6
min



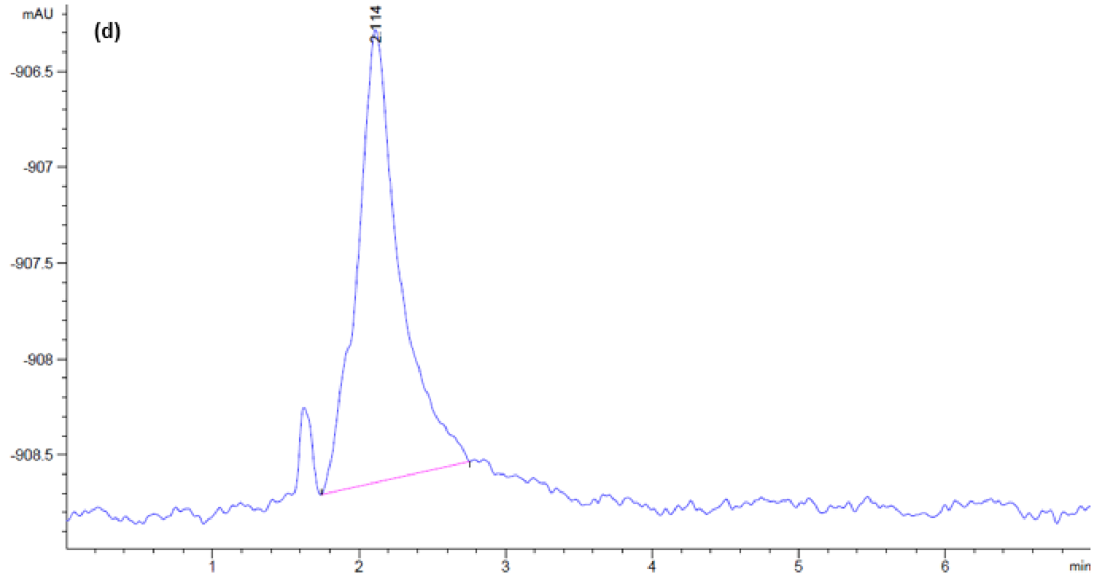
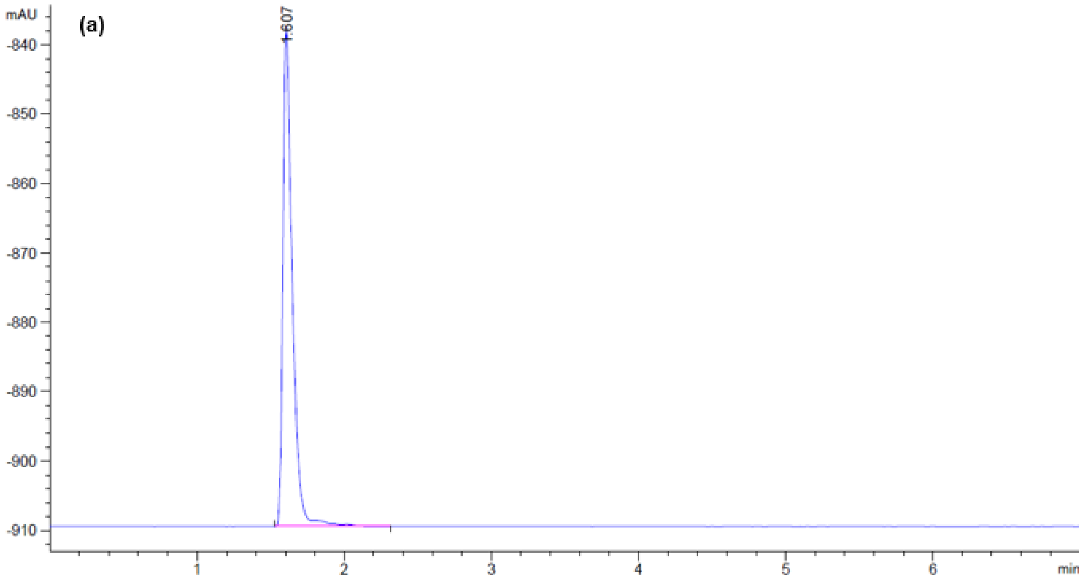
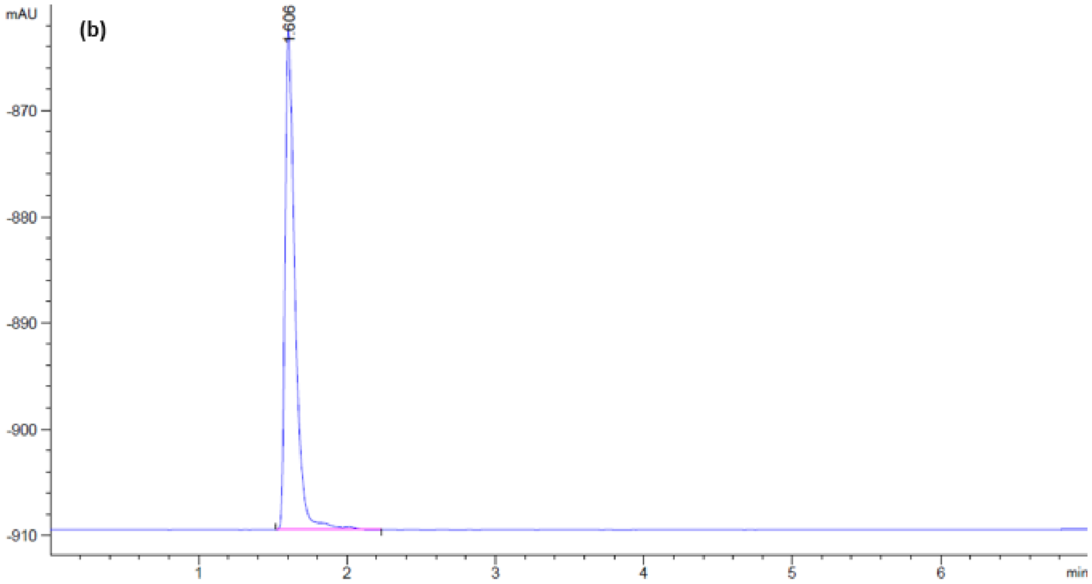
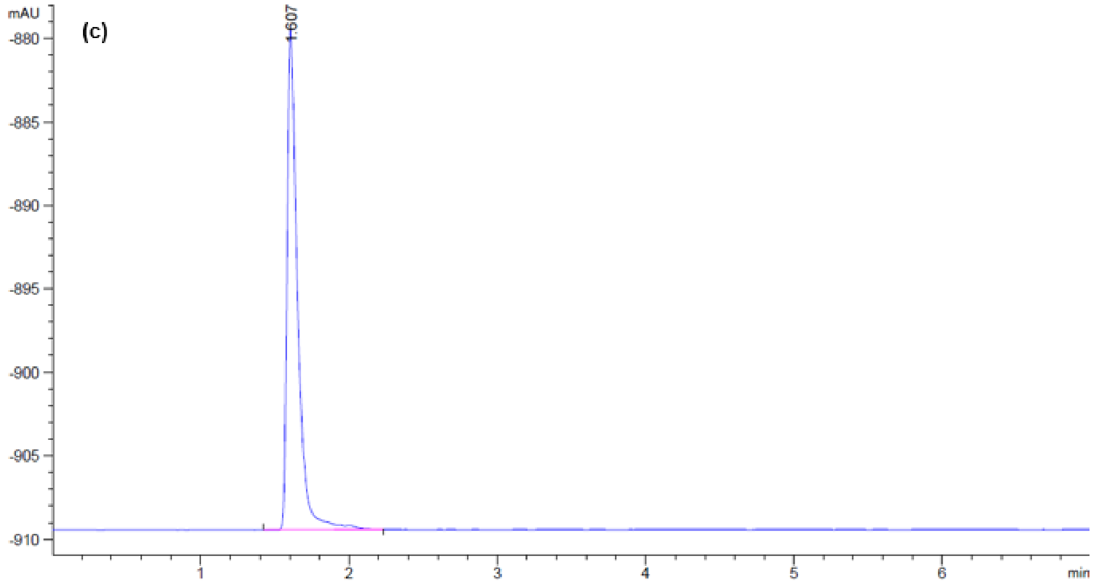


Figure E.4 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 15 September 2015 for basic red 46 after treatment. Note: (a), *Lemna minor* L. and algae ponds; (b), algae ponds; (c), *Lemna minor* L. ponds; (d), control ponds.


mAU
(a)
1.607
-840
-850
-860
-870
-880
-890
-900
-910
1
2
3
4
5
6
min


mAU
(b)
1.606
-870
-880
-890
-900
-910
1
2
3
4
5
6
min



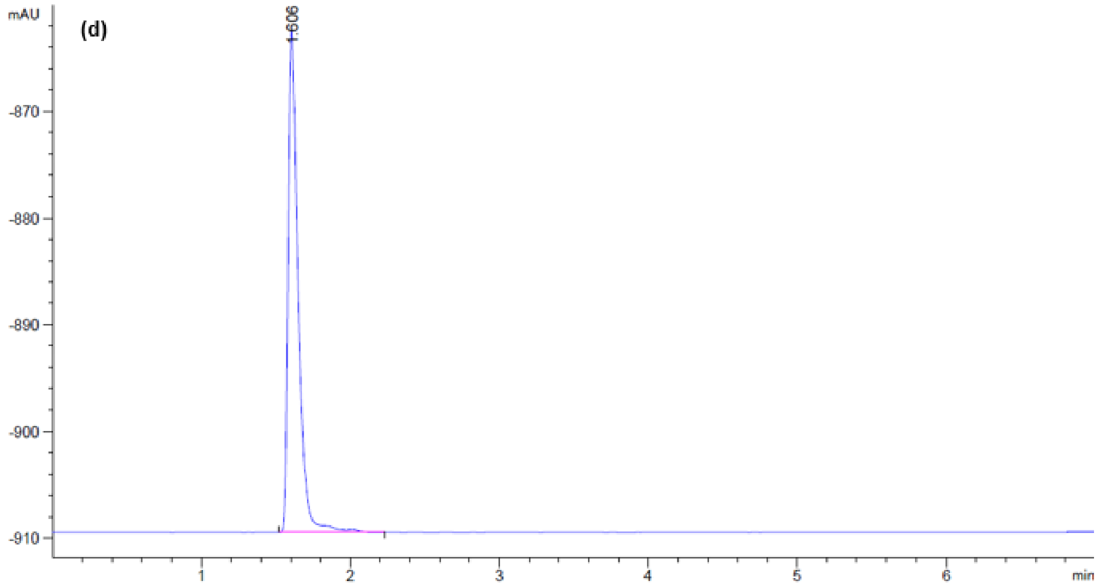


Figure E.5 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 15 September 2015 for direct orange after treatment. Note: (a), *Lemna minor* L. and algae ponds; (b), algae ponds; (c), *Lemna minor* L. ponds; (d), control ponds.

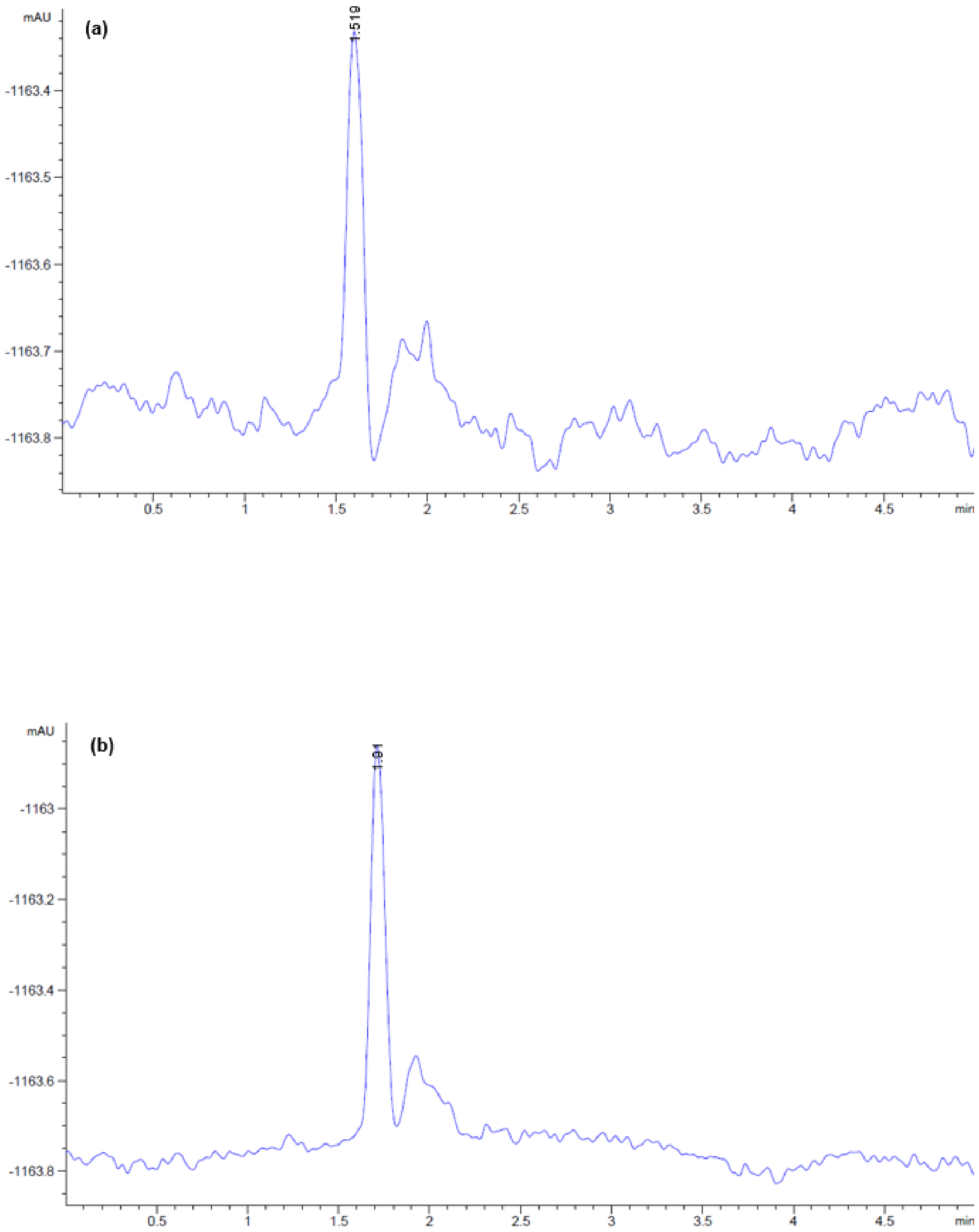
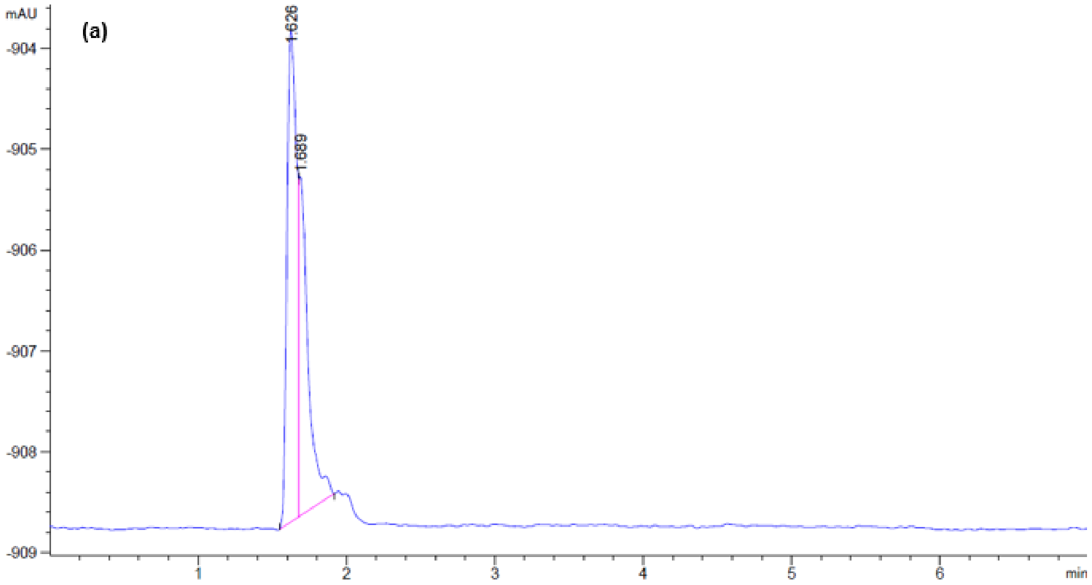


Figure E.6 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 2 February 2016 for acid blue 113 after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.



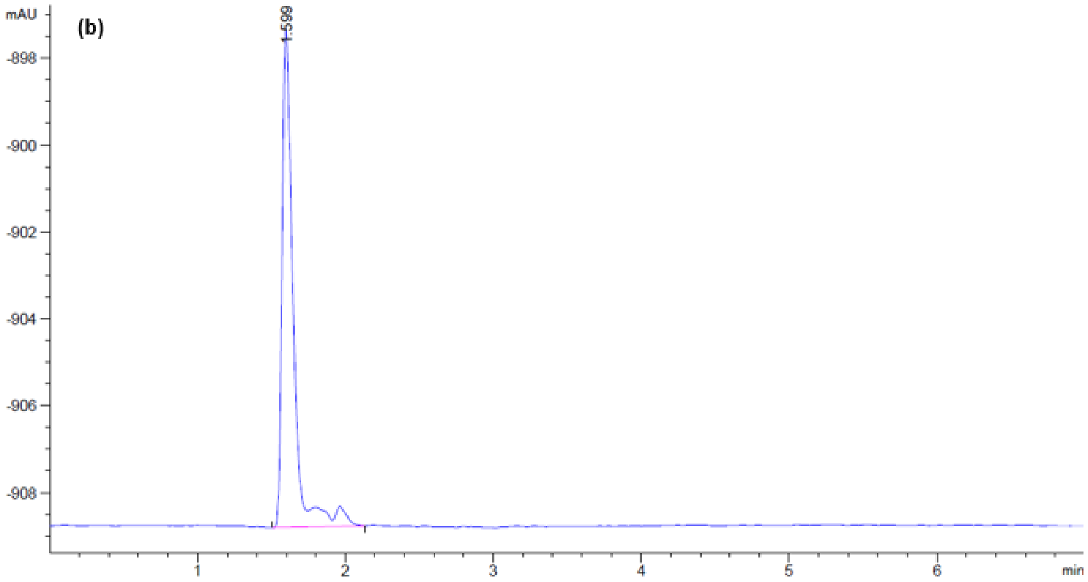


Figure E.7 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 2 February 2016 for reactive blue 198 after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.

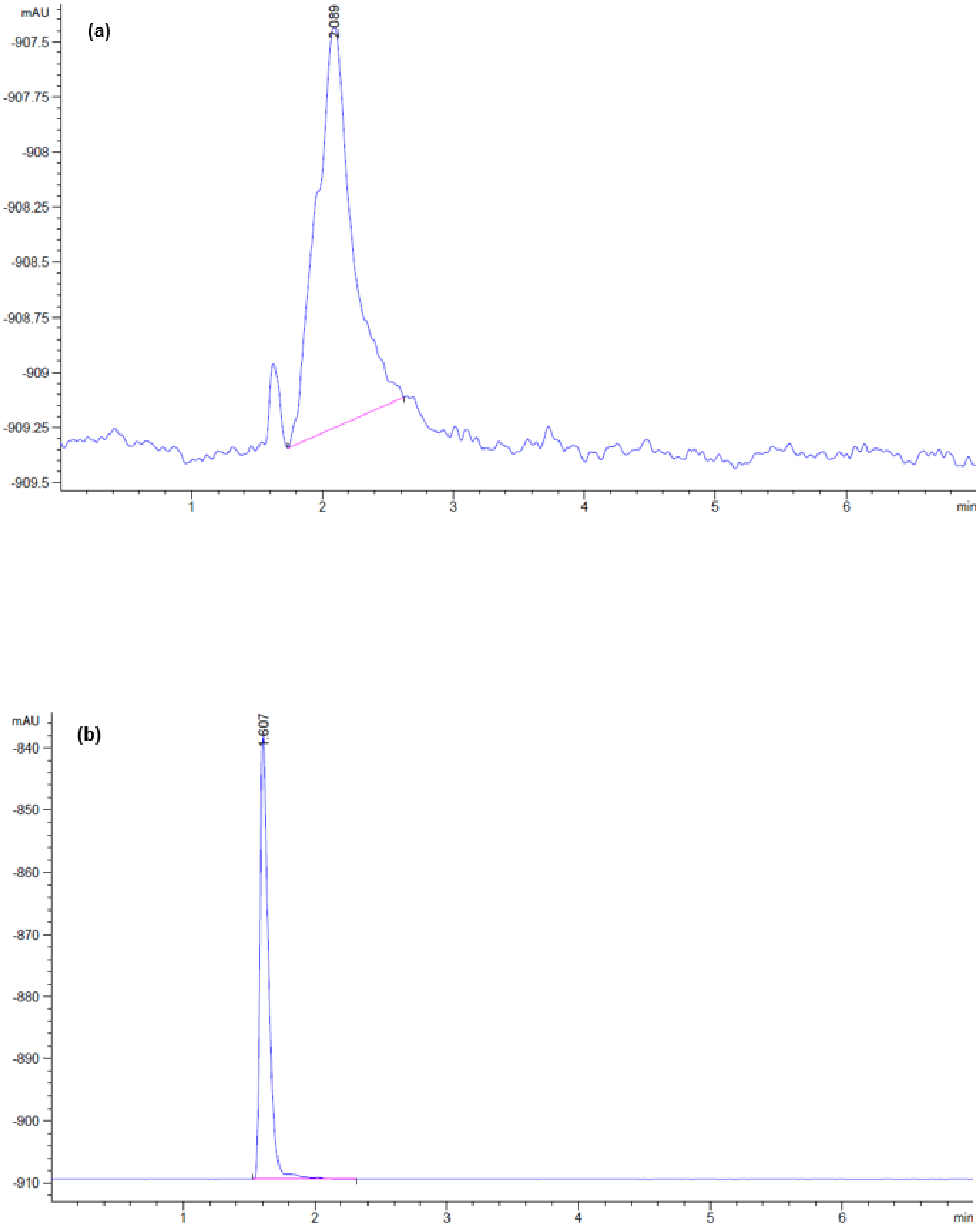


Figure E.8 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 2 February 2016 for basic red 46 after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.

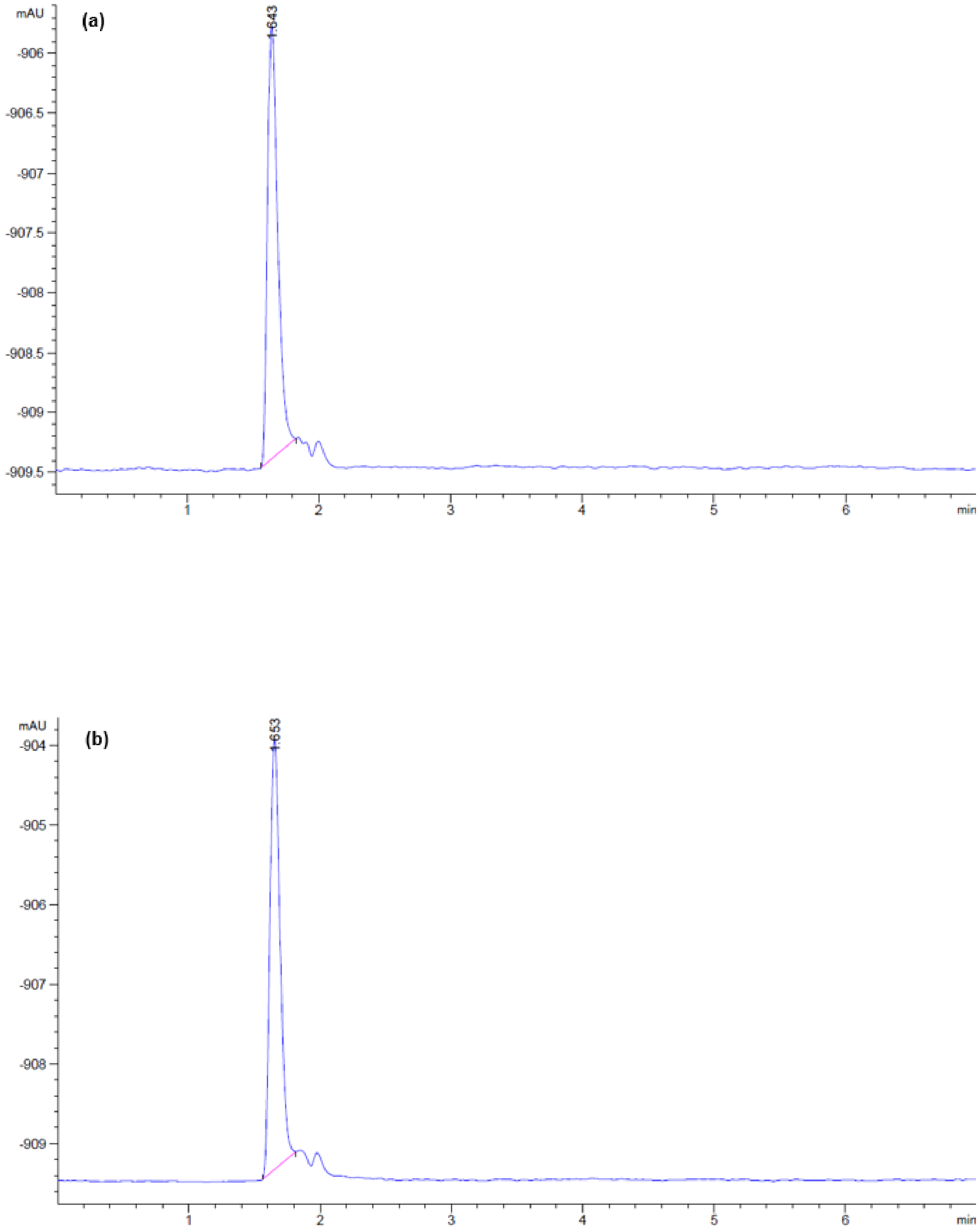
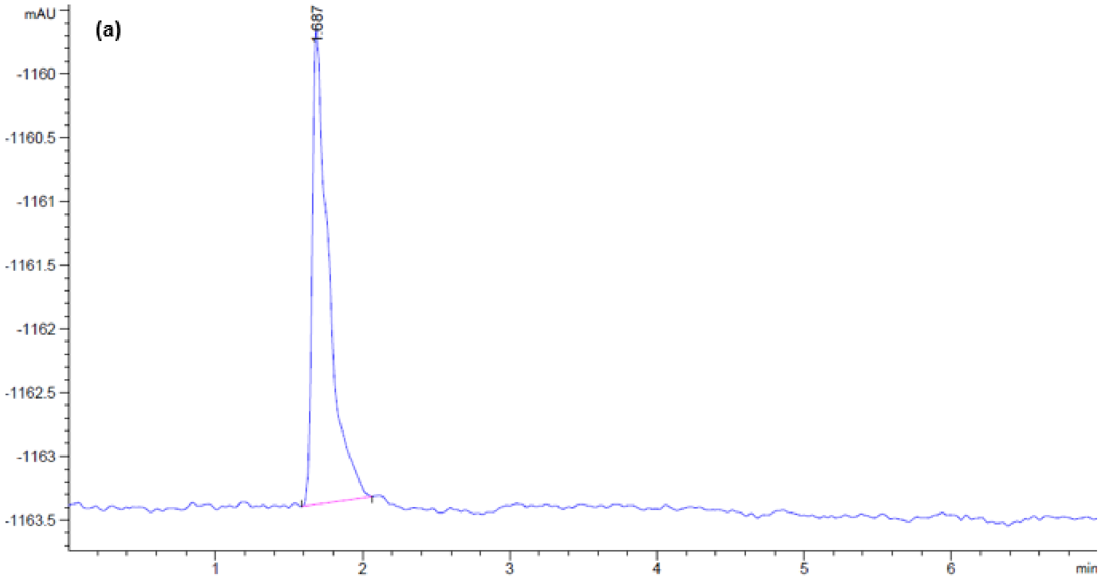
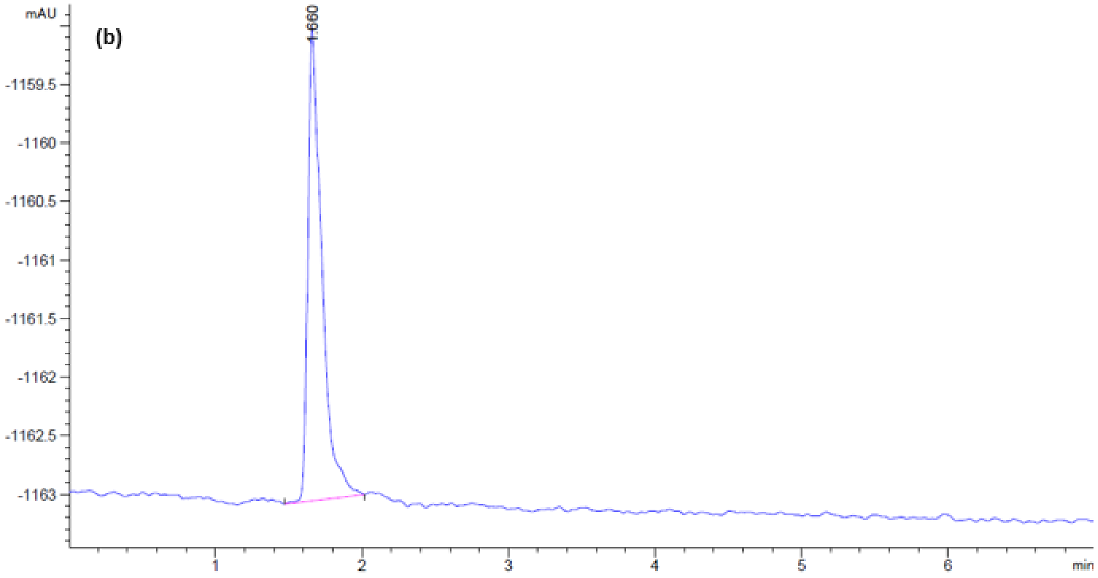
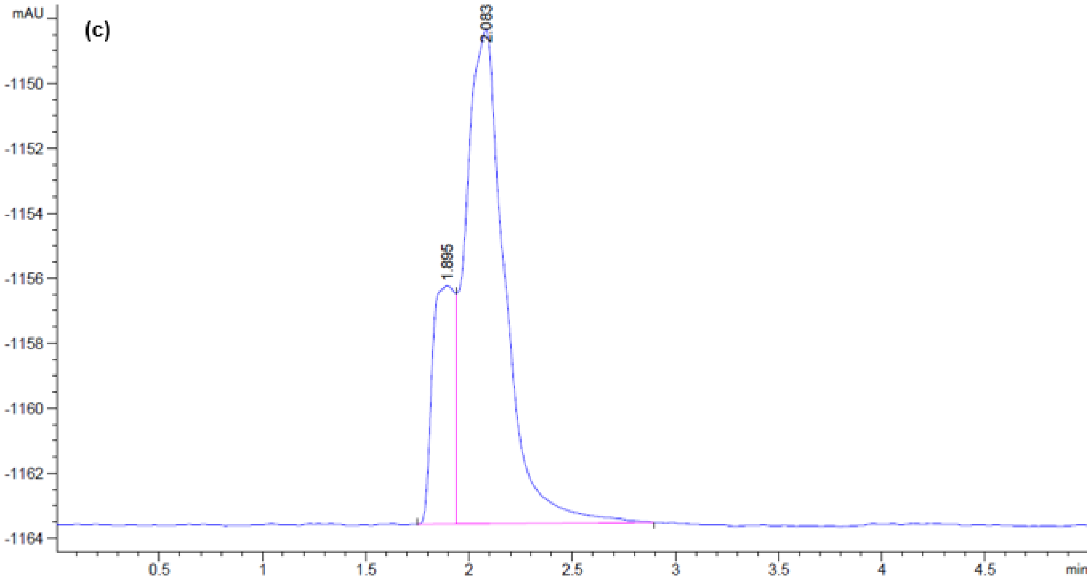
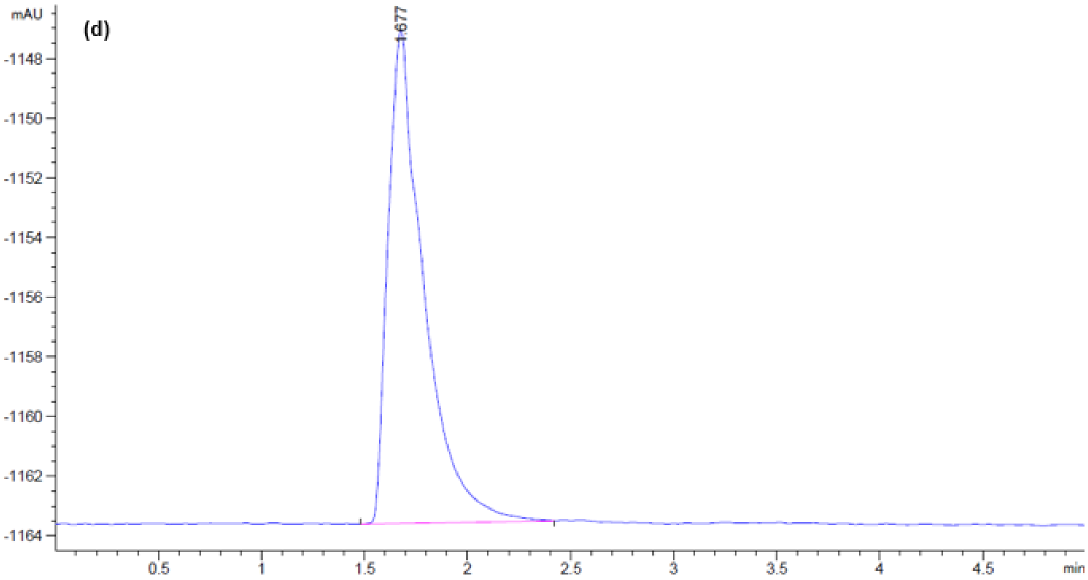


Figure E.9 HPLC analysis at the end of the experiment rig, which was operated between 15 December 2014 and 2 February 2016 for direct orange 46 after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.


(a)
1.687
mAU
-1160
-1160.5
-1161
-1161.5
-1162
-1162.5
-1163
-1163.5
1
2
3
4
5
6
min


(b)
1.660
mAU
-1159.5
-1160
-1160.5
-1161
-1161.5
-1162
-1162.5
-1163
1
2
3
4
5
6
min


mAU
(c)
2.083
1.895
-1150
-1152
-1154
-1156
-1158
-1160
-1162
-1164
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min


mAU
(d)
1.677
-1148
-1150
-1152
-1154
-1156
-1158
-1160
-1162
-1164
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

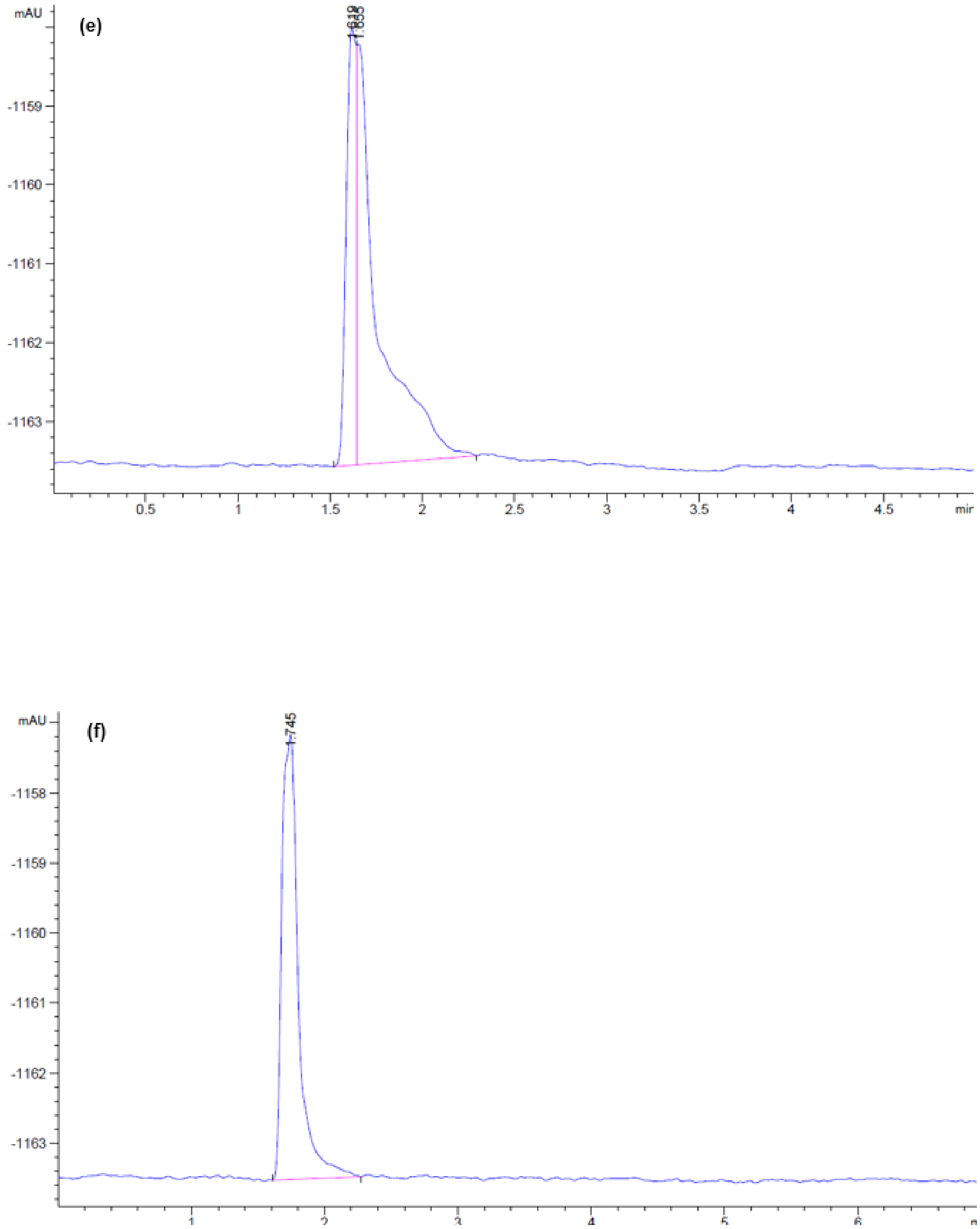
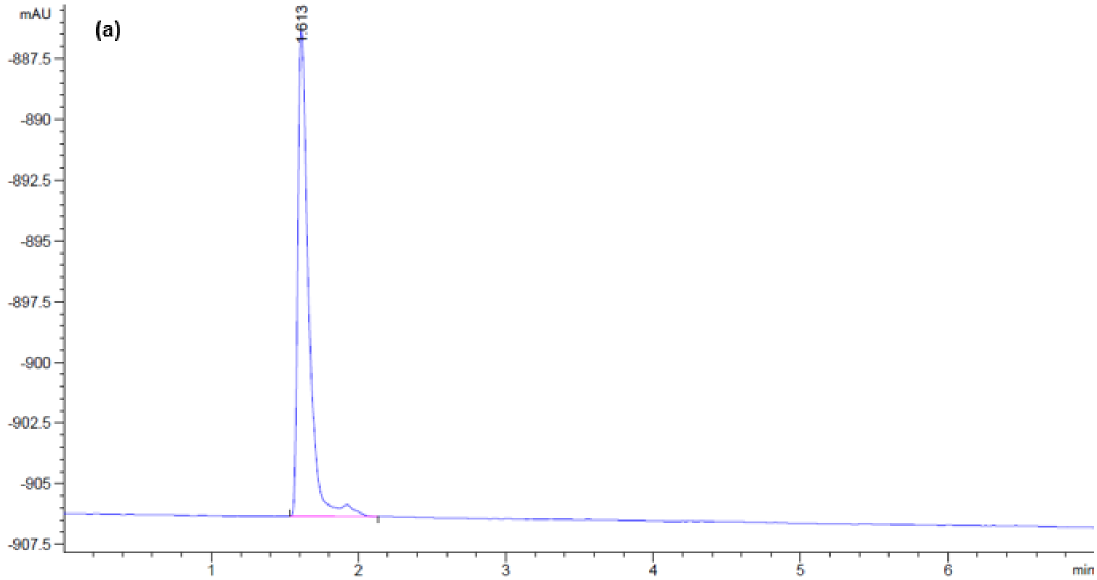
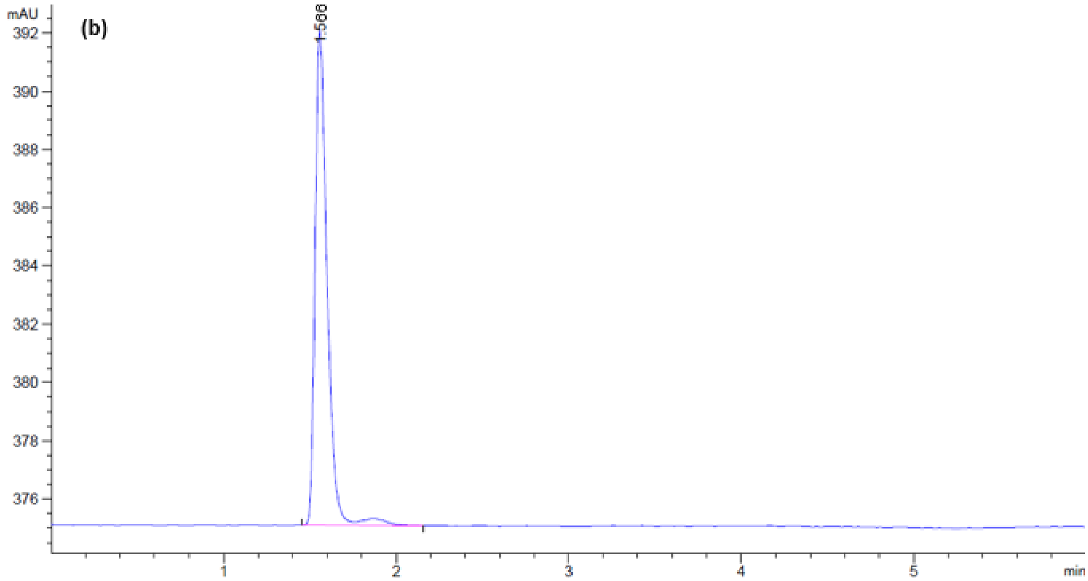
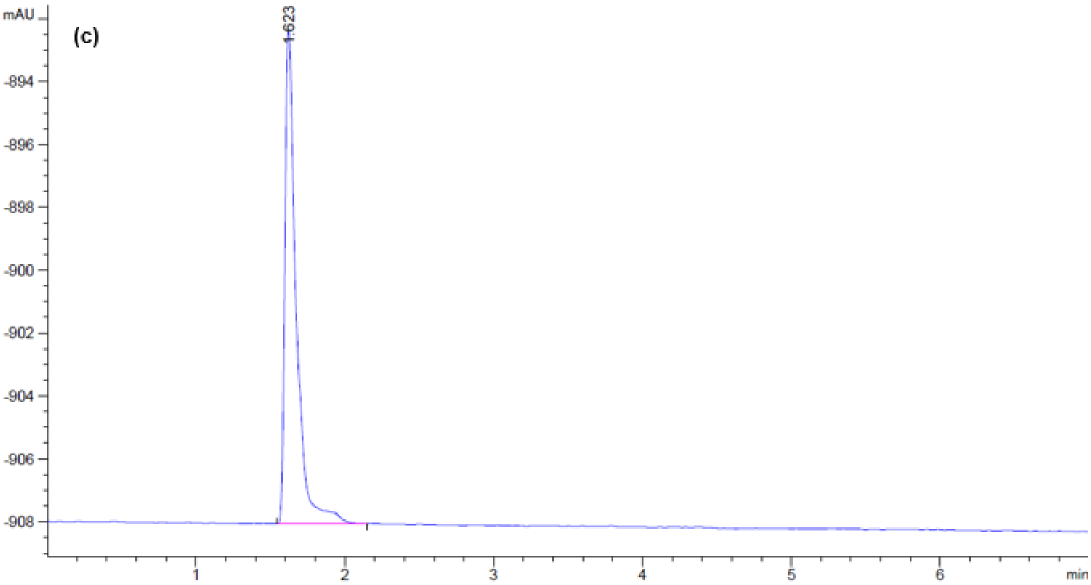
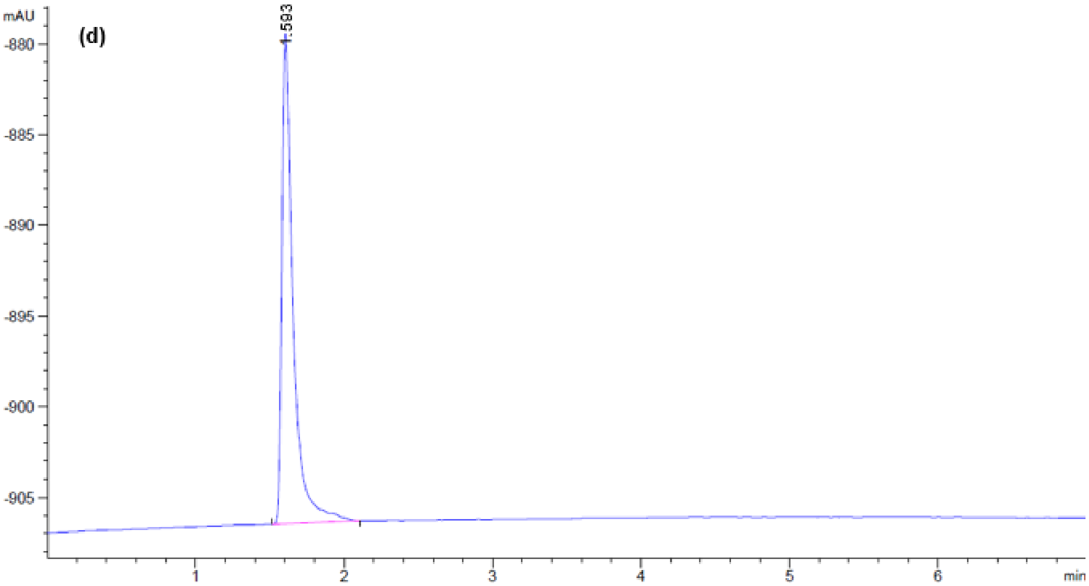


Figure E.10 HPLC analysis at the end of experiment rig, which was operated between 1 October 2015 and 19 January 2016 for acid blue 113 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
(a)
1.613
-887.5
-890
-892.5
-895
-897.5
-900
-902.5
-905
-907.5
1
2
3
4
5
6
min


mAU
(b)
1.566
392
390
388
386
384
382
380
378
376
1
2
3
4
5
min


mAU
(c)
1.623
-894
-896
-898
-900
-902
-904
-906
-908
1
2
3
4
5
6
min


mAU
(d)
1.593
-880
-885
-890
-895
-900
-905
1
2
3
4
5
6
min

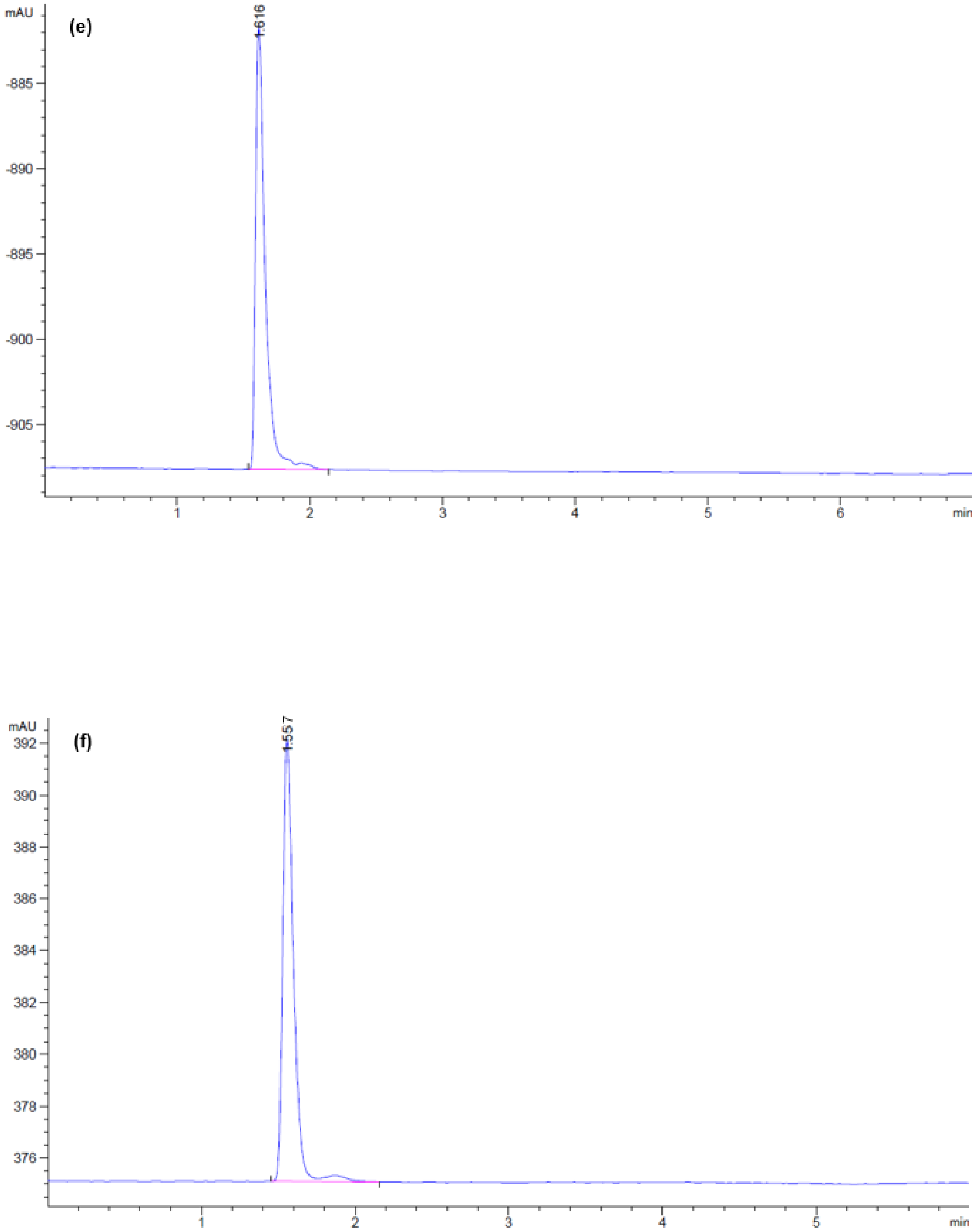
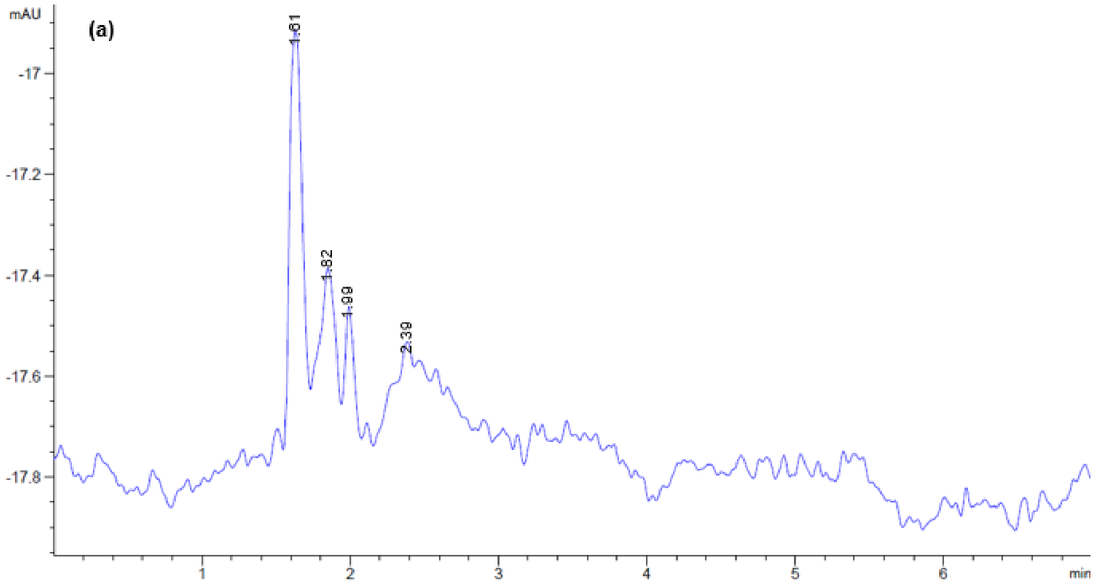
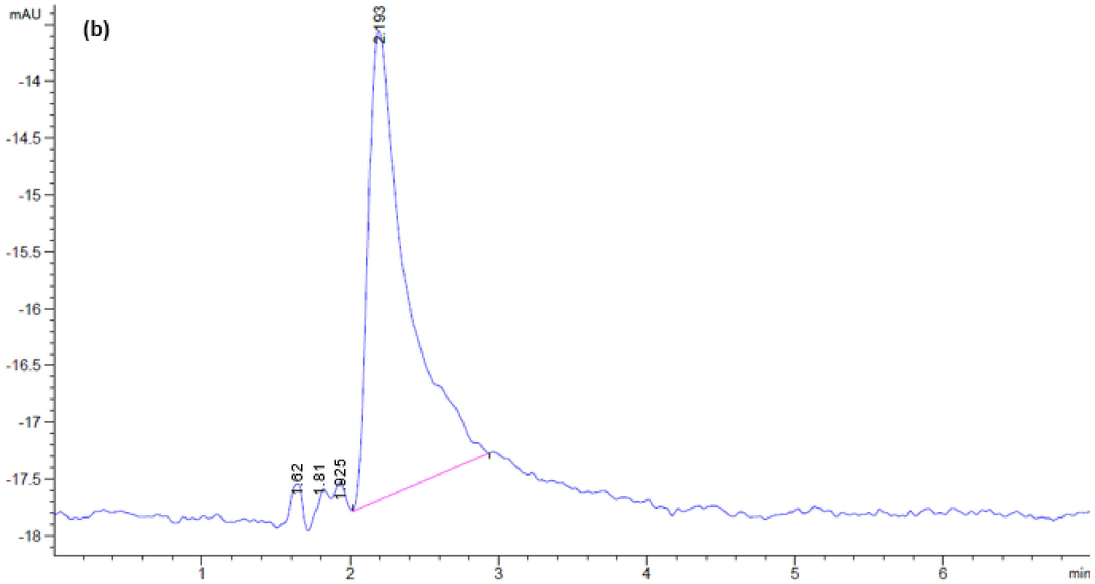
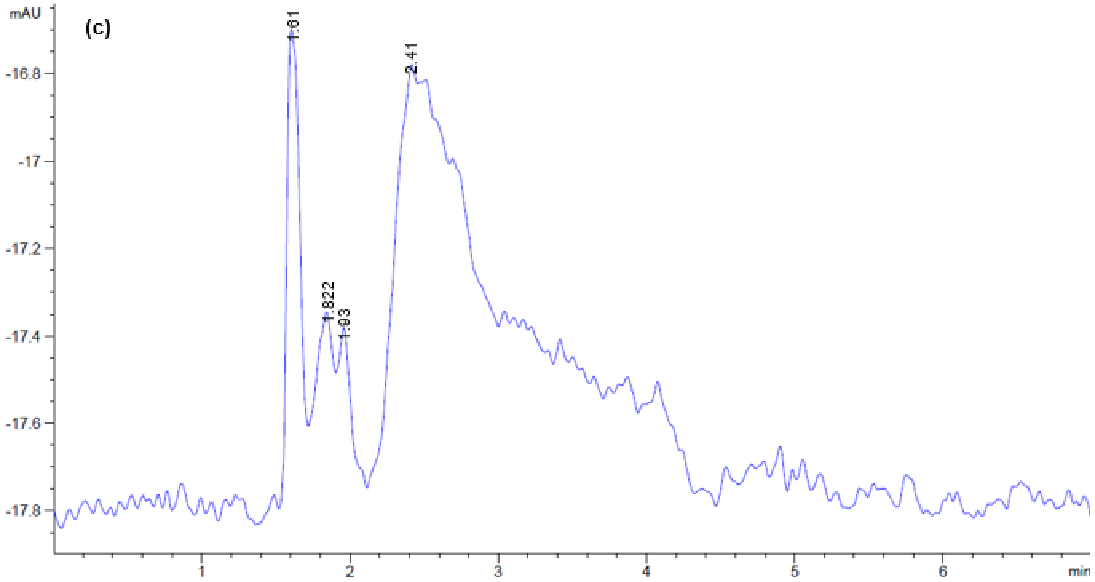


Figure E.11 HPLC analysis at the end of experiment rig, which was operated between 1 October 2015 and 19 January 2016 for reactive blue 198 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
(a)
1.61
1.82
1.99
2.39
-17
-17.2
-17.4
-17.6
-17.8
1
2
3
4
5
6
min


mAU
(b)
2.193
1.62
1.81
1.925
-14
-14.5
-15
-15.5
-16
-16.5
-17
-17.5
-18
1
2
3
4
5
6
min


mAU
(c)
1.61
2.41
1.822
1.93
-16.8
-17
-17.2
-17.4
-17.6
-17.8
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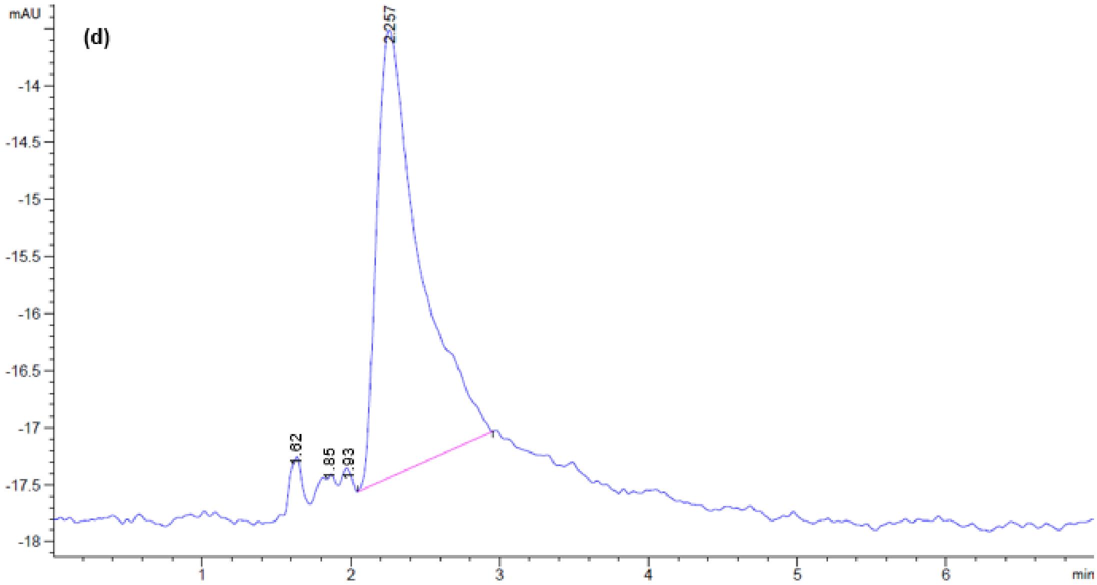
2
3
4
5
6
min


mAU
(d)
2.257
1.62
1.85
1.93
-14
-14.5
-15
-15.5
-16
-16.5
-17
-17.5
-18
1
2
3
4
5
6
min

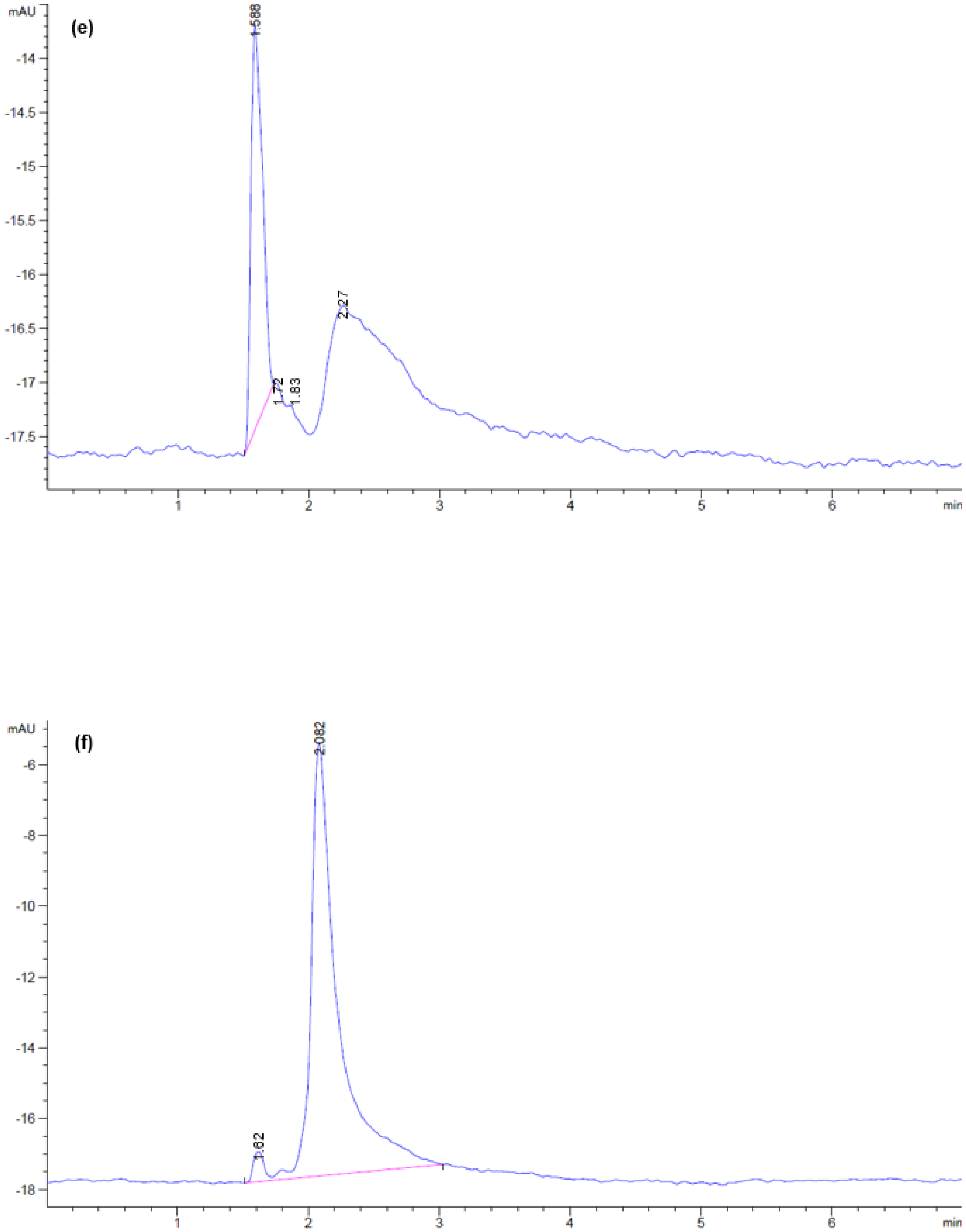
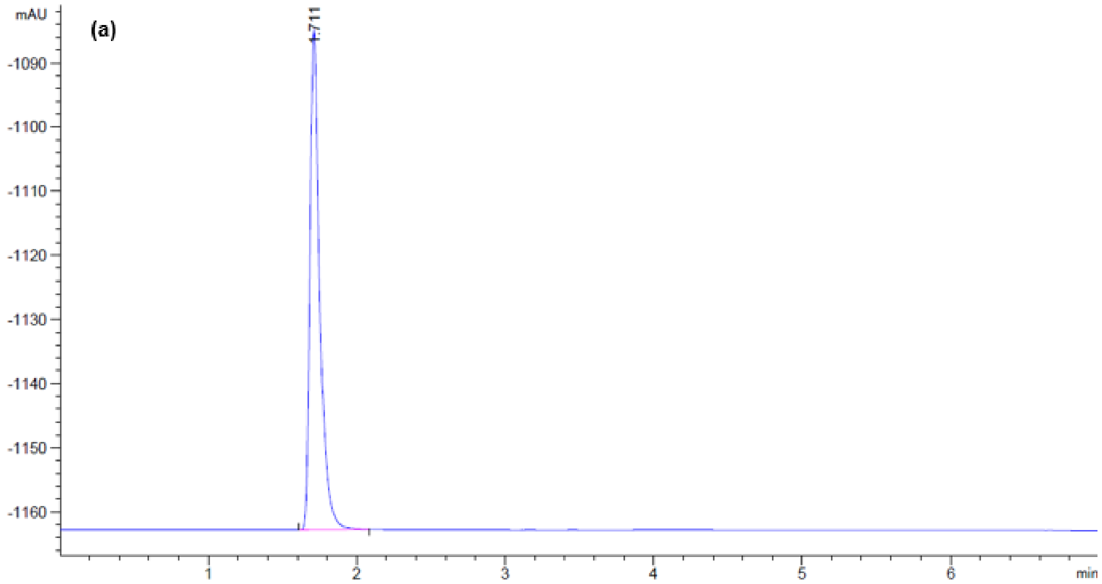
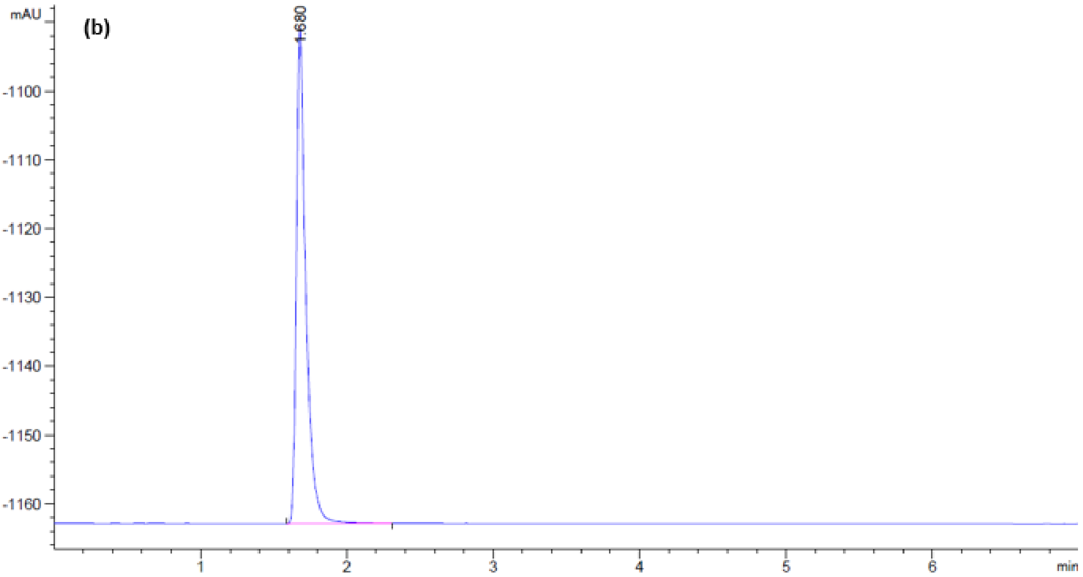
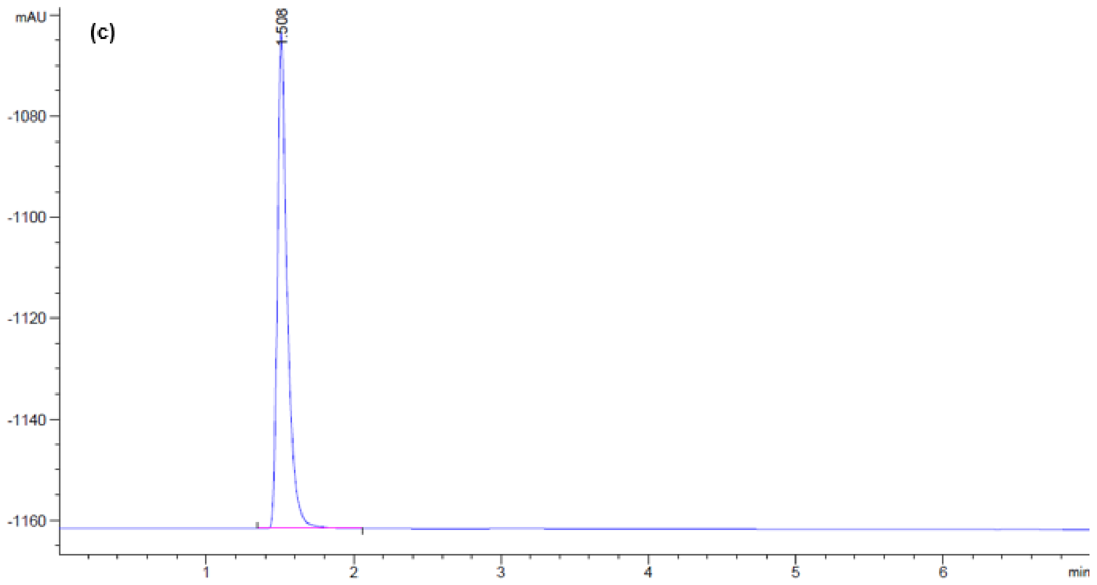
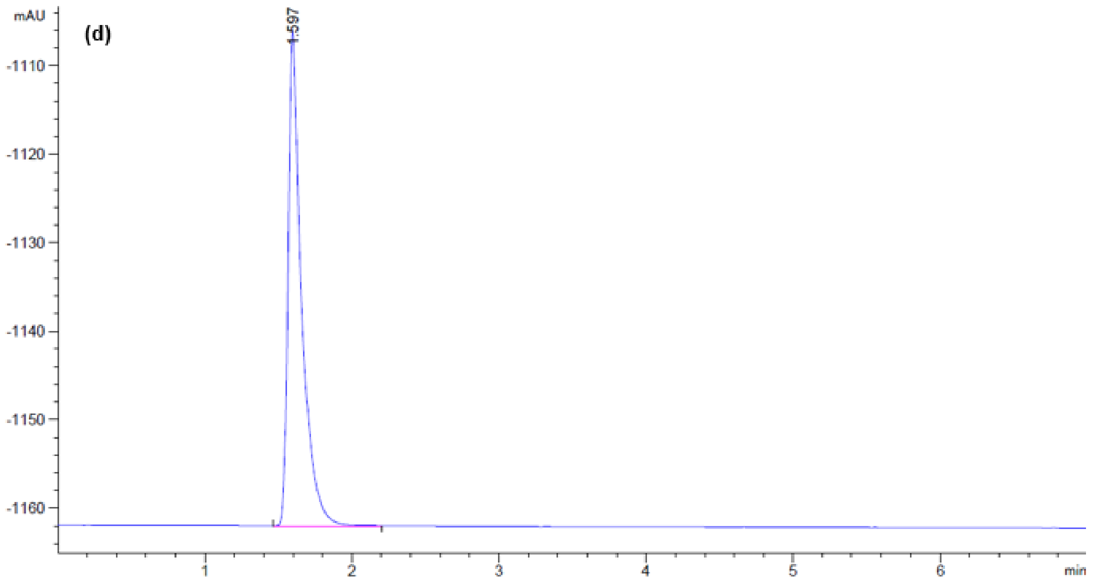


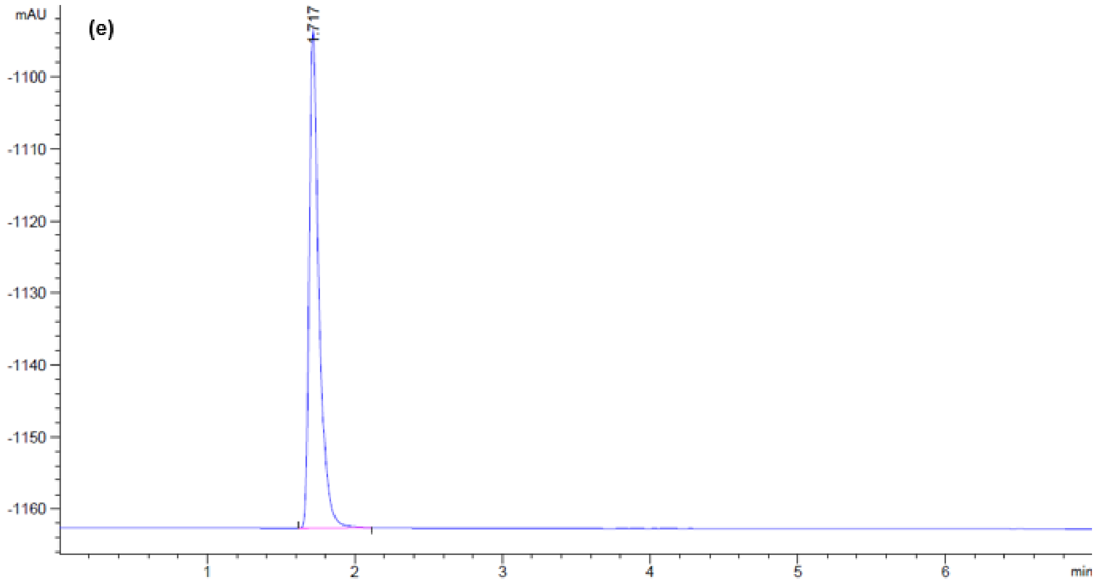
Figure E.12 HPLC analysis at the end of experiment rig, which was operated between 1 October 2015 and 19 January 2016 for basic red 46 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
(a)
1.711
-1090
-1100
-1110
-1120
-1130
-1140
-1150
-1160
1
2
3
4
5
6
min


mAU
(b)
1.680
-1100
-1110
-1120
-1130
-1140
-1150
-1160
1
2
3
4
5
6
min


(c)
mAU
1.508
-1080
-1100
-1120
-1140
-1160
1
2
3
4
5
6
min


(d)
mAU
1.597
-1110
-1120
-1130
-1140
-1150
-1160
1
2
3
4
5
6
min



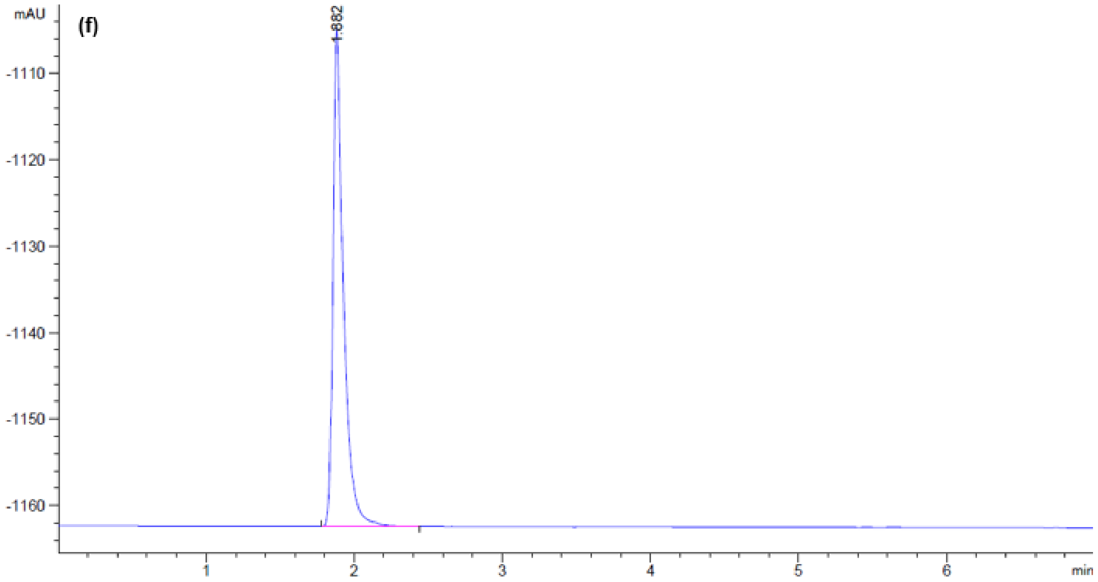
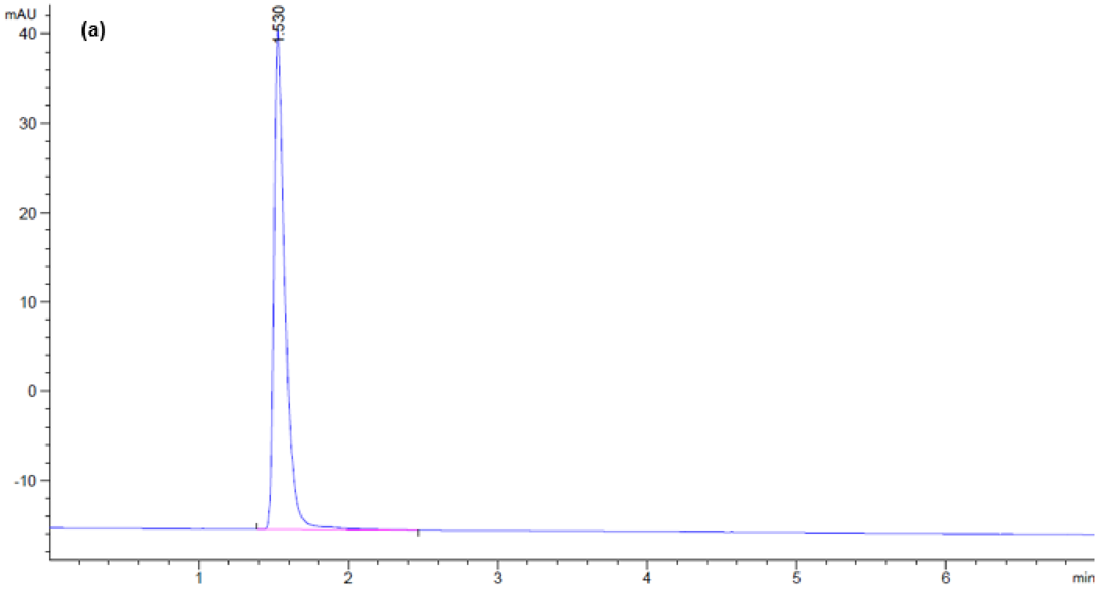
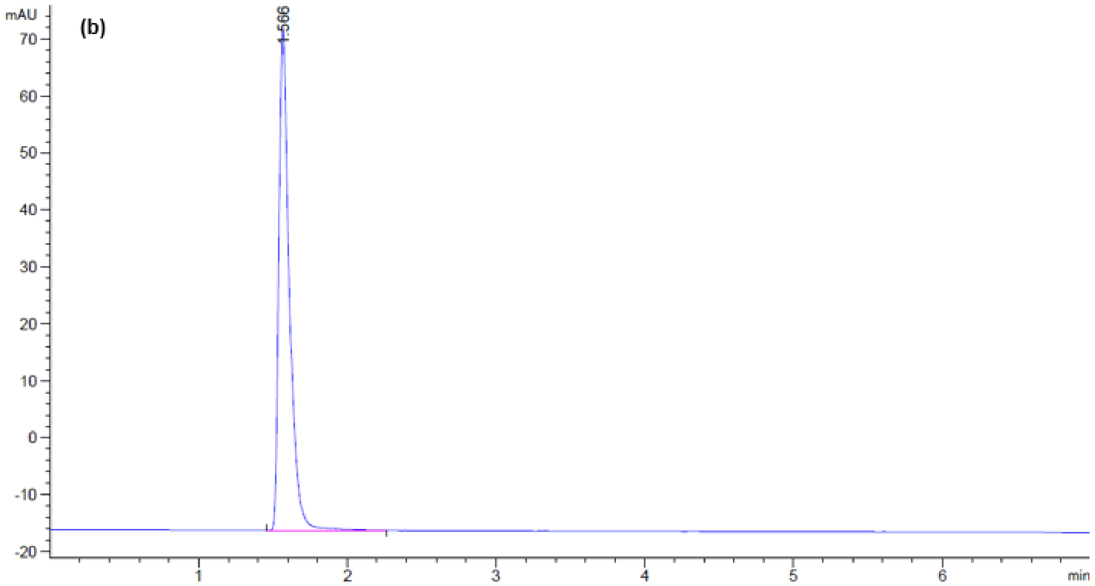
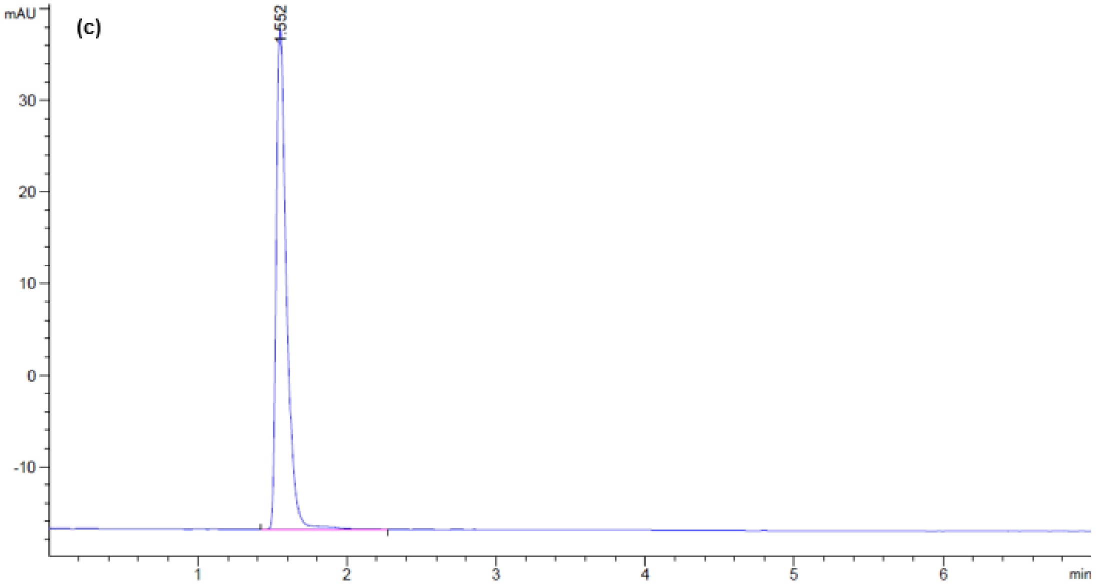
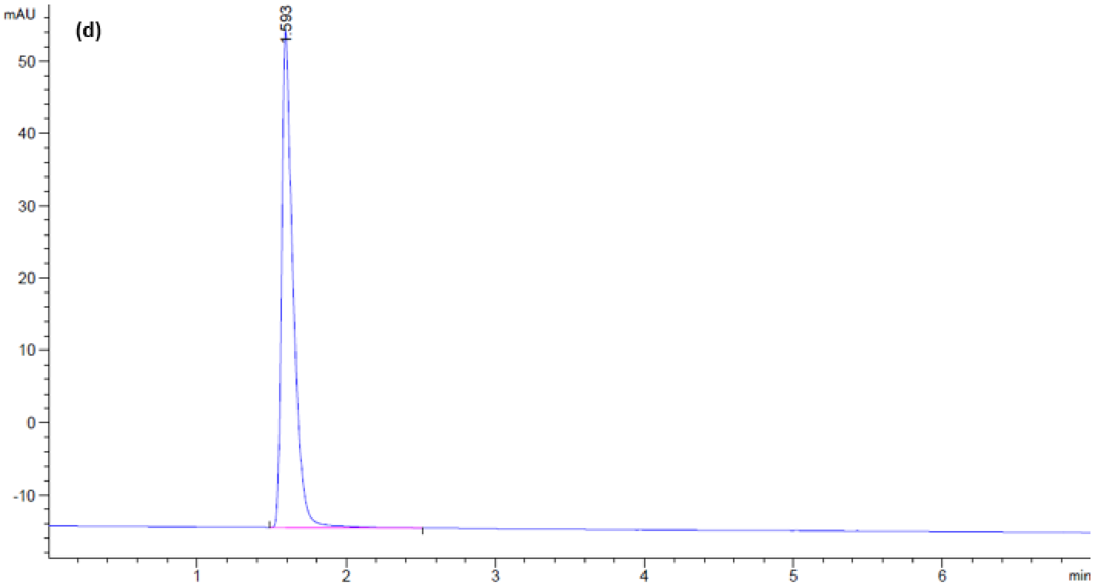


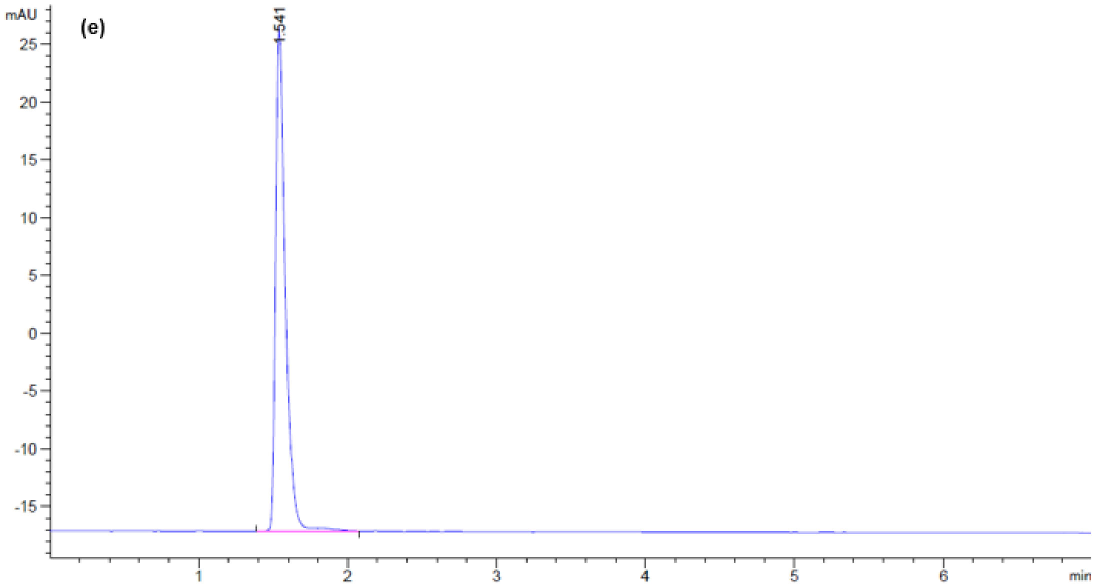
Figure E.13 HPLC analysis at the end of experiment rig, which was operated between 1 October 2015 and 19 January 2016 for direct orange 46 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
40
30
20
10
0
-10
(a)
1.530
1
2
3
4
5
6
min


mAU
70
60
50
40
30
20
10
0
-10
-20
(b)
1.566
1
2
3
4
5
6
min


mAU
(c)
1.552
30
20
10
0
-10
1
2
3
4
5
6
min


mAU
(d)
1.593
50
40
30
20
10
0
-10
1
2
3
4
5
6
min



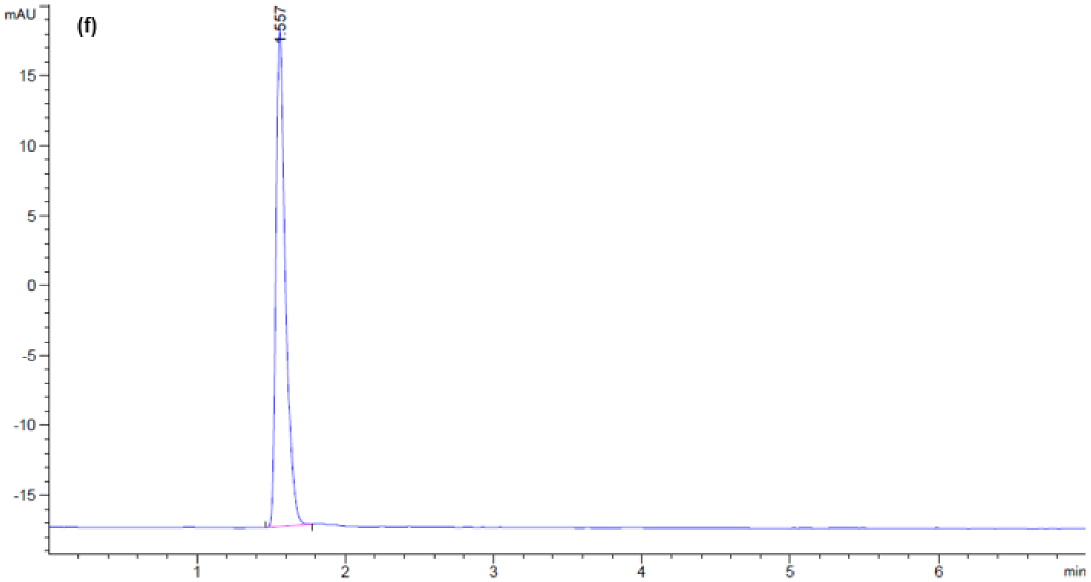
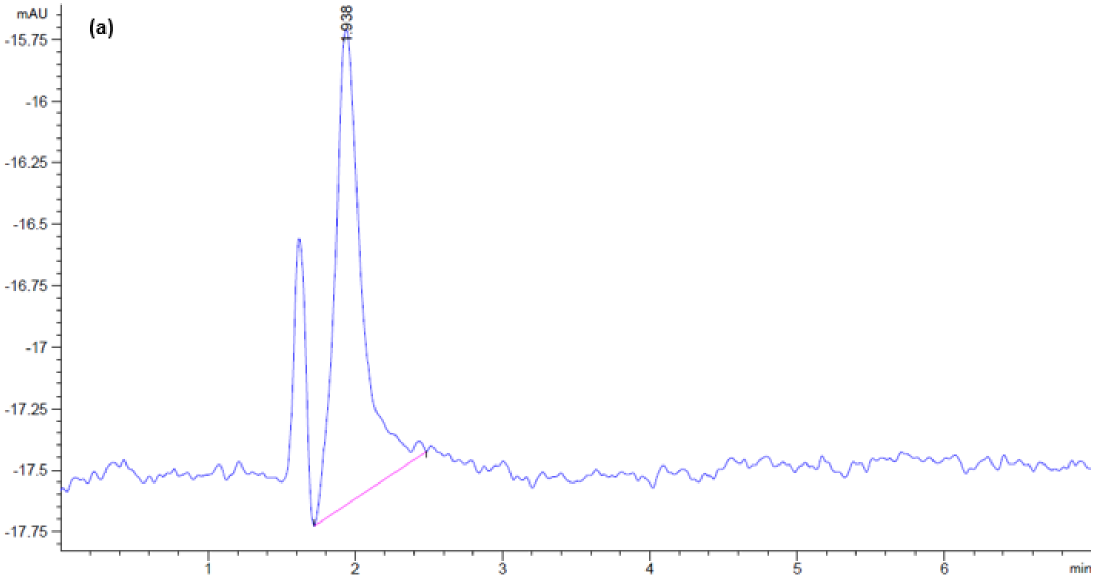
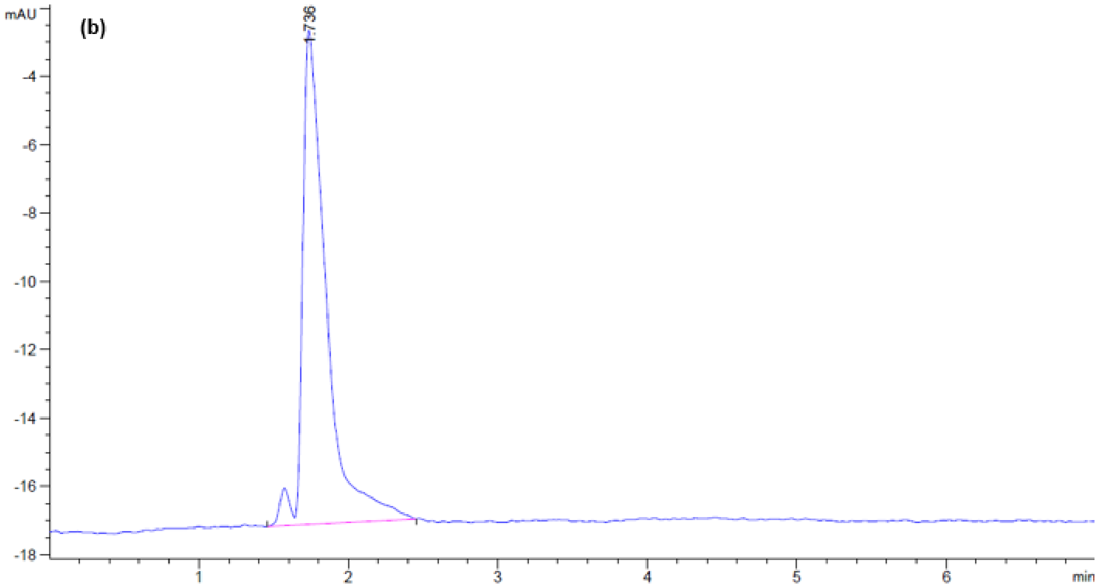
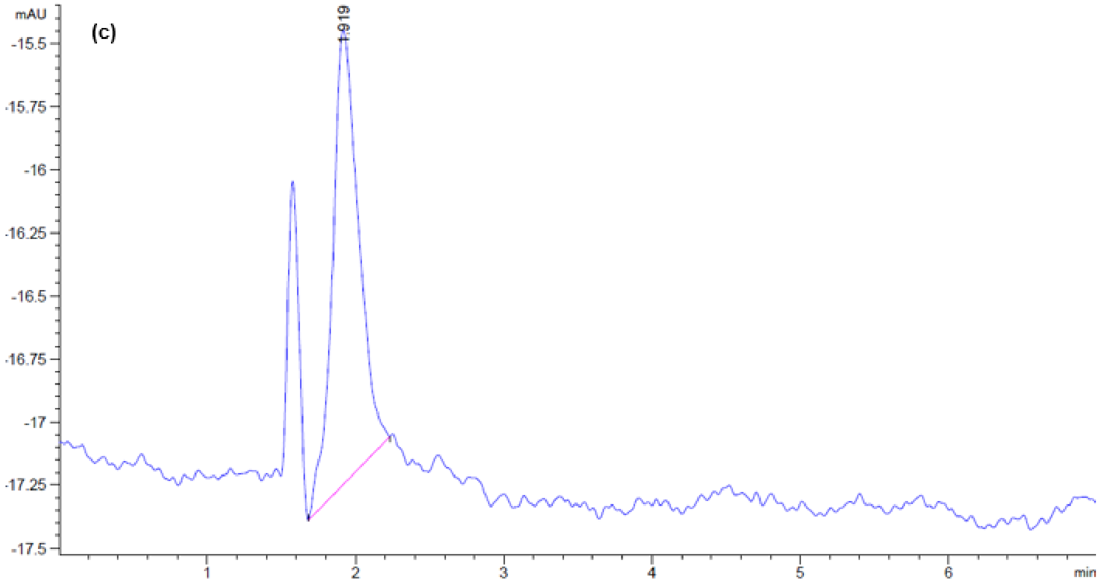
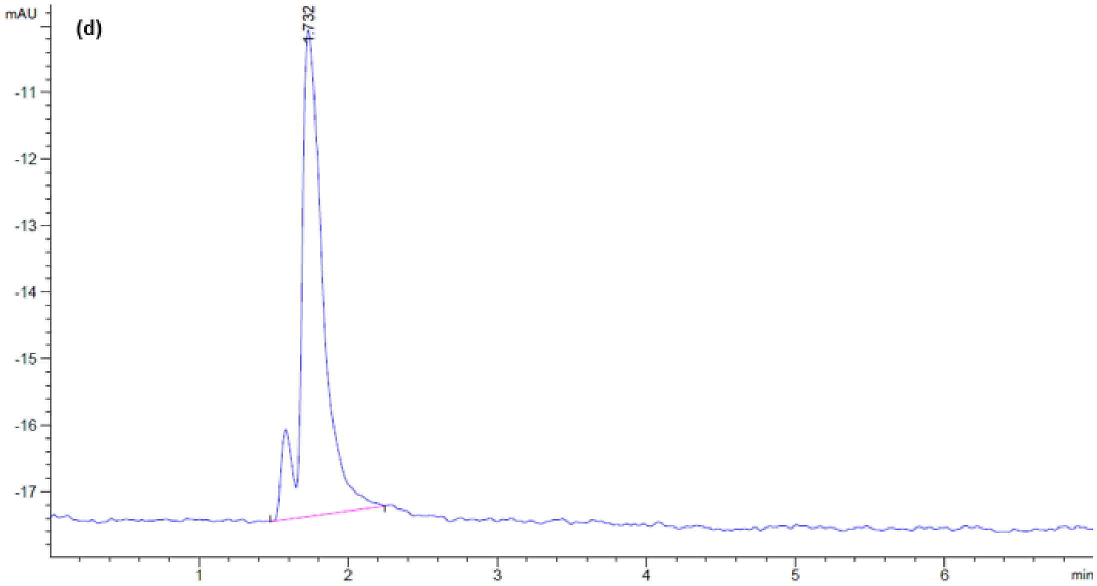


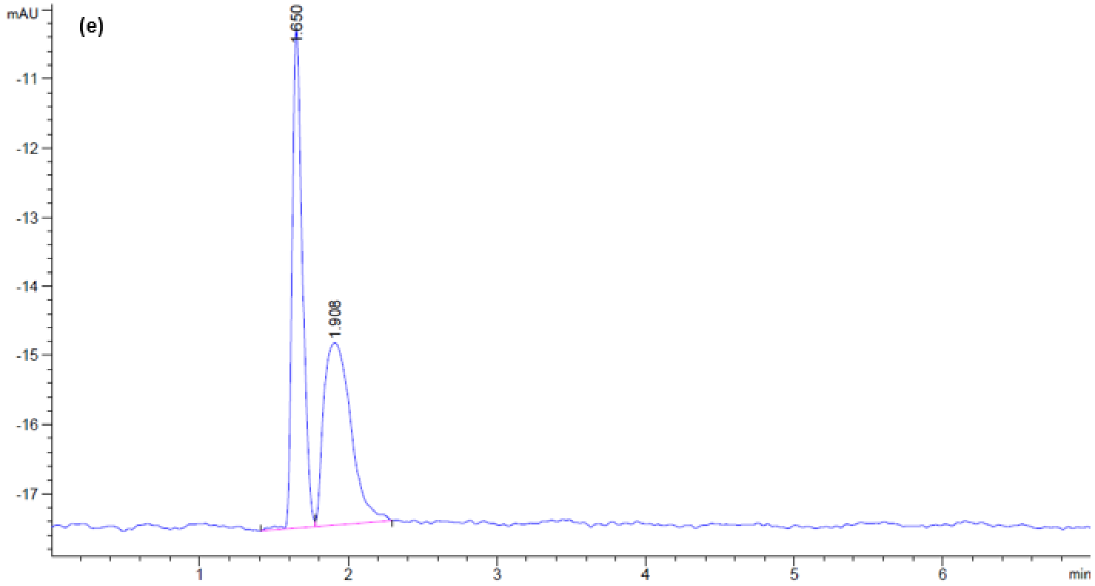
Figure E.14 HPLC analysis at the end of experiment rig, which was operated between 20 January 2016 and 18 October 2016 for reactive blue 198 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
(a)
1.938
-15.75
-16
-16.25
-16.5
-16.75
-17
-17.25
-17.5
-17.75
1
2
3
4
5
6
min


mAU
(b)
1.736
-4
-6
-8
-10
-12
-14
-16
-18
1
2
3
4
5
6
min


mAU
(c)
1.919
-15.5
-15.75
-16
-16.25
-16.5
-16.75
-17
-17.25
-17.5
1
2
3
4
5
6
min


mAU
(d)
1.732
-11
-12
-13
-14
-15
-16
-17
1
2
3
4
5
6
min



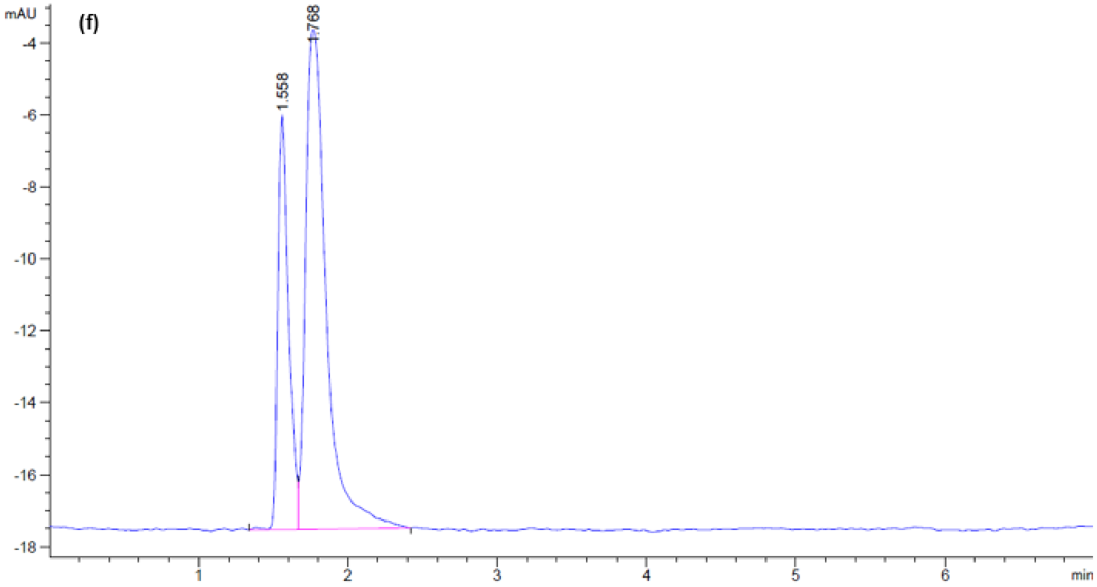
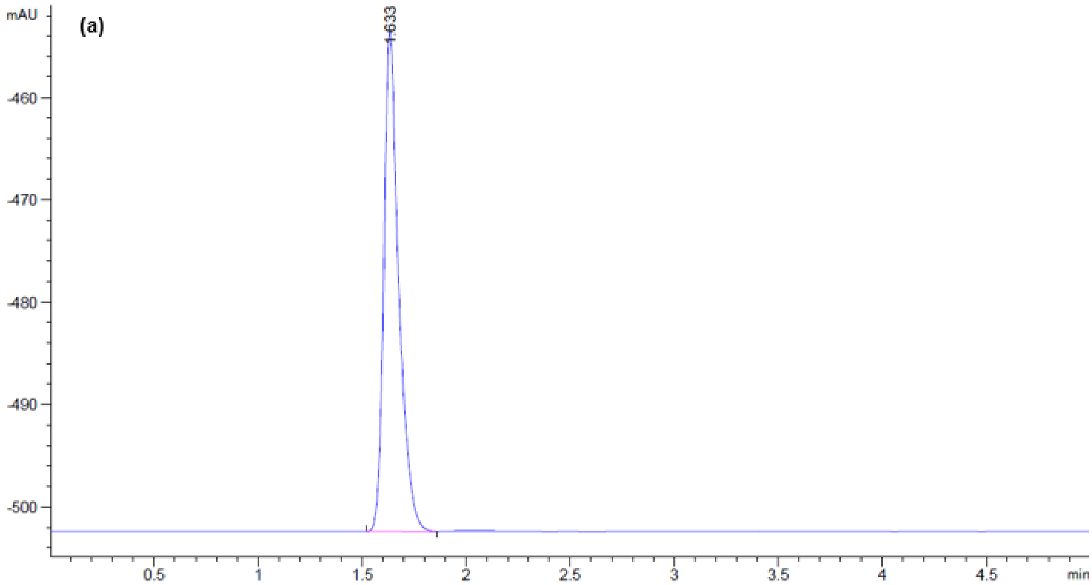
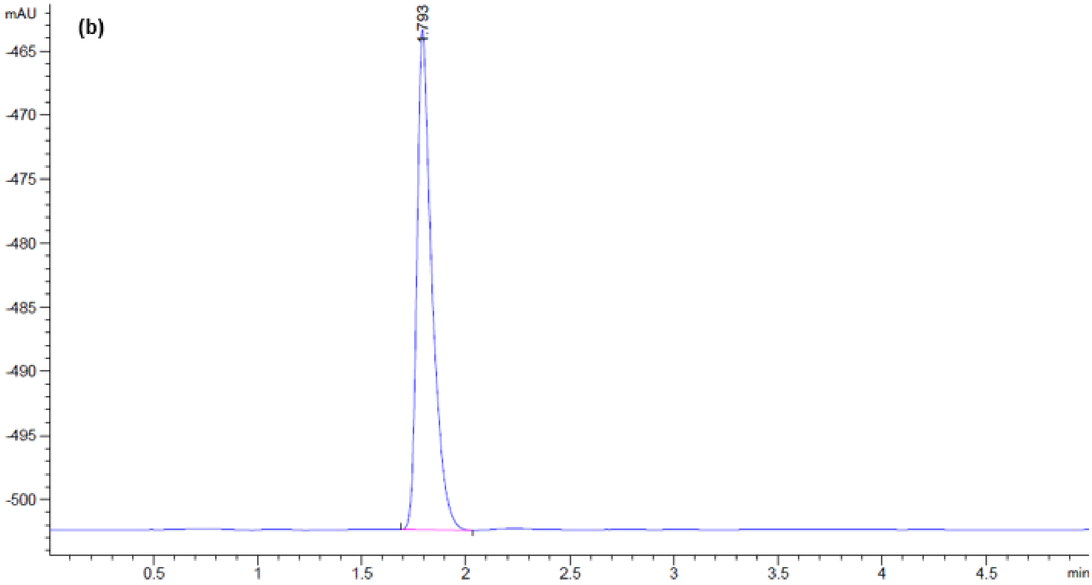


Figure E.15 HPLC analysis at the end of experiment rig, which was operated between 20 January 2016 and 18 October 2016 for basic red 46 after treatment. Note: (a), *Lemna minor* L. ponds receiving inflow at normal pH; (b), control ponds receiving inflow at normal pH; (c), *Lemna minor* L. ponds receiving inflow at pH of 9; (d), control ponds receiving inflow at pH of 9; (e), *Lemna minor* L. ponds receiving inflow at pH of 6; (f), control ponds receiving inflow at pH of 6.


mAU
(a)
1.633
-460
-470
-480
-490
-500
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min


mAU
(b)
1.793
-465
-470
-475
-480
-485
-490
-495
-500
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

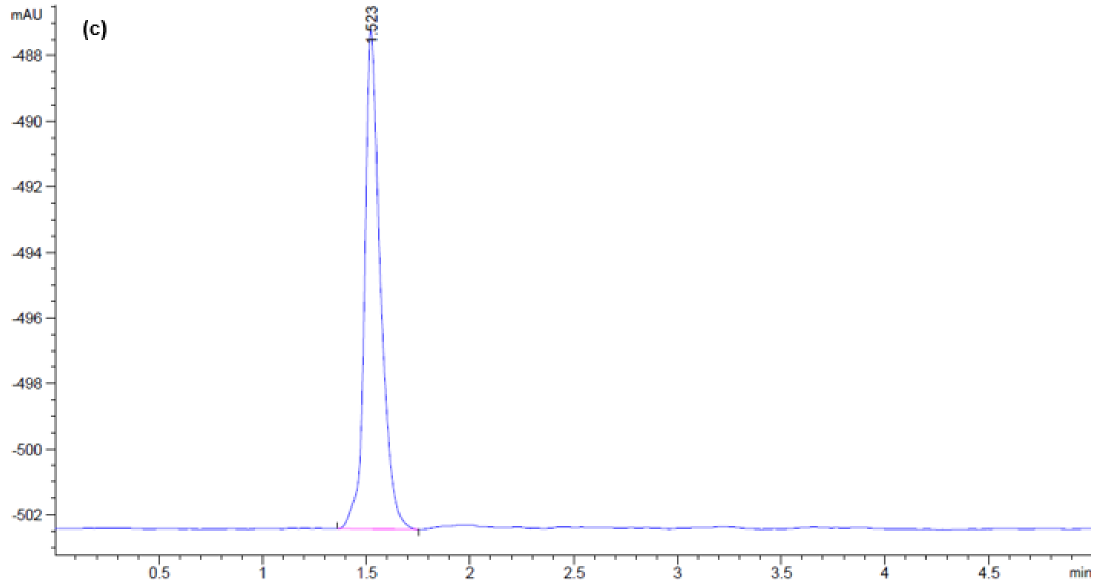
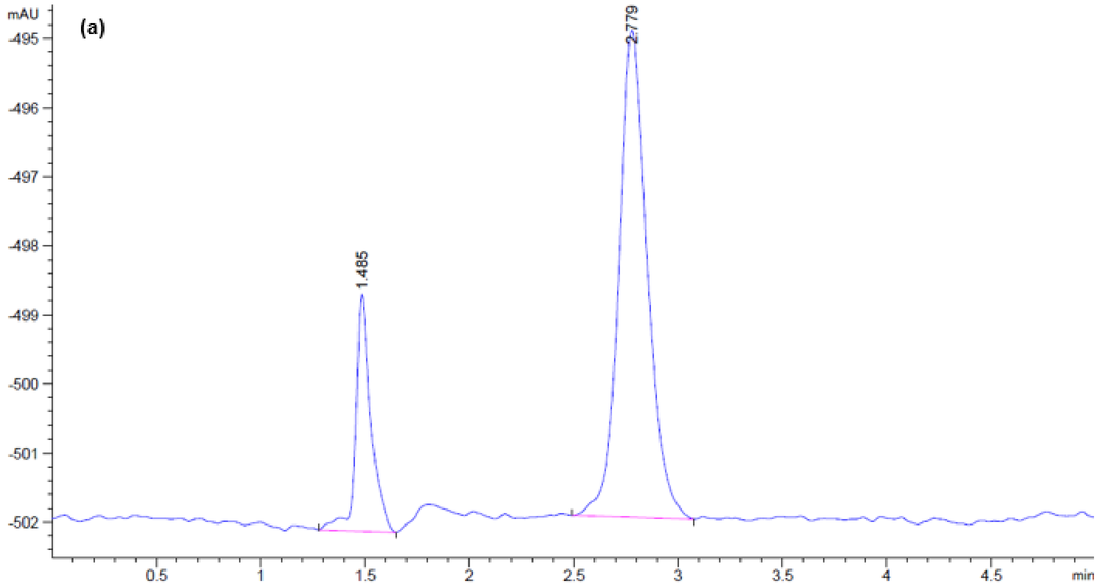


Figure E.16 HPLC analysis at the end of experiment rig, which was operated between 18 October 2016 and 30 June 2017 for reactive blue 198 after treatment. Note: (a), *Lemna minor* L. and algae ponds; (b), algae ponds; (c), control ponds.



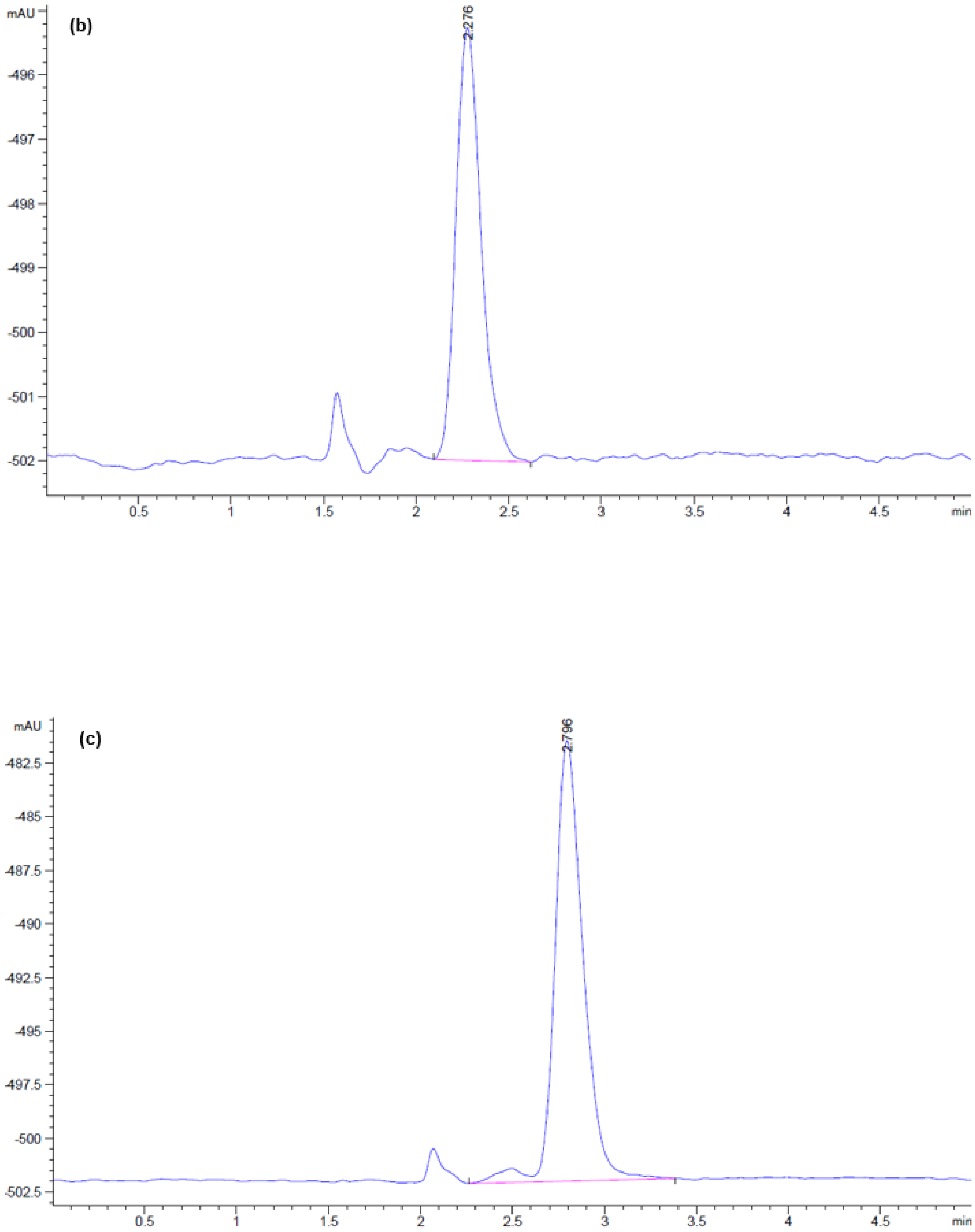
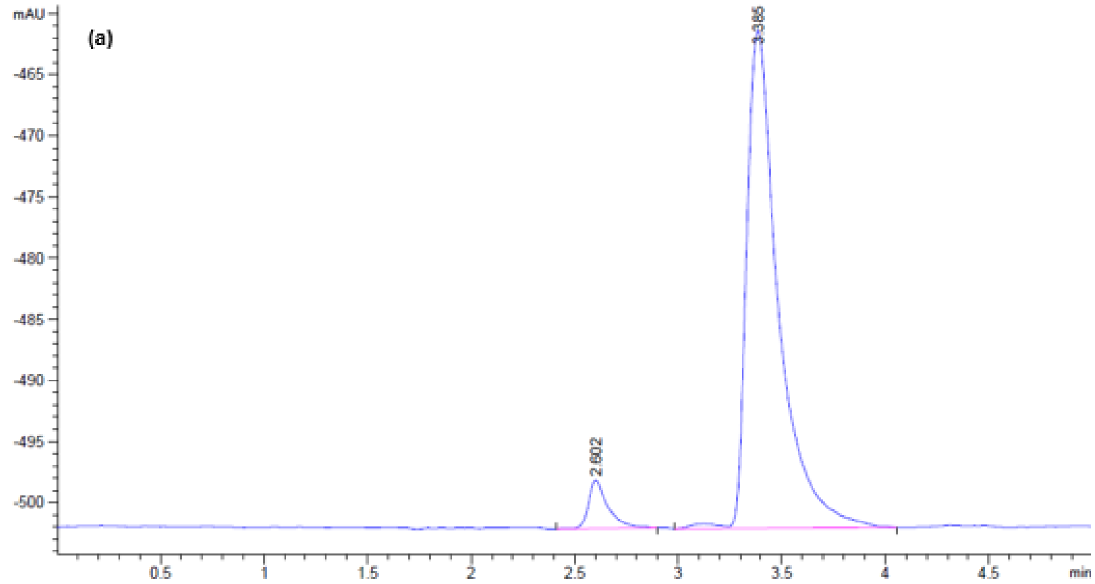
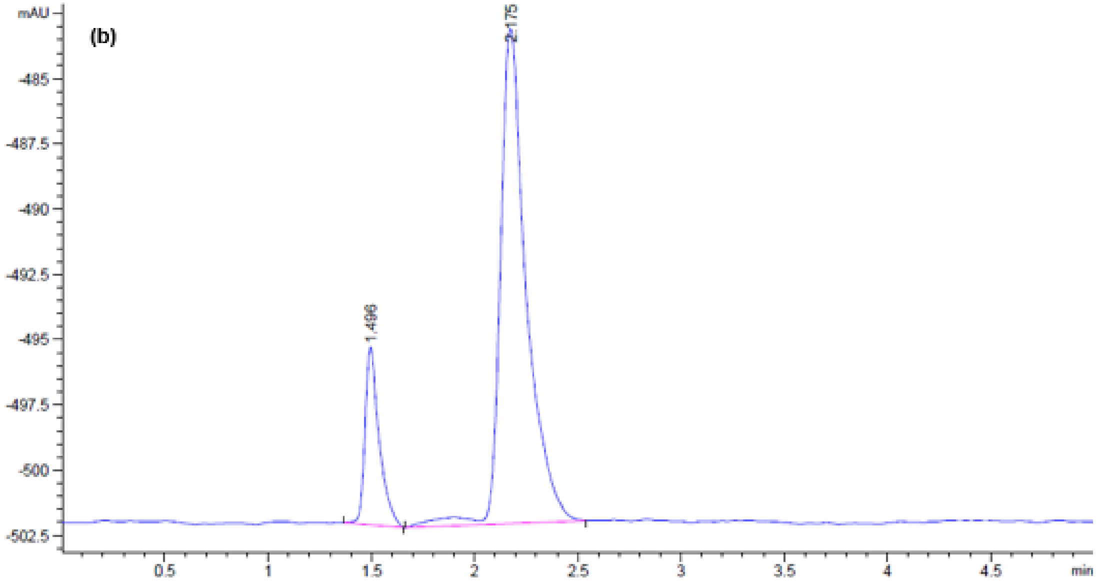


Figure E.17 HPLC analysis at the end of experiment rig, which was operated between 18 October 2016 and 30 June 2017 for basic red 46 after treatment. Note: (a), *Lemna minor L*. and algae ponds; (b), algae ponds; (c), control ponds.


mAU
(a)
-465
-470
-475
-480
-485
-490
-495
-500
3.385
2.602
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min


mAU
(b)
-485
-487.5
-490
-492.5
-495
-497.5
-500
-502.5
2.175
1.496
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

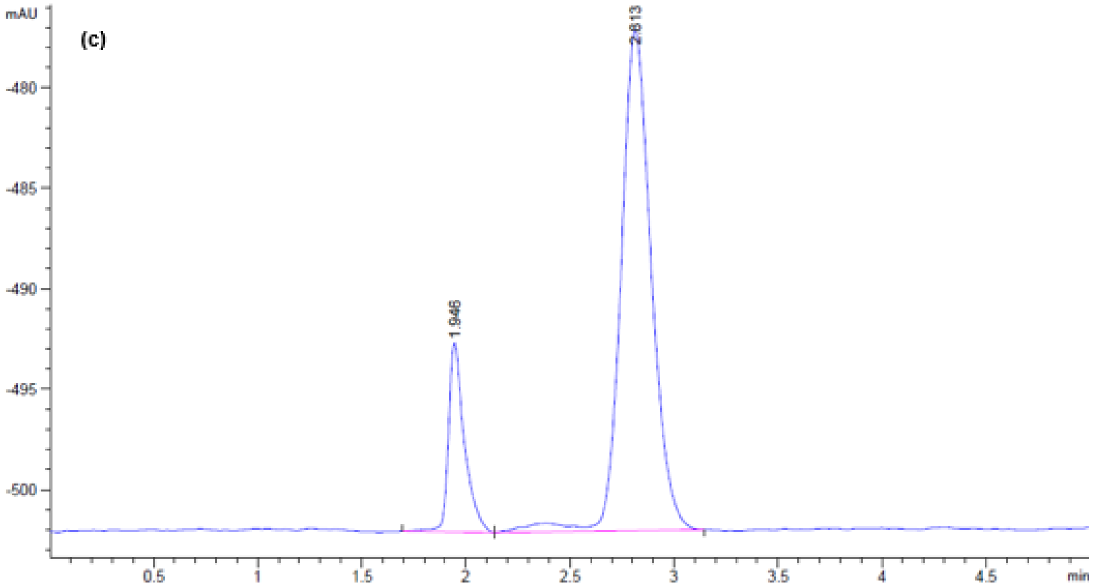
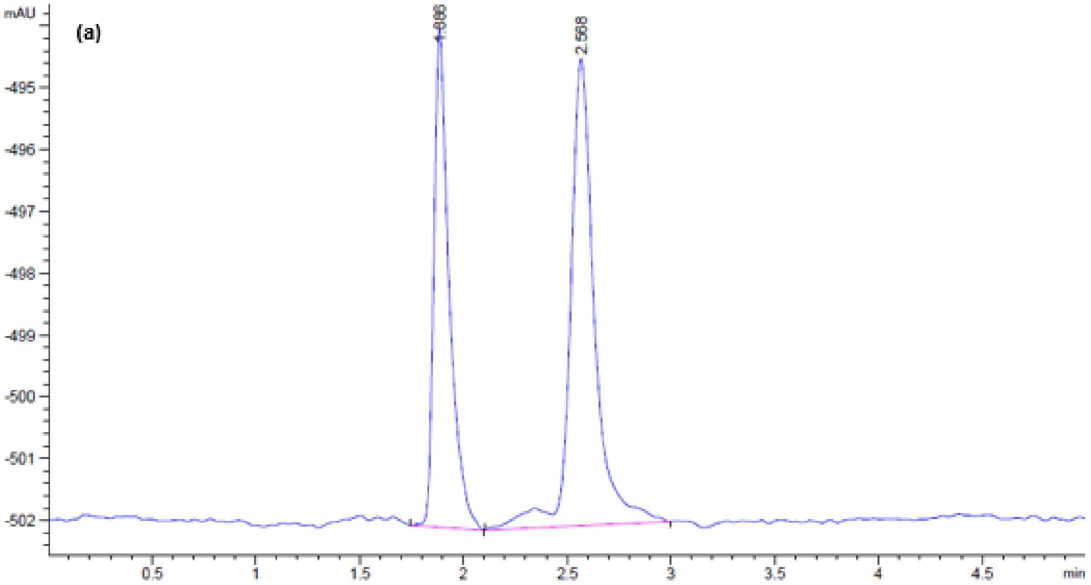


Figure E.18 HPLC analysis of the dye mixtures solution before treatment. Note: (a), mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); (b), mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198); (c), mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198).



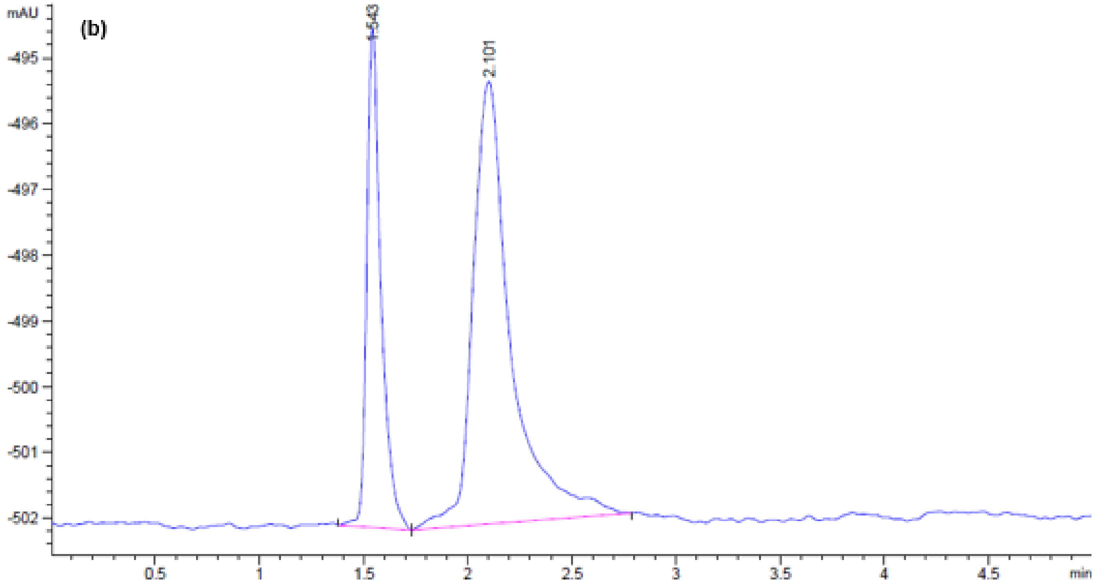
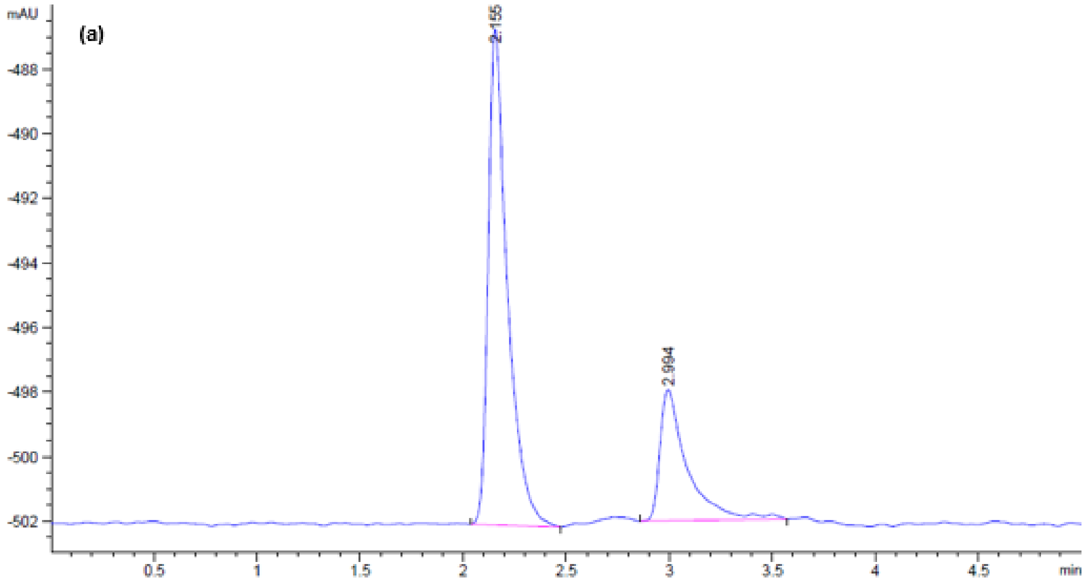


Figure E.19 HPLC analysis at the end of experiment rig, which was operated between 14 October 2016 and 27 June 2017 for mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198) after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.



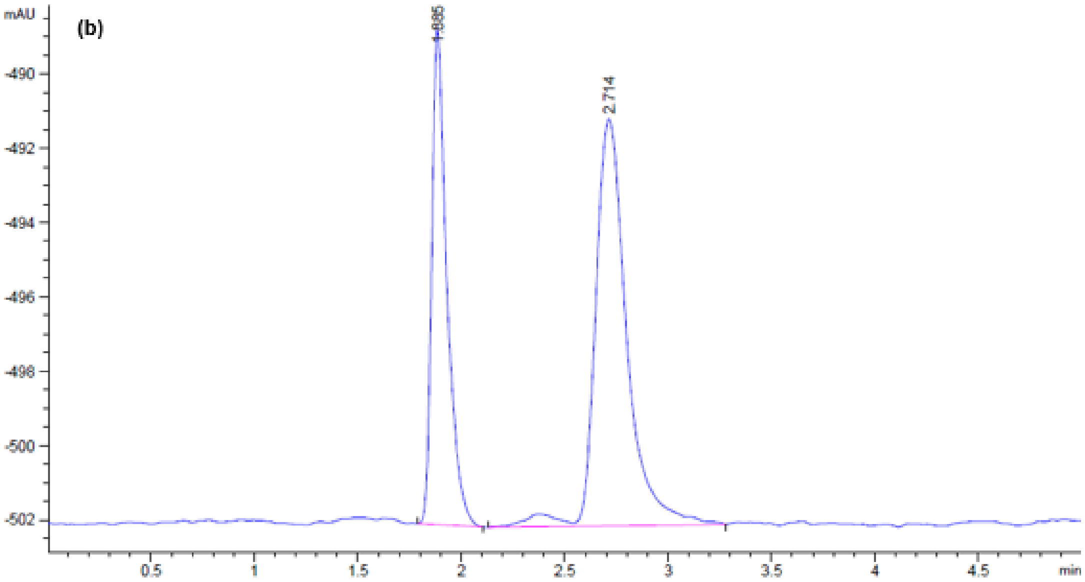
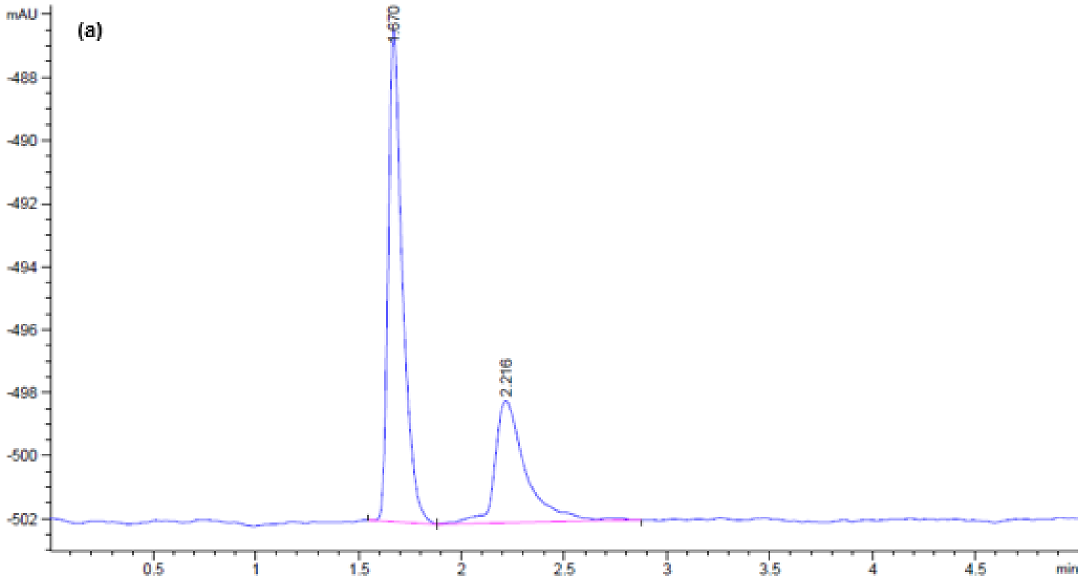


Figure E.20 HPLC analysis at the end of experiment rig, which was operated between 14 October 2016 and 27 June 2017 for mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue 198) after treatment. Note: (a), *Lemna minor* L. ponds; (b), control ponds.



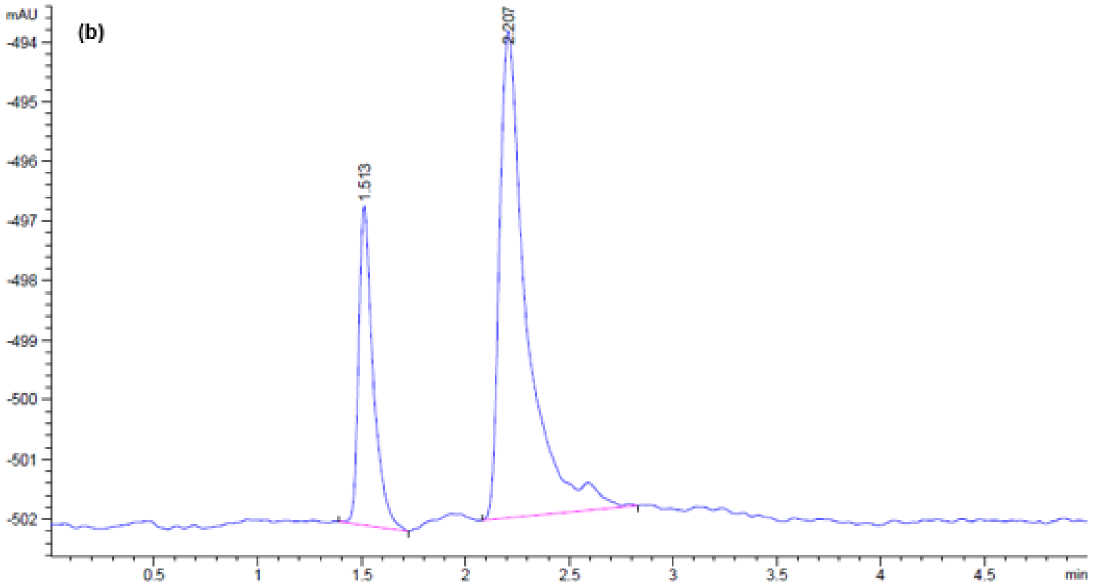


Figure E.21 HPLC analysis at the end of experiment rig, which was operated between 14 October 2016 and 27 June 2017 for mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198) after treatment. Note: (a), *Lemna minor L*. ponds; (b), control ponds.

Appendix F Pictures of the inflow and outflow samples, and plant

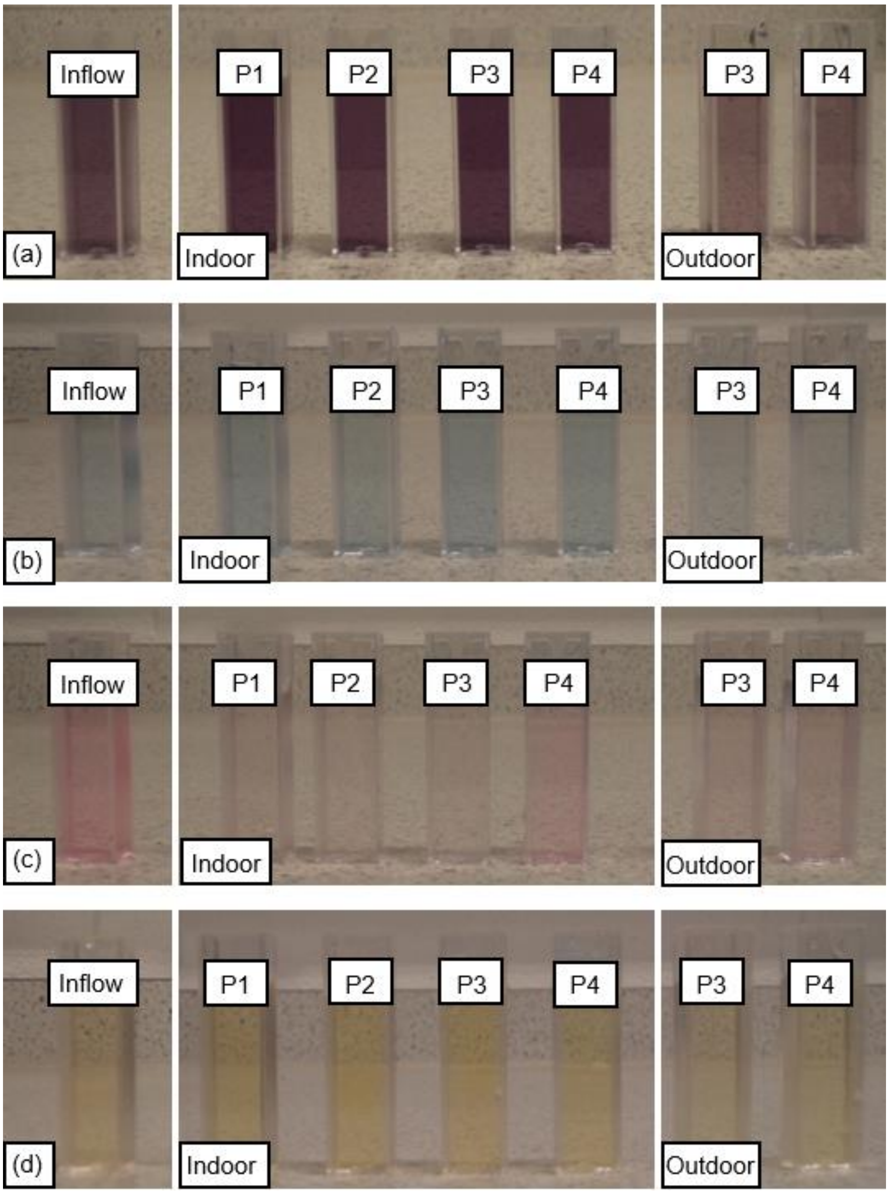


Figure F.1 Inflow and outflow filtered samples of both indoor and outdoor set-ups of the first experiment. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d) direct orange 46; P1, *Lemna minor* and algae ponds; P2, algae ponds; P3, *Lemna minor* ponds; P4, control ponds.

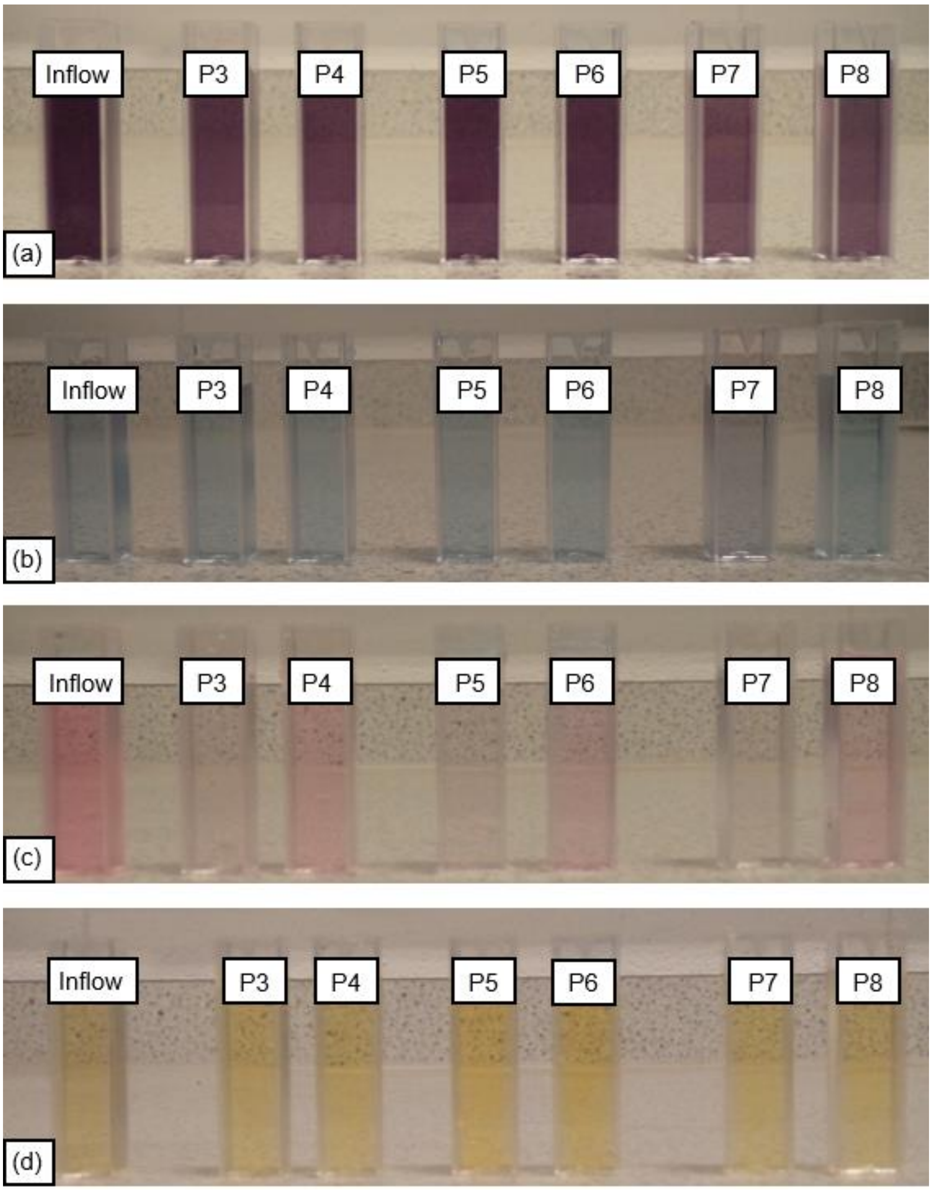


Figure F.2 Inflow and outflow filtered samples of second experiment between 1 October 2015 and 19 January 2016. Note: (a), acid blue 113; (b), reactive blue 198; (c), basic red 46; (d), direct orange 46; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

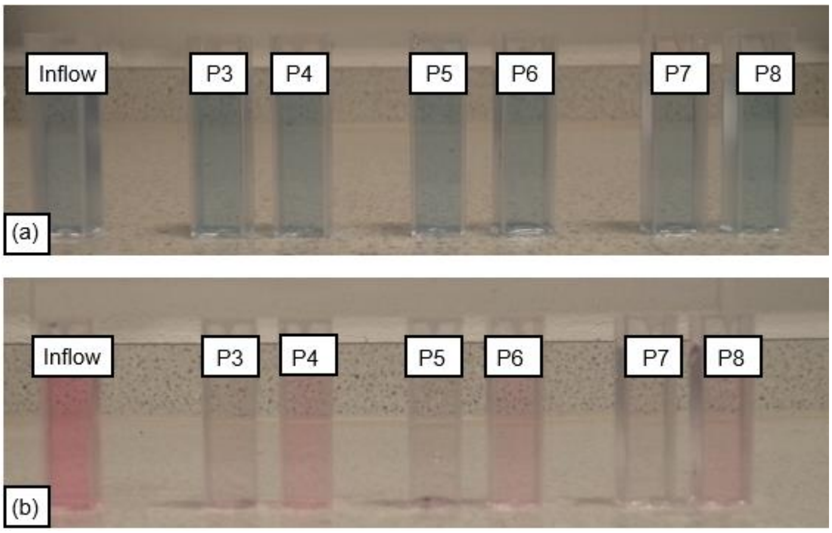


Figure F.3 Inflow and outflow filtered samples of the third experiment (first phase), which operated between 18 October 2016 and 30 June 2017. Note: (a), reactive blue 198; (b), basic red 46; P3, *Lemna minor* L. ponds receiving inflow at normal pH; P4, control ponds receiving inflow at normal pH; P5, *Lemna minor* L. ponds receiving inflow at pH of 9; P6, control ponds receiving inflow at pH of 9; P7, *Lemna minor* L. ponds receiving inflow at pH of 6; P8, control ponds receiving inflow at pH of 6.

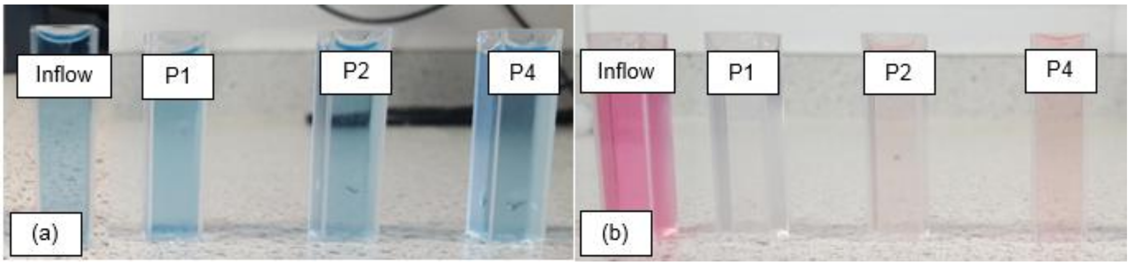


Figure F.4 Inflow and outflow filtered samples of the third experiment (second phase), which operated between 18 October 2016 and 30 June 2017. Note: (a), ponds treating the dye reactive blue 198; (b), ponds treating the dye basic red 46; P1, *Lemna minor* L. and algae ponds; P2, algae ponds; P4, control ponds.

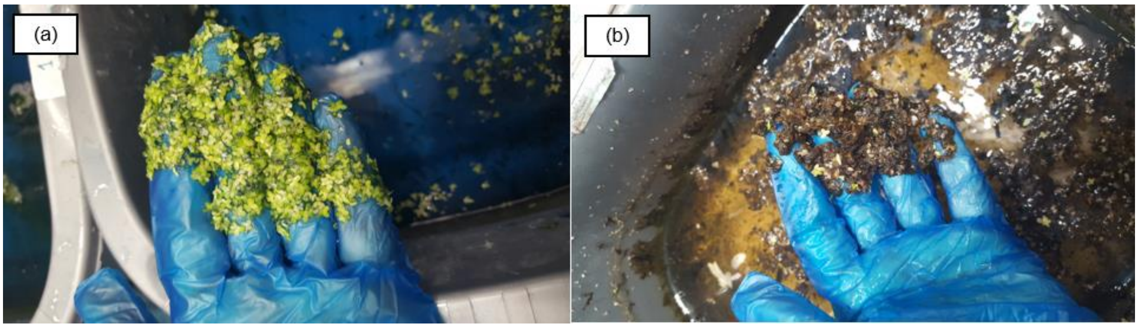


Figure F.5 *Lemna minor* L. state at the end of third experiment (second phase), which operated between 18 October 2016 and 30 June 2017. Note: (a), ponds treating the dye reactive blue 198; (b), ponds treating the dye basic red 46.

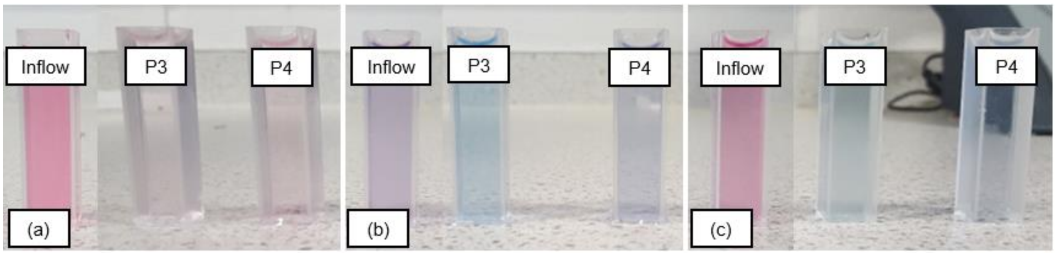


Figure F.6 Inflow and outflow filtered samples of the fourth experiment between 14 October 2016 and 27 June 2017. Note: (a), mixture 1 (8 mg/l of basic red 46 + 2 mg/l of reactive blue 198); (b), mixture 2 (2 mg/l of basic red 46 + 8 mg/l of reactive blue198); (c), mixture 3 (5 mg/l of basic red 46 + 5 mg/l of reactive blue 198); P3, *Lemna minor* L. ponds; P4, control ponds.

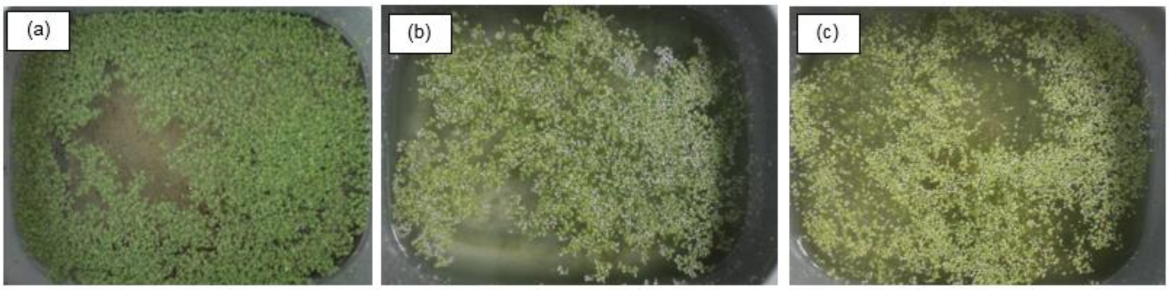


Figure F.7 *Lemna minor* L. state at the end of first and fourth experiment in ponds without dye. Note: (a), ponds containing synthetic wastewater (tap water and TNC complete fertilizer/first experiment); (b), ponds containing only dechlorinated tap water (fourth experiment); (c) ponds containing synthetic textile wastewater after dilution (fourth experiment).