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URBAN WASTEWATER TREATMENT WITH MATURE CONSTRUCTED WETLANDS

Design and operation of experimental vertical-flow constructed wetland applied for treatment of urban wastewater (with/without diesel spills contamination), and recycling of effluent for agriculture purposes

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Sincere thanks also go to the civil engineering team (Dr Akintunde, Dr Abdulkadir Sani, and Umar Iliyasu) whose contribution in this work. The research work includes two parts: the first part, carried out by the author, was the performance assessment of the wetland system to treat urban wastewater with and without hydrocarbon contamination, which is the novel work of this thesis. This assessment, conducted by the author, involved operating the wetland system and the hydrocarbon dosage details, as well as collecting and analysing all detailed data related to wetland filters (water quality parameters, from the third week of September 2013 until the third week of March 2016). Moreover, water quality data from January 2011 until the third week of September 2013 was obtained by Dr Akintunde and Dr Abdulkadir Sani who worked previously on my wetland system. Finally, Umar Iliyasu cooperated in wetland data collection from February 2016 and continues lab work till now. The second part was the evaluation of the effect of wetland system effluent on growing crops, which involved planting vegetables in two environments: the greenhouse, which was conducted by the author, and the lab environment, which was shared with Suhad Almuktar, on experimental chilli plants in lab G19/Cockcroft building.

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DECLARATION: LIST OF ORIGINAL PAPERS AND CONFERENCES

PAPERS

The novel approaches and findings of this thesis have been published (six papers) or submitted (one paper) in peer-reviewed journals, which will be referred to in the text as appropriate. The original published papers are listed below.

I. **Al-Isawi, R.H.K.**, Sani, A., Almuktar, S., & Scholz, M. (2015). Vertical-flow constructed wetlands treating domestic wastewater contaminated by hydrocarbons. *Water Science and Technology* 71 (6), 938–946. Rawaa Al-Isawi undertook the full experimental work relating to wetland filters (including operating the system, and data collection) for three seasons after the period of hydrocarbon dosage (Sept 2013-Jul 2014). She also conducted the full hydrocarbon analysis, prepared a comprehensive data analysis and the full draft paper. Abdulkadir Sani provided the water quality data for three seasons before the period of hydrocarbon dosage (Sept 2012-Jul 2013), and contributed to the editing of the second draft paper. Miklas Scholz reviewed and edited the final paper. Almuktar did not have any scientific contribution in this paper.

II. **Al-Isawi, R.H.K.**, Scholz, M., Wang, Y. & Sani, A. (2015). Clogging of vertical-flow constructed wetlands treating urban wastewater contaminated with a diesel spill. *Environmental Science and Pollution Research* 22(17), 12779–12803, doi:10.1007/s11356-014-3732-8. Rawaa Al-Isawi conducted the comprehensive hydrocarbon work, collected and analysed the hydrocarbon data, as well as the

related wetland data (water quality parameters and wetland plants) from the third week of September 2013 until June 2014. She also conducted the whole data analysis, presented the results in tables and figures, and prepared a full draft paper. Miklas Scholz finalized the paper, while Yu Wang finalized the mathematical model. Abdulkadir Sani provided the water quality data from January 2012 until the end of September 2013.

III. **Al-Isawi, R.H.K.**, Scholz, M., & Al-Faraj, F.A.M. (2016). Assessment of diesel-contaminated domestic wastewater treated by constructed wetlands for irrigation of chillies grown in a greenhouse. *Environmental Science and Pollution Research* 23(24), 25003-25023. doi: 10.1007/s11356-016-7706-x. Rawaa Al-Isawi undertook the full collection and analysis of the data for both the wetland system and the chilli plants system, and prepared a full draft paper. Miklas Scholz performed editing and revision of the paper. Furat Al-Faraj partly edited the draft of the journal paper and participated in the drafting of some tables and figures.

IV. **Al-Isawi, R.H.K.**, Almuktar, S.A.A.-A.N., & Scholz, M. (2016). Recycling of river, rain, gully pot and grey waters for irrigating chillies. *Environmental Monitoring and Assessment* 188(5), 1-12, doi:10.1007/s10661-016-5285-4. Rawaa Al-Isawi undertook the full data analysis of water quality parameters used for irrigated plants inside the laboratory and the greenhouse, collected and analysed the chilli plants data inside the greenhouse, and prepared a comprehensive draft paper. Suhad Almuktar collected and analysed the data on chilli plants inside the laboratory and partly contributed to the first draft paper. Miklas Scholz reviewed and edited the final paper.

V. **Al-Isawi, R.H.K.**, Sanak, R., & Scholz, M. (2017). Comparative study of domestic wastewater treatment by mature vertical-flow constructed wetlands and artificial ponds. Ecological Engineering, 100, (8-18). DOI: org/10.1016/j.ecoleng.2016.12.017. Rawaa Al-Isawi undertook the collecting and analysing of the wetland system data and prepared the first and final draft paper. She also contributed to the ponds experimental work, and analysis of most of the ponds data. Sanak Ray collected the ponds experimental data and partly analysed the ponds data and contributed in the initial draft paper, while Miklas Scholz finalized the paper.

A collaborative research project based on the PhD thesis was conducted and published in the following peer-reviewed journals:

I. Almuktar, S.A.A.-A.N., Scholz, M., **Al-Isawi, R.H.K.**, & Sani A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for irrigating chillies and sweet peppers. *Agricultural Water Management* 149, 1–22, doi.org/10.1016/j.agwat.2014.10.025. Suhad Almuktar performed the analysis of the vegetables data in Lab G19 and ICP test as well as contributed to revision and editing of an initial and final draft paper. Rawaa Al-Isawi undertook the full experimental work relating to the wetland filters (including operating the system, data collection and analysis) and participated in the chilli plants laboratory work. Al-Isawi undertook themeasurements and analysis of water quality parameters data used for irrigated chilli plants. Al-Isawi also contributed to the revision and editing of an initial and final draft paper and produced some of the figures and

tables. While Miklas Scholz prepared and wrote the first draft and the final paper. Abdulkadir Sani took part in the initial experimental set-up, as well as in the first and second draft of the journal paper and in the final paper revision.

II. Almuktar, S.A.A.-A.N., Scholz, M., **Al-Isawi, R.H.K.**, & Sani A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for watering of vegetables. *Water Practice & Technology* 01/2015; 10(2), 1–20, doi:10.2166/wpt.2015.052. Suhad Almuktar collected and analysed the crop experimental data, ICP test, contributed to the revision and editing of an initial and final draft paper. Rawaa Al-Isawi carried out the comprehensive experimental work relating to the wetland filters (including operating the system, data collection and analysis) and participated in the crop (chilli) plants experimental work. Al-Isawi undertook the measurements and analysis of water quality parameters data used for irrigated chilli plants. Al-Isawi also contributed to the revision and editing of an initial and final draft paper and produced some of the figures and tables, while Miklas Scholz edited and finalized the final paper. Abdulkadir Sani participated in the earlier set-up of the experiment, the draft of the paper and in the final revision after the authors review.

CONFERENCES

I. **Al-Isawi, R.**, Scholz, M., & Sani, A. 2014. Vertical-flow constructed wetlands treating domestic wastewater contaminated by hydrocarbon. *IWA 14th International Conference on Wetland Systems for Water Pollution Control*

*(ICWS2014):*12-16 October 2014, Shanghai, China. **Web: www.iwawetland2014.org**.

II. **Al-Isawi, R.**, Scholz, M., & Wang, Y. 2015. Modelling of hydrocarbon treatment in vertical-flow constructed wetlands treating urban waste water. Abstract Booklet of WETPOL 2015 - 6th International Symposium on Wetland Pollutant Dynamics and Control, and Annual Conference of the Constructed Wetland Association (13-18 September 2015), York, UK, pp. 210-211. **Web: www.wetpol.org/2015/.**

III. Almuktar, S.A.A.-A.N., Scholz, M., **Al-Isawi, R.H.K.**, & Sani A. 2015. Recycling of domestic wastewater treated by vertical-flow wetlands for watering of vegetables. *IWA 14th International Conference on Wetland Systems for Water Pollution Control (ICWS2014):*12-16 October 2014, Shanghai, China. **Web: www.iwawetland2014.org**.

IV. **Al-Isawi, R.H.K.**, Sani, A., & Scholz, M. 2015. Hydrocarbon removal and seasonal variability in vertical-flow constructed wetlands treating urban wastewater. *The 5th World Sustainability Forum* (WSF 2015) (7-9 September, 2015), Basel, Switzerland, p. 178. **Web: www.sciforum.net/conference/wsf-5.**

V. **Al-Isawi, R.H.K.**, & Scholz, M. 2015. Impact of different irrigation waters on the growth of chilli grown in a greenhouse. *The 5th World Sustainability Forum* (WSF 2015) (7-9 September, 2015), Basel, Switzerland, p. 181. **Web: www.sciforum.net/conference/wsf-5.**

VI. **Al-Isawi, R.H.K.**, & Scholz, M. 2015. Seasonal assessment of vertical-flow constructed wetlands treating domestic wastewater contaminated with hydrocarbon. *Salford Postgraduate Annual Research Conference (SPARC)* 26-28

May 2015. University of Salford, MediaCity UK, Salford. **Web: www.pg.salford.ac.uk/sparc_conference.**

VII. **Al-Isawi, R.H.K.**, Scholz, M. & Iliyasu, U. 2016. Long-term performance of vertical-flow constructed wetland systems treating domestic wastewater contaminated by two dosages of diesel spills. *Poster presentation. 4th International Environment Conference 2016* (2-3 March, 2016). Ajman – United Arab Emirates. **Web: www.aiec2016.org/.**

VIII. **Al-Isawi, R.H.K.**, & Scholz, M. 2016. Assessing the impact of different irrigation waters on *Capsicum annuum* grown in a greenhouse. *Poster presentation. 4th International Environment Conference 2016* (2-3 March, 2016). Ajman – United Arab Emirates. **Web: www.aiec2016.org/.**

IX. Sanak, R., **Al-Isawi, R.H.K.**, & Scholz, M. 2016. Comparative study of domestic wastewater treatment with ponds and vertical-flow constructed wetlands. *Poster presentation. 4th International Environment Conference 2016* (2-3 March, 2016). Ajman – United Arab Emirates. **Web: www.aiec2016.org/.**

X. **Al-Isawi, R.H.K.**, & Scholz, M. 2016. Impact of diesel spills on water quality outflow parameters of reed beds treating urban wastewater. *Salford Postgraduate Annual Research Conference (SPARC)* 14-16 June 2016. University of Salford, MediaCity UK, Salford. **Web: www.pg.salford.ac.uk/sparc_conference.**

XI. Almuktar, S.A.A.-A.N., **Al-Isawi, R.H.K.**, & Scholz, M. 2015. Domestic wastewater treatment with wetlands and recycling of the effluent for irrigation of crops. *Workshop NW0915 - Management of Wastewater and Waste, (Institute of Environmental Management and Assessment IEMA2015):* 23

September 2015, The University of Salford, Peel Park Campus, Peel Building, Room PG9. **Web:events.iema.net/events/view/MTU2.**

XII. Uzomah, V., Sani, A., **Al-Isawi, R.**, Almuktar, S., & Scholz M. 2014. Decision support tool based on ecosystem service variables for retrofitting of sustainable drainage systems including wetlands. *IWA 14th International Conference on Wetland Systems for Water Pollution Control (ICWS2014):* 12-16 October 2014, Shanghai, China. **Web: www.iwawetland2014.org**.

XIII. **Al-Isawi, R.**, & Scholz, M. 2016. Vertical-flow constructed wetlands treating domestic wastewater contaminated by hydrocarbon. *The 2016 International conference on Water Resource and Environment (WRE2016)*, 23-26 July 2016, Shanghai, China.

ACRONYMS AND ABBREVIATIONS

µS/cm	Micro Siemens/centimetres
AG	Artificial grey water
Al	Aluminium
Al_2O_3	Aluminium oxide
A_w	Wetted cross sectional area
BAF	Biological aerated filter
BOD	Biochemical oxygen demand after five days
BTEX	Benzene, toluene, ethylbenzene, and xylenes
BS	British Standard
C	Carbon number
CA	Control A
Ca	Calcium
CB	Control B
Cd	Cadmium
COD	Chemical oxygen demand
Cr	Chromium
Cs	Combined system
Cu	Copper
CW	Constructed wetland
CWs	Constructed wetlands
D	Dispersion coefficient
D	Deionized water
d,α,β	Three empirical parameters
Da	Damköhler number
D_m	Biomass diffusion coefficient
DO	Dissolved oxygen
DRO	Diesel range organic
D_{vtot}	Total volumetric specific deposit
EC	Electrical conductivity
ε	Porosity of the porous substrate beds of CWs
f	Hindered settling velocity

F1	Wetland filter 1
F2	Wetland filter 2
F3	Wetland filter 3
F4	Wetland filter 4
F5	Wetland filter 5
F6	Wetland filter 6
F7	Wetland filter 7
F8	Wetland filter 8
FAO	Food and Agricultural Organisation
Fe	Iron
FWS	Free water surface flow
FWSF CWs	Free water surface flow constructed wetlands
GP	Gully pot water
GRO	Gasoline range organic
h_1	Water depth at the upstream point
h_2	Water depth at the downstream point
HF	Horizontal flow
HFCWs	Horizontal flow constructed wetlands
HRT	Hydraulic retention time
IBM SPSS	International Business Machine Statistical Package for Social Sciences
ICP–OES	Inductively Coupled Plasma –Optical Emission Spectrometer
ICW	Integrated constructed wetland
Is	Intensified systems
ITRC	Interstate Technology and Regulatory Council Wetlands Team
K	Potassium
K_0	Constant
k_2, k_3, k_4	Empirical parameters
K_2O	Potassium oxide
L	Distance between upstream and downstream point
M	Biomass density within a control volume
M_{bass}	Biomass concentration
M_{clog}	Biomass density (no further hydraulic conductivity reduction)
Mg	Magnesium
M_{max}	Maximum biomass density

Mn	Manganese
MTBE	Methyl-*tert*-butyl ether
mV	Redox potential unit (millivolt)
n	Empirical parameter
N	Nitrogen
Na	Sodium
n/a	Not applicable
n/d	Not detected
NH_4-N	Ammonia-nitrogen
nm	Not measured
NO_3-N	Nitrate-nitrogen
NTU	Nephelometric Turbidity Unit
O_2	Oxygen
OVPH	Other Volatile Petroleum Hydrocarbon
P	Phosphorus
p,x,y	Empirical parameters
P_2O_5	Phosphorus pentoxide
PAH	Polynuclear aromatic hydrocarbon
Pb	Lead
pH	Hydrogen power
PO_4-P	Ortho-phosphate-phosphorus
P-UASB	Packed-bed up-flow anaerobic sludge blanket reactor
Q	Flow rate
q_m	Maximum adsorption rate
R	Adsorption
$R^{\bullet}$	Sink term (due to physical adsorption)
RA	Rain water
RG	Real grey water
RV	River water
RZM	Root zone method
SAR	Sodium adsorption ratio
SF	Surface flow
SOAs	Secondary organic aerosols
SS	Suspended solids

SSF	Subsurface flow
SSF CWs	Subsurface-flow constructed wetlands
t	Time
T	Tab water
T+F	Tab water with fertilizer (0.7 ml/l)
T+WW	Tab water (one part) with wastewater (four parts)
TALPHA	Total aliphatic hydrocarbon
TAROM	Total aromatic hydrocarbon
TBD	Turbidity
TCOD	Total chemical oxygen demand
THC	Total Hydrocarbons
TPH	Total petroleum hydrocarbon
TSS	Total suspended solids
TVPH	Total Volatile Petroleum Hydrocarbon
u	Water flowing process (convection)
UK	United Kingdom
USA	United States of America
USEPA	United State Environmental Protection Agency
v	Sedimentation of settling velocity
VF	Vertical-flow
VF CWs	Vertical-flow constructed wetlands
VFWs	Vertical-flow wetlands
VPH	Volatile Petroleum Hydrocarbon
w_0	Terminal settling velocity
WW	Preliminarily treated wastewater
WHO	World Health Organization
z	Elevation
α	Dispersivity
φ	Concentration of suspended solids
φ_s	Constant
Ψ	Source/sink term

ABSTRACT

Despite the global acceptance for the application of vertical-flow constructed wetlands (VF CWs) as sustainable and cost-efficient technology in treating various types of wastewater, including urban wastewater, continuous loading of wetlands over time can lead to performance inefficiency and generate operational problems especially when high shock loads, such as petroleum hydrocarbon spills, are subjected to the system. Contamination with petroleum hydrocarbon compounds results in changing the structure, function and ecosystem service values of wetlands, which can eventually lead to clogging of the wetland substrate and affect the life time of the system. Sound knowledge of long-term performance in mature vertical-flow constructed wetlands linked with hydrocarbon treatment processes is needed to make guided judgments about the probable effects of a given suite of impacts and revise the management plans accordingly.

A study was conducted to compare the impact of different design (aggregate size) and operational (contact time, rest time and chemical oxygen demand (COD) loading) variables on the long-term and seasonal performance of vertical-flow constructed wetland filters operated in tidal flow between June 2011 and March 2016. Ten different vertical-flow wetland systems were planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed). Approximately 130 and 975 grams of diesel fuel (equivalent to 20 and 150 grams/litre, respectively) were each poured into four wetland filters on 26/09/2013 and 26/09/2014 respectively. Overall findings showed that the mature wetland system improved the water quality except for ortho-phosphate-phosphorus (PO_4-P), which reduced less over time. Findings also indicated that the wetland filter with the highest chemical oxygen demand (COD) loading but no diesel contamination performed the best in terms of COD and biochemical oxygen demand (BOD) removal. Filters contaminated

by diesel performed worse in terms of COD and BOD but considerably better regarding nitrate-nitrogen removal. The removal efficiencies dropped for those filters impacted by the diesel spills. Seasonal analysis for water quality from different wetlands showed clear seasonal outflow concentration trends (low in summer) for COD, and nitrate-nitrogen (NO_3-N) while effluent BOD showed high treatment performance in winter. No clear seasonal trend for ammonia-nitrogen (NH_4-N), PO_4-P or suspended solids (SS) was noted. Serious clogging phenomena, impacting negatively on the treatment performance and the hydraulic conductivity, were not observed. The simulation model confirms the experimental findings that notable wetland clogging restricting the operation did not occur. Moreover, results showed that small aggregate diameter, low inflow COD load, and high contact and rest time were most efficient in reducing SS accumulation within the wetland filter bed. With regard to the treatment performance of the hydrocarbon contaminants, results indicated that all wetland systems had a relatively good performance in treating petroleum hydrocarbon compounds and the evaluation showed that all the hydrocarbon components were highly degraded and their concentrations were reduced in all treated effluents of wetland filters with time. This indicates that VF CW zones provide appropriate conditions for high treatment capacity of diesel compounds spilled with urban wastewater by a combination of processes taking place in the wetland filters, thus minimizing hydrocarbon compounds within the filter.

A new experimental artificial ponds system, including: ponds with wastewater; ponds with wastewater and reeds; and ponds with wastewater, reeds and aeration, was operated in parallel with the mature experimental vertical-flow constructed wetland system, for the period between July 2015 and October 2015, to compare performance, design and operation variables between the two treatment technologies in the treatment of urban

wastewater. Findings showed that highest COD and SS removals were observed for wetlands in comparison to ponds. Moreover, mature wetlands were better in removing NH_4-N and PO_4-P than ponds unless the ponds were aerated. Both systems were linked with medium to high levels of BOD removal. The aerated pond system demonstrated better treatment performance in terms of NH_4-N and PO_4-P. The NO_3-N concentration increased in the aerated ponds reflecting the high oxygen availability.

Due to increasing water scarcity and droughts, which are key concerns worldwide, there is considerable interest in recycling various wastewater streams, such as treated urban wastewater, for irrigation in the agricultural sector. Recycling of effluents from various wetland filters (with/without diesel contamination) was assessed for the irrigation of chilli plants (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') grown in a greenhouse environment. Concerning chilli fruit numbers, findings showed that the highest fruit yields for all wetland filters were associated with those that received inflow wastewater with a high loading rate, reflecting the high nutrient availability in treated wastewater, which is of obvious importance for yield production. Findings also indicated that wetlands without hydrocarbon contamination, with small aggregate size, low contact time, and low inflow loading rate provided high marketable yields (expressed in economic return). In comparison, chillies irrigated by filters with hydrocarbon contamination, small aggregate size, high contact time and high loading rate also resulted in high marketable yields of chillies, which pointed out the role of high contact time and high inflow load for better diesel degradation rates.

The overall outcome of this research could considerably contribute to optimization of the design and development of long-term operation variables for constructed wetland technology particularly in petroleum industry applications. Statistically validated long-

term data interpretation can particularly help the wetland modelling community and wetland managers to define, with insight into long-term and seasonal factors, removal processes for individual water quality parameters to maximize wetlands treatment performance.

CHAPTER ONE: INTRODUCTION

1.1 Overview

This chapter demonstrates the value of water to life and assesses the wetland concept as a valuable water treatment technology for environmental and public health protection. The chapter is divided into five sections. Section 1.2 presents the background to using wetlands including their features, importance and processes, and the principle of their application. The problem statement is defined in section 1.3. The aim and objectives are explained in section 1.4 and the importance of this study is discussed in section 1.5. Finally, the thesis structure is presented in section 1.6.

1.2 Background

Water is an important environmental factor which is essential for all forms of life on Earth. As stated by the World Health Organization (WHO), each person needs approximately (50 - 100 litres) of water to maintain the most basics requirements for life (United Nations - Office of the High Commissioner for Human Rights, UN-OHCH-UNHabitat-WHO, 2010). Due to a rising demand for freshwater, water shortage-related problems have been growing in the world and fresh water resources are increasingly insufficient to satisfy the growing demand (Kiani & Abbasi, 2012; Anderson et al., 2014). The rapid rise in urbanization and industrialization due to demographic growth with its

accompanying economic expansion are putting unexpected pressure on the water courses, particularly in arid areas (Water, 2006; Food and Agricultural Organisation (FAO), 2012). During the last century, water consumption has been growing worldwide at more than twice the rate of the increase in inhabitants (FAO, 2016), and an increasing number of areas are reaching the limit at which water services can be delivered on a sustainable basis (Ki-moon & General, 2010). According to the report of The United Nations World Water Development (WWAP United Nations World Water Assessment Programme, 2015), excessive use of the planet's natural water resources has reached an alarming level during the last 50 years and in turn, this has led to an increasing number of areas suffering from shortage of water (Valipour et al., 2015).

Water scarcity and droughts have been increasingly becoming key concerns worldwide; not only in dry regions (Chartres & Varma, 2010), but also in regions where freshwater resources are plentiful (FAO, 2012). They are among the major problems challenging many societies and the world in the current century (Hoekstra et al., 2012; Vo et al., 2014). About 1.2 billion people live in areas of physical scarcity, and for 500 million people this state is imminent. Another 1.6 billion people face economic water shortage (Water, 2007). The water scarcity phenomenon is expected to be exacerbated as the rapid increase in urban areas puts heavy pressure on adjacent water courses (Mambretti & Brebbia, 2014; FAO, 2016). By 2025, it is estimated that 1.8 billion people will be living in regions with total water scarcity, and two-thirds of the world will be living under water-stressed conditions (Chartres & Varma, 2010; Mambretti & Brebbia, 2014). An increasing population combined with the current excessive water consumption will worsen the situation and is expected to be the cause of many conflicts in the future (Samsó, 2014).

Adding to all that, the freshwater on the planet is badly distributed geographically and a large quantity of it is wasted. Figure 1.1 shows how water is a scarce resource. Furthermore, climate change and global warming are estimated to further complicate the already complex relationship between world development and water demand and put water resources even more at stake (FAO, 2012; Vo et al., 2014).

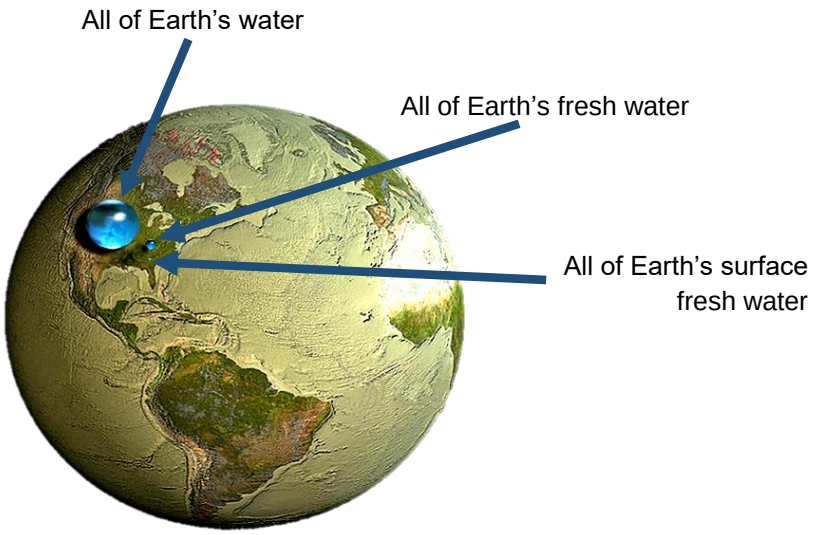


Figure 1.1: Spheres representing all of Earth's water (Credit: Howard Perlman, USGS; globe illustration by Jack Cook, Woods Hole Oceanographic Institution) (http://www.industrytap.com) indispensability.

The escalating environmental crisis due to diminishing fresh water availability has necessitated the use of wastewater as a viable alternative water source option (Angelakis & Snyder, 2015) to reduce anthropogenic impacts of water scarcity, rapid population growth, climate change effects, world fresh water supply shortage, and several other compelling factors that affect the sustainable availability of fresh water (Hamilton et al., 2005; Gross et al., 2007; Qadir et al., 2010; García-Orenes et al., 2015; Ramprasad & Kutty, 2016; Woltersdorf et al., 2016).

Presently, the pollution of water resources is considered one of the main global environmental issues that place pressure on the sustainability of ecosystems (Peasey et al., 2000; World Health Organization WHO, 2006). With the increasing urban populations, lack of suitable treatment technologies, and constrained budgets in many regions around the world, larger amounts of freshwater are being diverted to domestic, commercial, and industrial sectors, which generate greater volumes of wastewater. According to the World Water Development Report of 2012, over 80% of wastewater globally is not collected or treated; urban settlements are the major source of pollution, and in developing countries up to 90% of wastewater is released untreated into the receiving bodies. One of the main challenges ahead regarding water accessibility and quality is the discharging of municipal wastewater without any treatment or with only simple treatment (Qadir et al., 2010; Wu et al., 2015b). The discharge of poorly treated sewage wastewater has been recognized as a major contributor of pollutant releases into the environment (Scholz, 2015; Al-Isawi et al., 2016b; Valipour & Ahn, 2016). Release of pollutants with of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), turbidity, nitrogen compounds, phosphorus compounds, trace elements and heavy metals, and pathogens (Faulwetter et al., 2009; Abou-Elela et al., 2013; Vymazal, 2014), such that their concentrations exceed regulatory limits, to water resources can cause ecological and/or health problems (Scholz, 2006, 2010, 2015). Moreover, in many cases, it would be undesirable to use this water for human consumption, irrigation and aquatic life (Al-Isawi et al., 2016c). Improving and extending infrastructure can be very expensive and accordingly, in general, is not sustainable with rapid growth. Wastewater management therefore is emerging as a main global challenge (Alburquerque, 2013).

A further threat to the quality of receiving watercourses is chemical contamination, especially pollutants from oil products (Imfeld et al., 2009; Zhang et al., 2013; Vymazal, 2014; Al-Isawi et al., 2016b). More difficulties can be faced during the treatment of environmental services and ecosystem functions due to the increase of oily hydrocarbon contaminant residuals associated with various water users. Petroleum hydrocarbon pollution is, globally, considered one of the main environmental concerns that result in huge disturbances and disastrous consequences for the biotic and abiotic components of the ecosystem (Eke & Scholz, 2008; Vymazal, 2014; Zhang et al., 2014; Yavari et al., 2015). The environmental pollution is increasing gradually as petroleum hydrocarbon continues to be used as the principal source of energy (Al-Baldawi et al., 2013b; Al-Isawi et al., 2015b).

The discharge of wastewater contaminated with petroleum hydrocarbon compounds, such as diesel, directly into a (surface or ground) water body may cause detrimental effects on the environment and threaten human life. Diesel is one of the predominant energy sources that is used in various areas of human life to maintain economic and social development (Wang et al., 2011a; Agarry & Latinwo, 2015). It is a complex mixture of hydrocarbons produced by blending several fractions of crude oil distillates with brand-specific chemical additives (Owen & Coley, 1995). The chemical composition generally comprises of up to 25% aromatic compounds and around 75% saturated hydrocarbons (Toxicological Profile for Fuel Oils (TPFO), 1995). Diesel is found in the environment as a result of accidental release from an industrial site or transport vehicle. Hydrocarbon compounds from diesel spills mixing with urban runoff or industrial wastewater is a further recent challenge that affects the ecosystems around the world (García-Delgado et al., 2012; Scholz, 2015; Al-Isawi et al., 2016b). During the exploration and transportation

phases, spills of these hydrocarbons have increased, and, mixed with wastewater, pose a risk to the ecosystem, being toxic to many organisms and detrimental to human health (Moreira et al., 2011; Viggor et al., 2013; Al-Baldawi et al., 2015a; Yavari et al., 2015). Volatile organic compounds (VOCs) are usually harmful and carcinogenic and can cause severe environmental problems to the ecosystem (Ausma et al., 2002) and detrimental effects to human health (International Agency for Research on Cancer, 1988; Chen et al., 2012; Guittonny-Philippe et al., 2015a). Moreover, a group of diesel compounds contributes to the creation of photochemical ozone and secondary organic aerosols (SOAs), thus, leading to increasing global warming (Hu et al., 2009).

Most of the traditional treatment technologies used by the oil industry such as coalescence, flotation, centrifuges, hydro cyclones and various separators are expensive and not efficient concerning the removal of dissolved organic hydrocarbon components including aliphatics and aromatics in the dissolved water phase (Lin & Mendelssohn, 2009). The low efficiency and high cost of conventional treatment processes has produced economic pressures and has caused engineers to search for creative, cost effective and environmentally sound ways to control wastewater pollution by petroleum hydrocarbon compounds.

For all the reasons stated before, it is clear that our planet is undergoing serious environmental problems, and there is a need to explore sustainable treatment technologies which are efficient, and at the same time an economically reasonable solution to cope with the challenges of the future regarding water quality and accessibility (Abbasi et al., 2016). Due to their green, low or zero energy input, low investment, operation and maintenance costs, and sustainable credentials (Vymazal, 2007a, 2011b; Scholz, 2015; Sehar et al., 2016), constructed wetlands (CWs) are considered an alternative efficient

tool, and a reliable option for wastewater purification (Martinez-Guerra et al., 2015). They are promising green treatment alternatives to conventional wastewater treatment units and significantly reduce the use of energy intensive mechanical devices and technical complexity of mechanical treatment units (Korkusuz et al., 2005; Kadlec & Wallace, 2009; Vymazal, 2014; Wu et al., 2015f; Abbasi et al., 2016). They can be used effectively for several purposes with different configurations, scales and designs. This is because of their nutrient capturing capacity, simplicity, low construction cost, low energy demand, process stability, low excess sludge production, effectiveness and potential for creating biodiversity. They are capable of direct wastewater pollution control and yield high quality effluent with less energy dissipation, and with low environmental footprints (Chaikumbung et al., 2016).

Wetlands are constructed to imitate the optimal conditions occurring in natural treatment systems by encompassing biological, chemical and physical processes (Moshiri, 1993; Kadlec & Knight, 1996; Cooper et al., 1997; Vymazal et al., 1998; Mitsch & Gosselink, 2000; Scholz, 2010; Gikas & Tsihrintzis, 2014; Upadhyay et al., 2016) to remove various pollutants from different types of wastewater (Wu et al., 2015c; Jiang et al., 2016) with various loading rates and under a range of weather conditions (Kadlec & Wallace, 2009). Furthermore, these natural processes result in the efficient conversion of hazardous compounds (Xu et al., 2016). CWs are implemented for environmental pollution control, treating wastewaters including domestic wastewater, industrial effluents, urban and agricultural runoff, animal wastewater, sludge and mine drainage (Scholz & Lee, 2005; Scholz, 2010; Dong et al., 2012; Vymazal, 2014; Al-Isawi et al., 2015b; Scholz, 2015; Rozema et al., 2016a; Rozema et al., 2016b). The treatment efficiency of wetlands depends on various design and operation parameters including plant species, substrate

types, retention time, hydraulic loading and the quality of wastewater sources (Kadlec & Wallace, 2009). Constructed wetland studies show that removal percentages of COD, SS and BOD are generally high, whereas removal points of nutrients (particularly nitrogen and phosphorus) are often lower and less consistent (Vymazal, 2007a; Paing et al., 2015a). The purification capacity of wetlands to accumulate, retain, assimilate, and degrade various types of pollutants from wastewater in all environments has long been recognized (Mitsch & Gosselink, 2000).

Among the various types of constructed treatment wetlands, sub-surface vertical-flow constructed wetlands (SS-VFCWs), having a small footprint, signify the state-of-the-art design in wetland technology, attracting increasing interest for pollutant removal worldwide (Abou-Elela & Hellal, 2012; Chang et al., 2012; Stefanakis et al., 2014; Al-Isawi et al., 2015a; Wu et al., 2015d; Weedon et al., 2016). The main benefits of this design type are the lower area demand compared to that of other wetland systems (Scholz, 2010) and the fact that they provide sufficient oxygen within the bed for nitrification (Brix & Arias, 2005; Jia et al., 2010; Al-Isawi et al., 2015a; Al-Isawi et al., 2016b; Murphy et al., 2016). However, one of the main problems limiting the performance and efficiency of these systems is their contamination with petroleum hydrocarbon components, induced as a result of intensive petroleum exploration, production, transportation, distribution, utilization and refinement processes, which enter the wetland system and considerably damage the environment (Lin & Mendelssohn, 2009).

Wetlands treating industrial and domestic wastewaters are sometimes subject to permanent or one-off hydrocarbon contamination which results in changing the structure, function and ecosystem service values of the wetlands (Wang et al., 2013a). The inflow

wastewater characteristics and the physical state of the hydrocarbon impact on the efficiency and hydraulic properties of wetlands (Imfeld et al., 2009).

Wetlands contaminated with hydrocarbon compounds lead to smothering of soil particles and block air diffusion in the aggregate pores, thus causing anaerobic conditions and a reduction in permeability in the aggregate environment, affecting the diversity of micro-organisms (Khamehchiyan et al., 2007; Sutton et al., 2013). Moreover, the slow biodegradation of petroleum hydrocarbon compounds can result in accumulation and development of hydrophobicity in the wetland media and potentially lead to blockage and clogging of the system (Wu et al., 2015f). Clogging is one of the main factors which contributes to deterioration of the operation of vertical-flow filters for wastewater treatment. Some diesel components are water-insoluble, due to being mainly composed of hydrophobic organic compounds (Pazos et al., 2011). When these enter the wetland system, they gradually disturb its water quality (Al-Baldawi et al., 2013b; Al-Baldawi et al., 2014a; Al-Baldawi et al., 2015b). With long-term exposure, the substrate (the media of wetlands) may become clogged as a result of excessive formation of biomass from degradation of oily hydrocarbon pollutants and retention of insoluble hydrocarbon particles (Wu et al., 2015f).

The specific design concepts to treat a high dosage of petroleum hydrocarbon with constructed wetland systems have not been examined precisely. Sound knowledge of hydrocarbon treatment processes in vertical-flow constructed wetlands is needed to make guided judgments about the probable effects of a given suite of impacts. This will help the process design and operation of VF CWs, in terms of selecting and recommending the best values of many parameters whose relationships to hydrocarbon reduction with high water quality and healthy plants are poorly understood. This research focuses

specifically on a developing more thorough understanding of the science, underlying environmental variables, and mechanisms of diesel removal associated with vertical-flow constructed wetlands.

These hydrocarbons are, generally, more toxic to plants and contain higher concentrations of light hydrocarbon components (Lin & Mendelssohn, 2009; Liu et al., 2011; Al-Isawi et al., 2015a). Moreover, excessive hydrocarbon dosages may significantly suppress the growth variables of wetland plants, such as their stem density and shoot height (Ji et al., 2002; Wang et al., 2011b) and a high dosage could lead to the plants dying (Liu et al., 2011). Data describing the dose–response relationship of sub-surface flow constructed wetland (SSF CW) systems, and documenting the tolerance limits of wetland plants to petroleum hydrocarbon rarely exist.

Furthermore, most studies predominantly measured treatment performance of wetland systems and little attention was paid to the age effect of SSF CW systems (Tanner et al., 1998; Song et al., 2006; Mustafa et al., 2009; Dong et al., 2012) on the treatment performance. So far, there have been no substantial studies assessing the impact of mature constructed wetlands on the treatment performance based on efficiency comparisons with different types of artificial ponds. This research fills gaps in knowledge and understanding by evaluating the capability of mature wetlands, dissimilar in their designs and operation, in producing effluent that is treated appropriately before release into the environment, by comparing their efficiency with a new treatment system.

On the other hand, although constructed wetlands can be considered an effective, green and economic method to treat and control diverse contamination types, it is difficult to remove the hydrocarbon compounds completely from treated wastewater during the wetland operation. Most wetland systems release hydrocarbon with the treated outflow

water (Horner et al., 2012). The quantity and quality of the hydrocarbon compounds depend on the treatment efficiency of the wetland system (Lin & Mendelssohn, 2009; Vymazal, 2014; Al-Isawi et al., 2015b; Al-Isawi et al., 2016b). The continuous discharge of the effluent could pose a threat to natural ecosystems (Vymazal, 2014) and discarding of these petroleum hydrocarbons, even in small quantities, into water bodies may cause their concentration to exceed regulatory limits (Eke & Scholz, 2008; Guittonny-Philippe et al., 2015a). Nevertheless, the re-use of the treated wastewater for agriculture purposes, urban and industrial applications, recreational and ecosystem service needs, and artificial recharge of below-ground water (Metcalf et al., 2007; Al-Hamaiedeh & Bino, 2010; Marinho et al., 2013) might be a viable option to control such pollution to water courses.

Globally, agriculture is considered the biggest water consumer as it accounts for 65% of the water demand (Vo et al., 2014). The excessive use of water resources for agriculture has resulted in overexploitation of rivers, lakes and aquifers (Valipour et al., 2015). Instead of potable water and natural freshwater, treated wastewater can be applied for agriculture to tackle the challenge of increasing food production in water-scarce areas. Recycling of treated urban wastewater for irrigation has been considered as one of the promising strategies in the agriculture sector (Aiello et al., 2007; Cirelli et al., 2012; Norton-Brandão et al., 2013).

The smart reuse of treated wastewater in agriculture eliminates the need for using fertilizer and it makes it possible to expand agricultural land in arid areas; it is a relatively cheap disposal method for wastewater and offers good removal rates concerning microbial contamination (Peasey et al., 2000; World Health Organization WHO, 2006) and protects soil from contamination by nutrients and trace metals. Moreover, the benefits of constructed wetland systems for agricultural purposes are widely known. They produce

water with adequate quality for irrigation (Cui et al., 2003; Morari & Giardini, 2009; Becerra-Castro et al., 2015; Chaikumbung et al., 2016). This practice potentially increases agricultural yields, preserves freshwater, offsets the demand for chemical fertilizers, and reduces the costs of wastewater treatment by avoiding nutrient removal units (Murray & Ray, 2010). The application of treated wastewater in agriculture has not been efficiently managed (Cirelli et al., 2012; Sousa et al., 2016), particularly in developing countries (Al-Isawi et al., 2016b).

1.3 Problem statement

The above introduction identifies gaps in knowledge. The highlights suggest that the sustainable treatment of urban wastewater with sub-surface flow constructed wetland systems is a practical solution to remove pollutants of different types and concentrations (Saeed & Sun, 2012; Wu et al., 2014; Zhang et al., 2014; Wu et al., 2015a; Kim et al., 2016).

The investigation and application of these systems for hydrocarbon contaminated wastewater has gained increasing attention in recent years (Eke, 2008; Eke & Scholz, 2008; Scholz, 2010; De Biase et al., 2011; van Afferden et al., 2011). Despite the increasingly common application of such systems (Vymazal, 2011b), the contaminant removal processes have not been investigated in detail and analyses are limited to an assessment of the overall removal based on the inflow and outflow concentrations of contaminants.

The specific investigation and application of SSF CW systems for hydrocarbon contaminated wastewater has gained increasing attention from many authors during recent years in various studies (Omari et al., 2003; Eke & Scholz, 2008; Tang et al., 2009;

Wallace et al., 2011b; Wu et al., 2012; Al-Baldawi et al., 2013c; De Biase et al., 2013; Al-Baldawi et al., 2014a; Guittonny-Philippe et al., 2015a). However, few studies have assessed diesel as an example petroleum hydrocarbon to assess the performance of SSF CW systems (Omari et al., 2003; Al-Baldawi et al., 2013b; Al-Baldawi et al., 2013c; Al-Baldawi et al., 2014a; Al-Baldawi et al., 2015b). There is a significant gap in the previous research regarding the long-term performance of VF CWs treating urban wastewater contaminated with diesel.

The study seeks to provide a better understanding of the application of this technology and expand it to a new area by assessing the processes and verifying the effectiveness of vertical-flow constructed wetlands (tidal flow) at removing pollutants from simulated diesel contaminated wastewater. There is a lack of understanding of the complexity of function and internal interconnection processes derived from the application of diesel dosage within wetland filters, which can hinder their full deployment in the territory. This study provides an evaluation of internal workings of the constructed wetland components and the mutual relationships that exist within the system processes. The observations and results obtained are thus reported and the final findings will be helpful to regulators, operators and engineers to maintain good hydraulic and treatment performance.

Furthermore, various researchers including Kadlec and Wallace (2009), Vymazal (2011b) and Scholz (2015) have reviewed the effectiveness of vertical-flow constructed wetlands used for the treatment of urban wastewater. As the constructed wetlands reach the maturity stage, the treatment performance for pollutants removal may change. This research fills gaps in knowledge and understanding by evaluating the capability of mature wetlands, dissimilar in their designs and operation, in producing effluent that is treated appropriately before release into the environment, by comparing their efficiency with a

new treatment system. Three types of new artificial ponds were chosen in this study to assess the impact of mature wetland plants and the corresponding biofilm that develops around the gravel on nutrient removal, and water quality. Moreover, the impact of reeds and aeration on the treatment efficiency has been less well researched. Therefore, there is the research need to focus on the effect of reeds and aeration on the treatment performance of both mature wetlands and ponds.

Concerted efforts have been made to explore the use of treated wastewater as one of the most available water sources for agriculture (Aiello et al., 2007; Gross et al., 2007; Rousseau et al., 2008; Morari & Giardini, 2009; Cirelli et al., 2012; Norton-Brandão et al., 2013; Chen & Wong, 2016; Lavrnić & Mancini, 2016). However, investigation of use of domestic wastewater contaminated with diesel and treated by constructed wetlands for crop production has been less well researched. There is a lack of information about the optimum design and operational variables of vertical flow constructed wetlands to recycle this treated wastewater for agriculture purposes. This study offers the scientific evidence required for integrating wetland treatment technologies into food production. This will improve the resilience of communities to water scarcity and reduce pollution of the environment as well.

1.4 Aim and objectives

The wetlands technology has diverse applications and has been established as a cost effective and natural wastewater treatment technology around the world (Kadlec & Wallace, 2009; Vymazal, 2011b; Scholz, 2015). Constructed wetlands are widely used to treat various types of pollutants in wastewater (Scholz, 2010, 2015). A novel high dosage of hydrocarbon was introduced to a wetland system to study the effect of mature wetland

systems in treating this hydrocarbon dosage. The treatment mechanisms of wetlands technology consist of interconnected interactions of chemical, physical and biological processes and interactions between soil, plant rhizomes and the acclimatizing bacteria of the toxic effluents (Eke & Scholz, 2008). The use of constructed wetlands to treat and recycle urban wastewater contaminated with specific type of fuels such as diesel hydrocarbons is a relatively new ecological engineering technique, and therefore a clear understanding of the CW operations and functions is required.

The overarching aim of this study is to assess the capability of mature vertical-flow constructed wetlands with different design and operational variables in treating domestic wastewater with/without contamination by diesel spills and to evaluate their performance of these diverse mature wetlands for subsequent re-use of the treated effluent in irrigation of chillies (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne').

To achieve the research aim and address the key research gaps, the following objectives are set (Fig. 1.2 illustrates the aim and how objectives are linked to achieve it):

1. To assess the performance of different experimental vertical-flow constructed wetland filters treating domestic wastewater and to evaluate the annual and seasonal variability in water quality parameters (COD, BOD, nutrient, etc.) in wetland filters;

2. To assess the influence of design and operational parameters on clogging of different wetland filters treating domestic wastewater with/without diesel spills and evaluate a simulation model upgraded to investigate the impact of suspended solids (SS) sedimentation on the clogging processes of these wetland filters;

3. To investigate the efficiency of different wetland filters in treating hydrocarbon compounds and compared with those without diesel spill contamination and evaluate the hydrocarbon dose–responses on wetland plants growth;

4. To assess the impact of mature different wetland filters treating domestic wastewater without diesel spills on water quality parameters by comparing their treatment efficiencies with a new treatment ponds system;

5. To examine the influence of re-using differently treated wastewater on the growth of chilli plants taking into consideration the effect of boundary conditions on the growth environment, the amount of treated wastewater needed for irrigation and the economic viability of various experimental set-ups regarding their corresponding harvest.

Figure 1.2 below illustrates the methodology and how objectives are linked to achieve the aim.

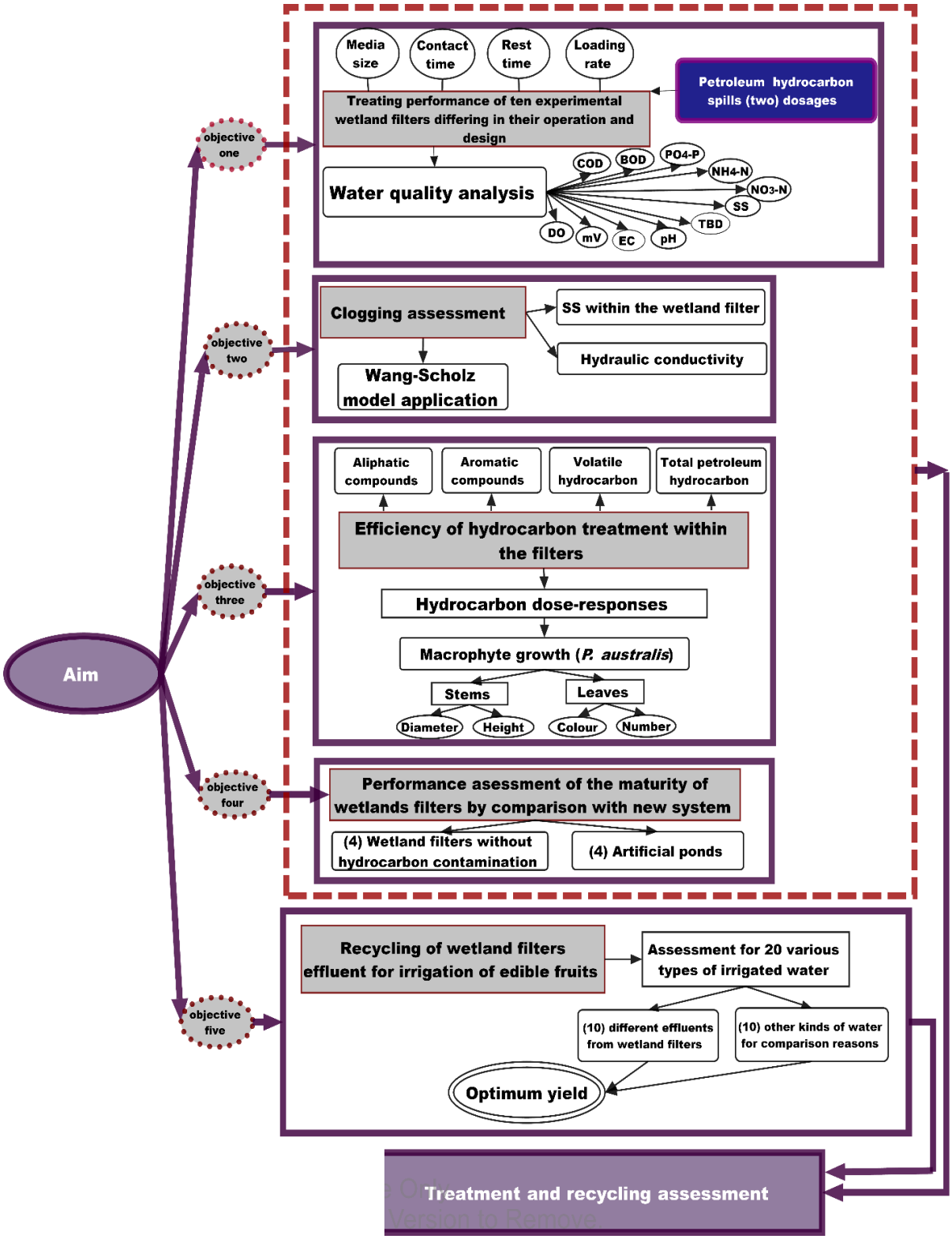


Figure 1.2: Aim and linked objectives diagram. Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate–phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; EC, electrical conductivity; mV, redox potential; DO, dissolved oxygen.

1.5 Importance of the study

Considering the problem statement, it becomes important to address key knowledge gaps with respect to the in-depth evaluation of the basic internal workings of the constructed wetland components and the interrelationships that exist within the system processes. The research uses data gathered from experimental-scale wetlands to assess long-term and seasonal removal efficiencies for water quality parameters and, subsequently, model different operational processes. This will help with formulation of proper wetland management plans by modellers and wetland mangers. Furthermore, this research covers the assessment of environmental, physical, chemical and microbial processes that affect the efficiency of petroleum hydrocarbon removal in each wetland and the comparison of different operational conditions. This enhances operational knowledge and understanding of treatment wetlands to control petroleum hydrocarbon spills that may be associated with the sewage discharged to municipal treatment plants and can also be discharged with industrial wastewater, from small factories and public utilities, and with domestic sewage. Moreover, the provided data can be used to design full-scale wetland systems, to be used in conjunction with petroleum and related water industries, for efficient hydrocarbon removal in wastewater treatment technology.

The study also provides a solid basis to support decision makers in making decisions regarding recycling treated municipal wastewater for crop irrigation. Findings will also assist community leaders in management of treated wastewater with/without diesel contamination for agricultural purposes. This study offers a promising solution to treat, and subsequently re-use, domestic wastewater in a more sustainable way, even when financial resources are limited. In addition, food grown on soil irrigated by pre-treated wastewater offers an additional economic return, which can help in solving food

shortages in many developed countries. Therefore, this study should be of interest to the international reader trying to protect the environment from pollution and solve wastewater treatment and food challenges at the same time.

1.6 Thesis structure

To meet the objectives and achieve the overall aim, and for easy flow, the thesis consists of five main chapters as described below:

Chapter 1: Introduction

This chapter starts with the background context, presents the problem statement and research gaps, defines the study aim and outlines objectives, identifies the importance of the study and lists the thesis chapters.

Chapter 2: Literature review

This chapter presents an overview of the background of the constructed wetlands and their types (Free surface flow, Sub-surface (Vertical and Horizontal) flow). An overview is also given of the constructed wetlands showing the role of key wetland components, pollutant removal mechanisms, clogging, and modelling. It also discusses the literature dealing with performance of pond systems and comparison with mature wetlands. Moreover, a portion of this chapter is dedicated to examining the existing literature dealing with hydrocarbon treatment mechanisms and, in addition, a review of the literature on recycling of treated wastewater for irrigation crops is provided.

Chapter 3: Materials and methods

This chapter describes the study site location, experimental set-up and operation methods. Experimental wetland filter design, construction, operation and controlled environmental conditions are also explained. Furthermore, the data collection procedure and subsequent analysis are also reported. Lastly, applications for re-using the outflow from different wetland filters are also described.

Chapter 4: Results and discussion

The overall treatment results and related discussions are presented in this chapter including water quality performance and the removal efficiency of each wetland filter for the whole operational period of the study. This chapter also focuses on the treatment performance for the hydrocarbon compounds from the experimental constructed wetland filters. The interactions between the hydrocarbon removal mechanisms and the role of macrophytes, filter media and nutrients are explained in detail. Furthermore, findings with regard to clogging of different wetland filters, and the modelling of the systems are described. The treatment performance of mature wetland filters in comparison with new pond systems is also presented. Furthermore, the growth of chillies in greenhouse using wastewater pre-treated by the constructed wetlands is explained in detail in this chapter.

Chapter 5: Conclusion and recommendations

The thesis concludes by summarizing the most important outcomes of the research and highlighting its main findings in this chapter, before recommending the possible directions in which this research can be continued.

CHAPTER TWO: CRITICAL LITERATURE REVIEW

2.1 Overview

This chapter presents a critical review of current information about natural and constructed wetland systems, including the types, components, and different removal mechanisms of contaminants in wetlands. The chapter is divided into ten sections as follows: section 2.1 represents an introduction to the chapter; section 2.2 describes the development of the constructed treatment wetlands; section 2.3 characterizes constructed wetland types associated with subsections for major wetland types; section 2.4 defines components of wetlands; and section 2.5 presents the removal mechanisms of contaminants with emphasis on various removal processes. Clogging processes within constructed wetlands are defined in section 2.6, and treatment modelling in constructed wetlands is presented in section 2.7 highlighting the application of constructed wetlands for suspended solids treatment. Section 2.8 presents the performance of mature constructed wetlands showing maturation impact on water quality parameters and performance in comparison with new ponds systems. Lastly, section 2.9 shows the application of wastewater for agricultural purposes with subsections emphasizing using treated wastewater for irrigation of edible crops. The chapter is summarized in section 2.10.

2.2 Development of constructed wetlands treatment

Wetlands are land areas where the water plays an important role in controlling the wetland environment with the contribution of plants and animal life (Ramsar, 1971, 2010a; Finlayson et al., 2011). The presence of these wetlands depends on the water level, partly or fully, covering the land surface for enough time to represent a transition between both terrestrial and aquatic system features (Smith, 1977; Cowardin et al., 1979; Scholz, 2010; Stefanakis et al., 2014). Wetlands vary broadly depending on different parameters and characteristics such as: regional and local differences in climate, soil types, water chemistry, geography, plant types, hydrology, and other factors, including human intervention (Russo, 2008), and in turn these parameters are responsible for the formulation of their status. Naturally occurring wetlands can be found in every climate from the tropics to the frozen tundra and on every continent except Antarctica (Vymazal et al., 1998).

Due to the variety of wetland types, the purposes of their use, and the problems associated with defining their boundaries, it is difficult to provide a precise definition of wetlands. The comprehensive definition of wetland was specified by US Fish and Wildlife Service as: "*Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water*" (Cowardin et al., 1979). In 1980, more than 100 countries over the world in the International Union for the Conservation of Nature and Natural Resources (IUCN), contributed to sign the Ramsar Convention which has adopted a definition on wetlands. This convention defined wetlands as: "*areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not*

exceed six meters" (Mitsch & Gosselink, 2000; Taylor, 2002; Ramsar, 2010a, 2010b). Guirguis (2004) explained the interaction processes that occur within wetland systems and with the aid of sunlight, water, animals, plants and micro-organisms are responsible together for water quality improvement within the systems.

From a historical perspective a large number of habitats are regarded as wetlands. A broad range of expressions has been used to characterize wetlands such as temporary shallow water bodies, marshes, swamps, lake margins (littorals), large river floodplains, coastal beaches, salt marshes, mangroves, peat, bogs, fens, sloughs, ponds, coral reefs, riparian area, pocosin, wet pasture, channel, seep, taiga, baylands, river, prairie pothole, wet meadow, intertidal mudflats, gulf, tundra, lagoon, lake, spring, estuary, sponge, stream, salt flat, creek, reservoir and beds of marine algae or sea grasses (Eke, 2008). In the nineteenth century scientists adapted the term "wetland" into common scientific usage as a euphemistic substitute for the terms mentioned above (Wright, 1907). However, some terms were still used by scientists such as mire, bog, and fen to describe specific kinds of wetland (Dennison & Berry, 1993; Mitsch & Gosselink, 2007).

Natural wetlands provide a series of multiple beneficial values for humankind including: ecological, socio-cultural, and economic. They are considered of special ecological importance, due to the diversity of species and population densities they support, their high productivity rate, and the particular habitats they include (Knight et al., 2001). They are considered to be ecologically multifunctional. These functions include water conservation (Lizotte et al., 2012), runoff regulation (Beutel et al., 2013; Ludwig & Wright, 2015), peat accumulation (Kleinen et al., 2012), carbon sequestration (Tuittila et al., 2013), pollution purification, toxic substance transformation (Paing et al., 2015b; Vymazal & Březinová, 2015), and disaster prevention for both droughts and floods (Li et

al., 2013a). Studies have shown the ability of wetlands to integrate with other systems to secure food processes and achieve more sustainable food production (Chen & Wong, 2016). The wetland has a powerful ecologically purifying effect, so it is also called “the kidney of the earth”, which plays an important role in supporting water resources conservation, adjusting the climate, degrading and absorbing pollution, protecting the biodiversity and providing the resources for human life production (Kadlec & Knight, 1996). Wetlands can also be known as “green” infrastructures as the ecosystem services produce a contribution toward mitigating the negative environmental impact of cities (Bai et al., 2013; Lundholm, 2015). Sometimes urban wetlands are called “biological supermarkets” which have the ecological functions for the extensive food chain and rich biodiversity they support and due to their operation as a host for wildlife (Mitsch & Gosselink, 1993; Barbier et al., 1997; Mitsch & Gosselink, 2007; Vymazal, 2011d). Wetlands are also considered as a “product treasury” providing materials and supplying substances and subsequently improving the community development and enhancing its economy (Lin et al., 2015). Generally, wetlands on the periphery of cities are defined as wetland reserves, which serve the main function of protecting the ecological environment in wetlands and conserving biodiversity (Bai et al., 2013; Li, 2014) and offer the possibility to recycle a high quality effluent for landscape irrigation or pond creation for educational and environmental purposes (Yu et al., 2015).

Natural wetlands have existed throughout human history and played a vital role in human life. The first civilizations, such as Egypt and Mesopotamia, recognized the values of natural wetlands when they were living close to the wetland areas, and took their advantages for supplying them with important resources (Scholz, 2010). Natural wetlands were also used by the Chinese thousands of years ago to clarify liquid effluent (Fujita,

1998). For centuries, natural wetlands have been distinguished as a convenient means for wastewater treatment by using them as places for wastewater discharge (Brix, 1994) because the wetland simply served as a beneficial recipient that was more adjacent than the nearest stream or other waterway (Reddy & Smith, 1987; Mitsch & Gosselink, 2007; Vymazal, 2011b). However, in most cases, this uncontrolled disposal of wastewater in wetlands led to filling their areas with nutrients, resulting in the devastation of many wetland sites (Vymazal, 2011b).

Over recent decades, the purification capacity of wetland systems to purify water has gradually been recognized both by the scientists and executives working with wetlands, and also by the public. This purification capacity stimulated interest in the potential to exploit these wetland capacities for a series of specific technological applications and since then they have become reliable and attractive options to treat various types of wastewater (Moshiri, 1993; Kadlec et al., 2000; Jackson & Myers, 2003; Vymazal et al., 2006; Vymazal, 2010, 2014; Vymazal & Březinová, 2015; Rozema et al., 2016a).

Constructed wetland systems are designed to mimic nature by providing wetland vegetation, soils, and their associated microbial assemblage processes (Kadlec, 1989), and imitating the treatment conditions, that originally existed in natural wetlands (IWA Specialist Group, 2000), which eliminate, transform, store and filter out pollutants as well as acting as sinks for nutrients (Hammer & Bastian, 1989; Vymazal, 2007b). They are managed and operated to take advantage of the physical, chemical and biological processes of natural wetlands to treat wastewater (Scholz, 2006) and for other purposes under a controlled environment (Hammer, 1989). At the early stage of constructed wetlands technology development, it is possible to observe their abilities in many different forms and applications, such as wastewater treatment and disposal, flood

protection, amelioration of water quality, fisheries, shoreline stability, and as reservoirs of biodiversity (Moshiri, 1993; Kadlec & Knight, 1996; Scholz, 2006; Kadlec & Wallace, 2009; Scholz, 2010, 2015).

The recognition of the ecological and economic benefits of wetlands gradually increased among the international communities when humans began to realize their values and tried to mimic water treatment processes presented in these natural wetlands, in an attempt to effectively address and manage various water quality problems (Vymazal, 2011b; Martinez-Guerra et al., 2015; Wu et al., 2015b). Research studies on the use of constructed wetlands for wastewater treatment began in Europe in the 1950s. The first experiments that relied on using wetland plants as an important component for wastewater treatment, "botanical treatment", were undertaken by Käthe Seidel in Germany at the Max Planck Institute in Plön (Seidel, 1965a). Bulrush (*Scirpus lacustris*) grown in artificial rooting environments were used to investigate the ability of this wetland plant to purify wastewater (Vymazal, 2005). From 1955 through the late 1970s, Seidel conducted numerous studies using wetland plants species to treat various types of wastewater (Vymazal, 2005, 2007b) including phenol wastewaters (Seidel, 1955, 1965a, 1966), dairy wastewater (Seidel, 1976), and livestock wastewater (Seidel, 1961). Furthermore, in the early 1960s, she investigated a method to improve anaerobic conditions of septic tank systems and to enhance the treatment of decentralized wastewater systems from low performance efficiency by using wetland plants in various types of sludge. She named this initial process a "hydrobotanical method" (Seidel, 1965b). To eliminate the anaerobic condition in the septic tank systems, she used a sandy soil layer with high hydraulic conductivity in a sealed module type and integrated a stage of initial sludge with a vertical percolation planted bed. This hydrobotanical method was subsequently considered as the

origin for the hybrid system which was known at the end of twentieth century (Vymazal, 2005, 2007b). Seidel's research and her discoveries marked the earliest documented engineered treatment constructed wetlands research in the western world (Eke, 2008). Moreover, literature pointed out that the first types of vertical-flow wetlands are those originated by Dr. Seidel in Germany (Cooper et al., 1996; Vymazal, 2005, 2009, 2011b). The period after the original design time showed a noticeable diminishing in using this type of wetland, however interest in using this system started to recover again six years later due to their capability to nitrify ammonia to nitrate better than that of horizontal-flow systems.

In the mid-1960s, Dr. Seidel collaborated with Dr. Reinhold Kickuth (Seidel's student) at the University of Göttingen, Germany, and developed horizontal sub-surface flow constructed wetlands HF CWs, commonly known as Root Zone Method (RZM) which were constructed with a cohesive heavy soil media (clay soils) (Vymazal, 2009, 2011b). This Kickuth's system was applied for a full-scale wetland system at Othfresen, Germany in 1974 (Kickuth, 1977, 1978, 1981; Vymazal, 2005, 2011b). Moreover, Kickuth continued with the experimental research and generalized this concept with his colleagues in Europe, resulting in around 200 municipal and industrial waste treatment systems (Moshiri, 1993). Interest in this (Root Zone Method) flow wetland had extended throughout Europe by the mid-1980s, especially when the UK Water Industry became familiar with the method which had then just started to be applied in Denmark (Cooper et al., 1996). Constructed wetland technology spread quickly after the 1990s and has been commonly used among scientists and researchers around the world because of its ability to remove nutrients efficiently from treated wastewater (Vymazal, 2011b).

In the United States of America (USA), the use of constructed wetlands for wastewater treatment has been applied since the late 1960s (Vymazal, 2011b). During the period between 1970 and 1980, land treatment alternatives were developed with the support of a significant research and development effort funded by numerous agencies in the USA such as the U.S. Environmental Protection Agency (USEPA) and the U.S. Army Corps of Engineers (USACE) (Moshiri, 1993; Vymazal, 2011b). A remarkable expansion of wetlands applications in the United States was witnessed after the Tennessee Valley Authority (TVA) published a design manual in 1993 based mainly on serving a one-family house (Wallace, 2004). The wetlands applications research was increased in the USA throughout the 1970s and 1980s, with significant federal involvement by the Tennessee Valley Authority (TVA) and the US Department of Agriculture (USDA) at the end of 1980 and beginning of 1990 respectively (USEPA, 2000; Vymazal, 2011b).

Nowadays, constructed wetlands technology is increasingly receiving global consideration and popularity for wastewater treatment and recycling. They can operate as a habitat for wildlife, and offer the possibility to recycle the high quality effluent for landscape irrigation or pond creation for educational and environmental purposes (EPA, 2000; USEPA, 2000; Scholz & Lee, 2005; Mara, 2009; Brix et al., 2010; Vymazal, 2010; Kushwah et al., 2011; Li, 2014; Ávila et al., 2015; Chouinard et al., 2015; Scholz, 2015; Chen & Wong, 2016; Tilak et al., 2016). Constructed wetland technology has widely spread in developed countries due to stricter discharge regulations, high economics rates of habitats, flexibility in changing on-site technologies use instead of centralized systems, and due to the vast experience and knowledge based on science and practical work (Vymazal, 2011b, 2013c; Gikas & Tsihrintzis, 2014; Lavrnić & Mancini, 2016; Rozema et al., 2016b). Recently, there has been an expansion in the variety of applications for

constructed treatment wetland technology for water quality improvement in some developing countries (Abou-Elela & Hellal, 2012; Saeed & Sun, 2012; Al-Baldawi et al., 2013b; Abou-Elela et al., 2015; Wu et al., 2015b; Zheng et al., 2015; Lu et al., 2016) as a result of the transmission of knowledge, practical collaboration and methodical co-operation by the researchers in industrialized countries (Kivaisi, 2001; Zhang et al., 2014). The increased use of constructed wetlands worldwide is mainly attributed to the growing awareness of and interest in technologies that support environmental protection, resource conservation and increased reliance on natural ecological processes in comparison to the more industrial looking, unattractive facilities, energy requirements and chemical intensive "mechanical" (conventional) systems (Kadlec & Wallace, 2009; Zhou et al., 2009; Stefanakis et al., 2014; Ayaz et al., 2015).

Today, constructed wetlands are being recognized and spread widely worldwide as an environmentally friendly, low-cost and reliable wastewater treatment technology, serving as a promising potential system for the treatment of wastewater from various sources (Vymazal, 2011b; Al-Baldawi et al., 2013c; Vymazal, 2014; Abou-Elela et al., 2015; Wu et al., 2015c; Wu et al., 2015b; Kim et al., 2016). While research and practical application of constructed wetlands as a suitable solution for the treatment of many types of wastewater have increased experience and knowledge over the years, some fundamental knowledge of the internal processes which lead to the observed experimental performance of wetlands is not yet fully understood (Kadlec & Wallace, 2009; Wallace, 2013; Stefanakis et al., 2014; Vymazal, 2014; Scholz, 2015). This could be attributed to the technology being a natural system, with variable performance, that depends on the complex interaction of different components of the wetland system with the nature of the

treatment processes, which are subject to seasonal change (Eke, 2008; Stefanakis & Tsihrintzis, 2012; Březinová & Vymazal, 2015; Xie et al., 2016).

2.3 Classification of constructed wetlands

2.3.1 Overall classification

Constructed wetlands (CWs) developments have increased dramatically and can be designed and constructed in numerous hydrologic modes and shapes that emphasize specific features to improve treatment performance capacity (Kadlec & Wallace, 2009; Wu et al., 2014; Wu et al., 2015c). However, most of these variants have evolved from the basic types of CWs, which are in relatively widespread use currently.

Constructed wetlands can be classified, according to their areas of application, into three main types: constructed wetlands for habitat creation, flood control, and wastewater treatment (Vymazal, 2013b; Stefanakis et al., 2014; Vymazal, 2014). Constructed wetlands for wastewater treatment technology can be further categorized (Kadlec & Knight, 1996; IWA, 2000; Kadlec et al., 2000; USEPA, 2000; Sharma et al., 2013; Stefanakis et al., 2014; Vymazal, 2014): according to the type of macrophytic growth (emergent, submerged, free floating and rooted with floating leaves), and according to the water flow regime, which is distinguished by the location of the hydraulic grade line (free water surface flow (FWS), sub-surface vertical (VF) or horizontal flow (HF)). Further types of constructed wetland systems (so-called hybrid systems or combined systems (C_S)) have been utilized from combinations of different types of wetland systems in sequence to maximize the treatment efficiency and minimize its cost (Cooper et al., 1999; Vymazal, 2013b; Vymazal & Kröpfelová, 2015). Furthermore, some literature pointed out another type of constructed wetland system called Intensified systems (IS)

which are basically used for wastewater with very high loads to achieve high removal efficiencies (Wu et al., 2015f; Wu et al., 2016b). The principal types of constructed wetland systems are shown in Figure 2.1.

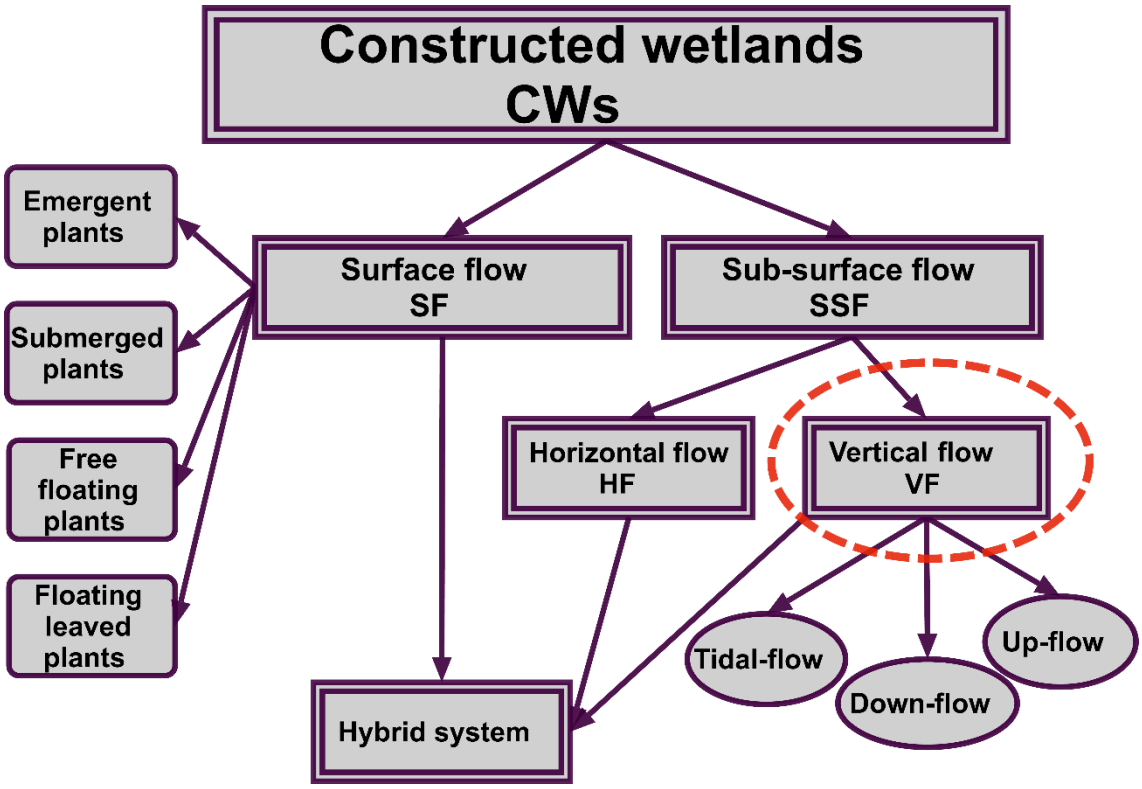


Figure 2.1: Classification of constructed wetlands for wastewater treatment. Dashed ellipse signifies the focus of this study.

2.3.2 Free water surface-flow constructed wetlands

The Surface Flow (SF) – also known as Free Water Surface Flow constructed wetlands (FWSF CWs) – system (Figure 2.2) mimics the hydrologic regime of natural wetlands, where water flows over the surface of the substrate from the inflow point to the outflow point and the flow of the water is in a relatively slow moving velocity mode (Vymazal et al., 1998; Vymazal, 2007a, 2011b). The FWSF CWs system design normally encompasses shallow channels provided with a barrier to prevent wastewater seepage to

the aquifers. Moreover, soil is selected as a substrate, or any other media, to fill up within the wetland to 0.4 m height to support the growth of wetland plants roots (Kadlec & Wallace, 2009; Vymazal, 2013a).

The wetland is flooded from the top and the water is distributed on the ground surface allowing the water to flow horizontally with slow velocity above the surface of the substrate layer, along the system, until collected at the outlet, creating a water column depth reaching to 40 cm (Vymazal et al., 2006) or even up to 80 cm (Crites et al., 2006). In some cases, and due to exposure of the surface water to the atmosphere and the sunlight, the water is completely lost by evapotranspiration and/or infiltration processes through the wetland media (USEPA, 1995; Knight et al., 1999).

The use of FWSF CW systems is more common in North America and they are applied almost exclusively for municipal wastewater treatment (Kadlec & Wallace, 2009). The first full-scale FWSF CW system for wastewater treatment was constructed in the Netherlands during the period 1967–1969 (Vymazal, 2010). The FWSF CW system can be planted with different types of macrophytes such as emergent, free floating, floating-leaved, bottom rooted or submersed macrophytes and thus, provide more wildlife habitat benefits (Vymazal et al., 1998).

Wetland treatment processes take place when the wastewater moves with low velocity through the wetland bed and subsequently comes into contact with the substrate and wetland plants parts, thus various pollutants are removed by a series of physical, biological, and chemical processes (Vymazal, 2007a). Most of the treatment processes occur in the lower layers of the wetland system by anaerobic microbes which is similar to the processes that are found in the natural pond system (Kadlec, 2001).

FWSF CWs have proved to be effective in the removal of suspended solids (SS) and biochemical oxygen demand (BOD). Removal of nitrogen (N), pathogens, and other pollutants (e.g., heavy metals) is relatively high, while phosphorus (P) removal is limited (Kadlec & Knight, 1996; Vymazal, 2007a; Kadlec & Wallace, 2009; Kotti et al., 2010; Zheng et al., 2016). The treatment of wastewater occurs when the water contacts with porous media and plant parts so SF wetlands usually need a higher surface area compared to other CW types (Vymazal et al., 2006). Moreover, this wetland type is not preferred in cold climates (Vymazal, 2007a). This is attributed to the tendency to freeze over in the wintertime, which results in considerably lower contaminant removal rates. Further reductions in removal efficiencies also arise from the lack of volatilization and oxygen transfer in cold weather (ITRC Interstate Technology and Regulatory Council, 2003). Furthermore, a drawback is the nature of the standing water which increases the possibility of mosquito breeding (Vymazal, 2013a).

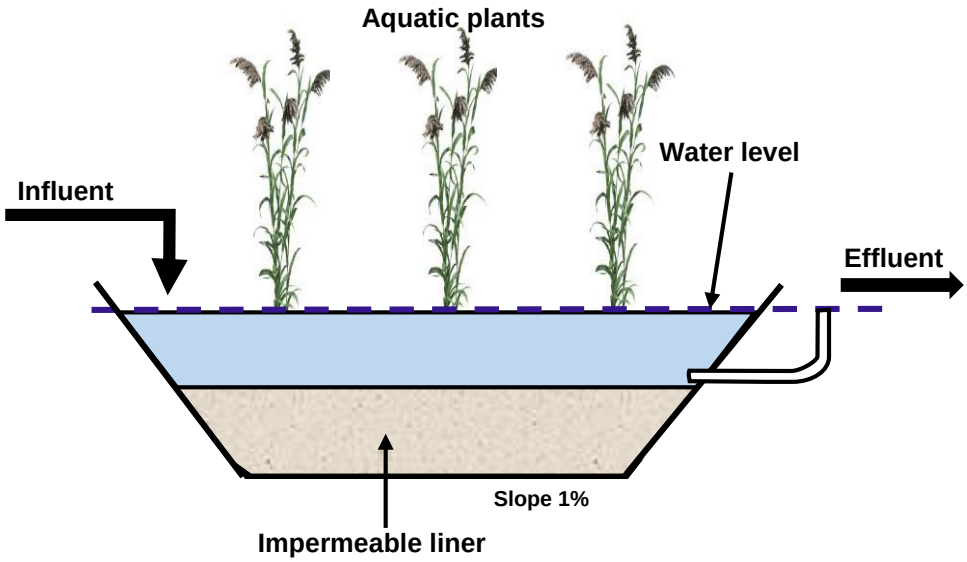


Figure 2.2: Typical configuration of a surface flow wetland system (SF).

2.3.3 Subsurface-flow constructed wetlands

Subsurface-flow constructed wetlands (SSF CWs) are also known as reed beds, rock-reed wetlands, gravel beds, vegetated submerged beds, and the root method. Reed beds and rock-reed wetlands use sand, gravel, or rock as substrates, while the root method uses soil (Kadlec & Knight, 1996). The media used as a substrate to construct the SSF CWs are, generally, from one or more different porous materials such as sand, soil, or gravel (Kadlec & Wallace, 2009). The wastewater enters the wetland system from the top and passes by an inlet distribution technique, then flows slowly under the surface of the substrate, passing through the shoots and/or root-zone of wetland plants until it reaches the outlet collection system. The water surface is usually kept under the surface of the ground, which may support different types of rooted emergent vegetation (Vymazal, 2009, 2011c).

The advantages of subsurface-flow systems include increasing treatment efficiencies for compounds such as nitrogen and carbon due to high oxygenation in their substrate (Fan et al., 2013a; Fan et al., 2013b; Nivala et al., 2013; Song et al., 2015; Zheng et al., 2016). SSF systems also have another advantage of requiring less land area for water treatment. Moreover, the substrate provides more surface area for bacterial biofilm growth over a surface-flow wetland, and this mean increasing treatment effectiveness with smaller land area requirements (Vymazal, 2001; Wu et al., 2015d) and, in turn, saving land area is important at many installations and translates into reduced capital cost for projects requiring a land purchase. SSF wetlands are also better suited for cold weather climates (Vymazal, 2014; Rozema et al., 2016a) since they are more insulated by the earth as well as suffering fewer pest problems as the water is kept under the surface of the wetland. Finally, many industrial waste streams, such as landfill leachate, can be treated in reed-

bed systems with minimal ecological risk, since an exposure pathway to hazardous substances does not exist for wildlife and most organisms (ITRC, 2003). However, these systems are generally not as suitable for wildlife habitat as surface-flow constructed wetlands. The SSF CW type may be divided into two groups, based on the direction of water flow through porous media: vertical and horizontal flow systems (Vymazal, 2007a, 2007b; Kadlec & Wallace, 2009; Langergraber et al., 2009).

2.3.3.1 Vertical-flow constructed wetlands

The vertical-flow constructed wetlands (VF CWs) system, uses a substrate media for growth of rooted wetland plants to efficiently treat various types of wastewater (Brix & Arias, 2005; Knowles et al., 2011; Li et al., 2013b; Huang et al., 2015b; Dogdu & Yalcuk, 2016; Rozema et al., 2016a; Rozema et al., 2016b). The surface of the wetlands is flooded with wastewater to a depth of several centimetres (3-5 cm), creating water ponding for a time, which then slowly moves and percolates downwards through the bed substrate planted with macrophytes (Figure 2.3). With this mode of operation, the wastewater passes through the granular media and flows by gravity vertically, undergoing filtration where it contacts a mixture of micro-organism populations living in association with the substrate particles and plant roots (Hoffmann & Platzer, 2010). As the water infiltrates through the system, pushing out the trapped air and sucking fresh air into the bed, air enters the substrate pores, increasing aeration availability and thus improving microbial activity (Fan et al., 2013a; Song et al., 2015). The two common types of filtering materials used to fill the bed system are sand or gravel with size gradation increment with depth (Vymazal et al., 2006). Gravel beds are very common systems used in North Africa, South Africa, Asia, Australia and New Zealand, while the sand bed systems originated in Europe and are currently used widely all over the world. The depth of the bed varies (between

450 and 120 cm) and the bottom of the bed has a small slope (1-2%) that allows percolation, collection, and drainage of treated water out of the system (Vymazal, 2011b).

VF wetlands can be saturated with water or dried by dosing the wastewater periodically into the system (Knowles et al., 2011; Wallace, 2013; Stefanakis et al., 2014; Vymazal & Kröpfelová, 2015). This filling and draining cycles technique (intermittently fed) for substrate media will enable the oxygen to be regenerated in all areas of the wetland providing suitable conditions for nitrification but denitrification is very limited in this system (Vymazal, 2007a).

The first VF CWs were originally developed by Seidel (1965a) when she applied them in the second step before HSF CWs and after an anaerobic septic tank (Vymazal et al., 2006; Vymazal & Kröpfelová, 2011). In the initial applications of the CWs, and due to the high operational cost of VF CWs systems, focus was given to the other types of constructed wetland technologies. Six years later, use of VF CWs technology was generally increased when the researchers realized its high nitrification ability and high ammonia nitrogen (NH_4-N) oxidizing capacity compared to HF systems (Cooper, 1999; Vymazal, 2001, 2009; Stefanakis et al., 2014; Vymazal, 2014). VF wetlands become more popular than the horizontal flow systems for many reasons. Firstly, they have much greater substrate aeration capacity resulting in good nitrification which in turn results in high removal efficiency for BOD, COD, ammonia, and bacteria (Cooper, 1999; USEPA, 2000; ITRC, 2003; Vymazal, 2007a; Kadlec & Wallace, 2009; Stefanakis & Tsihrintzis, 2012; Stefanakis et al., 2014). Secondly, they demand relatively small land requirements (1-2 m^2/capita) as compared with horizontal flow systems which need (5-10 m^2/capita) for secondary treatment (Stefanakis et al., 2014). Moreover, these types of wetlands have more equal root distribution and water-root contact and fewer problems of bad odour and

proliferation of insects, since they do not have a free water surface (Haberl et al., 1995; Cooper, 1999).

Research has shown that VF systems are good treatment technologies with regard to water quality parameters as they perform well in removal of chemical oxygen demand, biochemical oxygen demand, suspended solids, but are less efficient in the treatment of phosphorus because of the insufficient interaction between the wastewater and substrate media (Brix & Arias, 2005; Chang et al., 2012; Paing et al., 2015a). Furthermore, the nitrification process, that requires aerobic conditions, can be achieved well by these system (Langergraber et al., 2007; Vymazal, 2010; Zhi et al., 2015), while denitrification occurs within an anaerobic environment, which cannot be fulfilled simultaneously in conventional VF CWs (Fan et al., 2013b). Though some researchers referred to this system as a poor denitrifier (Vymazal, 2005; Scholz, 2010; Vymazal & Kröpfelová, 2011; Saeed & Sun, 2012; Wu et al., 2015a), several studies recently showed that VF CWs systems with intermittent loading regimes can denitrify well with modification (Gross et al., 2007; Fan et al., 2013a; Song et al., 2015; Wu et al., 2015a; Pan et al., 2016; Wu et al., 2016a). Although VF CWs with high organic loading rates and nutrients showed good treatment efficiencies, clogging can occur after long-term wastewater treatment (Knowles et al., 2011; Fu et al., 2013; Hua et al., 2013; Song et al., 2015). Some studies revealed that bioclogging can be mitigated when an intermittent operation process is applied in VF systems, because this operation process with loading and resting periods can effectively improve porosity and the hydraulic conductivity of the substrate media (Vymazal, 2005; Hua et al., 2014; Paing et al., 2015a; Wu et al., 2015a).

The use of VF CWs for wastewater treatment has been mainly attractive in Europe (particularly in Denmark, Austria, Germany, France, and the UK) and also in the USA

(USEPA, 1995; Kadlec & Wallace, 2009). They are preferably used for treatment of municipal and domestic wastewater and also, due to their high nitrification capacity, for other wastewater types that contain high ammonia nitrogen concentration (Kadlec & Wallace, 2009). The most common plant used in VF systems is reeds (*Phragmites australis*) as it is planted at the top of the wetland bed (Stefanakis et al., 2014). Recently, the application of this type of wetland has increased gradually to include many regions around the world (Brix & Arias, 2005; De Biase et al., 2011; Abou-Elela & Hellal, 2012; Song et al., 2015; Weerakoon et al., 2016; Yang et al., 2016a).

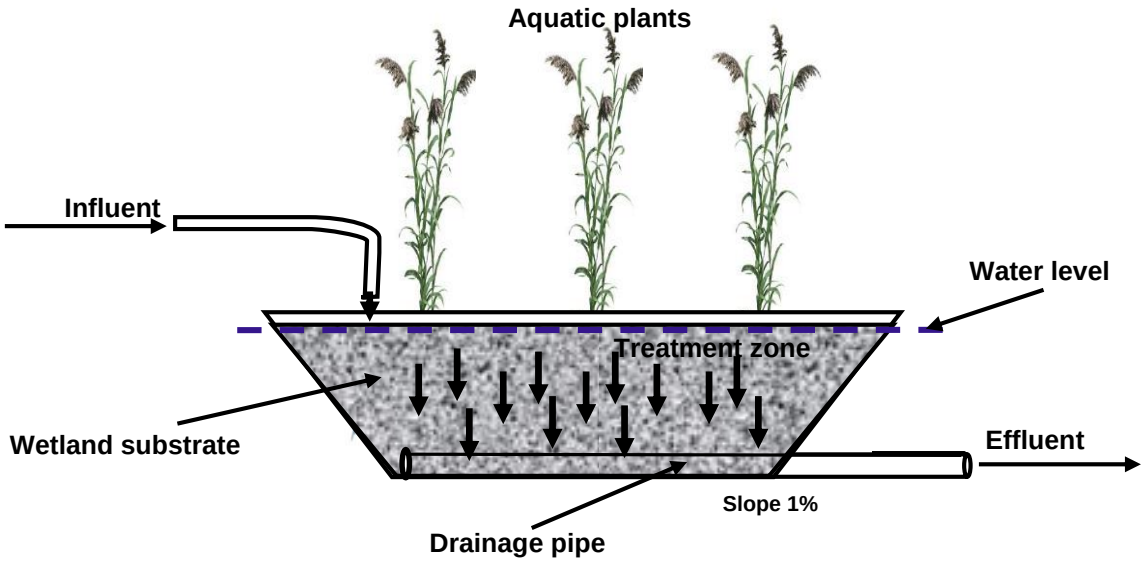


Figure 2.3: Schematic cross section of vertical-flow constructed wetlands (VF).

2.3.3.2 Horizontal flow constructed wetlands

Horizontal subsurface-flow constructed wetlands (HSSF CWs) are treatment systems designed in such a way that the wastewater is continuously fed in at the inlet, flows horizontally under the surface of the bed with a slow movement through the porous medium and the macrophyte roots till it reaches the outlet control zone where it is

collected (Vymazal, 2009, 2014; Vymazal & Březinová, 2015) (Figure 2.4). Typically, the removal of pollutants is accrued when the wastewater comes into contact with an interconnection of aerobic, anoxic and anaerobic zones of the wetland where various microbial, physical and chemical processes take place (Kadlec & Knight, 1996; Vymazal & Kröpfelová, 2015). The oxygen is provided to the substrate by leakage from the roots and rhizomes regions which represent the aerobic zones but the filtration bed is mostly anoxic or even anaerobic (Brix, 1987; Cooper et al., 1996; Vymazal, 2014). In this type of wastewater treatment, the water is not exposed to the atmosphere so the health risk for wildlife habitat and humans is minimized (Kadlec & Wallace, 2009). The material used for the substrate bed is gravel or a mixture of sand and gravel, and the depth of the substrate ranges between 30 and 80 cm, which usually supports the growth of the macrophyte (Vymazal et al., 2006; Vymazal & Březinová, 2015) with a slope (1-3%) applied in the bottom bed to enhance gravitational water flow (Kadlec & Wallace, 2009; Stefanakis et al., 2014).

Various literature has pointed out that the presence of plants with porous medium in HF systems enhances the development of biofilm layers, which in turn leads to improvement of the BOD and total suspended sediments (TSS) removal efficiency, but for complete oxidation of ammonia (nitrification) they demand a very large area due to the limited oxygen transfer within the wetland filter (Kadlec & Knight, 1996; Vymazal et al., 2006; Kadlec & Wallace, 2009; Gikas & Tsihrintzis, 2014). However, they are effective in denitrification (Cooper, 1999) and require a small area when compared with SFCWs systems, but have higher investment costs (Kadlec & Wallace, 2009).

The use of subsurface constructed wetlands with horizontal flow spread throughout Europe and the USA (Vymazal et al., 2006; Vymazal, 2011b, 2014) due to the small area

required and the excellent performance for pollutants removal as compared with SF CWs systems (USEPA, 1995; Kadlec & Wallace, 2009).

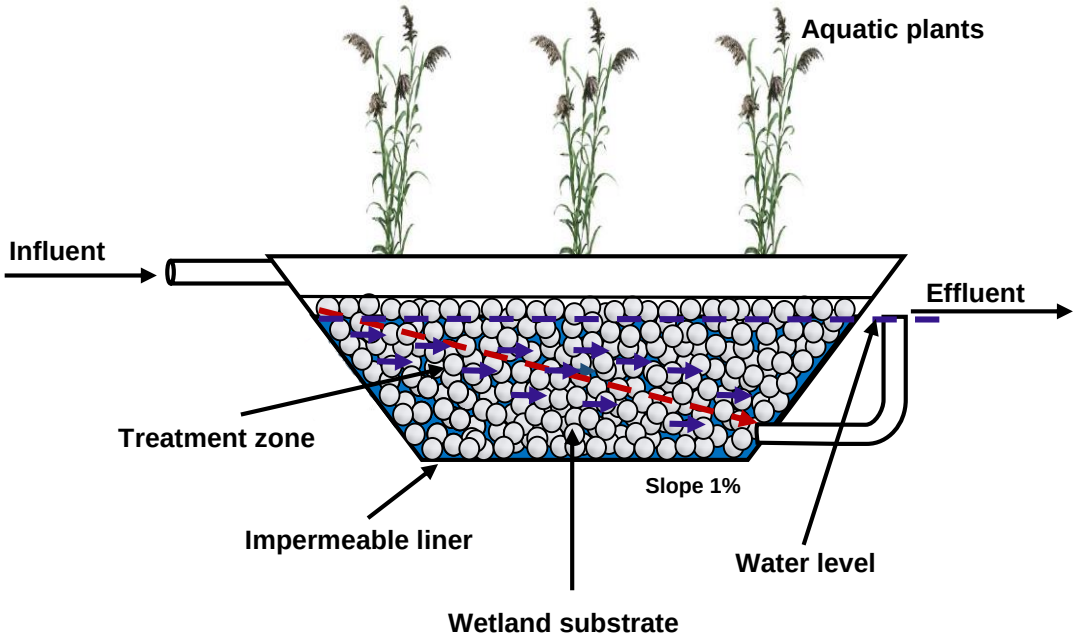


Figure 2.4: Schematic cross section of horizontal-flow constructed wetland (HF).

2.3.3.3 Hybrid constructed wetlands

A hybrid system also called a combined system, is a combination of two or more different systems to improve the overall wastewater treatment performance (Cooper, 1999; Vymazal, 2005; Vymazal & Kröpfelová, 2011, 2015; Ávila et al., 2016). It is mostly used to treat domestic or municipal sewage (Vymazal, 2013b; Stefanakis et al., 2014; Kim et al., 2016). The most common type of hybrid system comprises a combination of vertical flow and horizontal flow systems arranged in a staged manner (Cooper, 1999; Cooper et al., 1999; Vymazal, 2005; Vymazal et al., 2006; Vymazal & Kröpfelová, 2015; Upadhyay et al., 2016). In hybrid systems, the arrangement of the HF and VF systems (to complement each other) provides advantages. The concept of the combination of various types of filters was originated by Seidel in Germany in the 1960s; however, only a few

full-scale systems were built (e.g. Saint Bohaire in France or Oaklands Park in the UK) in the 1980s and early 1990s (Vymazal, 2005). In the late 1990s, the inability to achieve nitrification and denitrification processes together in a single horizontal flow or vertical flow system attracted researchers to consider using hybrid systems that combine various types of constructed wetlands (Vymazal, 2013b; Kim et al., 2016; Sehar et al., 2016). Vymazal (2013b) classified the combination of hybrid constructed wetlands into the following types: VF-HF systems, multistage VF-HF systems, VF hybrid systems, and hybrid constructed wetlands with FWSCW systems. However, VF-HF hybrid systems, with the VF beds placed first, is the most common arrangement used as it gives better treatment efficiency (Cooper, 1999) including achievement of a satisfactory removal of BOD, COD and bacteria, complete oxidation of ammonia to nitrate ions, and also a significant amount of total nitrogen can be removed (Vymazal, 2005; Kim et al., 2016).

2.3.3.4 Application of tidal vertical-flow constructed wetlands

The application of tidal flow mode in vertical-flow constructed wetland systems, as applied in this study, is mainly used to solve the oxygen transfer limitations in traditional CWs. The operation strategy of these systems rhythmically relies on the regular filling (temporary flooding) of the bed with wastewater (creating saturated conditions) followed by draining and creating unsaturated conditions (Stefanakis et al., 2014; Wu et al., 2014; Petitjean et al., 2016). This mode attracted significant attention due to its highly efficient treatment potential and relatively low operational cost (Kadlec & Wallace, 2009; Zhi et al., 2015; Kim et al., 2016). During the filling of the bed, air presented within the porous media is forced to escape the filter and the wetland progressively becomes saturated with wastewater. After an appointed period of time, when the bed remains completely submerged, the wastewater drain out starts, allowing fresh air from the atmosphere to

enter into the porous media, since the percolating wastewater runs as a passive air pump (Stefanakis et al., 2014).

Numerous studies have been conducted to test the impact of the tidal flow strategy on the efficiency performance of VF CWs systems to treat various types of wastewater. Li et al. (2015b) showed that the wetland systems that operated with a tidal flow system achieved high pollutant removal by exchanging modes between contact time (saturated period) and resting time (unsaturated period) which indicated the importance of both the contact time between the wastewater and the CW components (plant roots, substrate, biofilm) and also, the oxygen transfer into porous media during the treatment process (Austin et al., 2003; Sun et al., 2005; Song et al., 2015; Upadhyay et al., 2016).

Advanced treatment efficiency can be achieved by tidal flow wetlands as compared with conventional constructed wetlands as the former fully meet the oxygen demand in the bed. This strategy maximizes pollutant-biofilm contact due to raising the oxygen provision during the operation of the wetlands system and this in turn improves the removal of BOD through aerobic decomposition and removal of ammonium-N through nitrification (Wu et al., 2011b; Wu et al., 2014; Zhi et al., 2015; Kim et al., 2016).

2.4 Composition of wetland

2.4.1 Overview

Constructed wetlands are complicated artificial systems consisting of basic components for wetland characterization such as underlying strata, water, hydric soil, detritus, and macrophytes (vegetation) (Moshiri, 1993). However, other important components of wetlands such as the micro-organisms and invertebrates grow naturally. The interaction

of water, plants, animals and micro-organisms, and the environmental conditions play an important role in improving water quality (Scholz, 2006; Kadlec & Wallace, 2009). A proper understanding of the relationships and interactions between components is critical and can be manipulated in constructing a wetland in order to enhance internal processes and improve the efficiency of treatment performance (Scholz, 2010; Stefanakis et al., 2014; Scholz, 2015).

2.4.2 Water

Water is the main factor which controls the wetland environment and affects its aquatic life (Ramsar, 1971). The wetland can be constructed at any place in the landscape by making some changes either to the ground surface and/or basin to collect and retain water. The soil texture of wetlands should be hydric – saturated with water for a period of the growing season (Brix, 1993; Mitsch & Gosselink, 1993). Treatment wetland hydrology is very complex since the wastewater inflow with associated pollutants is regularly drawn through the wetland bed (Scholz, 2010; Wallace, 2013; Li et al., 2015). Hydrology determines the condition of the substrate saturation (constant or intermittent) where most of the general biogeochemical operations take place (Eke, 2008; Scholz, 2015; Morandeira & Kandus, 2016) and it is one of the most important design factors that determines the success or failure of a wetland's construction because water affects all other functions in the wetland (Kadlec & Knight, 1996; USEPA, 2000).

The hydrological characteristics of wetlands can be expressed by two features: the hydro period, which represents the time during which the substrate is flooding, and the flooding depth of the wetland filter (Moshiri, 1993; Kadlec & Knight, 1996). The average time that water stays in the wetland bed is known as the hydraulic retention time (HRT), which is a very important variable considered in designing, evaluating and operating a

constructed wetland and assessing the performance efficiency for pollutants removal (Hammer, 1989; Ghosh & Gopal, 2010; Stefanakis et al., 2014). Due to the continuous feeding operations for the constructed wetlands systems, substrate media will be developed and in turn provide a suitable environment for predominant plants species to exist in the saturated media (Mitsch & Gosselink, 2000; ITRC, 2003).

2.4.3 Macrophytes

Wetland plants are an important component of a wetland system (Tanner, 1996; Lee & Scholz, 2007; Vymazal, 2013c, 2013a; Butterworth et al., 2016) and references to CWs as a green technology may be due to the presence of their green vegetation (Stefanakis et al., 2014). Plants that grow in wetlands may include submerged plants, plants that emerge from the water's surface, floating mats of vegetation, small shrubs and grasses, mosses, trees and shrubs (Cowardin et al., 1979; Vymazal, 2013a). The role of higher wetland plants (vascular) and algae (non-vascular) is important in CW treatments. The development of roots within the wetland filter medium contributes to decomposition of organic matter and avoids clogging by providing channels for the water to pass through in the intermittent loading vertical-flow system. Moreover, algae play a vital role by increasing the dissolved oxygen (DO) content of the water during photosynthesis processes (Brisson & Chazarenc, 2009; Huang et al., 2010; Lai et al., 2011; Bhatia & Goyal, 2014).

Macrophytes are the common plant species that are used in treatment wetlands (Coleman et al., 2001; Scholz, 2006; Vymazal, 2011a, 2013a; Zheng et al., 2016) due to their tissue ability to assimilate pollutants, and also provide a surface area for the microbial community to grow (Huang et al., 2010; Lai et al., 2011; Vymazal, 2011a; Morandeira & Kandus, 2016). Furthermore, some wetland plants release sufficient oxygen via their roots

to create an aerobic condition within the root zone and thus support the micro-organism's activities (Cooper et al., 1996; Lai et al., 2011; Li et al., 2011; Butterworth et al., 2016). However, the metabolism depends on the availability of light, oxygen, temperature, nitrogen and phosphorus (Riis et al., 2012; Wang et al., 2015; Yang et al., 2016b). The most common plants in wetlands are cattail (*Typha spp*), common reed (*Phragmites spp*), rush (*Juncus spp*) and bulrush (*Scirpus spp*). However, the most frequently used plant species in Europe is *P. australis* (IWA, 2000; Brix & Arias, 2005). It has been reported as an "engine" for nutrient uptake from domestic wastewater, acting as a catalyst for purification by increasing the diversity in the rhizosphere, and enhancing a variety of biological and chemical reactions that support purification (Vymazal, 2007a). Moreover, it has shown extreme tolerance to most toxic compounds contained in all wastewater types (Stefanakis et al., 2014). This plant is an invasive species and it is tolerant of growing even in saline water, and also has the ability to grow in temperate climates as well as in tropical regions (Lismore, 2005; Stefanakis et al., 2014; Zheng et al., 2015). Moreover, this plant is characterized by growing quickly and providing a good insulation with long-term operation of wetlands systems (Vymazal & Krőpfelová, 2005). Moreover, the maximum growth for its above-ground biomass in CWs is between 1652 and 5070 g m−2. The authors also pointed out that the maximum biomass occurs after 3-5 years and the depths of underground biomass reach up to one metre. A comparative vegetation assessment of free water surface and horizontal subsurface flow systems (Zheng et al., 2016) showed that the capability of *P. australis* for nutrients uptake accounted for a higher proportion of the nitrogen removal in FWS, and for a higher proportion of the phosphorous removal in SSF. Furthermore, a study was conducted by Carballeira et al. (2016) to examine the influence of the presence of four plant species (*T Juncus effuses*, *Phragmites australis*, *Iris pseudacorus*, and *Typha latifolia L.*) on the treatment efficiency

of SSF CWs systems used for domestic wastewater treatment. Findings showed that *Phragmites australis* and *T Juncus effusus* were more tolerant and produced higher biomass than *Iris pseudacorus* and *Typha latifolia L*. under stressing conditions (high loading rate).

The role of wetland plants in purifying wastewater within wetlands systems is a controversial issue (Scholz, 2006) as some researchers have documented that macrophytes have the potential to improve pollutant removal efficiencies (Cooper et al., 1996; Kadlec et al., 2000; Lee & Scholz, 2007; Vymazal et al., 2010; Li et al., 2013d; Mburu et al., 2015; Morandeira & Kandus, 2016), while others did not detect any considerable difference in the removal efficiencies for some water quality parameters between planted and unplanted wetlands systems (Scholz et al., 2002; Scholz & Xu, 2002; Torrens et al., 2009; Abou-Elela et al., 2014). For instance, Abou-Elela et al. (2014) found that CWs filters without vegetation were efficient in the removal of COD, BOD and TSS, but they lacked efficiency in pathogen and nutrient removal.

Despite the contradiction in the scientific findings, today, numerous studies point to the positive effects of CWs plants on the wetland system operation and performance (Molle et al., 2006; Lee & Scholz, 2007; Brisson & Chazarenc, 2009; Wen et al., 2010; Fangli et al., 2011; Vymazal, 2013c; Mander & Chazarenc, 2015; Mburu et al., 2015). Plants can play an indirect role in treatment of contaminants in constructed wetlands. For example, the growth of roots within filter media helps to decompose organic matter (Lai et al., 2011) and prevents clogging by providing channels for the water to pass through (Molle et al., 2006). The macrophytes transport oxygen into the rhizosphere, which stimulates both aerobic decomposition of organic matter and the growth of nitrifying bacteria (Brix, 1997; Scholz, 2010).

2.4.4 Substrate

Substrates (also called aggregates or wetland media) are used to construct wetlands and include one of the following: soil, sand, gravel, rock, and organic materials. The selection of filter media plays a key role in CWs considerably affecting the treatment performance of wetland filters (Stottmeister et al., 2003; Babatunde et al., 2008; Rolland et al., 2009; Ge et al., 2014; Zhao et al., 2016). In addition to the capability of providing a suitable area to support wetland plants and micro-organisms to biodegrade pollutants (Ge et al., 2015; Ge et al., 2016a), substrates are able to sediment, filtrate, and adsorb most wastewater contaminants within wetlands systems (Akratos & Tsihrintzis, 2007; Stefanakis & Tsihrintzis, 2012; Ge et al., 2016a).

Studies conducted on SSF CWs have shown that the proper choice of filter media characteristics, including particle size, surface area, porosity, hydraulic conductivity, pH and organic matter content, plays an active role in achieving optimal conditions for pollutants interception within wetland filters and, subsequently, avoiding potential clogging of the media pores which in turn affects system treatment performance (Babatunde et al., 2008; Meng et al., 2014; Song et al., 2015).

Soil media has been used as a filler media in CWs because its material effectively supports macrophyte growth and enables the microbial biofilm layer to thrive (Meng et al., 2014; Stefanakis et al., 2014). However, soil media has a crucial influence on the hydraulic operation of the wetland filter (Stottmeister et al., 2003). Clogging problems have been created because of the small pore diameters of soil media which possess a low permeability for the applied hydraulic and organic load (Brix & Arias, 2005; Wallace & Knight, 2006).

A number of studies on wetland filter media have been conducted to assess the possibility of improving the adsorption capacity of media with different types of substrates. Using a mixture of sand and gravel as a filler is recommended to improve hydraulic conditions and the removal of contaminants (IWA, 2000). Korkusuz et al. (2005) showed that the treatment performances for a blast furnace granulated iron slag-filled wetland were better than that of the gravel-filled wetland in terms of removal of phosphorus and production of nitrate. A study of Saeed and Sun (2011) indicated that high removal efficiencies for nutrients can be achieved in the VF wetland column with organic mulch substrate which demonstrated the potential of using organic media in VF systems to enhance pollutant degradations. A recent study (Wu et al., 2016b) showed the effectiveness of using a novel substrate, named sludge-ceramsite (prepared from dehydrated sewage sludge and clay), for intensifying organics and nitrogen removal in SSF CWs treating domestic wastewater. Another study conducted by Lu et al. (2016) to assess the degradation of pollutants in different constructed wetland fillers, namely maifanite, steel slag, bamboo charcoal and limestone, to treat rural household sewage showed a very high effluent water quality for all filters that meet the discharge standard of pollutants for municipal wastewater treatment plants.

However, there have been contradictory views about the function of some of these expensive filter adsorption media, such as granular activated carbon, in the treatment process of CWs to improve the removal performance, and sometimes no additional benefit can be gained by using these expensive media (Scholz & Xu, 2002).

2.4.5 Micro-organisms

Microbes which live ubiquitously in soils are the key player in wetlands. A fundamental characteristic of wetlands is that their functions are largely regulated by micro-organisms

and their metabolisms (Wetzel, 1993). Microbes are responsible for and function in all the energy transformations of the ecological food web in the CWs by using the influent wastewater as a fuel which provides energy stored in organic molecules. Numerous studies have documented different micro-organism communities found in both aerobic and anaerobic layers of wetlands, including various forms of bacteria, fungi, algae and protozoa (Moshiri, 1993; Cooper et al., 1996; Kadlec & Knight, 1996; Imfeld et al., 2009; Meng et al., 2014).

Microbial activities have been recognized as a major contributor to the removal of wastewater contaminants, therefore understanding the functional diversity and metabolic characteristics of the intrinsic microbial community is a key point to improve the treatment performance of constructed wetlands (Valipour & Ahn, 2016).

Biological removal processes are probably the most important pathway for contaminant removal in wetlands (ITRC, 2003). Micro-organisms naturally live in water, soil, and on the roots of wetland plants feeding on organic materials and/or nutrients leading to the destruction, elimination or conversion of the pollutants into various biologically useful forms, or completely removing them from the wastewater due to their enzymes enabling them to use the contaminants as food (USEPA, 2000). The microbial transformation of nutrients is anaerobic (bacteria that flourish in the absence of oxygen) and aerobic (oxygen-needing bacteria). Moreover, some bacterial species are facultative anaerobes, that is, they are able to function under both aerobic and anaerobic conditions depending on changing environmental conditions (USEPA, 1995, 2000). Other types of bacteria are plant roots bacteria, transforming microbial which include predation and natural die off of micro-organisms.

Micro-organisms have an essential role in the biogeochemical processes that occur in CWs (Dong et al., 2012; Meng et al., 2014). Microbial transformation of organic contaminants normally occurs because the organisms can use the contaminants for their own growth and reproduction. Organic contaminants 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 (Das & Chandran, 2011). Literature also shows that many of the widely distributed micro-organisms in nature possess the ability to utilize hydrocarbons as the single source of carbon (energy) in their metabolism. The utilization of hydrocarbons by micro-organisms is highly dependent on the chemical nature of the components within the petroleum hydrocarbon materials, and environmental conditions (Atlas, 1981). The microbial community associated with the plant rhizosphere creates an environment which enhances the degradation of many volatile organic compounds (Pardue et al., 2000). Constructed wetlands depend on the indigenous micro-organisms in the presence of sufficient oxygen and nutrients to breakdown hydrocarbons and other organic contaminants.

2.5 Removal mechanisms of a constructed wetland

2.5.1 Overview

Constructed wetland systems have successfully served as natural water treatment systems. CWs are designed to imitate the optimal treatment conditions found in natural wetlands, which filter out pollutants and act as sinks for nutrients. Although the mechanical treatment of the CWs system is simple, the processes that remove contaminants are interconnected. CWs consist of soil, water, plants and micro-organisms

and there are many interactions between them making the design and operation of CWs to optimize the pollutants removal efficiency very complex. The governing mechanisms and their reactions are basically dependent upon the characteristics of the wetland, inflow parameters, and the interaction processes inside the wetland. The characteristics of inflow parameters mainly include the quantity and quality of wastewater and the hydrologic cycle of the system (USEPA, 2000; Garcia et al., 2010; Norton, 2014; Wu et al., 2014).

Constructed wetlands play a vital role in filtering out pollutants and act as a sink for nutrients by three removal processes (physical, chemical and biochemical) which combine to purify the effluent water by pollutant removal. As the wastewater flows through the wetland system, a simultaneous or sequential separation and transformation of wastewater contaminants occurs through a combination of various removal mechanisms (Kadlec & Knight, 1996; EPA, 2000; Scholz & Lee, 2005; Scholz, 2006; Saeed & Sun, 2012; Yan & Xu, 2014), including chemical transformation of pollutants (i.e. ammonification of nitrogen), settlement of suspended minute solid particles to the base of the system, filtration and chemical precipitation via the interaction of the outflow and the substrate media and litter, adsorption to soil particles, breakdown, transformation and uptake of pollutants and nutrients by micro-organisms and plants, absorption and ion exchange on the surface of the plants, substrate, sediment and litter, microbial transformations which include predation and natural die off of micro-organisms and settling of suspended particulate matter (Hammer, 1989; Moshiri, 1993; Kadlec & Knight, 1996; IWA, 2000; Stefanakis & Tsihrintzis, 2012; Meng et al., 2014; Kim et al., 2015). Research findings also illustrate that the biochemical oxygen demand for 5-days (BOD), chemical oxygen demand (COD), suspended solids (SS), and pathogens are

removed efficiently, while the removal values for nutrients are relatively low and variable (Vymazal, 2007a; Scholz, 2010; Vymazal, 2011c).

The predominant treatment mechanisms and their sequence of reaction are dependent on the external input parameters to the system, the internal interactions, and the characteristics of the wetland, specific contaminant, site conditions, remedial objectives, and regulatory issues (ITRC, 2003). The external input parameters most often of concern include the wastewater quality and quantity and the system hydrological cycle (USEPA, 2000).

For municipal wastewater, removal of pollutants from the wastewater is carried out using various technologies; however, biological processes are often the most economically sustainable treatment options (Zanetti et al., 2012; Abou-Elela et al., 2013; Gikas & Tsihrintzis, 2014; Sehar et al., 2016). As these biological treatment processes, such as assimilation, biodegradation, metabolism, adsorption, flocculation, precipitation and ion-exchange, depend on using common plants and micro-organisms to remove pollutant loads from wastewater, they can often be considered as environmentally friendly (Wu et al., 2015d). Various types of wetland systems provide natural biological processes in addition to physical and chemical processes, and these combined developments are responsible for pollutant removal from wastewater (Scholz, 2006; Vymazal, 2011b; Wu et al., 2014).

In conclusion, all types of constructed wetlands, including horizontal, vertical or a combination of the two, are proven to treat various kinds of pollutants in treated wastewater with high removal efficiency (Haberl et al., 1995; Vymazal, 2007b, 2014; Sultana et al., 2015).

2.5.2 Particle removal

The wastewater influent applied to wetland systems contains suspended solids (SS) which comprise various sizes and compositions, include organic and inorganic forms, and flow according to the water flow (Kadlec & Knight, 1996).

Wetlands have the ability to provide highly efficient mechanical removal of contaminants associated with SS in wastewater. Settling and sedimentation, adsorption, and microbial degradation achieve efficient removal of particulate matter and suspended solids in treatment wetlands systems (Kadlec & Knight, 1996; ITRC, 2003; Kadlec, 2009; Abou-Elela & Hellal, 2012; Abou-Elela et al., 2013).

In VF CWs, gravitational settling (sedimentation) and filtration are the major removal mechanisms for SS (Garcia et al., 2010; Hua et al., 2013). As the wastewater passes vertically, it percolates through the pores of the filter media and the water flows gradually with low velocity. The solids are trapped within the media pores either mechanically or by adhesion to various pollutants such as nutrients, pathogens, heavy metals, and organic matter (Kadlec & Wallace, 2009).

Numerous studies have been conducted to assess the performance efficiency of VF CW systems, and have concluded that these systems are efficient to reduce SS (Gikas & Tsihrintzis, 2012; Bhatia & Goyal, 2014; Paing et al., 2015b; Song et al., 2015; Rozema et al., 2016a).

Long-term operation and continuous influent wastewater application to VF CWs systems have been shown to accumulate suspended solids above the wetland bed creating a litter layer, and also within the substrate pores (physical blocking) and onto the surface of the media grains (Manios et al., 2003; Stefanakis et al., 2014). The non-biodegradable

mineral contents are possibly the major parameter causing substrate clogging and leading to a decrease in the hydraulic conductivity (Tilak et al., 2016). However, modification of some design and operational parameters, such as intermittent loading and application of resting periods between loadings, enables good aeration of the bed and oxidation of the accumulated organic solids, which also prevents the bed clogging (Fan et al., 2013b; Bhatia & Goyal, 2014; Johari et al., 2016).

2.5.3 Organic compounds removal

In wastewaters, there is a large variety of organic compounds, including: dissolved organic matter and particulate organic matter, which are commonly expressed by the biodegradable part (BOD) and the total organic matter part (COD) (Stefanakis et al., 2014). Organic matter contains about 45-50% carbon, which is used by micro-organisms as a source of energy and converted into carbon dioxide in the root zone by the macrophytes which supply the oxygen necessary (DeBusk, 1999). Hydrocarbons and other priority organic compounds are another group of contaminants that has the potential to affect the habitat value of treatment wetlands. Toxic organics can be subjected to wetland treatment via the same mechanism as natural organic compounds.

Generally, the major processes for elimination of COD and BOD organic matter in CWs systems include volatilization, photochemical oxidation, sedimentation, sorption, and biodegradation (ITRC, 2003). The soluble organic matter can be decomposed via both aerobic and anaerobic processes (Song et al., 2006; Garcia et al., 2010). Oxygen for aerobic degradation can be provided by atmospheric oxygen diffusion, convection, and/or macrophyte root transfer into the plant rhizosphere (Cooper et al., 1996). Anaerobic organics removal can proceed inside the media pores, lacking oxygen. Organic matter

accumulation in wetlands provides carbon and nutrients as energy to micro-organisms for denitrification.

EPA (1993) reported that the reduction of coarse organic matter in constructed wetlands is achieved rapidly via gravity settling in the pore openings of the substrate media, and the main elimination for the BOD organic material is achieved by aerobic degradation and sedimentation/filtration processes.

BOD is a measure of the oxygen required by the micro-organisms to oxidize the organic matter. In vertical flow constructed wetlands, aerobic decomposition by micro-organisms is usually considered the main removal process (Sun et al., 1999; Vymazal, 2007a; Saeed & Sun, 2012; Stefanakis & Tsihrintzis, 2012) having the potential to achieve high BOD removal (Abou-Elela et al., 2013; Scholz, 2015; Dogdu & Yalcuk, 2016), particularly due to the application of a "cycle of wet and dry" feeding mode that provides high oxygen availability for aerobic micro-organisms. Furthermore, organic matter can also be removed via adsorption/absorption processes. EPA (1993) revealed that the capacity for adsorption relied on the surface substrate, wetland plants, and organic matter characteristics. Saeed and Sun (2011) tested the ability of different types of media in VF constructed wetlands to remove pollutants and findings showed higher removal efficiencies in VF wetland columns with organic mulch substrate.

The pollutants removal is critically dependent on the type of compound, chemical/biological condition of the wastewater; environmental factors of the wastewater, such as pH, light intensity, temperature, nutrient availability, electron acceptor availability and oxygen availability; and operational strategies, i.e. presence of organic carbon, hydraulic load, feeding mode, retention time, pollutant loading, recirculation, and plant harvesting (Scholz, 2010; Saeed & Sun, 2012).

The accumulated organic matter may lead to media clogging of pore spaces in wetlands and may ultimately lead to a reduction in wastewater retention time and decline in the performance of nutrients removal (Nguyen, 2000). Moreover, operational parameters, such as COD and TSS loading rates, potentially contribute to clogging problem in wetlands system (Zhao et al., 2009; Hua et al., 2013; Song et al., 2015).

2.5.4 Nutrient removal

Removal of nitrogen (N) and phosphorus (P) nutrients in wastewater treatment, is considered an important issue because releasing large and uncontrolled amounts of nutrients to surface water resources can deteriorate the quality of effluent, resulting in serious health and environmental consequences (Kadlec & Wallace, 2009). Discharge undesirable amounts of nutrients in the receiving water can cause a damage to aquatic life, being toxic to fish, and changing the dissolved oxygen (DO) rate to inadequate levels for the living organisms, and lead to the materialization of the eutrophication of surface waters (Kadlec & Wallace, 2009; Ye & Li, 2009; Chen et al., 2011; Wu et al., 2011a; Ding et al., 2015).

Typically, wastewater contains two forms of nitrogen: organic and inorganic (Kadlec & Knight, 1996; Vymazal, 2007a; Garcia et al., 2010), and in particular, the composition of domestic wastewater consists of about 60% ammonia nitrogen and 40% organic nitrogen (Stefanakis et al., 2014). Studies have reported on the ability of different types of constructed wetlands systems to remove various forms of nitrogen compounds from wastewater. However, nitrogen removal in some cases is far from satisfactory when facing the increasingly strict discharge standards for nutrients (Wu et al., 2015a).

Numerous studies have pointed out that processes which contribute to nitrogen reduction in constructed wetlands are nitrification, ammonia volatilization, fixation, nitrate ammonification, ammonification, denitrification, organic nitrogen burial, anammox, plant and microbial uptake, and ammonia adsorption (Vymazal, 2007a; Kadlec & Wallace, 2009; Choudhary et al., 2011). On the other hand, some authors have reported that the optimal and economic nitrogen treatment within constructed wetlands is mainly accomplished by nitrification and denitrification processes (Brix, 1994; Lee et al., 2009) which are considered universally important in the cycling and bioavailability of nitrogen in wetland systems (Mitsch & Gosselink, 1993; Kadlec & Knight, 1996; DeBusk, 1999). These coupled processes require both aerobic and anaerobic environments, therefore nitrification/denitrification can occur simultaneously only in a soil which has both aerobic and anaerobic zones (Cooper et al., 1996); firstly, ammonia is oxidized to nitrate by the nitrification process, then the resulting nitrate is reduced to gaseous nitrogen by the denitrification process. Numerous studies on most traditional constructed wetlands systems have reported that either nitrification by nitrifying bacteria or the denitrification process by heterotrophic denitrifying bacteria causes low nitrogen removal efficiency in constructed wetlands. In constructed wetlands technology, maintaining a combination of both nitrification and denitrification processes is a main reduction pathway for nitrogen compounds (Vymazal, 2007a; Ye & Li, 2009; Garcia et al., 2010). The combination of vertical and horizontal SSF CWs has been successfully used to facilitate more effective nitrogen treatment, and these hybrid systems are particularly effective for achieving total nitrogen elimination (Cooper et al., 1999; Vymazal, 2007a; Molle et al., 2008; Vymazal, 2013b; Kim et al., 2016).

The availably of dissolved oxygen (DO) is the key in nitrogen transformation because of nitrifying bacteria competing with organics for limited DO (Fan et al., 2013b; Yang et al., 2016a). Therefore, VF constructed wetlands, due to the good aeration within substrate media created during the feeding mode, have been noted to provide a greater nitrification process than HF constructed wetlands (Kadlec et al., 2000; Brix & Arias, 2005; Abou-Elela et al., 2013), while the enzyme needed for denitrification may be suppressed in the presence of dissolved oxygen (IWA, 2000). However, using organic carbon sources is generally considered a controlling factor in the denitrification process. Song et al. (2016) found that constructed wetlands with added organic carbon sources and ferrous iron can be used together to complete the denitrification process. Authors have reported (Kadlec & Wallace, 2009; Saeed & Sun, 2012; Fan et al., 2013a; Song et al., 2015) that nitrogen removal in many constructed wetland systems without adequate active or passive aeration is insufficient, mainly because of the lack of available oxygen used for aerobic biological degradation. Therefore, artificial aeration (mainly continuous aeration and intermittent aeration) have been proven to be an alternative to provide sufficient oxygen, which can facilitate effective nitrification and thus subsequently guarantee denitrification for complete total nitrogen (TN) elimination (Wu et al., 2016b).

Saeed and Sun (2012) documented numerous environmental (e.g. pH, dissolved oxygen, temperature etc.) and operating (e.g. hydraulic and pollutant loading, detention time, influent feed mode, recirculation, organic carbon addition etc.) parameters which impact the performance of nitrogen removal processes within wetland systems. For instance, the performance of nitrifying bacteria can be affected by environmental parameters such as pH, dissolved oxygen and temperature (IWA, 2000). Moreover, Kadlec (1999a) reported that the removal of nitrogen compounds is affected annually by metrological parameters

such as temperature, humidity, and precipitation. Vymazal (2007) observed that generally nitrogen removal processes depend on the type of constructed wetlands, for example total nitrogen removal was found to be in small quantities in a single stage wetland except in a wide treatment surface area.

Wetlands treatment processes have been recognised (Kadlec, 1999a; Vymazal, 2001; Lee et al., 2009; Gikas & Tsihrintzis, 2012) to respond to seasonal variation. The removal processes of nitrogen within constructed wetlands varies seasonally, with lower values in cold months (Kuschk et al., 2003; Kim et al., 2016). The temperature variations cause changes in microbial activity, which in turn creates changes in microbially-mediated water quality improvement (Kuschk et al., 2003; Fan et al., 2016; Xie et al., 2016). The study of Xie et al. (2016) showed that the bacterial numbers and species responsible for ammonification, nitrification and denitrification varied seasonally, with denitrifying bacteria changing the most and nitrifying bacteria changing the least, with also higher numbers in the warm seasons (summer and autumn) and lower numbers in the cold seasons (spring and winter).

Phosphorus (P) is present in various types of wastewater and represents a macronutrient of special importance for biological organisms in several ecosystems (Ding et al. 2015). However, high concentrations of P are of concern to designers and researchers due to their toxic and harming effects on the receiving waters and species present. High concentrations of P are noted to be the most common cause of eutrophication in water bodies (Ding et al., 2015). In wetlands systems, phosphorus generally exists in two forms, inorganic phosphorus compounds and organic phosphorus compounds, but orthophosphate is the general form considered, as reported by (Vymazal, 2007a), as a main link between organic and inorganic phosphorus cycling in wetlands because it is the

only form of phosphorus that can utilized directly by macrophytes. The main sources of phosphorus (P) are: untreated or insufficiently treated wastewater; agricultural practices; and domestic, urban, and industrial runoff.

The removal of phosphorus during wastewater treatment in CWs occurs through physical, chemical and biological processes including adsorption, desorption and precipitation reactions, along with biological uptake, dissolution, plant and microbial uptake, fragmentation, leaching, mineralization, sedimentation (peat accretion) and burial (Moshiri, 1993; Kadlec & Knight, 1996; Bridgham et al., 2001; Vymazal, 2007a; Kadlec & Wallace, 2009; Li et al., 2013e).

In SSF CWs, adsorption and precipitation is widely known to be the most important removal pathway (Vymazal, 2010). The P sorption capacity of substrates is influenced by their physicochemical features such as mineral content, particle size, and specific surface area (Brix et al., 2001; Ge et al., 2016a). Phosphorus is bound in the media of the substrate, mainly as a consequence of adsorption and precipitation reactions with calcium (Ca), aluminium (Al) and iron (Fe) in the sand or gravel media (Moshiri, 1993; Kadlec & Wallace, 2009). The capacity of CWs to remove P may therefore be dependent on the contents of these minerals in the substrate. However, the common materials used for SSF CWs, such as washed gravel or crushed rock, provide low capacity for sorption and precipitation (Vymazal, 2011c; Paing et al., 2015a). Numerous studies have demonstrated that phosphorus removal in most traditional CWs is often low unless special substrates with high P-sorption capacities are used (Brix et al., 2001; Ge et al., 2016a). These techniques are often very efficient at removing P initially, but their performance decreases over time because the P-sorption capacity of the media is being used up, also they may not provide cheap and durable solutions on a long term basis (Li et al., 2013e; Kim et al.,

2015). Furthermore, long term sustainable removal of phosphorus compounds in CWs system can be accomplished through accumulation on and burial in the bed sediments.

Phosphorus represents an important nutrient for wetland plant growth. The cycling of P in CWs can occur by macrophyte growth, death and decay, returning the phosphorus back to the water filter. However, phosphorus is retained in those plant parts that withstand decay. It is this retention that plays an important role in the long-term storage of phosphorus (Kadlec, 1999b). Furthermore, the amount of phosphorus that can be removed by harvesting the plant biomass usually constitutes only an insignificant fraction of the amount of phosphorus loaded into the system with associated wastewater (Brix, 1997). Another study of Lantzke et al. (1999) showed that the plant harvesting reduced additional phosphorus in the range of 10-20%. Furthermore, it suggested that orthophosphate removal from wastewater by planted vertical-flow wetlands (VFWs) occurs through three parallel paths, including: sorption to media, biofilm assimilation, and macrophyte uptake. The quantity of P removed by the three paths is substrate > macrophyte > biofilm, in the short term, but macrophyte > substrate > biofilm, in long term. The deposits, which progressively accumulate at the surface of VF CWs, are usually removed every 10-15 years (Kim et al., 2015).

Wetland design and operation variables also determine the extent to which the phosphorus can be removed. Therefore, the appropriate selection of macrophyte, water depth, and hydraulic residence time can play a vital role in enhancing phosphorus removal efficiency within CWs systems (Bridgham et al., 2001; Liang et al., 2011; Li et al., 2013b; Wang et al., 2013b; Johari et al., 2016). For instance, Wang et al. (2013b) noted that phosphorus adsorption capacities in vertical-flow wetlands is influenced by hydraulic loading rate and the effluent P concentrations are more dependent on influent concentrations. Richardson

and Qian (1999) demonstrated that no change could be occurring in the ecological structure, dynamics, and function of a wetland ecosystem that received a reasonable phosphorus loading rate of 1 g m^{-2} yr^{-1} (area dependent) for a long-term operation.

The phosphorus transformation processes in CWs which result from biological activity and precipitation and adsorption through the media substrate may lead to P compounds accumulation and in turn cause clogging in the substrate pores (Kadlec & Wallace, 2009; Knowles et al., 2011). Furthermore, different cations can precipitate phosphate under certain conditions in wetland environments such as apatite, hydroxyapatite, variscite, strengite, vivianite and wavellite (Reddy & D'angelo, 1994).

2.5.5 Hydrocarbon removal

One of the main environmental problems today is the pollution by hydrocarbon that results from human activities. Exploration, production, refining, storage, transportation, distribution and utilization of petroleum hydrocarbons have brought about frequent occurrences of water and soil contamination with hydrocarbon (Agarry & Latinwo, 2015). Moreover, accidental releases of hydrocarbon products are of particular concern in the environment (Michel & Rutherford, 2013; Akpor et al., 2014). The components of hydrocarbon have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Wake, 2005). Hydrocarbons consist of a broad range of compounds, both naturally occurring and human-activities developed, whose characteristics are mainly specified by the arrangement of carbon and hydrogen compounds (ITRC, 2003). Chemically, they can be divided into two very broad families – the aliphatics and the aromatics. In wetland systems research, hydrocarbons are commonly investigated as total petroleum hydrocarbons (TPH), total hydrocarbons (THC), volatile organic contaminants (VOCs), and diesel and gasoline range organics (DRO & GRO) (Imfeld et al., 2009). The

classes of compounds are susceptible to the degradation processes typical to constructed wetlands. Total petroleum hydrocarbon (TPH) is one of the most widespread brands of hazardous organic pollutants in surface and groundwater and it is usually used as indicator for contamination from hydrocarbon compounds.

Petroleum hydrocarbon wastewaters also contain pollutants such as COD, BOD, nitrogen and phosphorus (Knight et al., 1999). However, the major focus of the petroleum industry is on assessing the efficiency of hydrocarbon removal. Nevertheless, COD and even BOD removal efficiencies for wetlands treating toxic hydrocarbons are comparable to wetlands treating other types of wastewater (Knight et al., 1999; Ji et al., 2007).

Diesel is one of the toxic hydrocarbon compounds, and its toxicity results from the presence of aromatic hydrocarbons such as BTEX (which represents benzene, toluene, ethylbenzene and xylene) and MTBE (methyl-*tert*-butyl-ether). The fractions of diesel hydrocarbon range from C8 to C26, 60-90% alkanes and cycloalkanes, while alkenes are about < 5% and aromatics rate is 10-30% (Van Epps, 2006). The presence of co-carcinogens in diesel compounds, such as C10-C20 alkenes and alkylated benzenes, poses a great threat to human life (Lohi et al., 2008).

Generally, wetland environments are known for their capabilities to naturally degrade hydrocarbon compounds (Wemple & Hendricks, 2000) by the combination of chemical, biological and physical processes. However, the variation in these processes depends on the nature of the hydrocarbon contaminants, operational and design variables of the wetland, wetland plants (macrophytes), and climatic and environmental conditions. Both surface and sub-surface flow constructed wetlands have been used to treat wastewater contaminated with hydrocarbon compounds (Knight et al., 1999; Brovelli et al., 2011; Cao et al., 2012). Operation of the first constructed wetlands to attenuate hydrocarbon-

contaminated wastewater began in the 1970s with free-flow constructed wetlands in the Mandam, North Dakota (Litchfield & Schatz, 1989), while using sub-surface flow constructed wetlands to treat hydrocarbon compounds was carried out by Seidel in Germany in 1973 to treat industrial organic compounds (Seidel, 1973) subsequently the procedure was successfully applied for a full-scale treatment system at the Mobil Oil AG terminal in Bremen, Germany (Vymazal et al., 1998).

The main hydrocarbon treatment processes which occur in constructed wetland systems are volatilization, sorption and sedimentation, plant uptake, phytodegradation and biodegradation (Kadlec & Knight, 1996; Moore et al., 1997; Imfeld et al., 2009; Das & Chandran, 2011). More than 90% of the hydrocarbon removal rate has been observed in the porous mineral substrate matrix of the constructed wetlands (Salmon et al., 1998). Sorption processes are assigned to remove 10% of hydrocarbon compounds, 25% is estimated for the volatilization process, and microbial degradation and eventual plant uptake are assumed to account for 60% of observed losses.

A comprehensive survey regarding previous related works studying the removal of various forms of hydrocarbons from different contaminated environments is presented in Table 2.1. The survey is categorized according to the authors, types and components of wetlands, kinds of hydrocarbon and the mechanism treatment process pathways, individually or in combination, monitored in the system.

Table 2.1: Survey of the related work of petroleum hydrocarbon treatment efficiency in different contaminated areas.

ID	Reference	Wetland		Wetland plant		Monitoring period	Hydrocarbon		Mechanism treatment	Inflow	Findings
		wetland type	wetland age	plants types	additives		concentration	measurements			
1	Al-Baldawi et al., 2013d	FS CWs (experimental)	new (not mature)	*Scirpus grossus*	nothing	72 days	(0,8700,17400,26100) mg/l diesel	total petroleum hydrocarbons (TPH)	phytoremediation	synthetic wastewater contaminated with diesel	*S. grossus* had the ability to reduce the TPH by 70.0 and 80.2% for diesel concentrations of 8700 mg/l and 17,400 mg/l, respectively. At a diesel concentration of 26,100 mg/l, *S. grossus* died after 14 days.
2	Al-Baldawi et al., 2013b	SSF CWs (H) (pilot)	new (not mature)	*Scirpus grossus*	nothing	72 days	(0, 0.1, 0.2, 0.25)% (Vdiesel/Vwater)	TPH	phytoremediation	tap water+diesel	TPH removal efficiencies were 82,71,67% for diesel concentrations of 0.1,0.2,0.25% respectively. Also, *S. grossus* plants play an important role for restoring 0.1% diesel-contaminated water.
3	Al-Baldawi et al., 2013a	SSF CWs (H) (experimental)	new (not mature)	*Scirpus grossus*	nothing	72 days	(0, 8700, 17,400, 26,100) mg/l diesel	TPH	phytoremediation	tap water+diesel	The maximum removal of TPH occurred at the diesel concentration of 17,400 mg/l at 91.5%. *S. grossus* could effectively promote the degradation of (TPH) when the concentration of diesel in water was up to17,400 mg/l.
4	Al-Baldawi et al., 2013c	SFCWs & SSFCWs (H) (experimental)	new (not mature)	*Scirpus grossus*	nothing	72 days	(1, 2, 3)% (Vdiesel/Vwater)	TPH	phytoremediation	synthetic wastewater contaminated with diesel	Subsurface flow system was more efficient than the free flow system in removing TPH from the synthetic wastewater, with average removal efficiencies of 91.5% and 80.2%, respectively.
5	Al-Baldawi et al., 2014	SSF CWs(H)	new (not mature)	*Scirpus grossus*	aeration	72 days	(0.0, 0.1, 0.175, 0.25)% (Vdiesel/Vwater)	TPH	phytoremediation	synthetic wastewater contaminated with diesel	The optimum conditions were diesel concentration= 0.25% (Vdiesel/Vwater), retention time= 63 days and with no aeration. The removal efficiency was 76.3%.
6	Al-Baldawi et al., 2015	SF CWs (experimental)	new (not mature)	*Scirpus grossus*	nothing	72 days	(0.1%, 0.175%, 0.25%) (Vdiesel/Vwater)	TPH	phytoremediation	synthetic wastewater contaminated with diesel	*S. grossus* has the ability to enhance diesel removal with the help of rhizobacteria and the adsorption of diesel, representing an environmentally friendly, alternative technology for the remediation of water contaminated with diesel.
7	Aslam et al., 2007	SSF CWs (V) (pilot)	new (not mature)	*Phragmites karka*	nothing	1 year	pre-treated refinery wastewater from nearest drain	(COD, BOD) as indirect measurement	bioremediation	settled refinery wastewater	The compost wetland gave better performance than the gravel-based one.
8	Bergier, 2011	SSF CWs (H)	new (not mature)	*Phragmites australis*	nothing	2 months	(6177.33-96.02) $\mu g/dm^3$	total C7-C30 aliphatic hydrocarbons	bioremediation	stormwater & oil	The highest removal effectiveness was observed for the hydrocarbons with the highest carbon atom numbers (from 51% for C20 to 92-93% for C26–C30). Hydrocarbons C14 to C18 were removed with the lowest effectiveness (26-32%). Moreover, Reed (*Phragmites australis*) showed resistance to oil derivatives influence.
9	Cao et al., 2012	Simulation experiments (pots)	matured	*Bulrush, Galingale, Wild rice, Reed*	fertilizer for plant growth	14 days	16,000 mg/kg	TPH	phytoremediation	distilled water+diesel	The tolerance of reed roots to diesel was strong compared with other plants.

ID	Reference	Wetland		Wetland plant		Monitoring period	Hydrocarbon		Mechanism treatment	Inflow	Findings
		wetland type	wetland age	plants types	additives		concentration	measurements			
10	Chen et al., 2012	SSF CWs(H) (pilot)	matured	*Phragmites australis*	nutrients (chemical materials) to enhance plant growth	3 years and 5 moths	10 mg/l, 2 mg/l (low and high chlorinated hydrocarbon)	chlorinate hydrocarbon concentration	bioremediation	groundwater +(chlorinated hydrocarbon)	The vegetation in the constructed wetlands had a significant influence on the removal efficiency of highly chlorinated hydrocarbons (52-68%).
11	Cottin and Merlin, 2008	SSFCWs (experimental) (V)	new (not mature)	n/a	nothing	2 months	3 mg/l PAHs	polycyclic aromatic hydrocarbons (PAHs)	bioremediation	runoff water+PAHs	Organic matter in compost layer could degrade the PAHs from runoff and adsorption is the main process for hydrocarbon removal (>95%).
12	Couto et al., 2011	Simulation experiments (pots)	new (not mature)	*H. portulacoides, S. maritimus, J. maritimus*	nothing	14 months	30 mg/kg	petroleum hydrocarbons (PHC)	biological remediation	turbine oil, crude oil	The presence of plants increases the efficiency of hydrocarbon removal. After 7 months: the removal is 100% in the presence of *S. maritimus* vs. 63% in its absence.
13	DeBiase et al., 2011	SSF CWs (experimental) (V)	new (not mature)	n/a	aeration	7 months	benzene= 20 mg/l MTBE= 3.9 mg/l	benzene & MTBE	biodegradation & volatilization	groundwater & benzene, MTBE & ammonium	Benzene and MTBE concentrations are very low due to aerobic biodegradation in the filter.
14	Eke & Scholz, 2008	SSF CWs (V) (experimental)	new (not mature)	Common Reeds	fertilizer for plant growth (some filters)	24 months	1 g/l	benzene	biochemical processes	tap water+benzene	The results showed good hydrocarbon removal efficiency (85-95%).
15	Fountoulakis et al., 2009	SSF CWs, FS CWs (pilot)	mature	*Phragmites australis and Arudo donax*	n/a	3 years	n/a	(PAHs) and linear alkyl benzene sulfonates (LAS)	absorption	primary treated sewage wastewater	PAHs and LAS decreased with increasing water temperature and the performance of the SSF wetland is significantly better than the FWS wetland.
16	Gessner et al., 2005	FS CWs (pilot)	new (not mature)	*Catail, Bulrush, Pondweed, Coontail*	nothing	1 year	0.34 mg/l (Diesel)	diesel range organic	volatilization & biodegradation	industrial wastewater	The reduction of organic diesel was 67%.
17	Ghobrial, 2008	SSF CWs (pilot)	new (not mature)	*Reeds*	activated carbon, iron (electron accepter)	2 months	(5 mg/l BTEX), (20 mg/l MTBE)	n/a	phytoremediation	groundwater +(BTEX & MTBE)	Leaves plants contribute to the bioremediation process through transpiration and petroleum hydrocarbons can significantly reduce the availability of plant nutrients in soil.

ID	Reference	Wetland		Wetland plant		Monitoring period	Hydrocarbon		Mechanism treatment	Inflow	Findings
		wetland type	wetland age	plants types	additives		concentration	measurements			
18	Ranieri et al., 2011	(SSF CWs) (H) (pilot)	mature	*Phragmites australis, Typha latifoglia*	nothing	(one season) winter	0.5 mg/l	BTEX	evapotranspiration	raw water (heavy metal + benzene)	BTEX removal ranged from 46% to 55%. The unplanted field removal was 20% lower than others.
19	Guittonny-Philippe et al., 2015a	n/a	not mature	*Various helophyte species*	nothing	113 days	organic pollutant mixture	total hydrocarbon (THC)	plant uptake	industrial wastewaters	Contaminants altered the biological characteristics of the treatment environment.
20	Hagahmed et al., 2014	Reed beds	new (not mature)	*Phragmites australis*	nothing	n/a	produced water from oil field	COD	biodegradation	water from oil industry	The use of multiple loops control in the constructed wetland enhanced the oil degradation and kept all the treatment units under control. Also, the removal efficiency for hydrocarbon was more than 90%.
21	Ji et al., 2002	SSF CWs (pilot)	new (not mature)	*Common Reeds*	nothing	2 years	(15-48) mg/l (Heavy Oil)	mineral oil	physio-chemistry & biochemistry	heavy oil produced water	Removal efficiency of mineral oil was 78-89%.
22	Ji et al., 2007	SF CWs (pilot)	new (not mature)	*Common Reeds*	nothing	3 years	(15-30) mg/l (Heavy oil)	mineral oil	physio-chemistry &biochemistry	heavy oil produced water	Removal efficiency of mineral oil was 91.6-92.8%.
23	Lin and Mendelssohn, 1998	Marsh (natural)	matured	*Spartina alterniflora and Spartina patens*	fertilizer for plant growth	30 months	(0,4,8,16,24) l/m^2	oil concentration	biostimulation & phytoremediation	crude oil	Fertilization can restore oil contaminated wetlands and accelerate oil degradation.
24	Lin and Mendelssohn, 2009	Durnal tidal regime (12h)	new (not mature)	*Juncus roemerianus*	fertilizer for plant growth	1 year	0, 20, 40, 80, 160, 320, and 640 mg/g	TPH	phytoremediation	(0, 40) mg/g dry sediment	High dosages significantly suppressed the growth of plants, measured by plant stem density, plant shoot height, above-ground biomass and below-ground biomass. The diesel tolerance limit of *J. roemerianus* was estimated between 160 and 320 mg/g.
25	Liu et al., 2011	Pot experiments	new (not mature)	*Scirpus triqueter*	bioaugmentation	60 days	(1000, 5000, 10,000, 15,000, 20,000) mg/kg (diesel)	diesel concentration	phytoremediation	running water	Saturated hydrocarbons were more degraded than aromatic ones with removal efficiencies of 67-72%. The plants had the ability to increase the hydrocarbon degradation.
26	Mills et al., 2004	Marshes	matured	*Variety of natural plants*	inorganic nutrients & electron accepter	140 days	40 mg/kg crude oil	saturated and aromatic hydrocarbon	biodegradation	natural marshes water+crude oil (spills)	The treatment efficiency for saturated hydrocarbons was more than for aromatic ones. Moreover, the additions have the ability to decrease the wetland recovery time.
27	Moore et al., 1997	Natural wetlands	matured	*Variety of natural plants*	nothing	n/a	natural oil activates	oil & gas concentration	volatilization & biodegradation & sorption	oil and gas with groundwater	The presence of peat in wetlands enhances the natural removal processes for hydrocarbon.

ID	Reference	Wetland		Wetland plant		Monitoring period	Hydrocarbon		Mechanism treatment	Inflow	Findings
		wetland type	wetland age	plants types	additives		concentration	measurements			
28	Najafi and Kashi, 2012	3000 ml glass vessel	new (not mature)	*Nothing*	bio-enzyme & aeration	10 days	COD= 440 mg/ml, PAHS=16 µg/l, TPH= 770 mg/l	COD, PAHs, TPH	biodegradation	crude oil	Bio-enzyme removed TPH/PAH, and COD by about 99.98% and 100%, after 10 days, respectively.
29	Omari et al., 2003	SSF CWs(H) (experimental)	new (not mature)	*Typha*	fertilizer	2 years	different diesel concentrations	hydrocarbon removal (alkane)	phytoremediation	water+diesel	The wetland filters planted with *Typha* showed a better performance in hydrocarbon removal as compared with unplanted ones.
30	Page et al., 2002	SF CWs	mature	*Various wetland plants*	n/a	99 days	1:10 and 1:20 dispersant-to-oil ratio	saturated and aromatic hydrocarbon	biodegradation and physical flushing	Arabian medium crude oil	No differences when comparing dispersed-oil treatment to the oiled control.
31	Wallace, 2011	SSF CWs (pilot)(SF&V) (2 wetland system)	matured	*Salix, Phragmites, Schoenoplectus, Juncus, Cornus*	sod and aeration	first project (10 years) & second project (4 years)	≤ 0.5 mg/l	benzene, BTEX, TPH, MTBE	biodegradation	refinery petroleum	The wetland systems are very effective for long-term operation under cold climate conditions, with more than 95% hydrocarbon removal efficiency.
32	Wallace and Kadlec, 2005	SSF CWs (pilot)(v), FCW	new (not mature)	*Salix, Phragmites, Schoenoplectus, Juncus, Cornus*	sod and aeration	5 months	≤ 0.5 mg/l (benzene, BTEX, gasoline)	benzene, BTEX, TPH, MTBE	biodegradation	refinery effluent	Both sod and aeration improved treatment performance in very cold temperatures (ice).
33	Wang et al., 2011a	Experimental pots	new (not mature)	*Reeds*	nothing	50 days	diesel (5000, 10,000, 15,000, 20,000) mg/kg	diesel concentration	phytoremediation	diesel	There was no visible toxic effect on the growth of reed at all diesel concentrations, but 15,000 mg/kg diesel concentration is optimal for reed growth.
34	Wang et al., 2011b	Simulation experiments (pots)	new (not mature)	*Carex phocota*	oil degrader (biostimulation)	60 days	diesel (5000, 10,000, 15,000, 20,000) mg/kg	diesel concentration	bioremediation & phytoremediation	refinery petroleum	The metabolization between micro-organisms and plants gives a good degradation rate of diesel. Moreover, it was found that the optimal concentration of diesel in wetlands was (15,000 mg/kg), as it is served as a nutrient to micro-organisms.
35	Wang et al., 2013a	Natural marshes	matured	*Calamagrostis angustifolia*	nothing	3 months	natural from nearest oil resources	TPH	bioremediation & phytoremediation	natural marshes water+crude oil (spills)	Crude oil contamination affects the soil physical and chemical properties. Wetland plants has the potential to simultaneously restore and remediate the petroleum hydrocarbon-contaminated wetlands
36	Wei et al., 2015	SSF CWs (H)	new (not mature)	*Juncus effuses, L.*	microbial electrochemical technology (MET-CW)	400 days	benzene= 12 mg/l MTBE= 3 mg/l	benzene, MTBE	bioremediation	groundwater contaminated & benzene, (MTBE), (NH4)	Benzene and MTBE were nearly completely removed after 125 days. Pollutant removal efficiencies reached steady state after around 150 days.
37	Zhang et al., 2013	Simulation experiments (pots)	new (not mature)	*Scirpus triqueter, reed, Herba caricis phacotae, Sagittaria sagittifolia*	nothing	30 & 60 days	1000, 5000, 10,000, 15,000, 20,000 mg/kg (diesel)	diesel concentration	bioremediation & phytoremediation	running water	Wetland plants with oil degrader showed a good capability to degrade diesel at optimal concentration of 15,000 mg/kg.

In the volatilization process, an emission for the pollutants accrues directly from the wastewater to the atmosphere. The pollutants which have the capability to volatilize are found with a vapour pressure (> 2.7 hPa) at 25 ºC (Imfeld et al., 2009). Some of the wetland plants absorb the pollutants, such as MTBE, through their roots and transfer them to the atmosphere via their transpiration stream by the phytovolatilization process (Ma & Burken, 2003). Phytovolatilization may be of particular relevance in SSFCWs systems, where direct volatilization is limited because of low diffusion amounts of pollutants through the unsaturated zone, in addition to laminar flow in water saturated soil zones, that may lead to relatively slow mass transfers (Kadlec & Wallace, 2009). Authors found that various types of volatile hydrocarbon contaminant groups are effectively treated in constructed wetlands such as chlorinated solvents, BTEX and MTBE (Wallace & Kadlec, 2005; Vymazal, 2009; De Biase et al., 2011). However, some of the fuel compounds could not be removed easily by volatilization, such as recalcitrant branched and aromatic hydrocarbon compounds (Wang et al., 2011b; Li et al., 2012; Li et al., 2013c).

Sorption is another process which can effectively eradicate hydrocarbon contaminants in wetlands systems. Sorption of a chemical to soil or sediment may result from the physical or chemical adhesion of molecules to the surfaces of solid bodies, or from partitioning of dissolved molecules between the aqueous phase and soil organic matter (Imfeld et al., 2009). In the early periods of wetland operation, the retention of hydrocarbon pollutants by the sorption process in the substrate beds is relatively high as long as the substrate materials does not reach the sorption–desorption equilibrium (Omari et al., 2003) and the wetland substrate media acts as a sink providing enough capacity to bind the pollutants (Tang et al., 2009). When the sorption process reaches the steady state condition, the system will reach the saturation stage and the

contaminant can release by reversible sorption processes and no further contaminant losses will occur (Imfeld et al., 2009). Moreover, these released pollutants can later be consumed by micro-organisms via biodegradation processes.

A further removal process for hydrocarbon compounds in constructed wetlands is the physical sedimentation process. It occurs with the settling downward of hydrocarbon particles within wetland aggregate media (Thurston, 1999; Imfeld et al., 2009). Knight et al. (1999), in their review, revealed that effluent concentrations reflect internal wetland solids processes more than influent concentrations do, and this is due to the high stochastic element of wetland processes that leads to different expeditions occurring for hydrocarbon compounds. Moreover, performance for TSS reduction in petroleum wastewaters is generally in line with other treatment wetlands.

Uptake of the hydrocarbon contaminants via wetland plants is an important process to eliminate the hydrocarbon compounds in wetland systems. Uptake of organic chemicals into plant tissue is predominantly affected by the lipophilic nature of organic pollutants, which can be characterized by the octanol water partition coefficient (Kow) (Ryan et al., 1988). Wetland plants, such as reed, are known for their ability to take up highly lipophilic compounds (Imfeld et al., 2009).

Microbial degradation and plant bioremediation are considered as attractive biological technologies treating various hydrocarbon compounds from different types of wastewater (Chen et al., 2012; Al-Baldawi et al., 2013c; Zhang et al., 2013; Al-Baldawi et al., 2014a; Truu et al., 2015). They can be used effectively to remove petroleum hydrocarbons from arising from both natural sources and human activities. They have advantage over various mechanical

remediation practices due to their low cost and less destruction of the environment (Venosa & Zhu, 2003; Mitsch, 2010).

In the microbial degradation process, the micro-organisms have the metabolic ability to transform or mineralize hydrocarbon pollutants into less harmful and non-hazardous substances, which are then integrated into natural biogeochemical cycles (Margesin & Schinner, 2001). The micro-organisms utilize the hydrocarbon as carbon for their energy source (Das & Chandran, 2011; Al-Baldawi et al., 2013e; Guittonny-Philippe et al., 2015a). The general removal order of hydrocarbons in the environment is n-alkanes, iso-alkanes, cycloalkanes, 1~3ring aromatics, polycyclic aromatics, asphaltenes and resins (Greenwood et al., 2008). The carbon number is the key factor that affects the degradation of hydrocarbon contaminants in constructed wetlands (Liu et al., 2012). The intensity of hydrocarbon biodegradation depends on many factors including: temperature, pH value, microbial population, degree of acclimation, accessibility of nutrients, oxygen availability in the contamination environment, composition and concentration of the contaminants, chemical and physical characteristics of the contaminant compounds, and the pollution history of the contaminated environment (Singh & Ward, 2004; Das & Chandran, 2011; Wang et al., 2013a).

Previous studies examined the impact of weather conditions on the biodegradation process in contaminated areas. Siron et al. (1995) explained that the biodegradation process is reduced under cold conditions due to a decrease in the ability of micro-organisms to grow and thrive in the wetlands environment. Another study in New York (Wallace et al., 2011a) demonstrated the ability of two full-scale treatment wetlands in removing hydrocarbon pollutants under cold conditions. These wetlands, used to remediate the groundwater from hydrocarbon pollutants, were operated under -35 ºC and -20 ºC, respectively. Both systems showed use of an aeration

system during the winter months resulted in improving the removal of total petroleum hydrocarbons (TPH) and BTEX compounds from constructed wetlands.

The presence of nutrients in contaminated areas has shown their positive impact in increasing the activities of micro-organisms and their ability to accelerate the biodegradation process (Pezeshki et al., 2000; Ji et al., 2002; Tao & Yu, 2013), even under cold climate conditions (Margesin & Schinner, 2001; Wallace et al., 2011a). Furthermore, researchers have revealed that the availability of oxygen is a key factor to enhance hydrocarbon removal by microbial degradation. Kadlec (2001) and Al-Baldawi et al. (2013e) explained that the positive role of aeration enhances both volatilization and aerobic degradation of hydrocarbons in sub-surface flow wetlands. Moreover, the micro-organisms in wetlands responsible for hydrocarbon degradation are sensitive to pH fluctuations. For example, wetlands with high amounts of ammonium result in an increase of acidification which in turn decreases the ability of micro-organisms for hydrocarbon degradation (Tao & Yu, 2013). Hawrot and Nowak (2006), studied the effects of different types of field soil treatments (fertilization (N:P:K), stirring, and bioaugmentation) on diesel fuel removal efficiency in soil contaminated with 5% diesel fuel concentration. The results demonstrated that the best efficiency rate (89%) was obtained after the application of fertilizer and a stirrer to the contaminated soil.

Grass species have frequently been suggested as effective plants for treating hydrocarbon pollutants in constructed wetlands due to their fibrous root system (Yavari et al., 2015) which has a large surface area per unit volume near the surface of the soil (Lee et al., 2008). The study of Glick (2010) elucidated the capability of plant species to degrade, transform, assimilate, metabolize, or detoxify various hydrocarbon pollutants and remove their toxicity effect from the environment via a biological phytodegradation process. A number of studies have

demonstrated the potential of phytoremediation to clean-up petroleum hydrocarbon compounds from contaminated wetland soil (Lin & Mendelssohn, 1998; Liste & Alexander, 2000; Widdowson et al., 2005; Lin & Mendelssohn, 2009; Wang et al., 2013a). This method provides the potential for cost reduction, and is less harmful to the environment than conventional treatment technologies, such as activated sludge (Kurzbaum et al., 2010).

Studies on the role of various types of wetland plants have shown the ability of plants (macrophytes) to enhance the degradation and remediation of hydrocarbon compounds in contaminated environments (Omari et al., 2003; Liu et al., 2011; Al-Baldawi et al., 2013a; Al-Baldawi et al., 2013d; Al-Baldawi et al., 2015a; Truu et al., 2015). The study of Wass and Fox (1993) revealed that wetland vegetation played a significant role in the removal of oil and grease in storm water in Arizona, United States treated by sub-surface flow wetlands. A study by (Lin & Mendelssohn, 2009) showed that the wetland plant *Juncus roemerianus* enhanced the oil degradation rate in a constructed wetland with a diesel concentration of 40 mg/g, while a high diesel dosage, more than 320 mg/g, had a detrimental impact on the growth of the wetland plants, significantly suppressing variables such as plant stem density, shoot height, and above- and below-ground biomass. Wang et al. (2013a) demonstrated the ability of *Calamagrostis angustifolia* to restore and remediate the petroleum hydrocarbon contaminated wetlands in Momoge National Nature Reserve in Jilin Province, China. In their review, Yavari et al. (2015) elucidated that different types of macrophytes can be successfully used as oil hydrocarbon phytoremediators. One of the features that makes wetland plants suitable for phytoremediation is their capability to grow promptly. They are invasive and quickly become abundant. Thus, they can be replaced with new growth soon after any damage caused by hydrocarbon contamination (Bhatia & Goyal, 2014). Caudle and Maricle (2015) assessed the impact of

spilled motor oil on inland salt marsh communities (*Distichlis spicata*) in a greenhouse experiment. After 10 week of experiment, it was noticed that *Distichlis spicata* plants were very sensitive to spilled hydrocarbon compounds. The oil exposure led to a 91% decrease in photosynthesis, 83% in chlorophyll concentration, and 34% of the above-ground biomass of the plants.

Wetland plants are used to restore and remediate hydrocarbon contaminated wetlands. Studying the response of wetland plants to hydrocarbon pollutants is essential for successful restoration and remediation of oil-impacted habitats. Chlorophyll levels and the moisture of leaves and stems of wetland plants were used as indices by Ghobrial (2008) to assess the phytoremediation process of petroleum contaminants in SSF CWs. The results of monitoring the above-ground part of *Phragmites australis* in SSF CWs indicated that the reeds *Phragmites australis* were very tolerant to hydrocarbon contamination and able to absorb, uptake and convert organic contaminants to less toxic metabolites via their leaves which contributed to hydrocarbon bioremediation through the transpiration process. Wang et al. (2011b) studied the tolerance mechanism of reeds to different dosages of diesel in soils. The results showed redundancy of chlorophyll content under lower concentrations of diesel (≤ 15,000 mg/kg soil). Results suggested that the low diesel concentration served as nutrition to the plant's growth while under high diesel dosage (more than 15,000 mg/kg), there was a remarkable reduction in the removal efficiency of diesel concentration in the contaminated area.

The combination of both bioremediation and phytoremediation has been proposed as an effectual option or practical technique to clean up wetlands that are polluted with hydrocarbon compounds. Studies showed that the co-metabolization of plants and oil degraders in wetlands could be reasonably matched to increase the diesel biodegradation rate and control the diesel

contamination of wetland systems (Wang et al., 2011a; Liu et al., 2012; Zhang et al., 2013). The efficiency of the hydrocarbon contaminant-degradation process is affected by the plant–microbe interactions in the rhizosphere area. The mechanism of rhizosphere degradation of hydrocarbon is suggested to happen through the plant rhizosphere zone when organic compounds are exuded by plants through their roots (Phillips et al., 2008). These organic exudates, act as substrates, leading to increase the density, diversity and activity of specific micro-organisms in the surrounding rhizosphere in the soil, thus improving the degradation of toxic organic compounds (Anderson et al., 1993). The extent and intensity effects of the rhizosphere on hydrocarbon removal are decreased with increasing distance from the root surface (Joner & Leyval, 2003). The combination of microbial community with the plant rhizosphere creates a good environment for hydrocarbon degradation (Wang et al., 2011b; Zhang et al., 2013; Hou et al., 2016). In their study, Pardue et al. (2000) showed that the combination of the microbial community with the wetland plant rhizosphere in the root zone plays a significant role in degrading a variety of volatile organic compounds.

The fibrous roots of some aquatic plants can provide a larger surface and denser rhizospheres for microbial colonization (White Jr et al., 2006). Omari et al. (2003) studied the differences in the diesel removal efficiencies of planted and unplanted sub-surface flow beds with different depths. Findings showed higher hydrocarbon removal efficiencies for the planted beds as compared with control ones and this is due to the ability of *Typha* to provide adequate oxygen from the air to the wetland soil through the plant roots' hairs which in turn helps to enhance the mechanism of hydrocarbon removal. Moreover, adding a fertilizer dosage to the wetland system resulted in a significant increase in the wetland plants growth, thus leading to increase the hydrocarbon removal efficiency. Muratova et al. (2003) and Salminen et al. (2004) showed that

plants with aerenchyma such as reeds (*Phragmites australis*) can successfully be used in the rhizoremediation process in the wetland system due to their roots' ability to release oxygen into the rhizosphere zone which, in turn, leads to enhancing the aerobic degradation of pollutants. Cao et al. (2012) found in their study that the root of reeds (*Phragmites australis*) can significantly enhance the tolerance of soil micro-organisms for diesel pollutants and improve the biodegradation ability of soil micro-organisms for these pollutants.

The effect of different diesel concentrations on the performance of both free-surface flow and sub-surface flow constructed wetlands has recently studied by (Al-Baldawi et al., 2013a; Al-Baldawi et al., 2013d). The two studies assessed the ability of bulrush (*Scirpus grossus*) in these two systems to phytoremediate diesel contaminants in simulated wastewater at four different concentrations (0, 8700, 17,400, and 26,100 mg/l). The authors revealed that after 72 days of treatment, TPH removal efficiency was 80.2% and 91.5% for FS and SSF wetland systems, respectively. Another study by (Al-Baldawi et al., 2013c), to compare the performance of these two flow systems regarding the process of hydrocarbon phytoremediation, revealed that the TPH removal efficiencies, the water quality parameters (including temperature, dissolved oxygen, redox potential and pH), and wetland plant growth were more efficient in the SSF than the SF system. Furthermore, Al-Baldawi et al. (2014b) investigated the optimum conditions for hydrocarbon removal from horizontal (SSF CWs) treated diesel contaminated water and found that TPH removal efficiency was 72.5% with diesel concentration of 0.25% (VDiesel/VWater) under the best retention time of 63 days with no aeration.

Al-Sbani et al. (2016) studied the ability of the *Lepironia articulate* plant to resist the toxicity of diesel and degrade polycyclic aromatic hydrocarbons (PAHs) from wastewater. During an 80-day experiment, L. articulate was exposed to different diesel concentrations of 1%, 2%, 3%

and 5% (VDiesel/VWater) in a batch sub-surface flow (SSF) system. The result indicated a significant difference ($p < 0.05$) between the treatments with and without plants, and higher performance was observed for PAHs removal with plants. The removal rates were 96.6%, 90.3%, 79.9% and 79.6% removal for 1%, 2%, 3% and 5% (VDiesel/VWater) diesel concentration, respectively, with plants.

2.5.6 Heavy metal removal

The main mechanisms for the removal of metal from urban and industrial wastewater treated by constructed wetlands are filtration and sedimentation, precipitation, adsorption, and uptake by helophytes and micro-organisms (DeBusk, 1999; Stottmeister et al., 2003; Choudhary et al., 2011). However, the studies of Sheoran and Sheoran (2006) and Guittonny-Philippe et al. (2014) showed that all of these reduction pathways depend on each other, making the total process of the heavy metals removal mechanism very complicated in wetlands.

Filtration and sedimentation are the main processes in removal of heavy metals from wastewater in CWs. Sinicrope et al. (1992) and Noller et al. (1994) reported that the removal of cadmium, lead, silver and zinc can be achieved by filtration. The removal efficiencies were reported to be 75-99.7% cadmium, 26% lead, 75.9% silver and 66.7% zinc. Sedimentation is a physical process that allows the heavy metals stack with large particles to sink through porous aggregate (Walker & Hurl, 2002). Precipitation depends on the solubility product (Ksp) of the metal, pH of the wastewater, and concentration of metal ions and relevant anions (Sobolewski, 1999; Imfeld et al., 2009; Gill et al., 2014; Caicedo et al., 2015). When the values of the concentration of cations and anions are such that their product exceeds Ksp, precipitation takes place (Sheoran & Sheoran, 2006). In this way, heavy metals are removed from wastewater and trapped in the wetland sediments.

Heavy metals in CWs may be adsorbed to soil or sediment, or may be chelated or complexed with organic matter. In addition to adsorption of heavy metals, oxide formation is also an important mechanism for metal removal from wastewater (Wieder, 1989). Biological removal is another important pathway for heavy metal removal in CWs; it includes plant and microbial uptake. The rate of metal removal by plants varies widely, depending on plant growth rate, plant species and concentration of the heavy metals in the wastewater (Sheoran & Sheoran, 2006). Barley et al. (2005) reported that the highest metal concentrations were observed in root plants.

Metal removal in these CWs mostly occurs due to the bioaccumulation in plant parts, phytoextraction and phytostabilization (Martinez-Guerra et al., 2015). Macrophytes also play an important role in the metals elimination process by assimilating them into the tissues, increasing environmental diversity in the rhizosphere, performing as catalysts for decontamination reactions, and enhancing a range of chemical and biological reactions (Morari et al., 2015). Vegetated treatment wetlands also offer a promising way to remediate water contaminated with inorganic compounds, like metals and metalloids, their uptake by various macrophytes being the protuberant contaminants removal mechanism. For instance, common reed (*Phragmites australis*) has the potential to extract and accumulate chromium from tannery wastewater (Calheiros et al., 2012). Han and Tao (2014) investigated copper (Cu) removal mechanisms and efficiency in a wetland planted with *Phragmites australis*. The plant uptake only accounted for 4.4% of total Cu removal with a preferred accumulation in below-ground biomass. In another study (Gill et al., 2014) of heavy metal removal from road runoff treated by CWs, *Phragmites australis* was found to survive better than *Typha latifolia*, even though the two species were planted in similar conditions in the constructed wetlands.

2.6 Clogging within constructed wetlands

Clogging is a process that develops from excessive formation of accumulated pollutants which build-up a biofilm within substrate pores of the wetland filter and leads to blockage of the filter media and, subsequently, diminishes the hydraulic conductivity over the operational period of the CWs system (Knowles et al., 2010; Knowles et al., 2011; Nivala et al., 2012; Fu et al., 2013). Two decades of treatment wetland literature have proven that clogging can limit the asset lifetime of SSF treatment wetlands and may threaten the widespread feasibility of the technology (Platzer & Mauch, 1997; Langergraber et al., 2009; Zhao et al., 2009; Pedescoll et al., 2011; Nivala et al., 2012; Song et al., 2015; Petitjean et al., 2016). Wetland scientists have widely acknowledged, from their research, that clogging of the filter surface is by far the biggest operational problem of SSF CWs (Austin et al., 2006; Sun et al., 2007; Babatunde et al., 2008; Giraldi et al., 2010; de la Varga et al., 2013; Hua et al., 2013; Song et al., 2015; Rozema et al., 2016a). Clogging reduces the infiltration capacity as well as the oxygen supply into a wetland system, leading to extremely fast decrease in the treatment performance (Prochaska et al., 2007; Turon et al., 2009; Knowles et al., 2011; Wu et al., 2015f).

The application of vertical-flow constructed wetlands is often limited by physical and biological clogging (Aslam et al., 2007; Lianfang et al., 2009; Nivala & Rousseau, 2009; Scholz, 2010; Sani et al., 2013b; Scholz, 2015). The deposition of organic and inorganic solids at the wetland surface leads to a clogging mat and deposition of solids within pores results in substrate clogging. The intrusion of solids that might be caught at the surface via screening and filtration depends on the aggregate size of the filter material (Pedescoll et al., 2009; Knowles et al., 2010; Pedescoll et al., 2011; Stefanakis & Tsihrintzis, 2012; Fu et al., 2013).

A layer responsible for clogging usually develops from retained solids and from biological processes within the biofilm (Nivala et al., 2012; Meng et al., 2014; Fu et al., 2015; Kim & Forquet, 2016; Valipour & Ahn, 2016). Clogging results in low hydraulic conductivity but also enhances the treatment efficiency of some wetland systems (Pedescoll et al., 2009; Fu et al., 2013; Vymazal, 2014; Wei et al., 2015). The aggregates and biomass within wetland systems provide surface area for the attachment of decomposing biological matter (Knowles et al., 2011; Nivala et al., 2012; de la Varga et al., 2013; Mander & Chazarenc, 2015; Wei et al., 2015; Kim & Forquet, 2016).

Microbial biomass growth decreases the hydraulic conductivity, because cells and their extracellular polymeric substances plug the pores between wetland aggregates. Biological clogging is enhanced if nutrient loadings are high (Soares et al., 1991; Soleimani et al., 2009; Hua et al., 2014; Samsó et al., 2016). Wetland plant decay may also lead to clogging of the top aggregate layer by decomposing plant litter (Scholz & Xu, 2002; Fu et al., 2013; Petitjean et al., 2016), particularly if the top biomass is not harvested.

Wetlands treating industrial and domestic wastewater are sometimes subject to permanent contamination such as oily hydrocarbon compounds (Al-Baldawi et al., 2013b; Vymazal, 2014; Al-Isawi et al., 2015a; Mander & Chazarenc, 2015). Diesel is one of the toxic fuel compounds with an adverse impact on the water environment (Mariano et al., 2008; Michel & Rutherford, 2013; Truu et al., 2015). Some diesel components are water-insoluble, gradually entering the wetland system, accumulating inside the pore spaces and thus may lead to blockage of the filter substrate. Khamehchiyan et al. (2007) and Sutton et al. (2013) showed that hydrocarbon compounds lead to smothering of soil particles and block air diffusion in the aggregate pores,

thus causing anaerobic conditions and a reduction in permeability in the aggregate environment, affecting the diversity of micro-organisms.

2.7 Treatment wetland models

The use of vertical-flow constructed wetlands for wastewater treatment has significantly increased due to their high capacity to oxidize organic matter and nitrogen compounds. However, the operation process is complex as they are usually intermittently fed with wastewater in order to enhance the quality of wastewater distribution through the bed, provide high oxygen diffusion, and limit clogging at the same time (Langergraber, 2007; Hua et al., 2013). Literature has shown that numerical models can be very helpful to attain a better understanding of the processes happening in CWs in order to optimize the design and operation criteria of constructed wetlands and make this technology fully reliable (Langergraber, 2008; Langergraber et al., 2009; Mander & Chazarenc, 2015; Meyer et al., 2015; Bustillo-Lecompte et al., 2016).

The majority of the treatment models for VF CWs are based on the model developed by Van Genuchten (1982), which describes one-dimensional flow and mass transport under unsaturated-saturated conditions. This model was initially developed to describe water flow and pollutant transport in groundwater and particularly in the vadose zone, but it was also later used to describe VF CW processes, due to the similar conditions encountered within the systems (Giraldi & Iannelli, 2009; Giraldi et al., 2010). Moreover, Kadlec and Knight (1996) initially used, first-order models, “black box”, using a first-order rate constant, to describe the effects of materials production, sedimentation, retention time, and temperature on pollutant removal in treated water.

Austin (2006) developed a model to predict the clogging phenomenon in a tidal flow VF CW. This model used the Damköhler number (Da), which expresses the ratio of reaction rate of the mass transport related to biofilm growth in the CW porous media. According to this model, when Da < 1, the biofilm growth is limited, while when Da > 1, mass transport is limited, and this means clogging may occur.

Langergraber et al. (2009) recommend a multi-component reactive transport model to simulate both transport and reaction of the main constituents of municipal wastewater in the sub-surface flow processes within wetlands. However, this model needs more detailed knowledge of various process variable interactions within wetland filters. Furthermore, Demaret et al. (2009) proposed a simple biological clogging model, which takes account of both the effect of biomass growth on hydraulic conductivity (Eq. 2.1) and spatial diffusion (Eq. 2.2). The biomass diffusion coefficient (DM) is estimated by using a mesoscopic biofilm model (Eq. 2.3).

$$K = \begin{cases} K_0 \dfrac{\left(1 - \dfrac{M}{M_{clog}}\right)^b + a}{1 + a} & M < M_{clog} \\ K_0 \dfrac{a}{1 + a} & M > M_{clog} \end{cases} \qquad \text{.....(2.1)}$$

where K is the hydraulic conductivity within the substrate of the wetlands, M is the biomass density within a control volume, K_0 is a constant, a and b are two empirical parameters, and M_{clog} is the biomass density beyond which no further reduction of hydraulic conductivity is observed.

$$\varepsilon \frac{\partial M}{\partial t} = \nabla(D_M \nabla M) + \varepsilon\left(\frac{k_3 CM}{k_2 + C} - k_4 M\right) \quad \text{.................................... (2.2)}$$

where ε is the porosity of the porous substrate beds of the wetlands, M is the biomass density within a control volume, t is time, D_M is the biomass diffusion coefficient, k_2, k_3 and k_4 are empirical parameters, and C is the concentration of the dissolved substrates (soluble reactive components) in water.

$$D_M = d \frac{\left(\frac{M}{\varepsilon M_{\max}}\right)^{\alpha}}{\left(1 - \frac{M}{\varepsilon M_{\max}}\right)^{\beta}} \quad \text{.. (2.3)}$$

where DM is the biomass diffusion coefficient, d, α and β are empirical parameters, M is the biomass density within a control volume, ε is the porosity of the porous substrate beds of the wetlands, and M_{max} denotes the maximum biomass density.

Giraldi et al. (2010) developed a reactive transport model for vertical-flow constructed wetlands (such as those studied in this paper), which is based on the Activated Sludge Model 1 (Henze 2000). Clogging is described by both the porosity reduction due to biological growth and the filtration of particulate components using the total volumetric specific deposit (D_{vtot}). The impact of porosity reduction on the hydraulic conductivity is estimated by Eq. 2.4 (Carman 1956).

$$K = \frac{K_0}{\left[\left(1 + p\frac{D_{vtot}}{\varepsilon_0}\right)^{x}\left(1 - \frac{D_{vtot}}{\varepsilon_0}\right)^{y}\right]} \quad \text{.. (2.4)}$$

Where K is the hydraulic conductivity, K_0 represents K when the filter is clean during the start-up phase, p, x and y are empirical parameters, D_{vtot} is the total volumetric specific deposit, and ε_0 is the porosity of the porous substrate beds during the start-up phase of the treatment wetland.

In recent years, numerous concerted efforts have been made to develop many numerical models as tools to help in understanding various processes which occur in SSF wetland systems (Nivala et al., 2012; Samsó, 2014; Haydar et al., 2015; Huang et al., 2015a; Meyer et al., 2015; Rajabzadeh et al., 2015; Bustillo-Lecompte et al., 2016; Samsó et al., 2016). The review study of Meyer et al. (2015) compares numerous constructed wetlands models applied to describe various CWs functions. These models are categorized to help the users to choose the most suitable model simulation. Meyer and Dittmer (2015) describe the retention soil within wetlands by using a simple model, which is basically a combination of VSSF CWs and retention basins. This model can predict pollutant discharges for a long period. The results of the study are useful for ecological engineers designing CWs to meet specific discharge requirements. Samsó et al. (2015) developed a BIO_PORE model to explain the transport of sulphur, organic matter, and nitrogen in sub-surface flow constructed wetlands. The purpose of this study was to define the impact of bacteria growth on the effluent pollutant concentrations predicted by the model. The high concentrations of organic matter and nutrients transported within the porous media of wetland filters leads to high bacterial biomass production, which decreases the flow capacity of the porous material (bioclogging) (Samsó et al., 2016).

2.8 Performance of constructed wetlands by comparison with ponds

Constructed wetlands treatment technology, widely applied for the treatment of various types of wastewater, has found efficient performance in the removal of pollutants in the wastewater and is simple to construct, operate, and maintain with low cost, low energy demand, effectiveness, and potential for creating biodiversity (Zhao et al., 2010; Mburu et al., 2013; Wang et al., 2016). However, literatures have noted the necessity to monitor, control and predict the treatment processes of constructed wetlands over time (Scholz, 2003; Song et al., 2006; Mustafa et al., 2009; Dong et al., 2012; Fan et al., 2016) to maintain meeting environmental and sustainability policies, and regulatory requirements, such as secondary wastewater treatment standards (USEPA, 2000; ITRC, 2003; Scholz, 2006, 2010, 2015). Although, from the technical point of view, a constructed wetland system seems like a simple structure, it is a very fragile system because it has to be hydro-technically effective for a long time, i.e. high amounts of water can be forced through it in addition to maintaining good internal conditions for water treatment. The monitoring study of the performance of integrated constructed wetland (ICW) mesocosms by Dong et al. (2012) showed a gradual reduction in their overall treatment performance with time and a relative decrease in the removal efficiency for contaminants of treated water. In contrast, the performance treatment of constructed wetlands used to treat farmyard dirty water from a dairy farm near Dunhill (Ireland) (Mustafa et al., 2009) were evaluated through physical, chemical and microbiological parameters analysis on data collected over approximately seven years. Findings showed the overall removal efficiencies were relatively high if compared to the international literature (BOD (97.6%), COD (94.9%), SS (93.7%), NH_4-N (99%), NO_3-N (74%) and molybdate reactive phosphorus (91.8%)).

Recently, there has been attention towards using pond systems as a sustainable technology to treat various types of wastewater using wetland plants as a cheap, effective and environmentally friendly method (Mara, 2009; EPA, 2011; Sekomo et al., 2012; Butler et al., 2015; Rühmland et al., 2015). Ponds are classified for man-made pits or basins constructed in or on the ground surface with earthen (or other man-made material) dikes for water retention (Adeola, 2007). Ponds are designed to enhance the development of natural ecosystem processes that are either anaerobic (providing conditions for bacteria that grow in the absence of oxygen [O_2] environments), aerobic (promoting the growth of O_2 producing and/or requiring organisms, such as algae and bacteria), or facultative, which is a combination of both the aerobic and anaerobic (EPA, 2011), which are managed to reduce contaminant concentrations to meet water quality requirements. In certain cases, ponds are used as the only means of wastewater treatment prior to discharge to receiving watercourses while in other cases they act as a storage facility prior to a treatment stage (Valero & Mara, 2007).

Ponds have been used to treat different forms of wastewater, including municipal wastewater (Mburu et al., 2013; Rühmland et al., 2015), storm water runoff (Semadeni-Davies, 2006; Chang et al., 2013) and industrial effluent (Sekomo et al., 2012). Municipal wastewater discharge may become a major environmental problem as it is considered one of the primary sources of nitrogen and phosphorus in watersheds. Receiving water courses become eutrophic due to the existence of a high amount of nutrients, subsequently promoting enormous plant growth that leads to the depletion of oxygen in the water environment (Chang et al., 2012; Butler et al., 2015). Ponds serve as a convenient means of wastewater and sewage management. They are mainly used for SS, BOD and nutrient removal (Ge et al., 2016b) although there are many cases where they are also used for pathogen removal (Pearson et al., 1987). The study of

Valero and Mara (2007) on ponds system treated wastewater showed that nitrogen removal can be achieved to low levels (< 5 mg ammonium N per litre) in warm summer months in England.

The pioneer research for treating wastewater with pond systems was carried out by scientists in the United States (Oswald in the USA and Marais in southern Africa) (Caldwell, 1946; Dildine & Franzmathes, 1970; Mara, 2009). These two works established the basic foundation of the present treatment ponds. The period from the mid-to-late 1970s was characterized by using large numbers of full-scale pond systems in France, Germany and the USA. Research of treatment ponds expanded greatly in several universities around the world, and much more is now known about pathogen removal (Von Sperling, 2005) and nitrogen transformation in pond systems (Valero & Mara, 2007).

Recently, the application of constructed treatment wetlands and facultative ponds in treating domestic sewage has attracted a lot of attention considering that both score highly regarding process simplicity (in terms of required equipment) and reliability and they offer an environmentally sound method for the removal of nutrients and various pollutants (Semadeni-Davies, 2006; Tsalkatidou et al., 2009; Greenway, 2010; Chang et al., 2012; Ávila et al., 2013; Mburu et al., 2013; Butler et al., 2015). However, despite these above-mentioned removal abilities associated with both SSF CWs and pond systems, different evaluation study research proposed that SSF CWs are better than ponds in some technical works. For example, Kadlec (2009) stated that substrate beds of SSF wetlands systems perform satisfactorily in wastewater particle and turbidity removal to produce clear water. In contrast, effluent pond systems are, usually, associated with high algae production (Tsalkatidou et al., 2009). Furthermore, in SSF CWs, there is no wastewater exposed on the surface therefore they do not encourage mosquito breeding (ITRC, 2003; Choudhary et al., 2011). Moreover, they do not have open wastewater

bodies, so there is no possibility of accidental contact with sewage (Bohorquez et al., 2015; Dogdu & Yalcuk, 2016).

Constructed wetlands do not produce sludge (except for the sludge produced from the pre-treatment step upstream of the SSF CW). In ponds, on the other hand, sludge accumulates over time, and the sludge has to be removed after approximately 10 years (this is often neglected in developing countries and the ponds are abandoned instead) (ITRC, 2003; Kadlec, 2009; Choudhary et al., 2011). Moreover, SSF CWs operating with well-functioning performance are associated with limited odour generation, whereas in most treatment pond systems, odour generation is common (Abis & Mara, 2003; Greenway & Jenkins, 2004; Mara, 2009). With regard to choice of either CWs or pond systems in terms of aesthetic view, greenery for CWs can be placed near entrances and gathering places, as well as being used as green belts around communities, since most people will only see a beautiful garden. While ponds, due to their open water surface, are much more difficult to integrate in a neighbourhood, especially an urban neighbourhood (Vymazal, 2007b; Bai et al., 2013; Prasad et al., 2016).

However, wastewater treatment engineers and planners are aware of the advantages of constructed wetlands over waste stabilization pond systems. Research with regard to an economical comparison evaluation study, based on land area requirements, performance and costs between HF constructed wetlands and facultative ponds for small rural communities in the United Kingdom, showed that constructed wetlands require more land and incur greater cost than facultative waste stabilization ponds (Mara, 2006).

Various researchers, including Kadlec and Wallace (2009), Scholz (2010), Vymazal (2011a), and Scholz (2015), have reviewed the effectiveness of constructed vertical-flow wetlands used for the treatment of urban wastewater. However, the impact of reeds and aeration on the

treatment performance and contaminant removal efficiency of both mature wetlands and pond systems has been less well researched (Abis & Mara, 2003). Moreover, studies assessing the impact of mature constructed wetlands on the treatment performance, based on efficiency comparisons with new different types of artificial ponds, are also less documented.

2.9 Recycling of wastewater for agricultural purposes

2.9.1 Application of wastewater for irrigated edible crops

Recycling of treated wastewater has been increasingly considered as a promising practical alternative that would contribute to addressing the current deficit and future availability of water resources, particularly in regions of scarce waters (Boyden & Rababah, 1996; Chu et al., 2004; Toze, 2006; Metcalf et al., 2007; Qadir et al., 2010; FAO, 2012; Singh et al., 2012; Hamid et al., 2013; Anderson et al., 2014; Missimer et al., 2014; Vo et al., 2014; Angelakis & Snyder, 2015; Nyomora, 2015; Woltersdorf et al., 2016). Adequate reuse of wastewater is a necessity to protect public health, the environment and water resources. Treated wastewater can be used for agriculture, aquaculture, urban and industrial applications, recreational and ecosystem service purposes, and artificial recharge of groundwater (Yadav et al., 2002; Benetti, 2008; Al-Hamaiedeh & Bino, 2010; Sou/Dakouré et al., 2013; Lopes et al., 2015; Al-Isawi et al., 2016c; Mohapatra et al., 2016; Ramprasad & Kutty, 2016). Literature indicates the promising use of recycling of treated wastewater for irrigation in the agriculture sector (Aiello et al., 2007; Cirelli et al., 2012). Nutrients embodied in treated wastewater can increase yields as much or more than a combination of tap water and chemical fertilizer (Lopez et al., 2006; World Health Organization WHO, 2006; Ma et al., 2011; Kiani & Abbasi, 2012; Nyomora, 2015) and can improve farm productivity (Raschid-Sally et al., 2005). However, contaminated wastewater

with undesirable concentrations of hydrocarbons from oil spills associated with urban runoff or industrial discharge are more recent challenges for agricultural application purposes (García-Delgado et al., 2012; Almuktar et al., 2015b, 2015a; Scholz, 2015).

Generally, applied research on constructed wetland systems highlights the fact that treated domestic wastewater effluent directed to irrigation may contain readily absorbable useful minerals, nutrients and easily biodegradable organics, with a quality that is compatible with the permissible limits of using water bodies for irrigation. The study conducted by Abou-Elela and Hellal (2012) to assess the use of domestic wastewater treated by VF CWs system for irrigation purposes showed high removal efficiencies for water quality parameters (COD, BOD, SS, and nutrients) in the final outflow water. The results revealed the suitability of using VF CW treated effluent for irrigation in rural areas and small communities. Abou-Elela et al. (2015) evaluated the performance of a packed-bed up-flow anaerobic sludge blanket reactor (P-UASB) followed by a biological aerated filter (BAF) for the treatment of municipal wastewater for unrestricted irrigation. The overall removal efficiency of the integrated treatment system operated at an average organic loading rate of 1.54 kg COD/m3/day was 89% of total chemical oxygen demand (TCOD), 92% of BOD and 95% of TSS. Moreover, the treatment system had a small footprint, was cost effective, could treat wastewater of low to medium strength, and produced an effluent suitable for reuse in unrestricted irrigation.

Wetland treatment systems have been successfully constructed to purify and control domestic wastewater contaminated with hydrocarbons (diesel fuel spillages) (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). However, it is difficult to remove the hydrocarbon compounds completely from treated wastewater during the wetland operation (Eke & Scholz, 2008; Tang et al., 2009; De Biase et al., 2013; Stefanakis et al., 2016). In general, most wetland systems release

hydrocarbon with the treated outflow water (Wang et al., 2011b; Liu et al., 2012; Vymazal, 2014). The quantity and quality of the hydrocarbon compounds depend on the treatment efficiency of the wetland system (De Biase et al., 2011; Al-Baldawi et al., 2015a). The discharge of this effluent could pose a threat to natural ecosystems. Nevertheless, the re-use of the treated wastewater for other purposes might be a viable option (Sousa et al., 2016).

The benefits of wetland systems for agricultural purposes are widely known. Vertical-flow constructed wetlands have commonly been used to improve the usability of treated wastewater for irrigation purposes (Cui et al., 2003; Morari & Giardini, 2009; Chen & Wong, 2016; Lavrnić & Mancini, 2016). This practice potentially increases agricultural yields, preserves surface water, offsets chemical fertilizer demand, and reduces the costs of wastewater treatment by eliminating nutrient removal processes (Murray & Ray, 2010). However, further investigation is required to determine the optimal wetland system design for recycling of treated wastewater contaminated with hydrocarbon spills to maximize yield productivity of crops (Martinho et al., 2009).

The use of other types of water such as river water for irrigation of plants in the agriculture sector is rather common, e.g., Selvi et al. (2007) found that river water is suitable for the irrigation of crops. In comparison, poor quality water may negatively affect irrigated crops such as industrially polluted water streams (Banerjee & Gupta, 2010). Tsado et al. (2014) and Rahman et al. (2014) highlighted the importance of variables such as conductivity, total dissolve solids, the sodium adsorption ratio (SAR) and specific ion toxicities for assessing the quality of river water used for irrigation. Rainwater collection particularly in semi-arid areas can make a significant contribution to the irrigation of crops. Radaideh et al. (2009) assessed the suitability of rainwater collected in the northern region of Jordan. Findings indicated great

variations in water quality depending on the storage tanks used, catchment area characteristics and the availability of public sanitary systems. Gully pots are a common feature of many sewerage drainage systems in urban areas. Their main function is to retain solids from road runoff. They are used to minimise the challenges linked with sediment in downstream drainage structures, pumps, wastewater treatment plants and receiving watercourses. Gully pot water is regularly taken out of gully pots together with sediment and urban rubbish for subsequent treatment (Scholz, 2004). Grey water comprises wastewater from bath tubs, showers, wash basins, laundry facilities and kitchen sinks (Palmquist & Hanaeus, 2005). Detergents and soaps are the predominant components of grey water (Jefferson et al., 1999). Mohamed et al. (2013) assessed grey water reuse in garden irrigation. The soil analysis results showed that salinity and the organic content of the soil increased as a function of time, subsequently affecting the growth of plants. In comparison, Pinto et al. (2010) undertook a glasshouse experiment to assess the effect of grey water on the growth characteristics of silver beet plants compared to the control treatment of pure potable water. Results indicated that grey water irrigation had no negative effect on the plant dry biomass, number of leaves and water use. Travis et al. (2010) undertook a controlled experiment to study the effect of using raw and treated artificial grey water for irrigation purposes. Findings indicated that raw artificial grey water considerably increased hydrophobicity in both the sand and loam soils and subsequently affected plant growth. In comparison, treated artificial grey water was successfully used for irrigation without any negative impact on soil or plant developments.

2.9.2 Plant selection and the growing environment

In this study, chillies have been chosen to assess the usability of the effluent treated from constructed wetlands for irrigation purposes. Chilli (De Cayenne; *Capsicum annuum*

(Linnaeus) Longum Group ‘De Cayenne’) is a crop often seen as ideal for growing in greenhouses (Nickels, 2012; Jones 2013; Ramalho do Rêgo et al., 2016). The fruits of chilli can be found with conical shape. The colour of chilli fruit at the unripe stage is green, while when the plants are growing well, the ripe fruits color will be dark red (Wahyuni et al., 2011; Al-Isawi et al., 2016b).

This experiment deals with recycled wastewater and selection of plants is made from those grown at adequate distance above the ground, such as peppers, strawberry, sunflower, aubergine, to avoid pathogen contaminants being incorporated in products which could happen due to direct contact with the ground receiving treated wastewater (Peasey et al., 2000; Hamilton et al., 2005; World Health Organization WHO, 2006; FAO, 2012; Mohapatra et al., 2016). Chilli plant is usually easy-to-grow, cost-effective and has a good nutritional value (Nickels, 2012; Bortolin et al., 2016). This type of chilli (*Capsicum annuum)* is a good source of metabolites has an ability to promote health characteristics with provitamin A, vitamin C, vitamin E, flavonoids and capsaicinoids (Wahyuni et al., 2011).

Chillies grow well in climates, which are moist and warm and where the soils are rich in nutrients. This fruiting vegetable is commonly grown as an annual in temperate climatic regions (Al-Isawi et al., 2016c).

2.9.3 Nutrients and minerals

Heavy metals can be toxic to peppers, particularly if they are grown in acidic soil (FAO, 2003). FAO (1994) classified treated wastewater for recycling. Acceptable ranges for ammonia-nitrogen, ortho-phosphate-phosphorous and potassium were from 0 to 5, 0 to 2 and 0 to 2 mg/l in that order. Pescod (1992) highlighted that there is no restriction for irrigation water, if nitrate-

nitrogen concentrations are below 5.0 mg/l. There are slight to moderate restrictions between 5 and 30 mg/l and severe restrictions for values above 30.0 mg/l.

Johnson and Decoteau (1996) recommended that chillies should not be grown when nitrogen is higher than 280 kg/ha. Furthermore, Haifa Chemicals (2014) highlighted the following needs of pepper: nitrogen (390–920 kg/ha), phosphorus pentoxide (200–330 kg/ha), potassium oxide (640–1530 kg/ha), calcium oxide (100–210 kg/ha), magnesium oxide (60–150 kg/ha) and sulphur (40–50 kg/ha).

2.10 Summary

This chapter has documented the development of constructed wetland systems and provides the primary information on the technology. This chapter also describes the components and types of wetlands, as well as the removal mechanisms of pollutants in constructed wetlands. The chapter also covers the explanation of wetland removal mechanisms and numerical modelling. Furthermore, the chapter discuss the treatment of petroleum hydrocarbon compounds in wetland systems. The information about comparison performance of wetland systems with pond treatment systems is explained in this chapter. Finally, the chapter presents the potential for re-using various types of wastewater for agricultural purposes.

3

CHAPTER THREE: MATERIALS AND METHODS

3.1 Overview

The materials and methods used in this study are demonstrated in this chapter. Section 3.1 provides description of chapter structure. A description of the experimental set-up of the constructed wetlands system, including wetland filter design and media composition, is discussed in section 3.2. Operation conditions such as hydraulic retention time, resting time and loading rate, and feeding mode are documented in section 3.3. Sections 3.4 and 3.5 illustrate the monitoring, sampling, and analysis of water quality parameters and growth measurements of the wetland plants. Sections 3.6 and 3.7, document the selection and analytical method used for the hydrocarbon compounds determination. Overall clogging measurements and the application of the Wang-Scholz model are presented in section 3.8. Section 3.9 presents the experimental set-up for artificial ponds as compared to constructed wetlands. The description for the experimental set-up of recycling the treated wastewater for irrigated chillies in greenhouse environment is presented in sections 3.10. Statistical analysis applied for data interpretation is explained in section 3.11. The research limitations are stated in section 3.12 and finally, a summary of the chapter is given in section 3.13.

3.2 Experimental set-up and boundary conditions of wetlands system

The vertical-flow constructed wetland set-up is located within a greenhouse (Fig. 3.1; door left open) on top of the roof of the Newton Building, which is part of the University of Salford, Greater Manchester, UK. The system comprises ten filters and has been in operation since 27 June 2011 to assess the treatment performance of different wetland filters in terms of substrate gravel size, contaminant inflow load, contact time, resting time and the composition of wastewater particles on the evolution of clogging. The laboratory set-up of vertical-flow constructed wetlands, planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed), was constructed from Pyrex tubes with an inner diameter of 19.5 cm and a height of 120 cm. The surface area of each wetland was approximately 300 cm^2. All filters were filled with siliceous (minimum of 30%) pea gravel (of 10 mm and 20 mm diameter, (Fig. 3.2)) up to a depth of 60 cm and operated between 27 June 2011 and 22 March 2016 (Sani et al., 2013a; Al-Isawi et al., 2015b). For more pictures showing the development of the constructed wetlands system during the operation period, readers may refer to Appendix A.

Figure 3.1: Laboratory set-up of the vertical-flow constructed wetlands in the greenhouse.

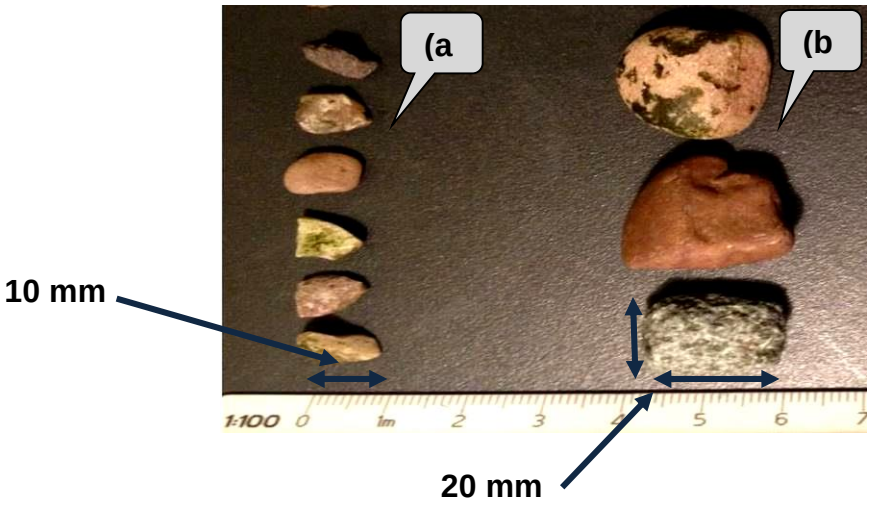


Figure 3.2: Substrate media, supplied by Travis Perkins Company, used for designing the wetland filters: (a) pea gravels of 10 millimetres used for Filters 3, 4, 5, 6, 7, 8 and both Controls A and B; (b) pea gravels of 20 millimetres used for Filters 1 and 2.

The statistical experimental set-up is illustrated in Figure 3.3, which shows the top view of the wetland system, and Figure 3.4 shows the side view of any wetland filter. Dead macrophyte plant material was harvested in winter and returned to the corresponding wetland filters by placing it on top of the litter zone (Al-Isawi et al., 2015a). The outlet valve is located at the bottom of each constructed wetland filter. Eight further valves are located on the sidewall of each wetland column at heights of 10, 20, 30, 40, 45, 50, 55 and 60 cm from the bottom (Al-Isawi et al., 2015a) (Fig. 3.4).

Four factors were investigated to examine the performance of the vertical-flow constructed wetlands: (1) gravel size; (2) contact time (also known as hydraulic retention time); (3) rest time; and (4) inflow COD load. Filters 1 and 2 are compared to Filters 3 and 4 to examine the influence of a larger aggregate diameter. Filters 5 and 6 are compared to Filters 3 and 4 to estimate the impact of a higher inflow COD load. The selection of a lower contact time was investigated through comparing Filter 7 with Filters 3 and 4, and finally, the impact of rest time was obtained through a comparison between Filter 7 and Filter 8.

An external cooling coil system including five Aqua Medic Titan chillers (A1-A5) (Fig. 3.3) (Aquacadabra, Barnehurst Road, Bexleyheath, UK) were connected with both wetland filters and two storage tanks (ST1 and ST2) filled with tap water. The chillers (Fig. 3.5) are usually used for adjusting temperatures within the system by circulating cold water ranging from 6-8 °C around the sub-base of the wetlands via a coolant, water pump and water tubing. The surrounding covers of the wetland filters were insulated with aluminium sheeting (Fig. 3.6) of 1 cm in thickness to prevent external heat transfer to the sub-base zone for the wetland (Tota-Maharaj et al., 2012).

These conditions were set and kept temperatures of the sub-base at approximately 9-11 °C simulating real scenarios of ground and soil conditions within the UK. The chillers, which are provided with a heat exchanger made from sea-water proof Titanium steel, have a one-phase cooling system containing the cooling medium R 134 a (FCKW-free) and work with capillary injection, after which, the heated water is passed by pumps through a pipe network (Fig. 3.6) which is installed around each wetland filter to subsequently transfer the heat of the piped water to the wetlands system. The water source heating and cooling system included reinforced 5-mm laboratory polypropylene tubes placed in the lower sub-base of the first constructed wetland system. The tubing was looped approximately 10 times within the saturated water zone of the wetland structure with a total length of approximately 10 metres. Both ends of the tube were located in a plastic storage tank water vessel. One end was connected to a pump and the other end used as an orifice for discharges. The heating system was applied to provide a suitable temperature for the stored water which, in turn, maintains the combined root system and debris layer of all the wetland filters at a semi-natural below-surface temperature of approximately 12 °C. This temperature simulates the temperature of the upper earth layer where the root system of the wetland plants of a real treatment system would be located.

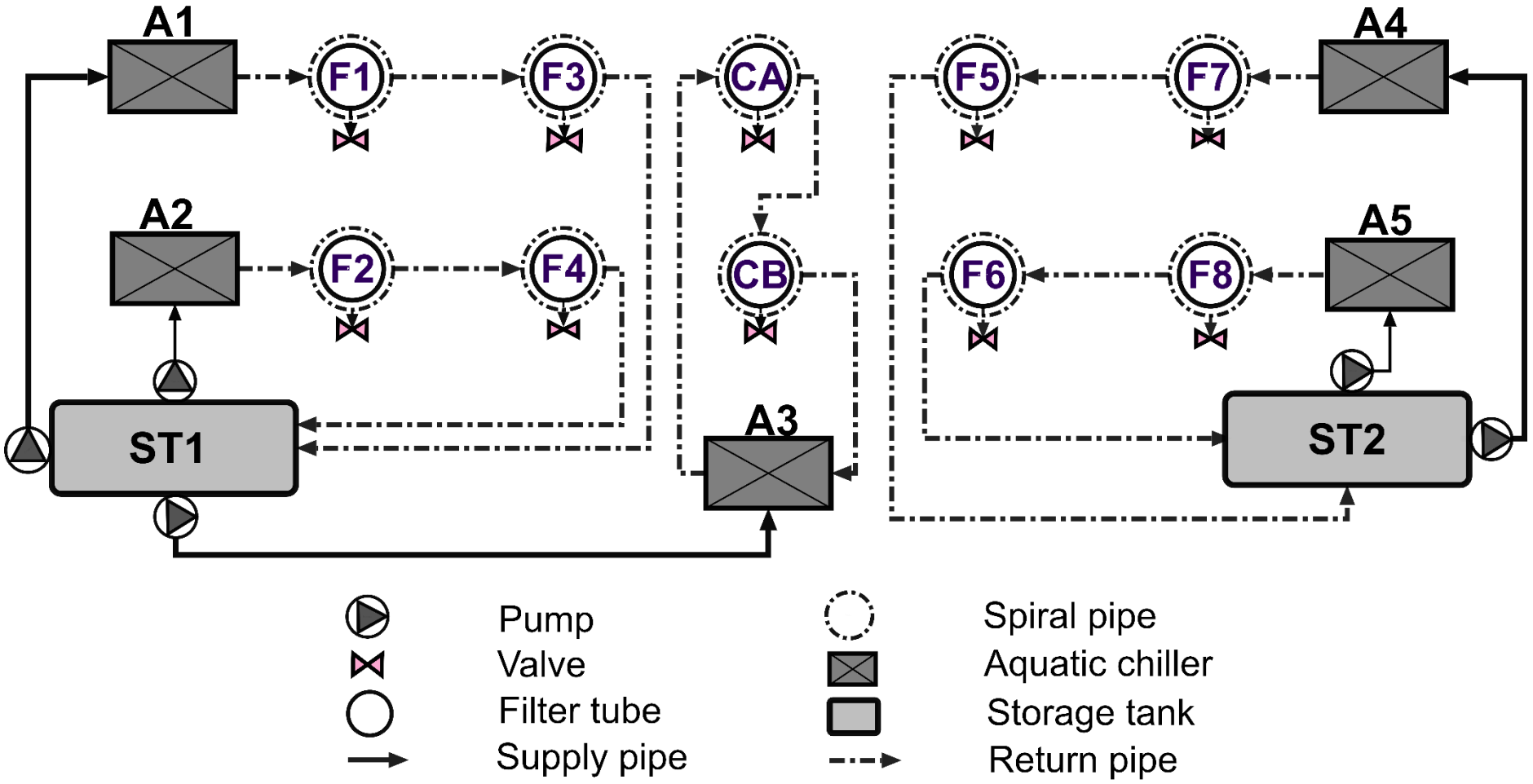


Figure 3.3: A schematic diagram (top view) of the experimental wetland system.

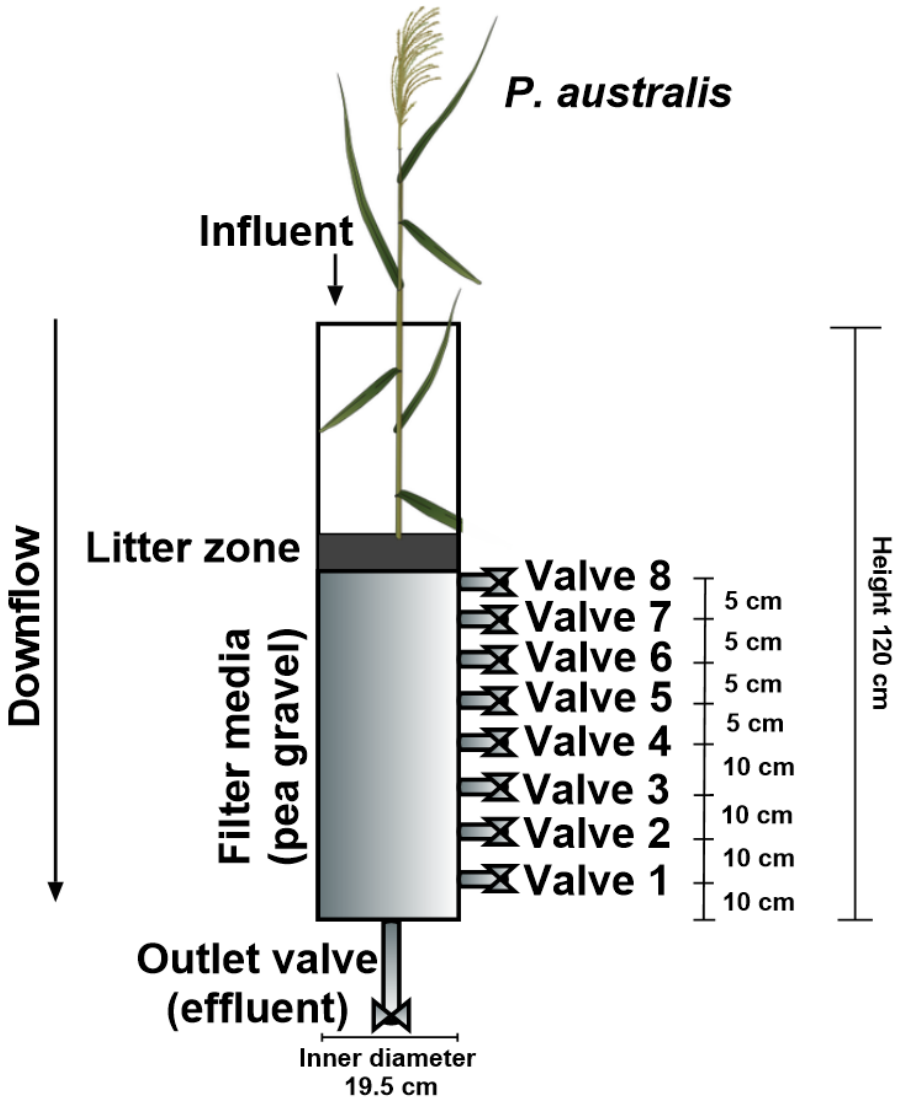


Figure 3.4: Cross-section of a wetland filter.

Figure 3.5: Aqua Medic cooling unit.

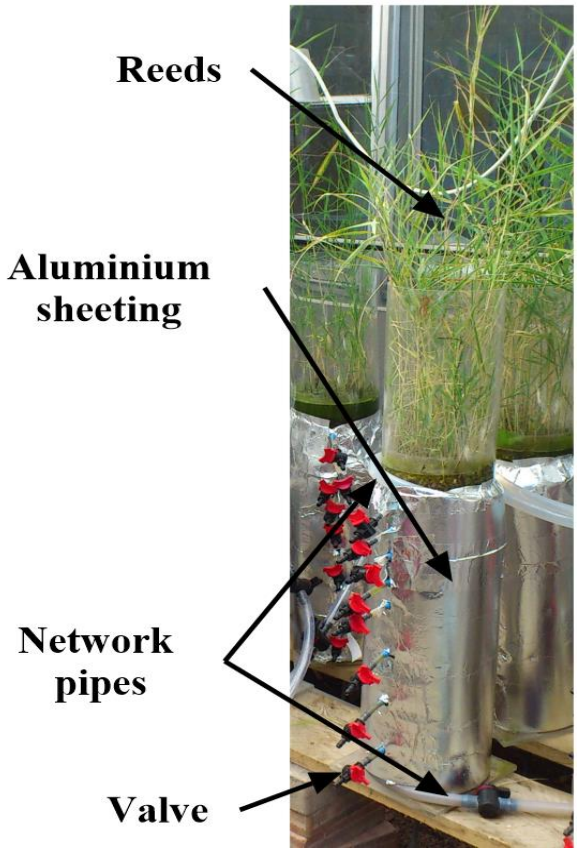


Figure 3.6: A constructed wetland filter.

In order to imitate diesel fuel (100% pure; no additives) spillage, two dosages of diesel fuel, were poured into Filter 1, 3, and 5 and into one of the two control filters (Control A) on 26 September 2013 and 26 September 2014, respectively (Table 3.1). The selection of these two diesel dosages is to test the long-term performance of VF CWs treating domestic wastewater and subject to diesel spillages (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). The first low dose of 130 grams (equivalent to an inflow concentration of 20 g/l) diesel fuel was poured into the filters to test the impact of hydrocarbon on the wetland filters during one year (acclimatization stage). Thereafter, a high dosage of diesel (975 grams; similar to an inflow concentration of 150 g/l)) was applied to assess the treatment performance of the wetlands system. Diesel fuel samples were purchased from a petrol station owned by Tesco Extra (Pendleton Way, Salford, UK). For more information

regarding the diesel fuel used in this research, as provided by European Chemicals Bureau : ecb.jrc.it), readers may refer to Appendix D.

Table 3.1: Overview of diesel dosages applied to filters (1, 3, 5, and Control A) within the running period.

Date	Diesel dosage		
	per water volume (g/l)	per filter (g)	Total quantity for filters (F1, F3, F5, Control A) (g)
26/9/2013	20	130	520
26/9/2014	150	975	3900

3.3 Operation method for vertical-flow constructed wetlands

The wetlands system has been operated for about five years (27 June 2011 to 22 March 2016) to assess the water quality and removal efficiency of the ten wetland filters. Batch flow mode design has been used to operate the wetland system. Two influent (inflow wastewater) types were applied to the wetland filters: concentrated wastewater (preliminary treated urban wastewater) and diluted wastewater (50% preliminary treated urban wastewater mixed with 50% de-chlorinated tap water [synthetic]). Wetland filters F5 and F6 are fed with concentrated wastewater without dilution. With exception of the controls (CA and CB) that receive only tap water, the remaining wetland filters are fed with raw wastewater diluted with tap water. Application is batch-wise, through the top surface of the filter layer; when the surface is completely flooded, the feeding is stopped, the wastewater is then held in the bed and, at a set time later, the wastewater is drained downwards. Water percolates gradually downward through the gravel media drainage network to reach the bottom of the wetland filter. Effluent is discharged from the outlets

at the bottom of VF CWs. After the water has drained from the filtration bed, the treatment cycle is complete and air can diffuse into the voids in the filtration material (Cooper, 2005; Vymazal, 2010).

All wetland filters received 6.5 l of inflow wastewater during the feeding mode, but were each operated differently. Table 3.2 shows an overview of the experimental set-up used in the study to test the impact of four variables. Filters 1 to 6 were tested after 72 hours of contact time and then left to rest for 48 hours, while Filters 7 and 8 were sampled after 36 hours of contact time and left to rest for 48 hours and 24 hours, respectively. This resting time enhances the oxygen transfer within the wetland filters by allowing air to refill the wetland systems, and the dosing cycling traps this air. The treatment processes are enhanced by the extensive rhizomatous root system of the wetland plants (*Phragmites australis*) which can transfer limited quantities of oxygen into the filter media, supporting the micro-organisms. Furthermore, with the exception of Filters 7 and 8, all filters had replicates till 25 September 2013, when petroleum hydrocarbon was applied (only the second replicates received diesel).

The preliminary treated urban wastewater used for the inflow water was obtained from the Davyhulme Sewage Works, one of the largest wastewater treatment plants in Europe (http://en.wikipedia.org/wiki/Davyhulme) (the treatment plant location can be found in Appendix B), operated by the water company United Utilities in Greater Manchester. Fresh wastewater was sampled regularly, nearly once a week, and was stored and aerated by standard aquarium air pumps in a cold room with temperature around 4±0.10 °C before use. The wastewater quality was rather variable, comprising of mainly domestic but also industrial wastewater, both diluted by surface water runoff.

Table 3.2: Comparison of the experimental vertical-flow wetland set-ups.

Design and/or operational variable	Unit	Filters 1 and 2	Filters 3 and 4	Filters 5 and 6	Filter 7	Filter 8	Control A and B
Aggregate diameter	mm	20	10	10	10	10	10
Contact time	h	72	72	72	36	36	72
Resting time	h	48	48	48	48	24	48
Chemical oxygen demand	mg/l	138.1	138.1	277.6	138.1	138.1	2.3
Nature of wastewater	-	WW+T	WW+T	WW	WW+T	WW+T	T

Note: Annually treated volumes of wastewater: Filters 1 to 6, 470 l/a; Filter 7, 624 l/a Filter 8, 858 l/a. On 26 September 2013 and 26 September 2014, 130 g and 975 g, respectively, of diesel were added to Filters 1, 3,5 and Control A, WW: preliminary treated wastewater, and T: tap water.

The COD was applied as the criterion to distinguish between low and high loads used in the wetlands system (Table 3.2). For raw domestic wastewater without dilution, an inflow target COD of approximately 277 mg/l (commonly between 100 and 660 mg/l) was applied for wetlands (Filters 5 and 6) characterized by a high loading rate. In order to simulate a low loading rate, the raw wastewater was diluted with 50% de-chlorinated tap water (synthetic) and used as inflow for Filters 1, 2, 3, 4, 7 and 8. The inflow target COD for these experimental wetlands was about 138 mg/l (roughly between 43 and 350 mg/l).

3.4 Water quality analysis

In each fill-draw wetland cycle, a sample of the influent wastewater used to supply each filter was taken, to measure all water quality parameters immediately after pouring it in the wetland filter; these water quality parameters were also measured when the effluent was discharged from the wetland filter at the end of the cycle. All of the water samples

were transported to the laboratory for analyses within 24 h. The procedure for water quality sampling and the appliances used for water quality parameter measurements were determined according to the American Public Health Association (APHA, 2005), unless stated otherwise. Routine water quality sampling of various variables (for sample numbers and frequencies, refer to data illustrations) was conducted to monitor the water quality and examine the performance of the treatment. A spectrophotometer manufactured by HACH Co. (model DR2800, Hach, Loveland, CO, USA) was applied for standard water quality analysis of COD, NH_4-N, NO_3-N, PO_4-P and SS.

The five-day BOD was determined for all water samples with the OxiTop IS 12-6 system, a manometric measurement device, supplied by the Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany. Nitrification was suppressed by adding 0.05 ml of 5 g/l N-Allylthiourea (WTW chemical solution No. NTH600) solution per 50 ml of sample liquid.

Turbidity was measured with a Turbicheck Turbidity Meter (Lovibond Water Testing, Tintometer Group, The Tintometer Limited, Lovibond House, Solar Way / Solstice Park, Amesbury, UK, www.lovibond.com). The redox potential for all water samples was measured using a VARIO pH meter (Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany). The electrical conductivity for all water samples was measured using a Mettler-Toledo AG (Schwerzenbach, Switzerland) conductivity meter. The pH was measured with a sensION+Benchtop Multi-Parameter Meter (Hach Lange, Düsseldorf, Germany). Water pH was within the allowable range of 4-9.5, suitable for the survival of most bacteria (Kadlec & Wallace, 2009). The dissolved oxygen was determined using a Hach Lange HQ30d dissolved oxygen meter (Salford, England, UK).

All analyses of water samples for trace elements were performed using a Varian 720-ES Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES; Agilent Technologies UK Ltd, Wharfedale Road, Wokingham, Berkshire, UK). The analysis was undertaken to determine nutrient and trace element concentrations. Water samples of 50 ml were preserved in glassware bottles at 4 °C (EPA, 1994). The samples were then acidified, if appropriate, by adding 1 ml of 70% concentrated nitric acid to dissolve any suspended material in order to extract heavy metals and to reduce the pH to below 2, which was required for analysis. The samples were then filtered through a filter paper with a diameter of 0.45 µm before analysis by ICP–OES.

According to standard laboratory methods, all meters and their sensors were regularly calibrated and maintained their necessary solutions to be ready for measurements accordingly. Calibration for all equipment used in water quality measurement was performed when necessary, as instructed in the user manuals. For more details about the water quality parameter measurement procedures conducted in this research, readers may refer to Appendix C.

Temperature data for the first year of operation of the wetland system were recorded outside and in the shade at an official weather station in Woodford located south-east of Salford. The raw data were supplied by the UK MetOffice (MetOffice, FitzRoy Road, Exeter, Devon, UK) (www.metoffice.gov.uk). Concerning the remaining operational periods, temperature measurements were monitored using a Thermometer-Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany) and performed by project team members inside and outside the greenhouse environment.

3.5 Wetland plant growth monitoring

In each wetland filter, wetland plants "*Phragmites australis*" growth parameters including: stem thickness and related density, canopy height and above ground biomass were monitored before and after the diesel spill (Al-Isawi et al., 2015a). Concerning the wet and dry weight parameters for plants in each filter, the above ground biomass plant was randomly selected from each filter. The biomass was sampled during the period of peak standing crop and completely rinsed with distilled water to remove the adhering water and sediments. The water was then absorbed in tissue paper to record the wet weight. Thereafter, the plant was dried in an oven at 70 °C for 72 h before determining the dry weight. The dead above-ground biomass for each wetland filter was harvested, cut into 2-cm long pieces at the end of each winter, and subsequently returned to the corresponding filters by placing it on top of the litter zone. Munsell colour charts were used to determine the leaf colour (Munsell, 1977) .

3.6 Petroleum hydrocarbon selection

In this study, diesel has been chosen as a model to assess the capability of the wetland system to remove petroleum hydrocarbon compounds (Al-Isawi et al., 2015b). Due to global technological development, use of diesel has increased, mainly as a fuel for many forms of transport (such as road vehicles, ships and trains) and also in electricity generators which use certain types of diesel (Agarry & Latinwo, 2015). Diesel spills are found in the environment as a result of accidental release from an industrial site, transport vehicle or drilling (Al-Baldawi et al., 2015a). Other activities involved in processing diesel can result in events such as pipeline breaks, well blowouts, tank leaks, and ship collisions. Water runoff from land also carries diesel into groundwater (Lee et al., 2005).

Diesel is considered as one of the most toxic and carcinogenic impacts on the ecosystem even in small concentrations (Wake, 2005) and its spills can create organic pollutants which can cause detrimental effects to human health (Astm, 1995; McMillen et al., 2001; Moreira et al., 2011). Moreover, diesel is a hazardous fuel because of its content of water-insoluble components and its gradual migration from water to the wetland beds (Zhang et al., 2013).

Some conventional treatment technologies, such as hydro cyclones, flotation, coalescence and centrifuges, are expensive, can lead to incomplete hydrocarbon decomposition such as diesel (Das & Chandran, 2011), and are not efficient in removing dissolved hydrocarbon compounds from polluted water (Lin & Mendelssohn, 2009).

Diesel composition is characterized by a low volatilization rate as compared to other types of fuels such as kerosene and gasoline and therefore, using biological treatment, micro-organisms and/or plants to assess diesel pollutant treatment is more appropriate (Truax et al., 1995).

Diesel compounds have long been recognized as a source of urban air pollution considered as one of the groups that contribute to photochemical ozone and secondary organic aerosols (SOAs) formation which in turn lead to increasing greenhouse effect, global warming, acid rain, smog, and shift in climatic conditions (Chauhan et al., 2016; Seinfeld & Pandis, 2016).

3.7 Petroleum hydrocarbon analysis

Total petroleum hydrocarbons (TPH) were determined by gas chromatography and flame ionization by Exova Health Sciences (Hillington Park, Glasgow, UK) according to their own accredited “TPH in Waters (with Aliphatic/Aromatic Splitting) Method” (Exova

Health Sciences, 2014) which is accredited to the British Standard (BS) method BS EN ISO IEC 17025 by the United Kingdom Accreditation Service and compatible with the International Organization for Standardization (ISO) standards (e.g., ISO17025), BS method BS DD 220 1994, and American Standard methods (United States Environmental Protection Agency (US EPA) Method 3510C and US EPA SW846 Method 8015). For more details of petroleum hydrocarbon measurements, see Appendix D.

In order to assess the natural volatilization process in the wetland filters, 500 ml of pure diesel was poured into an open round container of 10 cm diameter, and kept in the greenhouse to mimic the natural volatilization process after the simulated diesel spill in wetland filters. Another container of 500 ml of diesel was kept in a fume cupboard of the laboratory for comparison (Al-Isawi et al., 2015a).

3.8 Clogging tests and modelling

Overall hydraulic conductivity measurements to assess the severity of clogging were performed. Each column was regularly filled with wastewater to the top of the debris layer and subsequently emptied after a resting time of two hours to allow for air bubbles to escape the media. The time taken to drain each column and the associated water volume captured were noted.

The average hydraulic conductivity was calculated by using Eq. 3.1 (Darcy's Law). Darcy's law is generally applied to define the water flow through porous media. For a constant flow rate, the hydraulic gradient between an upstream and downstream point must increase as clogging decreases the hydraulic conductivity. For vertical-flow constructed wetlands, Darcy's law can be described by Eq. 3.2 (Hillel, 1998).

$$K = \frac{Q \times L}{A_w \times (h_1 - h_2)} \quad \text{..... (3.1)}$$

where K (m/d) is the saturated hydraulic conductivity of the media; A_w (m^2) is the wetted cross-sectional area of the reactor in the axial flow direction; Q (m^3/d) is the flow rate; L (m) is the distance between an upstream and a downstream point in the axial flow direction; h_1 (m) is the water depth at the upstream point; and h_2 (m) is the water depth at the downstream point.

$$u = -K \frac{\partial H}{\partial z} \quad \text{..... (3.2)}$$

where u is the velocity of the flowing solution, K is the hydraulic conductivity, H is the water head, and z is the elevation.

SS samples were taken from the inflow wastewaters, the layer of debris on top of each filter, the eight sampling ports of each column and the outflows. The results were used as input data for the upgraded mathematical Wang-Scholz model (Sani et al., 2013b; Al-Isawi et al., 2015a; Meyer et al., 2015), which is a one-dimensional model originally developed to simulate the evolution of the liquid-solid mixture, addressing the mechanisms of diffusion, sedimentation and adsorption. Solutions to the model were obtained by using finite elements. Simulations were performed for different time periods. Some coefficients were initially selected for sedimentation, damping, adsorption and diffusion. The modelling output was subsequently compared with the experimental findings.

Meyer et al. (2015) undertook a comparative review of the scope and aims of a wide range of constructed wetland models. The Wang-Scholz model was the most suitable model for this case study, because it is the only suitable model concerned with solid deposition in vertical-flow wetlands treating urban wastewater. Furthermore, the model has already been previously calibrated for the same wetland system (Sani et al., 2013b; Al-Isawi et al., 2015a) using earlier data.

The Wang-Scholz model (Eq. 3.3; Massoudieh et al. (2008)) was applied to simulate both settling and aggregation mechanisms. By neglecting the effect of the varied sizes of SS and the lateral flow along the walls of the filters, Eq. 3.3 can be simplified to Eq. 3.4. The mechanical dispersion of SS can be described with Eq. 3.5.

$$\frac{\partial \varphi_i}{\partial t} = D\frac{\partial^2 \varphi_i}{\partial z^2} - (u - v_i)\frac{\partial \varphi_i}{\partial z} \pm \psi_i + \frac{q(z)}{A}\varphi_{i,in} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

where φ_i is the concentration of SS with particle sizes of range i; t is time; D is the dispersion coefficient; z is the vertical elevation position; u is the vertically flowing water velocity (positive upward); v_i is the fall velocity or settling velocity of the SS of particle size i; ψ_i is the source or sink term of the SS of particle size i and is used to take account of the effect of the aggregation or break-up of particles; q(z) is the lateral inflow to the wetland; A is the wetland area; and $\varphi_{i,in}$ is the concentration of the SS of size i in the lateral flow.

Applying the model described by Eq. 3.3 for vertical-flow constructed wetland systems, the particles sizes and the lateral flow are not accounted for. As a result, the effects of aggregation and break-up of SS particles will be reflected by the dispersion coefficient and the settling velocity. A modified mass conservation governing model is expressed in

Eq. 3.4, which requires four sub-models for the dispersion coefficient D, the vertically flowing water velocity u, the fall velocity v and the source or sink term R.

$$\frac{\partial \varphi}{\partial t} = D\frac{\partial^2 \varphi}{\partial z^2} - (u - v)\frac{\partial \varphi}{\partial z} + \dot{R} \quad \text{..... (3.4)}$$

where φ is the concentration of SS particles of all sizes within the treated wastewater; t is time; D is the dispersion coefficient; z is the vertical elevation position; u is the vertically flowing water velocity (positive upward); v is the fall velocity or settling velocity of the SS; and $\dot{R}$ is the sink term of suspended solids particles due to the physical adsorption on the surface of the bulk mass (e.g., biomass, sediment and pebbles) within the constructed wetland bed.

$$D = \alpha \cdot u \quad \text{..... (3.5)}$$

where D is the dispersion in static water; α is the dispersivity; and u is the convection velocity of the flowing water, which, for continuous flow, may be estimated using Darcy's law (Eq. 3.2).

A simplified model step representing the settling velocity v of SS particles is shown in Eq. 3.6. Equation 3.7 (Richardson & Zaki, 1954) represents a hindered settling function. The parameter n (Eq. 3.7) has the value 5.1 (Rowe & Babcock, 2007) representing aggregate properties comparable to those in the experiment discussed in this research.

$$v = w_0 f \quad \text{..... (3.6)}$$

where *v* is the settling velocity of SS particles; w_0 is the average terminal settling velocity of isolated particles; and *f* is the hindered settling velocity.

$$f = (1-\varphi)^n \quad \text{..... (3.7)}$$

where f is the hindered settling velocity; φ is the total particle fraction or concentration; and n is an empirical parameter.

In order to solve Eq. 3.4, $\dot{R}$ needs to be known. The Monod reaction kinetic rate has been applied to simulate biomass growth in relevant systems (Langergraber, 2007; Soleimani et al., 2009). Equation 3.8 relates particle absorption to the growth of biomass. To solve Eq. (3.8), Eq. (2.2) was applied to estimate biomass growth.

$$\dot{R} = -M_{bss} q_m \frac{\varphi}{\varphi_s + \varphi} \quad \text{..... (3.8)}$$

where $\dot{R}$ is the sink term of suspended solids due to the physical adsorption on the surface biomass; M_{bss} is the biomass concentration (Eq. 2.2); q_m is the maximum adsorption rate; φ is the total particle fraction or concentration; and φ_s is a constant representing the particle concentration in wastewater when the growth rate is the half of the maximum value q_m.

The model introduced above has been applied to simulate the SS sedimentation processes within the experimental wetland filters. Values obtained from the above literature have been used for parameters where no measurements were available. Moreover, appropriate assumptions regarding the boundary conditions, which are subject to underlying mechanisms and the operation of the filters, have been made.

3.9 Experimental set-up for performance comparison between artificial ponds and constructed wetlands

3.9.1 Artificial pond set-ups

The pond system was located on top of the roof of the Newton Building, which is part of The University of Salford, Greater Manchester, UK, (Fig. 3.7). The set-up includes four types of treatment filters (Fig. 3.8). Table 3.3 shows an overview of the experimental set-up applied to test systematically the impact of three variables. Ponds 1 to 3 compared to Ponds 4 to 6 are used to test the impact of an elevated loading rate in terms of COD. The application of lower contact time is assessed between Ponds 1 to 3 and Ponds 7 to 9. Finally, the impact of a lower resting time is assessed by comparing performance differences between Ponds 7 to 9 and Ponds 10 to 12.

Each pond set comprising three ponds is different in design. The first pond contains only wastewater. The second pond comprises both wastewater and *P. australis*. The last one contains wastewater and *P. australis*, and is subjected to aeration. So, there are 12 ponds for 4 sets. In order to maintain experimental authenticity, another 12 ponds are used as corresponding replicates. During the start of the experiment, an equal quantity of *P. australis* (80 g of rhizomes i.e. wet weight) which contained around 90 nodes was introduced to the relevant ponds (2,3,5,6,8,9,11,12).

Twenty-four cylindrical buckets (partly buried to avoid overheating; see also Fig. 3.7) made-up of black plastic polymer with inner bottom and top diameters of 16 cm and 24 cm, respectively, and a height of 30 cm were used. The cylindrical buckets were placed inside large soil-filled concrete containers at 80% of their height, so as to simulate the

natural conditions of ponds and to avoid contamination by the surrounding soil due to rain-splashing activity.

3.9.2 Vertical-flow constructed wetland set-up

Five filters from the vertical-flow constructed wetlands are used in the comparison study. The set-up of these wetland filters, which are located within a greenhouse on top of the roof of the Newton Building, which is part of The University of Salford, Greater Manchester, is shown in Figure 3.7. The system comprises four filters (Fig. 3.8) and has been in operation since 27 June 2011.

Table 3.3 demonstrates the statistical experimental set-up applied to test the impact of three variables: (1) contact time; (2) rest time; and (3) loading rate. Wetland filter F6 can be compared to wetland filter F4 to assess the effect of a higher loading rate. The application of a lower contact rate is assessed through comparing wetland filter F7 with wetland filter F4. The impact of resting time is determined through a comparison between wetland filters F7 and F8. For more details about the wetlands system, see section 3.2.

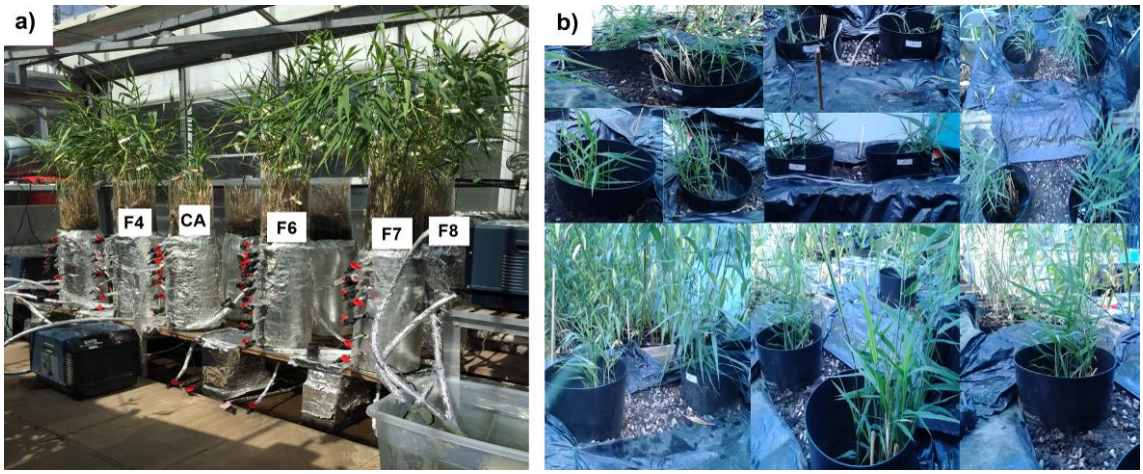


Figure 3.7: Photograph (taken on 16 September 2015) of the experimental set-up of both the vertical-flow constructed wetland and pond systems in Salford: (a) the wetland set-up (F4-Wetland filter 4; F6-Wetland filter 6; F7-Wetland filter 7; F8-Wetland filter 8; and CA-Control A received tap water) and (b) the artificial pond set-up.

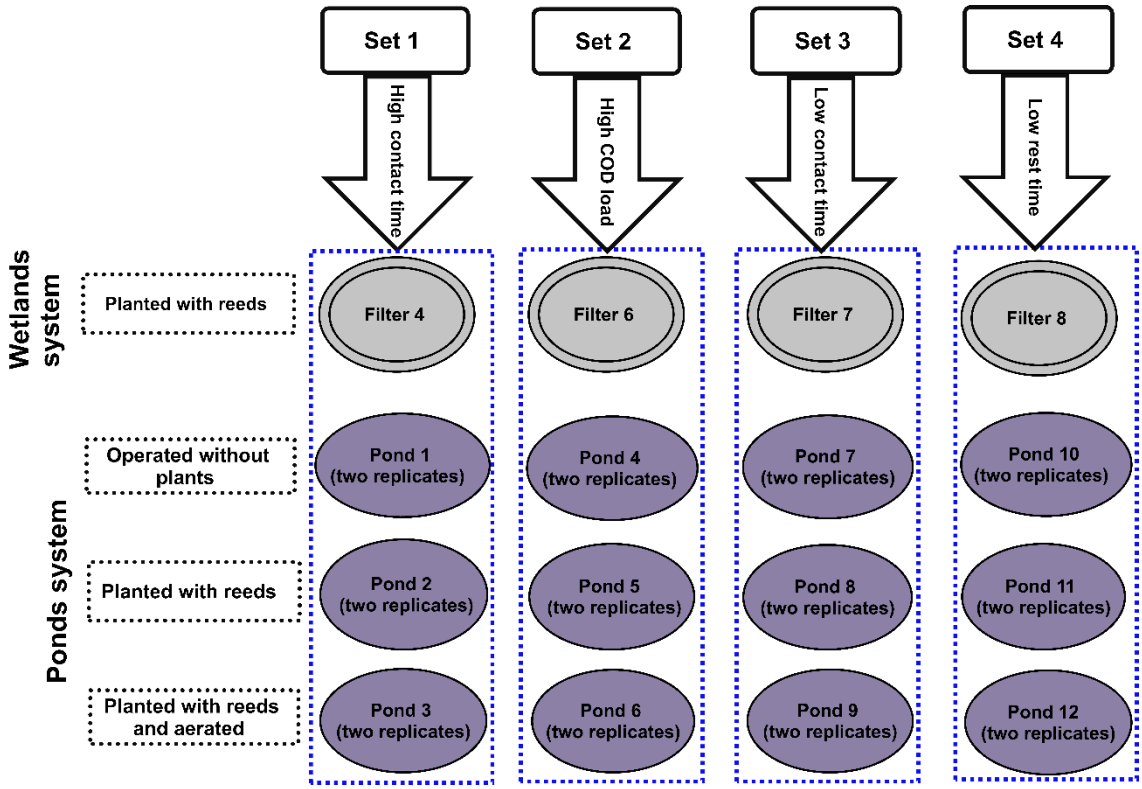


Figure 3.8: Set-up of both the vertical-flow constructed wetland and pond systems in Salford shows the comparison performance between four sets: (Set 1) high contact time; (Set 2) high COD load; (Set 3) low contact time; and (Set 4) low resting time.

Table 3.3: Comparison of the statistical experimental set-up for both wetland and pond systems.

Wetlands systems						
Design and operational parameter	Unit	Filter 4 (F4)	Filter 6 (F6)	Filter 7 (F7)	Filter 8 (F8)	Control A (CA)
Contact time	h	72	72	36	36	72
Resting time	h	48	48	48	24	48
Chemical oxygen demand	mg/l	203.5	404.8	203.5	203.5	2.3
Ponds systems						
Design and operational parameter	Unit	Ponds 1–3 (P1–3)	Ponds 4–6 (P4–6)	Ponds 7–9 (P7–9)	Ponds 10–12 (P10–12)	
Contact time	h	72	72	36	36	
Resting time	h	48	48	48	24	
Chemical oxygen demand	mg/l	203.5	404.8	203.5	203.5	

Note: All wetland filters were planted with *Phragmites australis*. The yearly average wastewater inflow volumes to the wetland systems were as follows: F4,6, 475 l/a; F7, 680 l/a; F8, 949 l/a. P1,4,7,10 were operated without plants. P2,5,8,11 were planted with *P. australis*. P3,6,9,12 were planted with *P. australis* and aerated. Each pond had two replicates.

3.9.3 Operation method for vertical-flow constructed wetlands and pond systems

The comparative study has been carried out for three months (13 July to 13 October 2015) to assess the effluent water quality and removal efficiency of mature wetlands and immature ponds. Both wetland and pond systems were fed with 6.5 l of inflow water during the feeding phase. Wetland operation was different between filters (Fig. 3.7 and Table 3.3). Wetland filters F4 and F6 were tested after 72 h of contact time and subsequently left to rest for 48 h, while Wetland filters F7 and F8 were sampled after 36 h of contact time and left to rest for 48 h and 24 h, respectively.

Pond operation was different between the four systems (Table 3.3). Ponds 1 to 3 and Ponds 4 to 6 were sampled after every 72 h of contact time and subsequently left to rest for 48 h, while Ponds 7 to 9 and Ponds 10 to 12 were tested after 36 h of contact time and left to rest for 48 and 24 h, respectively.

All water quality parameters were obtained during or directly after taking samples. The COD content of the inflow was the same for both wetlands and ponds (Table 3.3).

The preliminary treated domestic wastewater applied as the inflow water for both treatment systems (ponds and wetlands) was delivered by the Davyhulme Sewage Works. Fresh urban wastewater was obtained once per week, and was stored and aerated by common aquarium air pumps in a cold room with temperatures of around 4±0.10 °C before application. The water quality was rather variable, which comprised mainly domestic but also industrial wastewater, both diluted by surface water runoff.

The COD was applied as the criterion to distinguish between low and high loads applied to both systems (Table 3.3). An inflow COD (raw domestic wastewater without dilution) of approximately 405 mg/l (commonly between 236 and 629 mg/l) was applied for Wetland filter 6 characterized by a high loading rate. To attain low loading rate synthetically, the raw wastewater was diluted with 50% dechlorinated tap water and used as inflow for Wetland filters 4, 7 and 8. Inflow COD for these experimental wetlands was about 204 mg/l (roughly between 118 and 314 mg/l). In comparison, the same modality was used for the ponds system. An inflow COD of about 405 mg/l (frequently between 236 and 629 mg/l) was chosen for Ponds 4 to 6 which represent a high loading rate. The remaining Ponds 1 to 3, Ponds 7 to 9 and Ponds 10 to 12 received wastewater diluted with 50% dechlorinated tap water. The inflow COD for these systems was about 204 mg/l (roughly between 118 and 314 mg/l).

3.10 Experimental set-up and boundary conditions of chilli plants in greenhouse environment

3.10.1 Selection of fruiting vegetables

Chillies were chosen to assess the usability of the vertical constructed wetlands treating domestic wastewater for irrigation purposes. Chilli (De Cayenne; *Capsicum annuum* (Linnaeus) Longum Group 'De Cayenne') is a good crop, often seen as ideal, for growing in greenhouses. This plant is usually easy-to-grow, cost-effective and has a good nutritional value (Nickels, 2012).

The literature indicated that there is no risk of microbiological contamination for chillies, which are not growing in direct contact with soil and/or irrigation wastewater (Cirelli et al., 2012). This is particularly true for the edible parts (Norton-Brandão et al., 2013; Christou et al., 2014).

Chilli (De Cayenne), as part of the verve brand (product code: 362387), was supplied by B&Q plc (Chandlers Ford, Hampshire, England, UK). All seeds were bought on 10 February 2014. The chilli planting periods were: (a) germination period; (b) first planting after germination period; (c) first replanting period before fruiting; (d) second replanting period after the development of the first set of fruits; and (e), second replanting period after fruiting (i.e. second diesel spill on 26 September 2014). The first dose of diesel fuel was added on 26 September 2013 (Al-Isawi et al., 2015b).

3.10.2 Growing of chillies

Germination stage: In this experiment, 288 seeds were sown thinly in a propagator (verve; B&Q plc) into seed and cutting compost (verve; B&Q plc) and covered with 6

mm of compost on 12 February 2014. Each propagator contained 72 planting cells with an average depth of 5 cm (only planted up to about 4 cm; measured before initial watering) and square sides of approximately 3.5 cm. The compost comprised 58% sustainably sourced Sphagnum (peat moss) and unspecified amounts of composted bark, green compost, wood fibre and coir (normal fibre mined from the pod (outer shell) of coconuts), and oyster shells (optional), vermiculture (optional), clay (optional), charcoal (optional), perlite (optional), sand (optional), shingle (optional), wetting medium (to keep moisture). Essential nutrients and trace minerals (lasting for approximately six weeks) were also part of the product. The remaining 42% comprised among other components more than 48% non-peat composted organic material for example dolomitic limestone, fertilizer, and a combination of composted foliate waste and consumed brewery grains.

The propagators were located within a dark incubation room. The compost was kept moist until the seeds germinated. The transparent covers of the propagators were usually, kept above the propagator bases. In the period of plant germination, the temperature was maintained between 16.5 and 20.2 °C (average of 19.8 °C).

First planting after germination: Germination of some seeds was noticed on 10 March 2014. All pots were relocated to a lab fitted with OSRAMHQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights provided by OSRAM (North Industrial Road, Foshan, Guangdong, China) and supported by a H4000 Gear Unit, which was supplied by Philips (London Road, Croyden CR9 3QR). The bulbs were relatively similar to those applied by Boyden and Rababah (1996).

The lights were set on timers, simulating sunrise and sunset times in Greater Manchester (http://www.timeanddate.com). Light was measured using the lux meter ATP-DT-1300 (TIMSTAR, Road Three Industrial Estate, Winsford, Cheshire, England, UK) for the

range between 200 lux and 50,000 lux. Just above the top of the plants, values between 3,855 and 12,316 lux (mean of 6,921 lux) were recorded. Humidity and temperature were monitored by a Thermometer-Hygrometer-Station provided by wetterladen24.de (JM Handelspunkt, Geschwend, Germany). The temperature was controlled using an electrical heater, Rhino H029400 TQ3 2.8kW Thermo Quartz Infrared Heater 230V, supplied by Express Tools Ltd. (Alton Road, Bournemouth, England, UK). The humidity was artificially increased by five humidifiers (Challenge 3.0 L Ultrasonic Humidifier; Argos, Avebury Boulevard, Central Milton Keynes, England, UK). The observed relative humidity ranged between 68% (±10.7%) and 87% (±4.6%).

The temperatures above the plants ranged between 15.7 and 29.7 °C (average of 26.9 °C). The propagator covers were kept on top of the corresponding bases (gap of about 6 cm) until the first seedlings reached the covers on 15 March 2014 (Fig. 3.9).

Figure 3.9: Experimental set-up of chilli plants: first planting after germination period.

Second planting: The second planting of the strongest 120 chilli plants took place when the majority of seedlings had at least two true leaves, which was around 8 April 2014. The remaining weakest 168 chilli plants were not used. Thirty chilli plants either did not germinate or died before replanting. All plants were relocated into the greenhouse (same place as the wetland system is located) (Fig. 3.10). Temperature and relative humidity were monitored with the same device used in the first planting period.

Chillies were replanted individually into 10-litre plastic, round plant pots provided by scot plants (Hedgehogs Nursery, Crompton Road, Glenrothes, Scotland, UK). The pot dimensions were 22.0 cm for height, 22.0 cm for the bottom diameter and 28.5 cm for the top diameter. The top 2 cm were not planted. Chillies were planted to a depth of 17.5 cm and covered by 2.5 cm of bark (B&Q verve range) based on mixed wood. Some of these pots received wetland outflow water.

Chilli trees were firstly braced by small bamboo canes (diameter of approximately 0.3 cm; length of up to 30 cm) and afterwards, when chillies started maturation stage, larger bamboo canes were used (diameter average of 0.8 cm; range between 0.6 and 1.2 cm; length of up to 150 cm) if and when required. Moreover, to maintain plant stability, string was used to lightly tie the main stem beside the cane (Fig. 3.10).

Domestic cultivars were carefully chosen to maximize self-fertility. In an open-air setting, airstream or insects afford adequate motion to yield commercially feasible harvests (Jones, 2013). Thus, manual movement of the plants and physical pollen transference between plants was applied in this research.

The statistical evaluation of different types of irrigated water was used in this selected experimental set-up, such as the effect of minerals and nutrients in the wastewater on the

chilli plants growth, and enabling comparison with other contaminated and contamination-free water sources impacts.

At the end of the growth season, trace minerals and potentially poisonous pollutants for chilli fruits were analysed for a randomly selected number of fruits. Chilli plant analysis was performed (Plank, 1992) using a dried weight of >0.3 g for digestion. The dried samples were ground to a fine powder in a James Martin ZX809X Spice and Coffee Grinder (WAHL Global, Herne Bay Trade Park, Sea Street, Kent, UK). Samples were turned into white ash in a carbolite muffle furnace at 550 °C for 4 h. The ash samples were dissolved in 7 ml of 70 % concentrated nitric acid. Thereafter, the samples were diluted with deionised water up to 25 ml and transferred into 15-ml polystyrene tubes to be examined by a Varian 720-ES ICP-OES analysis.

Figure 3.10: Experimental set-up of chilli plants in the greenhouse environment (second planting period).

3.10.3 Irrigation water sources

Chillies were grown between 12 February and 24 December 2014. Table 3.4 outlines the experimental design regarding plant number allocations after replanting in compost covered by bark. Different water sources were collected and used as irrigation water within the greenhouse location (Fig. 3.11). The plants were grouped into four sets according to the sources of irrigated water:

Set 1: This set included 24 plants (each six plants were replicate) irrigated with outflow water from filters contaminated with diesel (Filters 1, 3 and 5 as well as Control A).

Set 2: This set included 36 plants (each six plants were replicate) irrigated with effluent from filters without diesel contamination (Filters 2, 4, 6, 7 and 8 as well as Control B).

Set 3: This set included 18 plants (each six plants were replicate) irrigated with contamination-free water sources. Tap water (T) was collected directly from the greenhouse taps in Newton Building at Salford University. Deionized water (D) was purified and distilled with (ULTRAPURE system) equipment which produces high purity water for lab purposes. These two types of irrigated water were used to monitor the depletion of nutrients and trace elements provided by the organic media. Tap water with fertilizer (0.7 ml/l) (T+F) which the liquid fertilizer (concentrated fruit and vegetable) was from the B&Q verve range with a nitrogen to phosphorus to potassium ratio of 4 to 4 to 4. The total nitrogen constituent was 4%. Nitric nitrogen and ureic nitrogen portions were 1.1% and 2.1%, respectively. Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) made up 4% each. However, the corresponding P and K contents were simply 1.7% and 3.3%, respectively. This type of irrigated water was used to assess the effect of artificial fertilizer on growth of plants.

Set 4: This set included 42 plants (each six plants were replicate) irrigated with different wastewater sources. Preliminarily treated wastewater (WW) was obtained from the United Utilities Davyhulme wastewater treatment plant (http://en.wikipedia.org/wiki/Davyhulme). Diluted wastewater (WW+T) was produced by mixing one part preliminarily treated wastewater (as described previously) with four parts tap water. River water (RV) was collected freshly directly from the River Irwell (located directly east to the main campus of The University of Salford). Rain water (RA) was collected from the roof of the greenhouse (located on top of the Newton Building, The University of Salford) via gutters discharging into a clean plastic tank. Gully pot water (GP) was randomly collected freshly from manholes located on The University of Salford campus. Gully pot waters were filtered using a sieve with a diameter size of 250 µm. Two grey water types were used for irrigation of the chillies: real grey water (RG) and artificial (synthetic) grey water (AG). The real grey water was collected freshly from the private property of the author (located in Withington, south-east of Salford) and used directly for irrigation purposes. In comparison, the artificial grey water was prepared according to the suggested recipe by Nghiem et al. (2006) by using the following compounds: humic acid (20 mg/l), cellulose (50 mg/l), kaolin (50 mg/l), calcium chloride (0.5 mM or 20 mg/l of calcium), sodium chloride (10 mM) and sodium bicarbonate (1mM at pH 8). All chemicals used for preparing the synthetic grey water were supplied by Fisher Scientific UK Ltd, Loughborough, England, UK. These types of irrigation wastewater were used to study the impact of high nutrients and trace elements, that are provided naturally with wastewater, on growth and production of plants.

Table 3.4: Experimental design in terms of chilli plant (in greenhouse) number allocations after replanting in compost covered by bark.

Inflow source	Chilli number	Diesel contaminated	Chemical oxygen demand	
			mean $g/m^2.day$	Standard deviation $g/m^2.day$
Filter 1 outflow	C1;C2;C3;C4;C5;C6	Yes	0.38	0.005
Filter 2 outflow	C7;C8;C9;C10;C11;C12	No	0.12	0.003
Filter 3 outflow	C13;C14;C15;C16;C17;C18	Yes	0.46	0.014
Filter 4 outflow	C19;C20;C21;C22;C23;C24	No	0.12	0.002
Filter 5 outflow	C25;C26;C27;C28;C29;C30	Yes	0.49	0.009
Filter 6 outflow	C31;C32;C33;C34;C35;C36	No	0.15	0.008
Filter 7 outflow	C37;C38;C39;C40;C41;C42	No	0.09	0.002
Filter 8 outflow	C43;C44;C45;C46;C47;C48	No	0.16	0.006
Control A outflow	C49;C50;C51;C52;C53;C54	Yes	0.28	0.009
Control B outflow	C55;C56;C57;C58;C59;C60	No	0.03	0.001
Deionized water	C61;C62;C63;C64;C65;C66	No	0.00	0.000
Tap water (100%)	C67;C68;C69;C70;C71;C72	No	0.01	0.003
Tap water with fertilizer (0.7 ml/l)	C73;C74;C75;C76;C77;C78	No	0.01	0.003
Wastewater (20%); tap water (80%)	C79;C80;C81;C82;C83;C84	No	0.17	0.669
Wastewater (100%)	C85;C86;C87;C88;C89;C90	No	0.89	0.012
River water	C91;C92;C93;C94;C95;C96	No	0.02	0.00
Rain water	C97;C98;C99;C100;C101;C102	No	0.05	0.00
Gully pot	C103;C104;C105;C106;C107; C108	No	0.06	0.001
Artificial grey water	C109;C110;C111;C112;C113; C114	No	0.92	0.011
Real grey water	C115;C116;C117;C118;C119; 120	No	0.27	0.005

Note: Original seed planting reference numbers; Chilli (C1–C120).

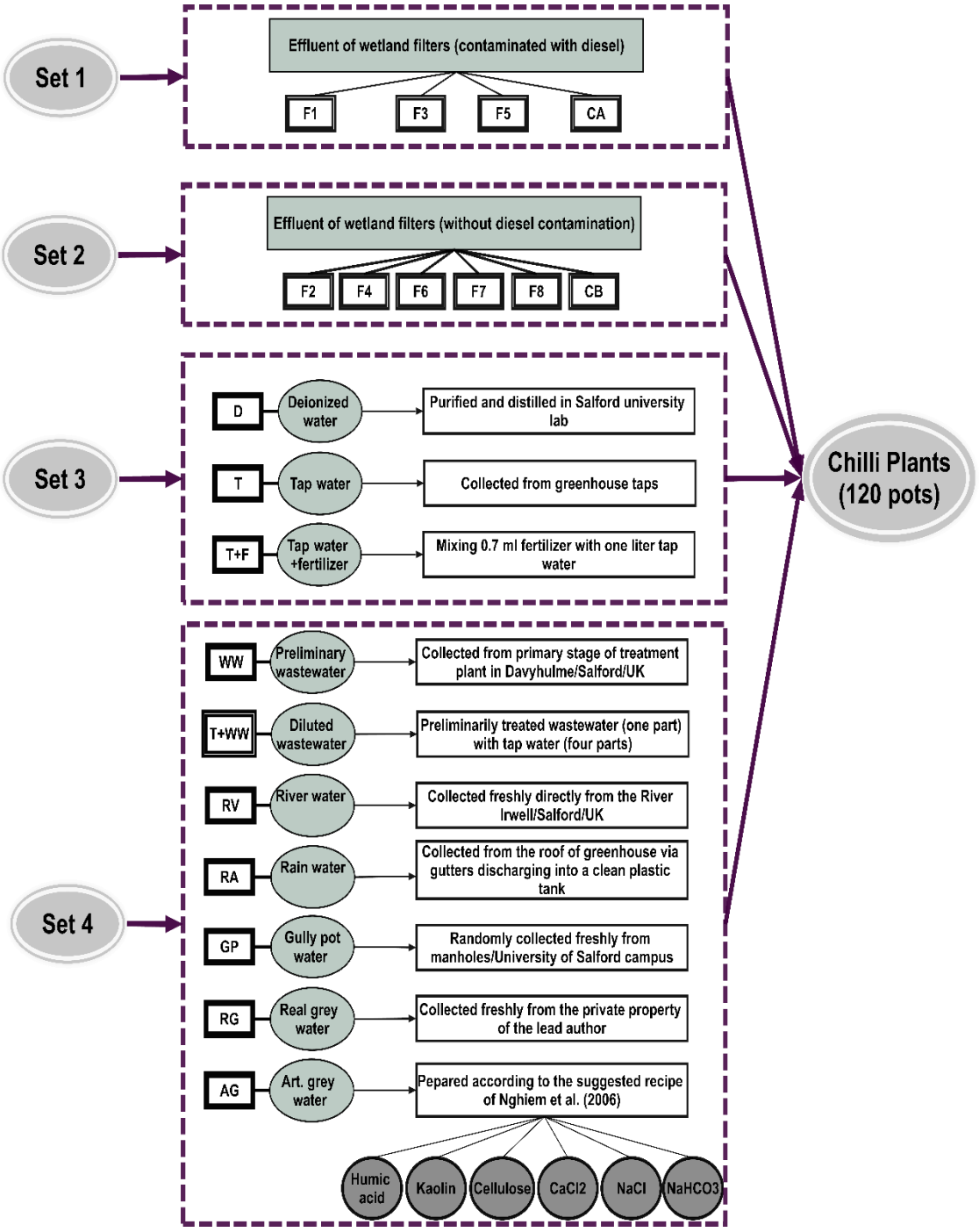


Figure 3.11: A schematic diagram for irrigation water sources used for chilli plants in greenhouse environment.

3.11 Statistical analysis

In the experiments of this study, the removal efficiencies for each wetland filter were calculated from the difference in concentration between the influent and effluent of the CWs. The pollutant concentration removal percentages (R (%)) in terms of all water quality parameters were chosen to be the response parameters to evaluate the treatment efficiency of the system and were calculated as

follows:

$$R\ (\%) = (C_{in} - C_{out})/C_{in} \times 100 \quad \ldots\ldots\ldots \quad (3.9)$$

where C_{in} is the influent pollutant concentration (mg/l) and C_{out} is the effluent pollutant concentration (mg/l).

After data collection, data were subjected to a normality test before validation and subsequent analysis. Because of high variability, the data were not normally distributed even after transformations with transformers such as arc sine, square root, log, etc. and as a result, simple statistical tools that will fit the abnormal distributed data such as non-parametric tools were sought and applied. Microsoft Excel (www.microsoft.com) was used for general data analysis unless stated otherwise. The IBM SPSS v22 (IBM Corp., 2013) package was applied to perform the correlation analysis between variables and to assess statistical differences between treatments. Matlab (www.mathworks.co.uk) was used to analyse the computed data from the Wang-Scholz model and compare them with experimental data.

3.12 Experimental research limitations

In this research, in spite of the fact that the experimental constructed wetlands used are not similar to large-scale systems used in practical field activities, some studies accomplished based on comparable wetland filters (Babatunde et al., 2011; Sani et al., 2013b; Sani et al., 2013a; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b) were noted to explain results appropriate to field scales and consequently passable by the scientific public.

The wetland filters evaluated in this experimental study are in the greenhouse environment with semi-controlled conditions and cannot be directly compared with other wetland systems in real field environments. However, the obtained results of the study can help and be used as a model serve in designing, operating and scaling of new wetland systems in different environments. Additionally, since wetlands in real life conditions employ a large land area combined with plentiful natural energy feeds to construct a self-sustaining structure, resulting in a favourable environment for numerous types of microbes due to their diverse microenvironments, the experimental wetland filters set-up applied in this research could not represent the real requirement of the massive land area involved in the real field scale. Additionally, real, large constructed wetlands may accommodate numerous types of animals which will have an effect on the processes happening in the wetland which are not accrued in these small wetlands.

Due to a lack of sufficient resources and space to construct the essential number of replicates for this experimental study, some of the wetland filters, like Filters 1 and 2, 3 and 4, 5 and 6, and Control A and B, are replicated while Filters 7 and 8 are not. Moreover, one filter from each replicates has subjected to petroleum hydrocarbon dosages after two

years of operation of the experiment wetland filters, so the wetlands system applied in this research could not represent the real wetland environment with full set-ups, however many studies have been performed using similar wetland filters (Almuktar et al., 2015b; Al-Isawi et al., 2016a; Al-Isawi et al., 2016b) and have been accepted by the scientific community.

The direct measurement of clogging was not appropriate within the small experimental wetlands system since this could affect and terminate the wetlands work. Moreover, the experimental wetlands are used frequently by other (under- and post-graduate) researchers for their assignments. Accordingly, indirect methods of clogging measurements are used such as hydraulic conductivity, suspended solids and turbidity concentrations, in different layers of the wetland filters and the outflow waters, to assess clogging evolution.

The results of growing chillies show a partially incomplete picture of the recycling of treated wastewater for irrigation purposes, because microbiological parameters were not fully studied. However, microbial contamination of chillies is improbable due to the fairly long distance between the chilli fruits and the possibly contaminated soil (Cirelli et al., 2012; Almuktar et al., 2015a; Al-Isawi et al., 2016c). Additionally, chilli plants receiving treated wastewater with constructed wetlands can be implied as safer than those receiving preliminary treated domestic wastewater. Moreover, domestic wastewater normally lacks the essential amount of potassium for growth of vegetables (Boyden & Rababah, 1996; Almuktar et al., 2015a; Al-Isawi et al., 2016c). Therefore, potassium could be supplemented at an optimal dosage for the growth of plants. However, the results in this research point out that potassium is adequate in the outflow waters of most wetlands.

3.13 Summary

This chapter describes the experimental wetlands system set-up including each wetland filter design and operation. It also explains the monitoring, sampling, and analysis of water quality parameters, wetland plants growth, clogging and modelling. Furthermore, a description of petroleum hydrocarbon selection in addition to the analytical method used for hydrocarbon compounds determination is elucidated. Comparison performance for wetland filters with new ponds is demonstrated. Lastly, the description for the experimental set up of recycling the treated wastewater for chilli irrigation in greenhouse environment is presented.

4

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Overview

This chapter documents the overall results and discussions for the whole experimental work. Section 4.2 presents the overall treatment performance results for the vertical-flow constructed wetlands system. Section 4.3 presents the variations in seasonal performances for experimental treatment wetlands. Section 4.4 presents the evaluation results of the clogging of each wetland filter based on water quality parameter variations. Important results of the study to examine and demonstrate the treatment performance of hydrocarbon compounds in the constructed wetland systems are documented in section 4.5. Comparison performance evaluation of the mature constructed wetland filters with artificial ponds is presented in section 4.6. The impact of design and operation variables of wetland filters on the growth of chillies and comparison with chillies irrigated from other water sources is explained in section 4.7.

This chapter documents the overall results of the variables involved in the study for performance evaluation of the experimental vertical-flow constructed wetlands. This chapter also aims at advancing the knowledge of treated oily wastewater with constructed wetlands and focuses on thorough understanding of the interaction of internal processes with the components of each wetland filter. Furthermore, the chapter also presents vital results of the study as it examines wetlands effluent performance in recycling as irrigation

for edible crops and presents a classification for the chilli fruits, and the monetary value of harvest plants.

4.2 Long-term treatment efficiency performance of wetland filters

The results and discussions presented in this section have been published in the paper shown below:

Al-Isawi, R.H.K., Scholz, M., Wang, Y. & Sani, A. (2015). Clogging of vertical-flow constructed wetlands treating urban wastewater contaminated with a diesel spill. *Environmental, Science and Pollution Research*. 22, 12779–12803, doi:10.1007/s11356-014-3732-8.

4.2.1 Inflow water quality

The inflow concentration values of water quality parameters examined in all wetland filters for the operation period (about 5 years) were analysed in this section:

- First experimental phase 27/06/11 to 25/09/11;
- Second experimental phase 26/09/11 to 25/09/12;
- Third experimental phase 26/09/12 to 25/09/13;
- Fourth experimental phase 26/09/13 to 25/09/14; and
- Fifth experimental phase 26/09/14 to 22/03/16).

Two petroleum hydrocarbon dosages represented by diesel fuel (one-off dose) were each applied to the wetland system on 26 September 2013 and on 26 September 2014, respectively. The concentrations of the two diesel dosages were 20 g/l and 150 g/l, respectively (Table 3.1). Table 4.1 shows the overall inflow water quality for the five

experimental phases (first experimental phase, second experimental phase; third experimental phase; fourth experimental phase; and fifth experimental phase). The data variability was relatively high, reflecting the use of real domestic wastewater (Sani et al., 2013a; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Variability of the influent can also be linked to shock loads to the sewers, weather conditions, seasonal variation, and dilution of the wastewater by precipitation. Synthetic wastewater was not used to allow for the establishment of a realistic microbial diversity and corresponding dynamics (Sani et al., 2013a; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Artificial wastewater would have been much more stable but difficult to justify for long-term experiments simulating industrial processes as accurately as possible on a small scale (Scholz, 2010, 2015). The chemical oxygen demand (COD) was used as a criterion to discriminate between low and high loads. An inflow target COD of about 277 mg/l (usually between 100 and 660 mg/l) was set for wetland filters with a high loading rate (Filters F5 and F6). The remaining Filters F1 to F4 and Filters F7 and F8 were established to receive raw wastewater diluted with tap water (synthetic). The target inflow COD for these filters was approximately 138 mg/l (usually between 43 and 350 mg/l). Moreover, the COD to biochemical oxygen demand (BOD) ratio of the influent (preliminary treated domestic wastewater) was about 1.85, which is slightly higher than the 1.14 reported in the literature (Stefanakis et al., 2014). This indicates that a substantial part of the organic matter will be easy-to-degrade biologically. Therefore, someone may conclude that the influent has a high biodegradability and can be classified as rather low-strength wastewater.

The average mean concentration values of the raw (undiluted) inflow wastewater for the whole experimental period for COD, BOD, ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), ortho-phosphate-phosphorus (PO_4-P), suspended solids (SS), turbidity (TBD),

pH, redox potential (Redox), electrical conductivity (Conductivity, EC), and dissolved oxygen (DO) were: 277 mg/l, 151 mg/l, 40 mg/l, 4.8 mg/l, 14 mg/l, 153 mg/l, 100 NTU (nephelometric turbidity units), 7.7, -44 mV, 863 µS/cm, and 7 mg/l, respectively. The water quality parameter values for each phase of the experiment period are shown in Table 4.1.

Table 4.1: Inflow water quality for Filters 5 and 6: raw (i.e. before dilution) preliminarily treated urban wastewater mixed with urban runoff (27/06/11 to 22/03/16).

Parameter	Unit	Number	Mean	Minimum	Maximum	Standard deviation
First experimental phase (27/06/2011 to 25/09/2011)						
COD	mg/l	34	356.5	90.0	620.0	185.88
BOD	mg/l	15	21.6	3.0	36.1	9.78
NH_4-N	mg/l	10	0.9	0.2	1.7	0.56
NO_3-N	mg/l	20	9.0	5.7	13.6	2.61
PO_4-P	mg/l	18	209.6	54.0	400.0	138.01
Second experimental phase (26/09/2011 to 25/09/2012)						
COD	mg/l	116	267.7	125.0	620.0	118.25
BOD	mg/l	28	103.3	42.0	150.0	32.60
NH_4-N	mg/l	84	45.2	14.9	86.0	22.66
NO_3-N	mg/l	72	3.4	0.3	14.4	3.86
PO_4-P	mg/l	80	17.0	2.4	40.0	10.88
SS	mg/l	98	77.0	2.4	294.8	68.73
TBD	NTU	36	303.4	90.0	450.0	103.54
pH	n/a	4	7.9	7.8	8.7	0.24
Third experimental phase (26/09/2012 to 25/09/2013)						
COD	mg/l	58	239.8	122.0	390.0	91.39
BOD	mg/l	117	151.2	40.0	330.0	67.83
NH_4-N	mg/l	60	59.1	0.1	131.8	23.44
NO_3-N	mg/l	54	7.7	0.3	20.9	5.94
PO_4-P	mg/l	50	13.0	2.9	32.1	9.11
SS	mg/l	132	232.5	18.0	760.0	177.47
TBD	NTU	98	120.7	6.7	457.0	94.43
pH	n/a	47	7.8	7.3	8.3	0.29
Fourth experimental phase (26/09/2013 to 25/09/2014)						
COD	mg/l	16	248.3	112.0	385.0	79.02
BOD	mg/l	56	114.1	10.0	360.0	78.45
NH_4-N	mg/l	24	35	3.1	70.0	18.06
NO_3-N	mg/l	22	2.6	0.3	14.0	3.32
PO_4-P	mg/l	22	15.3	3.4	27.6	7.08
SS	mg/l	64	142.3	27.0	474.0	98.13
TBD	NTU	56	82.7	11.6	391.0	76.30
pH	n/a	56	7.5	6.3	8.4	0.42
Redox	mV	34	-38.9	-69	3.0	17.56
EC	µS/cm	41	616.3	248.0	790.0	133.60
DO	mg/l	25	6.3	0.1	9.8	3.08
Fifth experimental phase (26/09/2014 to 22/3/2016)						
COD	mg/l	39	284.9	100.0	660.0	144.72
BOD	mg/l	91	181.0	30.0	360.0	68.45
NH_4-N	mg/l	42	26.4	0.0	61.0	15.06
NO_3-N	mg/l	45	5.6	0.2	21.1	7.02
PO_4-P	mg/l	46	11.3	3.8	50.5	7.55

Table 4.1 (cont.)

SS	mg/l	80	138.5	17.0	269.0	67.02
TBD	NTU	75	51.6	3.4	147.0	28.58
pH	n/a	75	7.7	6.6	8.4	0.35
Redox	mV	73	-46.6	-84.0	61.0	21.67
EC	µS/cm	88	983.6	44.0	2400.0	387.80
DO	mg/l	79	7.6	0.3	18.9	3.12

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; EC, electrical conductivity; DO, dissolved oxygen. NTU, nephelometric turbidity unit; and n/a, not applicable. BOD start of measurement on 2 July 2012; TBD start of measurement on 21 June 2012; pH start of measurement on 22 June 2012; EC and Redox start of measurement on 20 February 2014; and DO start of measurement on 01 May 2014.

4.2.2 Comparison of outflow water qualities

4.2.2.1 Comparison of oxygen demand variables (COD and BOD)

Tables (4.2 to 4.6) summarize the overall outflow water quality for all experimental phases. Generally, COD and BOD are used to assess organic matter removal in wetlands. Organic matter decomposition in constructed wetlands can be achieved by both aerobic and anaerobic microbial processes as well as by filtration and adsorption (Garcia et al., 2010; Saeed & Sun, 2012; Stefanakis et al., 2014). The relatively high BOD to COD ratio indicates that the wastewater is easily biodegradable (Table 4.1). Excluding the start-up period and diesel spills contamination, the removal efficiency for all wetland filters was relatively good and improved with time. This is evident by the fact that the microbial activities that are responsible for organic compound biodegradation are improved with time (microbial acclimatization) (Scholz, 2010; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Almuktar et al., 2015b; Scholz, 2015).

For the period after petroleum hydrocarbon spills contamination, a difference in COD values was noticed reflecting the degradation of hydrocarbon compounds that contributed to increase the organic strength of the wastewater. The results for COD removal efficiencies for wetland filters with petroleum hydrocarbon contamination (Filters 1, 3 and 5, and Control A) (Tables 4.5 and 4.6) showed remarkable drops in the periods after pouring diesel as compared with those in the period before hydrocarbon application. Diesel spills resulted in a sharp decline of the removal efficiency (Table 4.5 and 4.6) (Figure 4.1a) because diesel contributes artificially to the COD of the inflow water (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Findings also showed that the treatment performance of filters F1, F3 and F5, and Control A was very poor in the second diesel

spill period as compared with that in the first diesel spill period. This is explained by the impact of the high dosage of petroleum hydrocarbon (150 g/l) that was applied to these filters resulting in an indirect increase of the COD in the inflow wastewater (Table 4.6). However, an estimated third of the diesel volume is likely to evaporate (Scholz, 2010; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). The exact amount of removed diesel is a function of various factors such as mixing efficiency, temperature, plant presence and loading regime. Therefore, it would be flawed to assume that all diesel poured into the filters is actually associated with 100% of the COD equivalent of diesel. Therefore, the calculated removal efficiencies do not take account of the additional COD associated with the diesel spill. It follows that the removal rates for filters subjected to diesel spills are strictly speaking flawed. However, this would also be the case for accidental spills that often go unnoticed in real plant operations. Drops in such filter performances are frequently considered as 'natural fluctuations' (data noise) (Al-Isawi et al., 2015b).

Figure 4.1 provides an overview of the COD distribution for filters with and without diesel contamination over time. It can be noticed that petroleum hydrocarbons such as diesel are associated with high COD values (Figure 4.1a). Regulatory agencies for environmental pollution control put standard limit values on water quality parameters such as COD and BOD in secondary wastewater treatment. As the threshold limits produced by the agency of The Urban Waste Water Treatment (England and Wales) Regulations (UK Government, 1994), which performs the Council Directive 91/271/EEC Concerning Urban Waste Water Treatment (European Community, 1991) specify, the allowable limit for COD concentration in secondary wastewater treatment is 125 mg/l. Regarding the wetland filters without diesel contamination (F2, F4, F6, F7, and F8) (Tables 4.2 to 4.6; Figure 4.1b), the COD concentration values were acceptable and within

the allowable limits for these filters as shown in Figure 4.1b. The COD removal efficiency generally improved as the micro-organisms responsible for biodegradation acclimatized (Scholz, 2006, 2010; De Biase et al., 2011). Additionally, the treatment efficiencies of the experimental wetlands for the removal of organics are generally highly dependent on the oxygen available in the bed. In this study, it is suggested that tidal-flow mode is capable of providing sufficient oxygen diffusion into all wetlands filters (Wu et al., 2015f; Zhi et al., 2015; Weedon et al., 2016). This also explains the high treatment performance of wetland filters without any clogging phenomena being noticed during system operation period (Al-Isawi et al., 2015a).

The BOD removal efficiencies generally improved over time. This improvement can be attributed to the development of a mature biomass adjusted to the environmental boundary conditions of the wetland system (Sani et al., 2013a; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Almuktar et al., 2015a). The common UK threshold for BOD removal from secondary wastewater is 20 mg/l and 25 mg/l for sensitive and less sensitive areas, respectively (Royal Commission on Sewage Disposal, 1915). Figure 4.2 shows the variation in the outflow biochemical oxygen demand concentration values in all wetland filters. The fifth experimental phase showed a noticeable improvement with regard to BOD treatment efficiencies (Table 4.6). The effluents for wetland filters were relatively lower than the threshold value of 25 mg/l (Royal Commission on Sewage Disposal, 1915) (Figure 4.2). This could be explained by the positive effect of the wetland age on treatment performance, as the concentration values of BOD clearly decreased as the wetland filters became older (Figure 4.2). This is linked to the development of biomass within the wetland filter, as well as the development of the surface area of the filter layer

that provides a suitable spreading of the wastewater through the entire bed surface (Paing et al., 2015a).

Table 4.7 provides the statistical difference analysis between outflow water quality parameters of different filters using the non-parametric Mann-Whitney U-test. COD and BOD effluent analysis of wetland filters F5 and F6 (high inflow load) indicates clearly that they were statistical significantly different ($p \leq 0.05$) from wetland filters F3 and F4 (low inflow load) (Table 4.7). The analysis of COD and BOD effluent indicates that wetland filter F7 (low contact time) was statistically similar ($p \geq 0.05$) to wetland filters F3 and F4 (high contact time), however diesel application to F3 resulted in a significant performance difference as compared with F7. Wetland filters with large aggregate diameter were also analysed against wetland filters with small aggregate diameter and the COD and BOD results indicate that they were similar ($p \geq 0.05$) during the whole period, reflecting that aggregate size may not matter (Sani et al., 2013a; Al-Isawi et al., 2015a).

Table 4.2: Comparison of outflow water quality and air temperature for the start-up period (first experimental phase) (27/06/11 to 25/09/11).

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 1 and Filter 2 combined							
COD	mg/l	11	81.0	55.1	34.8	135.0	33.07
NH_4-N	mg/l	7	7.9	44.6	0.8	21.8	6.83
NO_3-N	mg/l	5	0.6	-17.4	0.4	1.3	0.26
PO_4-P	mg/l	10	2.0	58.1	0.2	3.3	0.88
SS	mg/l	9	25.7	75.1	6.0	85.0	23.02
Filter 3 and Filter 4 combined							
COD	mg/l	10	75.6	58.1	36.4	120.0	29.45
NH_4-N	mg/l	7	11.1	22.0	3.8	30.9	8.06
NO_3-N	mg/l	5	0.4	12.2	0.3	0.6	0.10
PO_4-P	mg/l	10	2.0	56.7	1.0	3.2	0.75
SS	mg/l	9	27.2	73.7	7.0	120.0	33.31
Filter 5 and Filter 6 combined							
COD	mg/l	11	167.9	53.0	84.2	452.0	104.66
NH_4-N	mg/l	7	28.0	-29.7	12.9	62.8	17.58
NO_3-N	mg/l	5	0.7	20.0	0.5	0.9	0.19
PO_4-P	mg/l	10	4.6	48.5	2.4	7.4	1.75
SS	mg/l	8	35.6	83.0	9.0	75.0	18.29
Filter 7							
COD	mg/l	11	102.3	43.4	58.2	255.0	55.55
NH_4-N	mg/l	7	18.2	-27.9	8.2	35.8	9.60
NO_3-N	mg/l	4	0.5	0.8	0.4	0.6	0.12
PO_4-P	mg/l	11	2.5	45.6	1.8	3.3	0.56
SS	mg/l	10	33.6	67.5	9.0	85.0	27.73
Filter 8							
COD	mg/l	13	345.5	54.2	90.0	620.0	185.88
NH_4-N	mg/l	9	11.7	18.0	6.5	18.7	4.35
NO_3-N	mg/l	6	0.3	29.7	0.2	0.5	0.09
PO_4-P	mg/l	13	2.2	53.1	1.4	3.9	0.66
SS	mg/l	14	18.6	81.8	7.0	45.0	10.97
Air temperature	°C	28	14.9	n/a	11.1	18.1	2.10

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; and n/a, not applicable.

Table 4.3: Comparison of outflow water quality and air temperature for the first year after the start-up period (second experimental phase) (26/09/11 to 25/09/12).

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 1 and Filter 2 combined							
COD	mg/l	52	56.7	57.5	5.0	135.0	29.22
BOD	mg/l	13	36.2	30.5	15.0	70.0	18.11
NH_4-N	mg/l	37	9.1	75.4	0.3	25.3	5.90
NO_3-N	mg/l	34	1.1	14.4	0.0	7.8	1.81
PO_4-P	mg/l	40	3.0	69.7	0.0	6.0	1.36
SS	mg/l	49	7.3	83.8	0.2	52.0	10.16
TBD	NTU	15	1.7	99.0	0.0	5.1	1.87
pH	n/a	14	7.0	n/a	5.8	7.4	0.37
Filter 3 and Filter 4 combined							
COD	mg/l	50	56.6	59.9	6.0	165.0	33.73
BOD	mg/l	13	32.2	38.1	10.0	65.0	19.96
NH_4-N	mg/l	37	6.9	81.3	0.1	31.2	5.68
NO_3-N	mg/l	34	1.6	-28.1	0.0	11.9	2.64
PO_4-P	mg/l	40	2.6	73.1	0.0	6.5	1.25
SS	mg/l	49	6.1	86.4	0.0	60.0	11.01
TBD	NTU	15	1.2	99.3	0.0	3.9	1.28
pH	n/a	14	7.0	n/a	5.8	7.4	0.38
Filter 5 and Filter 6 combined							
COD	mg/l	50	89.9	66.4	20.5	240.0	48.95
BOD	mg/l	13	41.5	59.8	0.0	130.0	37.44
NH_4-N	mg/l	41	15.7	65.3	0.9	35.8	8.65
NO_3-N	mg/l	37	3.1	9.9	0.0	21.2	4.53
PO_4-P	mg/l	40	4.5	73.5	0.0	8.2	2.25
SS	mg/l	47	11.1	85.6	1.4	84.0	15.95
TBD	NTU	15	4.9	98.4	0.0	12.1	3.57
pH	n/a	14	7.2	n/a	5.8	7.9	0.44
Filter 7							
COD	mg/l	57	59.1	56.2	10.9	158.0	31.04
BOD	mg/l	14	23.2	55.4	0.0	70.0	17.05
NH_4-N	mg/l	44	5.6	84.9	0.0	14.8	3.61
NO_3-N	mg/l	47	4.2	-232.8	0.0	14.6	4.00
PO_4-P	mg/l	47	2.5	74.3	0.0	4.9	1.16
SS	mg/l	55	7.2	84.2	0.0	50.0	9.85

Table 4.3 (cont.)

TBD	NTU	19	2.5	98.6	0.0	9.1	2.77
pH	n/a	20	7.3	n/a	5.8	8.1	0.44
Filter 8							
COD	mg/l	59	54.8	59.3	11.80	128.0	27.29
BOD	mg/l	14	16.1	69.1	0.0	55.0	14.03
NH_4-N	mg/l	46	5.5	85.1	0.2	13.7	3.41
NO_3-N	mg/l	45	3.3	-164.7	0.0	12.7	3.47
PO_4-P	mg/l	48	2.3	76.2	0.0	4.8	1.17
SS	mg/l	60	6.0	86.8	0.0	40.0	8.51
TBD	NTU	19	2.1	98.8	0.0	6.8	2.18
pH	n/a	26	7.2	n/a	5.7	7.8	0.48
Air temperature	°C	141	12.7	n/a	0.8	28.0	4.20

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; NTU, nephelometric turbidity unit; and n/a, not applicable.

Table 4.4: Comparison of outflow water quality and air temperature for the second year after the start-up period (third experimental phase) (26/09/12 to 25/09/13).

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 1 and Filter 2 combined							
COD	mg/l	29	64.4	49.3	39.1	117.0	17.07
BOD	mg/l	61	37.2	51.2	0.0	105.0	21.70
NH_4-N	mg/l	29	11.6	69.4	0.4	31.2	9.90
NO_3-N	mg/l	27	2.1	47.8	0.1	9.7	2.83
PO_4-P	mg/l	25	3.0	56.7	1.4	6.2	1.27
SS	mg/l	65	7.8	93.3	0.0	46.0	8.80
TBD	NTU	50	6.9	89.5	0.0	44.0	8.35
pH	n/a	55	6.7	n/a	6.0	7.2	0.27
Filter 3 and Filter 4 combined							
COD	mg/l	29	57.9	54.4	23.2	95.1	13.82
BOD	mg/l	61	33.8	55.6	0.0	150.0	25.72
NH_4-N	mg/l	29	8.4	77.8	0.2	28.0	8.21
NO_3-N	mg/l	27	3.0	26.4	0.1	10.5	3.15
PO_4-P	mg/l	25	2.5	62.8	1.3	6.0	0.97
SS	mg/l	65	5.8	95.0	0.0	26.0	5.28
TBD	NTU	50	6.5	90.1	0.0	63.4	8.53
pH	n/a	55	6.8	n/a	6.2	7.4	0.24
Filter 5 and Filter 6 combined							
COD	mg/l	29	81.3	66.0	32.1	126.0	20.92
BOD	mg/l	60	48.6	67.9	5.0	245.0	35.59
NH_4-N	mg/l	29	25.0	57.7	1.2	62.2	20.06
NO_3-N	mg/l	28	6.0	9.9	0.1	24.8	6.15
PO_4-P	mg/l	25	4.2	68.0	1.0	7.8	1.72
SS	mg/l	65	8.6	96.3	0.0	48.0	9.04
TBD	NTU	50	10.9	90.9	0.0	65.4	13.36
pH	n/a	55	7.0	n/a	6.0	7.4	0.23
Filter 7							
COD	mg/l	26	55.8	56.1	16.8	78.3	15.37
BOD	mg/l	65	28.28	63.0	0.0	75.0	16.13
NH_4-N	mg/l	32	8.0	79.0	0.4	27.2	6.69
NO_3-N	mg/l	30	5.6	-37.1	0.3	17.5	4.40
PO_4-P	mg/l	26	3.0	56.5	1.7	6.8	0.94
SS	mg/l	69	7.3	93.8	0.0	49.0	9.56
TBD	NTU	56	6.9	89.5	0.0	30.9	7.93
pH	n/a	63	6.9	n/a	6.2	7.5	0.27
Filter 8							
COD	mg/l	27	62.4	50.9	24.9	88.2	12.73
BOD	mg/l	73	27.9	63.4	0.0	68.0	17.30
NH_4-N	mg/l	31	10.8	71.6	0.1	30.6	9.04
NO_3-N	mg/l	29	4.9	-18.8	0.1	17.5	4.69
PO_4-P	mg/l	24	3.1	54.1	1.7	8.4	1.32
SS	mg/l	87	8.8	92.4	0.0	39.0	10.03

Table 4.4 (cont.)

TBD	NTU	61	8.6	87.0	0.0	53.1	10.93
pH	n/a	79	6.9	n/a	6.2	7.6	0.3
Air temperature	°C	306	13.1	n/a	1.0	29.0	3.5

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; NTU, nephelometric turbidity unit; and n/a, not applicable.

Table 4.5: Comparison of outflow water quality and air temperature for the fourth experimental phase (first dosage of diesel spill) (26/09/13 to 25/09/14).

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 1							
COD	mg/l	19	91.9	26.8	36.7	346.0	65.59
BOD	mg/l	56	25.8	54.6	0.0	80.0	16.39
NH_4-N	mg/l	22	5.8	69.5	1.1	29.0	5.82
NO_3-N	mg/l	20	0.4	70.2	0.2	0.9	0.22
PO_4-P	mg/l	19	4.3	44.4	1.1	10.8	2.89
SS	mg/l	59	11.4	83.7	0.0	52.0	10.21
TBD	NTU	57	9.2	81.2	3.0	28.4	5.61
pH	n/a	57	6.4	n/a	5.5	7.10	0.26
Redox	mV	33	23.3	n/a	13.0	35.0	5.41
EC	µS/cm	32	344.6	n/a	270.3	401.0	45.02
DO	mg/l	23	1.6	n/a	0.7	3.9	0.87
Filter 2							
COD	mg/l	16	38.6	69.3	16.1	93.2	23.60
BOD	mg/l	54	14.2	75.0	0.0	36.0	8.58
NH_4-N	mg/l	21	6.2	67.2	0.5	18.6	6.01
NO_3-N	mg/l	19	2.1	-60.5	0.1	8.6	2.68
PO_4-P	mg/l	19	3.4	56.4	1.7	5.6	1.34
SS	mg/l	59	6.7	90.4	0.0	49.0	9.31
TBD	NTU	56	5.5	88.7	2.0	26.1	5.61
pH	n/a	57	6.5	n/a	5.5	6.92	0.21
Redox	mV	33	9.8	n/a	2.0	22.0	4.96
EC	µS/cm	32	342.5	n/a	260.0	410.0	50.31
DO	mg/l	23	2.1	n/a	1.0	4.1	0.84
Filter 3							
COD	mg/l	19	100.3	20.1	53.2	332.0	61.53
BOD	mg/l	54	23.2	59.2	0.0	98.0	16.11
NH_4-N	mg/l	22	4.2	78.2	0.7	16.9	3.78
NO_3-N	mg/l	20	0.4	72.2	0.1	1.1	0.27
PO_4-P	mg/l	19	3.6	54.0	0.9	9.7	2.25
SS	mg/l	59	11.7	83.2	0.0	54.0	10.51
TBD	NTU	56	8.9	81.9	2.5	30.7	5.98
pH	n/a	57	6.5	n/a	6.1	7.0	0.18
Redox	mV	33	11.9	n/a	3.0	21.0	4.03
EC	µS/cm	32	409.7	n/a	305.7	531.0	66.39
DO	mg/l	23	2.0	n/a	0.6	3.9	1.00
Filter 4							
COD	mg/l	16	38.4	65.4	9.9	90.6	26.85
BOD	mg/l	53	14.2	75.0	0.0	49.0	10.28
NH_4-N	mg/l	21	3.4	82.2	0.1	15.2	3.86
NO_3-N	mg/l	19	1.7	-33.1	0.0	11.3	3.20
PO_4-P	mg/l	19	3.0	61.3	1.7	5.7	1.12
SS	mg/l	59	7.4	89.4	0.0	50.0	10.32

Table 4.5 (cont.)

TBD	NTU	56	5.8	88.1	1.3	27.3	5.42
pH	n/a	57	6.5	n/a	5.8	7.1	0.19
Redox	mV	33	11,0	n/a	2.0	17.0	3.61
EC	µS/cm	32	366.2	n/a	264.0	512.0	60.18
DO	mg/l	23	2.1	n/a	0.7	4.1	0.91
Filter 5							
COD	mg/l	19	114.2	54.0	60.5	356.0	88.21
BOD	mg/l	54	23.6	79.3	0.0	78.0	16.83
NH_4-N	mg/l	22	12.3	64.9	5.6	61.5	11.30
NO_3-N	mg/l	20	0.9	64.7	0.2	2.8	0.84
PO_4-P	mg/l	19	4.8	67.8	1.0	13.6	2.92
SS	mg/l	60	12	91.6	0.0	68.0	12.82
TBD	NTU	56	9.4	88.6	3.6	35.8	6.95
pH	n/a	57	6.7	n/a	6.4	7.1	0.21
Redox	mV	33	7.4	n/a	-9.0	16.0	4.46
EC	µS/cm	32	595.5	n/a	310.4	784.0	145.25
DO	mg/l	23	1.8	n/a	0.6	3.6	0.73
Filter 6							
COD	mg/l	15	42.5	82.9	6.5	139.0	34.36
BOD	mg/l	55	16.9	85.2	0.0	44.0	13.12
NH_4-N	mg/l	22	11.7	66.6	0.5	54.2	12.05
NO_3-N	mg/l	19	3.4	-45.5	0.2	17.9	4.60
PO_4-P	mg/l	19	5.1	66.3	1.7	13.5	3.69
SS	mg/l	60	7.4	94.8	0.0	41.0	8.85
TBD	NTU	56	6.2	92.6	1.0	27.0	5.51
pH	n/a	58	6.8	n/a	6.5	7.7	0.21
Redox	mV	33	3.6	n/a	-4.0	10.0	3.56
EC	µS/cm	32	570.8	n/a	320.0	751.0	145.66
DO	mg/l	23	2.0	n/a	0.6	4.4	1.10
Filter 7							
COD	mg/l	16	37.3	68.0	14.1	106.0	26.41
BOD	mg/l	64	12.5	78.0	0.0	42.0	8.71
NH_4-N	mg/l	23	4.6	76.0	0.1	20.7	6.36
NO_3-N	mg/l	19	2.7	-109.8	0.2	10.8	2.95
PO_4-P	mg/l	18	3.8	51.0	1.8	9.1	2.38
SS	mg/l	69	2.9	95.9	0.0	19.0	4.08
TBD	NTU	65	3.6	92.6	1.5	14.4	2.55
pH	n/a[c]	65	6.6	n/a	6.1	7.0	0.19
Redox	mV	40	7.6	n/a	0.0	18.0	4.48
EC	µS/cm	37	371.5	n/a	255.0	779.0	100.58
DO	mg/l	28	1.8	n/a	0.5	3.4	0.73
Filter 8							
COD	mg/l	16	37.3	70.3	14.1	106.0	26.41
BOD	mg/l	72	14.4	74.7	0.0	36.0	7.94
NH_4-N	mg/l	20	2.2	88.5	0.1	17.4	3.78
NO_3-N	mg/l	17	2.7	-108.9	0.2	10.8	3.45
PO_4-P	mg/l	17	3.5	54.6	1.9	7.9	2.11

Table 4.5 (cont.)

SS	mg/l	81	3.1	95.6	0.0	25.0	4.35
TBD	NTU	80	3.8	92.2	1.2	17.3	2.78
pH	n/a	81	6.5	n/a	6.2	7.7	0.20
Redox	mV	43	13.6	n/a	-5.0	20.0	5.26
EC	µS/cm	40	368.8	n/a	231.0	707.0	98.09
DO	mg/l	30	2.0	n/a	1.1	5.3	0.92
Control A							
COD	mg/l	18	80.1	nm	6.9	312.0	72.10
BOD	mg/l	54	13.0	nm	0.0	42.0	8.91
NH_4-N	mg/l	22	1.0	nm	0.0	4.6	1.49
NO_3-N	mg/l	20	0.4	nm	0.0	2.0	0.46
PO_4-P	mg/l	19	2.0	nm	0.9	4.3	0.79
SS	mg/l	59	8.9	nm	0.0	39.0	10.02
TBD	NTU	56	5.8	nm	2.2	21.3	4.36
pH	n/a[c]	58	6.7	n/a	6.4	7.1	0.17
Redox	mV	33	2.8	n/a	-11.0	8.0	3.52
EC	µS/cm	32	159.5	n/a	106.2	223.0	31.15
DO	mg/l	23	1.5	n/a	0.5	3.4	0.77
Control B							
COD	mg/l	16	20.6	nm	0.2	90.3	23.63
BOD	mg/l	55	8.8	nm	0.0	34.0	7.41
NH_4-N	mg/l	22	1.1	nm	0.0	6.9	1.64
NO_3-N	mg/l	19	0.3	nm	0.1	1.0	0.36
PO_4-P	mg/l	19	2.0	nm	1.0	4.2	0.61
SS	mg/l	59	3.9	nm	0.0	49.0	8.16
TBD	NTU	56	4.3	nm	1.1	27.5	4.68
pH	n/a	57	6.6	n/a	6.1	7.0	0.20
Redox	mV	33	12.8	n/a	7.0	19.0	3.04
EC	µS/cm	32	162.6	n/a	95.9	216.0	56.2
DO	mg/l	23	2.2	n/a	0.6	4.0	0.96
Air temperature	°C	311	11.3	n/a	2.0	27.0	3.8

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; EC, electrical conductivity; DO, dissolved oxygen. NTU, nephelometric turbidity unit; n/a, not applicable; and nm, not measured.

Table 4.6: Comparison of outflow water quality and air temperature for the fifth experimental phase (second dosage of diesel spill) (26/09/14 to 22/03/16).

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 1							
COD	mg/l	45	141.5	2.6	50.3.8	260.0	64.91
BOD	mg/l	93	37.1	59.7	0.0	98.0	25.01
NH_4-N	mg/l	46	5.1	61.1	0.6	26.0	4.84
NO_3-N	mg/l	47	1.5	50.1	0.1	11.2	2.70
PO_4-P	mg/l	50	5.9	-3.8	2.2	18.5	2.46
SS	mg/l	83	23.5	67.1	1.0	99.0	18.21
TBD	NTU	81	20.6	36.3	3.7	207.0	25.73
pH	n/a	85	6.5	n/a	5.4	8.5	0.58
Redox	mV	86	18.8	n/a	-20.0	88.3	19.84
EC	µS/cm	92	535.2	n/a	125.9	1412.0	205.10
DO	mg/l	85	3.1	n/a	0.5	9.1	1.60
Filter 2							
COD	mg/l	37	43.9	69.8	14.5	102.0	18.04
BOD	mg/l	94	12.5	85.4	0.0	72.0	11.08
NH_4-N	mg/l	43	3.9	70.6	0.0	13.3	3.54
NO_3-N	mg/l	43	2.2	29.2	0.0	13.8	3.39
PO_4-P	mg/l	44	4.1	28.1	1.5	8.3	1.56
SS	mg/l	83	12.2	82.9	0.0	60.0	9.91
TBD	NTU	81	9.8	69.5	1.9	34.1	6.28
pH	n/a	85	6.7	n/a	5.9	7.4	0.33
Redox	mV	86	1.8	n/a	-31.0	50.0	13.91
EC	µS/cm	92	517.0	n/a	167.2	1261.0	153.22
DO	mg/l	90	3.6	n/a	0.7	6.5	1.33
Filter 3							
COD	mg/l	45	189.4	-30.3	26.1	478.0	120.29
BOD	mg/l	92	32.2	65.0	0.0	98.0	24.64
NH_4-N	mg/l	48	3.3	74.1	-0.1	25.4	4.07
NO_3-N	mg/l	47	1.8	40.6	-0.1	17.0	3.09
PO_4-P	mg/l	50	5.6	1.7	2.8	15.4	2.08
SS	mg/l	82	22.5	68.7	2.0	104.0	15.56
TBD	NTU	80	17.8	45.0	4.0	93.9	13.46
pH	n/a	85	6.6	n/a	5.9	7.4	0.35
Redox	mV	85	14.1	n/a	-18.0	77.9	16.79
EC	µS/cm	92	614.6	n/a	155.6	1851.0	230.01
DO	mg/l	90	3.0	n/a	0.4	6.9	1.48
Filter 4							
COD	mg/l	34	38.3	73.7	16.6	68.6	12.44
BOD	mg/l	93	11.1	88.7	0.0	35.0	8.03
NH_4-N	mg/l	43	2.9	77.5	0.0	18.6	3.36
NO_3-N	mg/l	43	0.7	75.6	0.0	5.5	1.26
PO_4-P	mg/l	43	3.6	36.3	0.5	6.0	1.48
SS	mg/l	81	5.2	92.7	0.0	31.0	4.02
TBD	NTU	79	4.4	86.2	1.9	26.8	3.80

Table 4.6 (cont.)

pH	n/a	84	6.7	n/a	6.1	7.4	0.28
Redox	mV	85	2.5	n/a	-26.0	22.0	10.97
EC	µS/cm	89	547.4	n/a	98.5	1483.0	183.51
DO	mg/l	90	3.9	n/a	0.6	8.0	1.54
Filter 5							
COD	mg/l	44	202.3	29.0	63.5	454.0	114.41
BOD	mg/l	92	40.0	77.9	6.0	98.0	21.17
NH_4-N	mg/l	47	6.4	75.3	0.1	34.8	6.01
NO_3-N	mg/l	46	3.3	40.5	0.2	18.6	4.92
PO_4-P	mg/l	49	7.0	36.9	1.2	15.8	3.00
SS	mg/l	81	23.4	83.1	3.0	60.0	13.37
TBD	NTU	79	19.8	61.7	2.8	77.7	13.52
pH	n/a	84	6.5	n/a	5.9	7.2	0.32
Redox	mV	85	9.5	n/a	-30.0	70.9	15.81
EC	µS/cm	89	930.5	n/a	323.0	1573.0	272.72
DO	mg/l	88	3.3	n/a	0.4	22.0	2.63
Filter 6							
COD	mg/l	34	55.7	80.5	25.0	198.0	28.24
BOD	mg/l	93	18.9	89.5	0.0	64.0	14.65
NH_4-N	mg/l	45	6.8	74.1	0.1	23.7	5.86
NO_3-N	mg/l	42	5.0	10.2	0.1	11.8	4.16
PO_4-P	mg/l	41	5.1	53.9	1.2	12.9	2.63
SS	mg/l	9.5	9.5	93.2	0.0	41.0	8.42
TBD	NTU	75	7.8	85.0	1.9	28.2	6.04
pH	n/a	81	6.9	n/a	5.8	7.3	0.30
Redox	mV	79	-6.5	n/a	-32.0	15.0	11.29
EC	µS/cm	87	901	n/a	317.0	1575.0	269.73
DO	mg/l	87	3.7	n/a	0.6	7.4	1.35
Filter 7							
COD	mg/l	35	36.6	74.8	18.4	101.0	17.47
BOD	mg/l	105	9.9	89.2	0.0	40.0	7.80
NH_4-N	mg/l	43	3.3	74.9	0.1	13.6	3.96
NO_3-N	mg/l	41	4.4	-45.5	0.1	10.1	3.17
PO_4-P	mg/l	42	4.2	26.9	1.5	21.4	3.33
SS	mg/l	89	3.2	95.5	0.0	40.0	5.23
TBD	NTU	97	3.9	88.1	0.0	18.9	2.77
pH	n/a	84	6.8	n/a	6.0	8.0	0.38
Redox	mV	96	-3.7	n/a	-27.0	16.0	10.19
EC	µS/cm	100	542.8	n/a	118.7	1517.0	178.3
DO	mg/l	89	4.5	n/a	0.5	9.5	1.88
Filter 8							
COD	mg/l	34	48.0	67.0	23.0	177.0	24.2
BOD	mg/l	94	13.9	84.9	0.0	72.0	11.53
NH_4-N	mg/l	44	2.3	82.6	0.01	12.2	3.21
NO_3-N	mg/l	41	3.4	-11.2	0.2	8.9	2.32
PO_4-P	mg/l	43	3.8	34.3	0.1	11.3	1.97
SS	mg/l	102	4.5	93.7	0.0	69.0	7.82

Table 4.6 (cont.)

TBD	NTU	101	4.2	86.9	0.0	32.0	3.61
pH	n/a	94	6.8	n/a	5.5	7.6	0.39
Redox	mV	106	-1.5	n/a	-54.0	17.0	11.79
EC	µS/cm	104	572.9	n/a	205.0	987.0	156.78
DO	mg/l	108	4.6	n/a	0.6	11.0	2.29
Control A							
COD	mg/l	43	128.4	nm	18.3	386.0	73.41
BOD	mg/l	78	14.2	nm	0.0	68.0	9.49
NH_4-N	mg/l	45	0.6	nm	0.0	6.2	1.21
NO_3-N	mg/l	47	0.3	nm	0.0	6.1	0.87
PO_4-P	mg/l	50	2.6	nm	1.0	11.0	1.68
SS	mg/l	79	13.1	nm	1.0	52.0	9.80
TBD	NTU	81	8.3	nm	1.1	35.2	4.96
pH	n/a	88	6.8	n/a	6.0	7.1	0.18
Redox	mV	86	2.3	n/a	-17.0	65.0	13.81
EC	µS/cm	92	224.5	n/a	96.2	889.0	116.55
DO	mg/l	90	3.3	n/a	0.4	7.3	1.42
Control B							
COD	mg/l	30	12.4	nm	1.3	58.7	10.21
BOD	mg/l	78	3.8	nm	0.0	18.0	3.88
NH_4-N	mg/l	41	0.6	nm	0.1	7.7	1.95
NO_3-N	mg/l	34	0.3	nm	0.1	5.7	0.96
PO_4-P	mg/l	38	1.9	nm	0.9	4.7	0.81
SS	mg/l	77	3.1	nm	0.0	29.0	4.44
TBD	NTU	79	3.8	nm	1.6	12.4	1.96
pH	n/a	89	6.8	n/a	6.1	7.7	0.34
Redox	mV	82	-0.5	n/a	-27.0	14.0	9.51
EC	µS/cm	89	160.2	n/a	71.2	498.0	76.88
DO	mg/l	88	5.8	n/a	1.3	9.9	1.87
Air temperature	°C	325	10.5	n/a	4.0	25.0	5.35

Note: COD, chemical oxygen demand; BOD, biochemical oxygen demand; PO_4-P, ortho-phosphate-phosphorus; NH_4-N, ammonia-nitrogen; NO_3-N, nitrate-nitrogen; SS, suspended solids; TBD, turbidity; EC, electrical conductivity; DO, dissolved oxygen. NTU, nephelometric turbidity unit; n/a, not applicable; and nm, not measured.

Table 4.7: Overview of the statistically significant differences between outflow water quality variables of different wetland filters using the non-parametric Mann-Whitney U-test (27/07/11-22/03/16).

Parameter	Unit	Statistics	Aggregate diameter[a]	Contact time[b]	Resting time[c]	Chemical oxygen demand[d]
First to third experimental phase (27/07/11-25/09/13)						
COD	mg/l	*P-value*	0.355	0.526	0.804	<0.000
		h	0	0	0	1
BOD	mg/l	*P-value*	0.183	0.068	0.476	0.011
		h	0	0	0	1
NH_4-N	mg/l	*P-value*	0.079	0.856	0.676	<0.000
		h	0	0	0	1
NO_3-N	mg/l	*P-value*	0.237	<0.000	0.095	0.025
		h	0	1	0	1
PO_4-P	mg/l	*P-value*	0.080	0.134	0.241	<0.000
		h	0	0	0	1
SS	mg/l	*P-value*	0.025	0.483	0.519	<0.000
		h	1	0	0	1
TBD	mg/l	*P-value*	0.832	0.983	0.543	0.031
		h	0	0	0	1
pH	n/a[p]	*P-value*	0.005	0.055	0.658	0.004
		h	1	0	0	1
Parameter	Unit	*Statistics*	Aggregate diameter[e]	Contact time[f]	Resting time[g]	Chemical oxygen demand[h]
Fourth and fifth experimental phases (26/09/13-22/03/2016)						
COD	mg/l	*P-value*	0.775	0.015	0.403	0.200
		h	0	1	0	0
BOD	mg/l	*P-value*	0.554	0.006	0.372	0.520
		h	0	1	0	0
NH_4-N	mg/l	*P-value*	0.200	0.224	0.972	0.002
		h	0	0	0	1
NO_3-N	mg/l	*P-value*	0.406	0.001	0.691	0.079
		h	0	1	0	1
PO_4-P	mg/l	*P-value*	0.462	0.345	0.817	0.294
		h	0	0	0	0
SS	mg/l	*P-value*	0.505	<0.001	0.184	0.978
		h	0	1	0	0
TBD	mg/l	*P-value*	0.554	<0.001	0.005	0.680
		h	0	1	1	0
pH	n/a[p]	*P-value*	0.015	0.539	0.333	0.018
		h	1	0	0	1
Redox	mV	*P-value*	0.069	0.457	0.098	0.209
		h	0	0	0	0
Conductivity	µS/cm	*P-value*	0.102	0.133	0.699	0.003
		h	0	0	0	1
DO	mg/l	*P-value*	0.411	<0.000	0.391	0.079

Table 4.7 (cont.)

		h	0	1	0	0
Parameter	Unit	*Statistics*	Aggregate diameter[i]	Contact time[j]	Resting time[k]	Chemical oxygen demand[l]
Fourth and fifth experimental phases (26/09/13-22/03/2016)						
COD	mg/l	*P-value*	0.557	1.000	0.113	0.211
		h	0	0	0	0
BOD	mg/l	*P-value*	0.211	0.281	0.455	0.129
		h	0	0	0	0
NH_4-N	mg/l	*P-value*	0.455	0.418	0.972	0.121
		h	0	0	0	0
NO_3-N	mg/l	*P-value*	0.634	0.480	0.691	0.985
		h	0	0	0	0
PO_4-P	mg/l	*P-value*	0.753	0.600	0.832	0.611
		h	0	0	0	0
SS	mg/l	*P-value*	0.966	0.005	0.100	0.649
		h	0	1	0	0
TBD	mg/l	*P-value*	0.212	<0.000	0.005	0.937
		h	0	1	1	0
pH	n/a[p]	*P-value*	0.001	0.672	0.335	0.005
		h	1	0	0	1
Redox	mV	*P-value*	0.073	0.467	0.055	0.065
		h	0	0	0	0
Conductivity	µS/cm	*P-value*	0.200	0.033	0.911	0.002
		h	0	0	0	1
DO	mg/l	*P-value*	0.111	0.001	0.211	0.139
		h	0	1	0	0
The difference between the filters at the same period (the effect of hydrocarbon dosages) (26/09/13-22/03/16)						
Parameter	Unit	Statistics	The influence of hydrocarbon[m]	The influence of hydrocarbon[n]	The influence of hydrocarbon[o]	
COD	mg/l	*P-value*	0.031	0.021	0.019	
		h	1	1	1	
BOD	mg/l	*P-value*	0.007	0.001	0.100	
		h	1	1	0	
NH_4-N	mg/l	*P-value*	0.406	0.655	0.129	
		h	0	0	0	
NO_3-N	mg/l	*P-value*	0.029	0.015	0.140	
		h	1	0	0	
PO_4-P	mg/l	*P-value*	0.780	0.656	0.08	
		h	0	0	0	
SS	mg/l	*P-value*	0.001	0.000	0.016	
		h	1	1	1	
TBD	mg/l	*P-value*	0.007	0.001	0.000	
		h	1	1	1	
pH	n/a[p]	*P-value*	0.019	0.617	0.002	
		h	1	0	1	

Table 4.7 (cont.)

Redox	mV	*P-value*	0.103	0.617	0.502
		h	0	0	0
Conductivity	µs/cm	*P-value*	0.177	0.071	0.098
		h	0	0	0
DO	mg/l	*P-value*	<0.000	0.001	0.003
		h	1	1	1

[a]Comparison between the mean daily values of Filters 1 and 2, and the mean daily values of Filters 3 and 4
[b]Comparison between the mean daily values of Filters 3 and 4, and Filter 7
[c]Comparison between Filters 7 and 8
[d]Comparison between mean daily values of Filters 3 and 4, and mean daily values of Filters 5 and 6
[e]Comparison between the mean daily values of Filters 1 and 3
[f]Comparison between the mean daily values of Filters 3 and 7
[g]Comparison between Filters 7 and 8
[h]Comparison between mean daily values of Filters 3 and 5
[i]Comparison between the mean daily values of Filters 2 and 4
[j]Comparison between the mean daily values of Filters 4 and 7
[k]Comparison between Filters 7 and 8
[l]Comparison between mean daily values of Filters 4 and 6
[m]Comparison between Filters 1 and 2
[n]Comparison between Filters 3 and 4
[o]Comparison between Filters 5 and 6
[p]not applicable
Note: *P-value*, probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true; h, response indicator; if h=1, filters are statistically significantly different (*P-value* < 0.05) for the corresponding water quality parameter; if h=0, the difference is not significant.

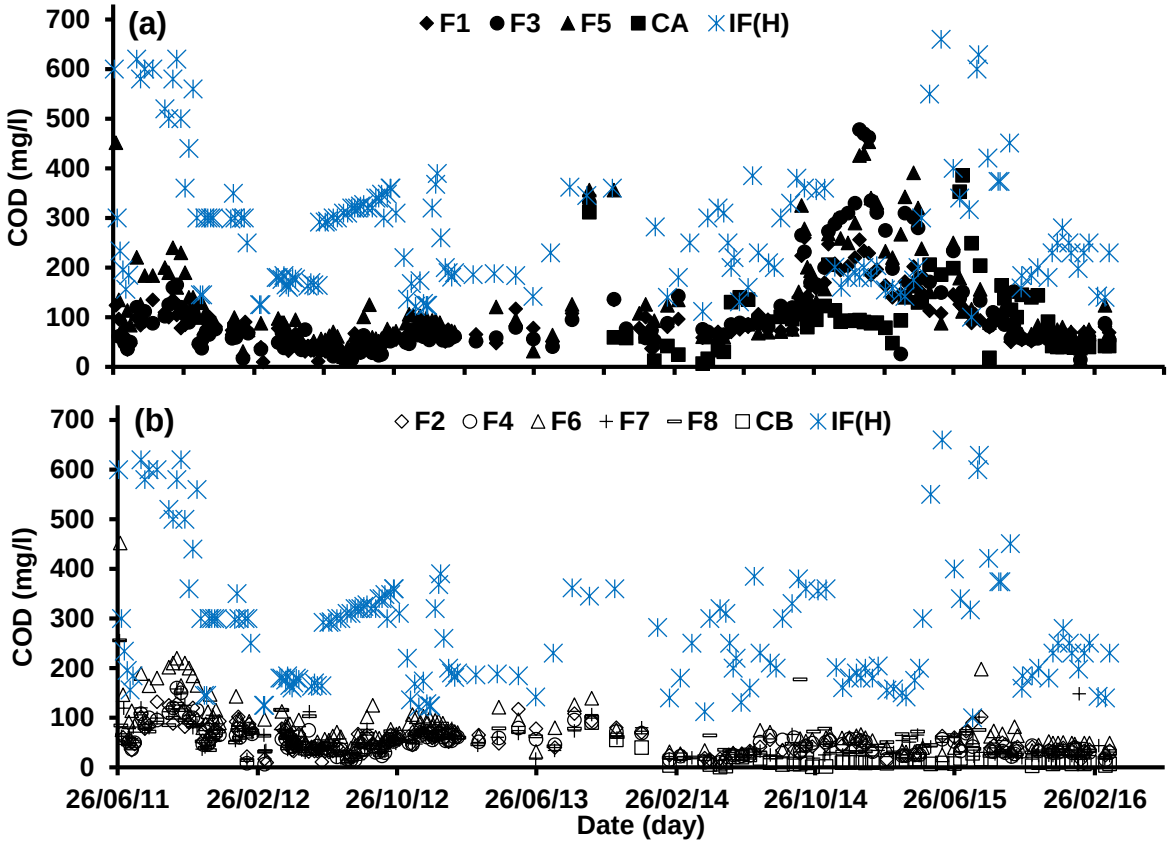


Figure 4.1: Overall variations in chemical oxygen demand (COD) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution). F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

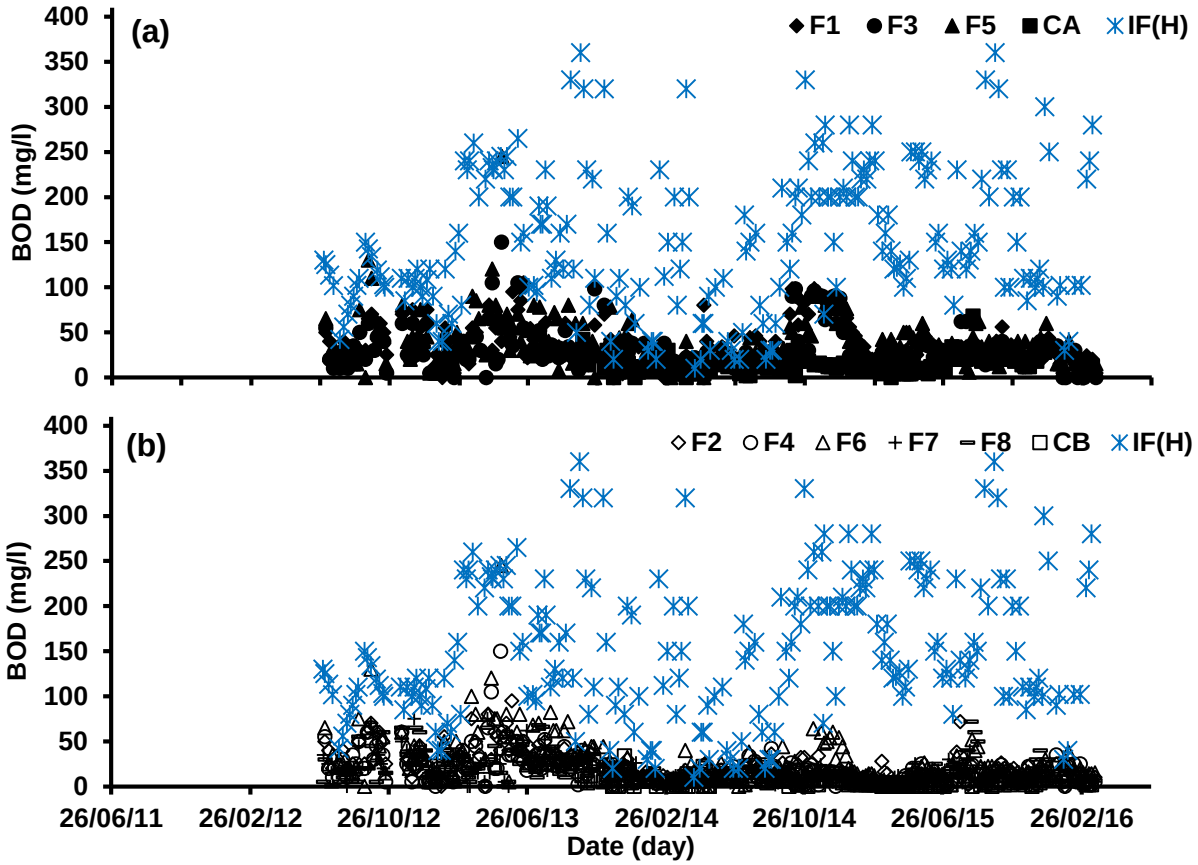


Figure 4.2: Overall variations in biochemical oxygen demand (BOD) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

4.2.2.2 Comparison of nutrients

The main removal mechanisms of nitrogen in constructed wetlands are microbial nitrification and denitrification. The removal mechanisms are two-step processes: Ammonium oxidation, where firstly, ammonium is oxidized to nitrite, and subsequently nitrite is oxidized to nitrate. Then the second step in which nitrate is reduced to gaseous nitrogen by the denitrification process. Aeration availability is a key factor to determine

high nitrogen removal performance within wetland systems (Vymazal, 2007a; Wu et al., 2011a; Fan et al., 2013b; Song et al., 2015; Yang et al., 2016a).

In this study, overall water quality parameters with regard to nutrients in the outflow of wetland filters in the five experimental phases are presented in Tables 4.2 to 4.6. There is an increase in the nitrogen removal efficiencies over time. This could reflect the role of the maturity of wetlands systems with well-established wetland plants growth that supports various types of bacteria species (Liang et al., 2011; Lavrova & Koumanova, 2013; Zheng et al., 2015; Kim et al., 2016; Murphy et al., 2016). Moreover, the tidal-flow mode contributes to greater re-oxygenation of the wetland bed which in turn provides high nitrification capacities within wetland filters (Wu et al., 2011a; Fan et al., 2013b; Wu et al., 2016b). The NH_4-N treatment performances (in terms of NH_4-N concentration) were generally better in wetland filters in the fourth and fifth phases than those in earlier periods (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Treatment performances (in terms of NH_4-N concentration values) for Filters 5 and 6 were less than those of the other filters. These wetland filters received concentrated wastewater (without dilution) containing high amounts of nutrients (treatment efficiency decreased with increasing nutrient loading) compared with others filters (that received fewer nutrients because the inflow wastewater was diluted with tap water). Filters 5 and 6 showed an increase in their treatment removal efficiencies in the fifth experimental phase (Table 4.6). These findings were also confirmed by (Merriman & Hunt Iii, 2014) who found improvement in nitrogen compound removal efficiencies for long-term operation of wetland systems. This might be reflecting the gradual improving capacity of the mature wetland system to treat the nutrient load efficiently (Lee et al., 2009; Al-Isawi et al., 2015a; Kim et al., 2016). The statistical analysis of NH_4-N effluent indicates that Filters 5 and 6 were statistically

significantly different ($p \leq 0.05$) from Filters 3 and 4 reflecting the impact of high inflow loads. However, aggregate size, resting time and contact time were not essential for the NH_4-N treatment performance (Table 4.7).

Nitrate-nitrogen removal efficiencies were higher for those filters treating petroleum hydrocarbons (Table 4.5 and 4.6). This is explained by the biodegradation processes of diesel spills in Filters 1, 3 and 5 (Table 4.5 and 4.6) reducing the availability of nutrients to micro-organisms and *P. australis*. However, as the biodegradation of diesel progresses, small amounts of remaining hydrocarbon promote the growth of some micro-organisms, which increases the degradation rate (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Furthermore, the lack of wetland plants in filters contaminated with hydrocarbon resulted in using an additional organic carbon source, to achieve the denitrification process. Moreover, the lack *P. australis* in filters contaminated with diesel led to promote the reduction of nitrate-nitrogen. This is in agreement with the study of Lavrova and Koumanova (2013) who found in their results that NO_3-N can be reduced effectively in vertical-flow CWs without plants and with a sufficient organic carbon source.

Figures 4.3 and 4.4 show the temporal variations in ammonia-nitrogen and nitrate-nitrogen for the filters with and without hydrocarbon contamination over time, respectively. A typical standard set by environment agencies for ammonia-nitrogen removal concerning secondary wastewater treatment is 20 mg/l (Royal Commission on Sewage Disposal, 1915). Ammonia-nitrogen values for Filters 5 and 6 (elevated loading rate) were frequently above this threshold (Figure 4.3) during the first three years of wetlands operation. Later, NH_4-N in all filters showed a decrease in their values highlighting the impact of hydrocarbon degradation (in the affected filters) and the impact of system maturity (in other filters). The traditional UK standard for NO_3-N removal from

secondary wastewater is 50 mg/l (Royal Commission on Sewage Disposal, 1915). Biodegradation is considered to be responsible for a high proportion of the nutrient removal in the wetland system (De Biase et al., 2011; Norton, 2014). Although the NO_3-N concentration in the inflow was relatively low, the outflow concentrations were rather high for most filters, highlighting the availability of high oxygen within the filter bed and a limit in the denitrification process. It follows that these filters can be considered as sources for NO_3-N. The NO_3-N values were lower for filters contaminated with hydrocarbon compared to those without hydrocarbon contamination. This demonstrates that the addition of carbon (via diesel) stimulated the removal of nitrogen, which is required by micro-organisms to degrade hydrocarbons (Al-Isawi et al., 2015a).

NO_3-N values in this experiment were relatively low and variable (particularly during the hydrocarbon treatment period). It can be noticed that after petroleum hydrocarbon compounds reduced in the affected filters with time, NO_3-N concentration values started gradually to increase in these filters. Findings of statistical analysis indicate that F5 and F6 (high inflow load) were statistically different ($p \leq 0.05$) to F3 and F4 (low inflow load). Analysis also shows that F7 was statistically different from F3 and F4, reflecting the impact of low contact time on treatment performance (Table 4.7). The presence of petroleum hydrocarbon compounds contributed to the significant difference ($p \leq 0.05$) between treatment performance of Filter F1 as compared with F2.

Furthermore, the high nitrogen compounds removal and the low outflow SS concentration noticed for all wetland filters did not show any significant media clogging or decline in the treatment performance over time which is surprising as the previous studies showed that high nutrient treatment performance of wetland filters led to accumulation of nitrogen compounds within the filter bed and could result in a clogging of wetland media with time

(Lavrova & Koumanova, 2013; Song et al., 2015). This can be explained by the impact of resting time to provide more oxygen that stimulated microbial degradation activities and led to improve hydraulic conductivity and treatment performance (Paing et al., 2015a; Wu et al., 2015f; Petitjean et al., 2016).

Regarding ortho-phosphate-phosphorus, the main removal mechanisms for phosphorus in constructed wetlands are: plant uptake, adsorption by wetland substrate, microbial uptake, accumulation around wetland media, and precipitation (Vymazal, 2007a, 2011b; Li et al., 2013e; Gikas & Tsihrintzis, 2014; Li et al., 2015a; Johari et al., 2016). Furthermore, literature has shown that removal efficiency of phosphorus compounds with constructed wetlands is generally poor (Choudhary et al., 2011; Lavrova & Koumanova, 2013; Ge et al., 2016a). In this research, the removal efficiency for PO_4-P was relatively high during the first three operation periods of the wetland system (Tables 4.2 to 4.4), slightly reduced in the fourth operation period (Table 4.5) and significantly dropped in the fifth one (Tables 4.6). This increasing in effluent PO_4-P concentrations over time could be explained by long-term operation of mature wetlands resulting in a saturation of their media by phosphate accumulation. These findings are in agreement with (Merriman & Hunt Iii, 2014), who found increases PO_4-P concentrations in the outflow with long-term wetlands operation.

Figure 4.5 shows the temporal variations in ortho-phosphate-phosphorus. A typical standard set by environment agencies for PO_4-P removal concerning secondary wastewater treatment is 2 mg/l (Royal Commission on Sewage Disposal, 1915). Ortho-phosphate-phosphorus values were relatively high and variable as compared with the threshold (Figure 4.5). Findings with regard to PO_4-P comparison between different treatment filters using the non-parametric Mann-Whitney U-test are shown in Table 4.7.

The results show that PO_4-P effluent of wetland filters 5 and 6 were statistically significantly different ($p \leq 0.05$) from wetland filters 3 and 4 reflecting the impact of high inflow load on treatment performance (Al-Isawi et al., 2015a). However, contact time, rest time and aggregate diameter showed no differences regarding PO_4-P treatment performance.

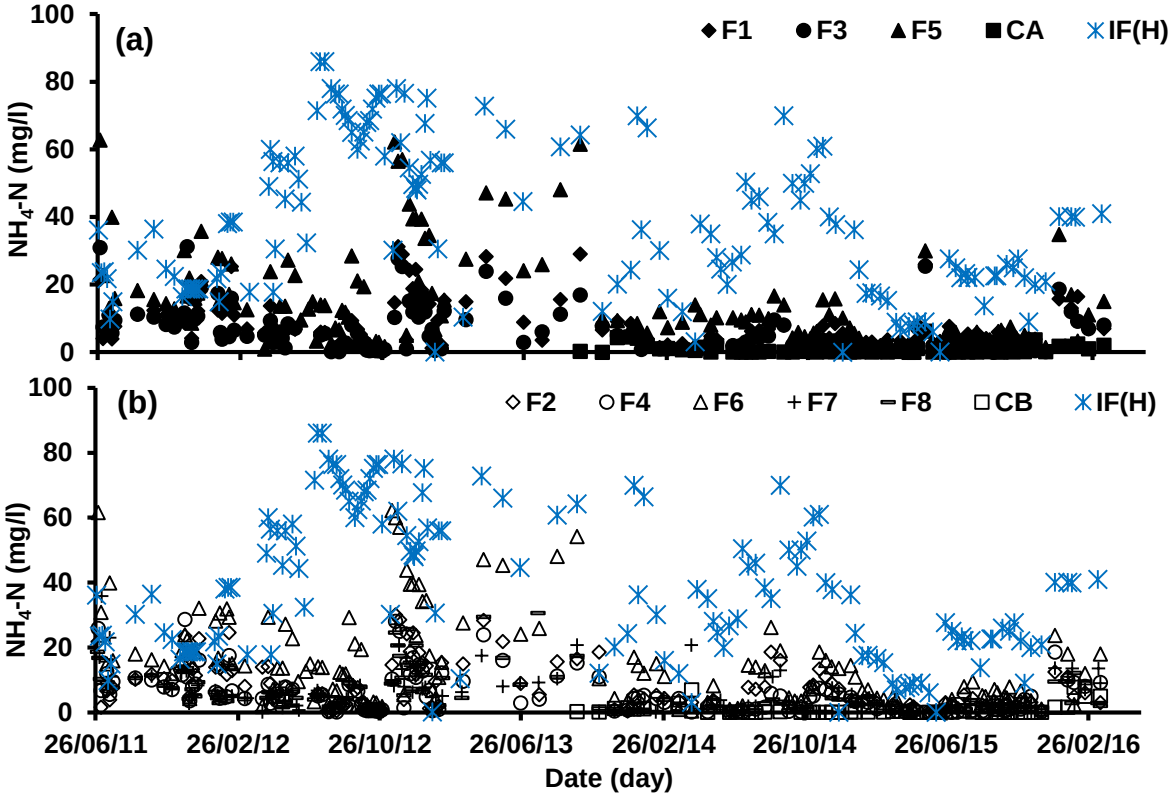


Figure 4.3: Overall variations in ammonia-nitrogen (NH_4-N) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

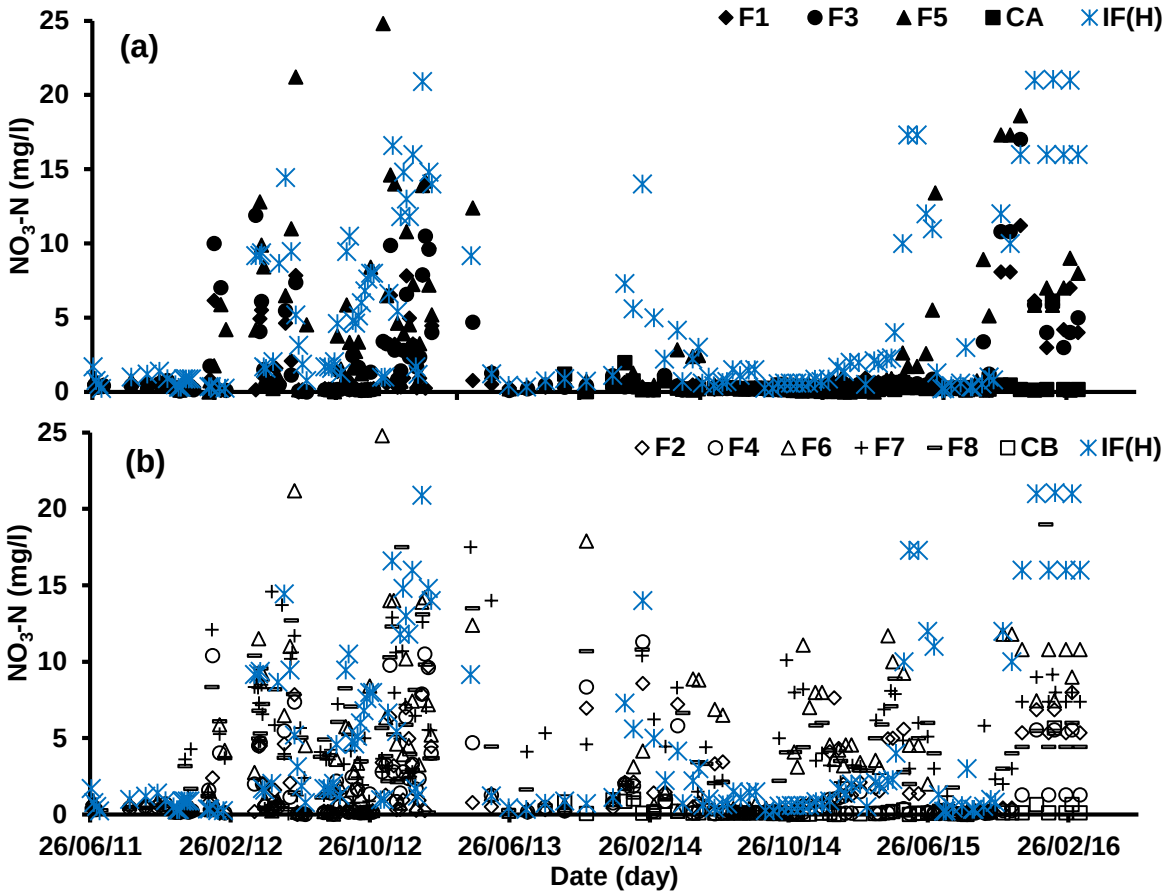


Figure 4.4: Overall variations in nitrate-nitrogen (NO_3-N) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

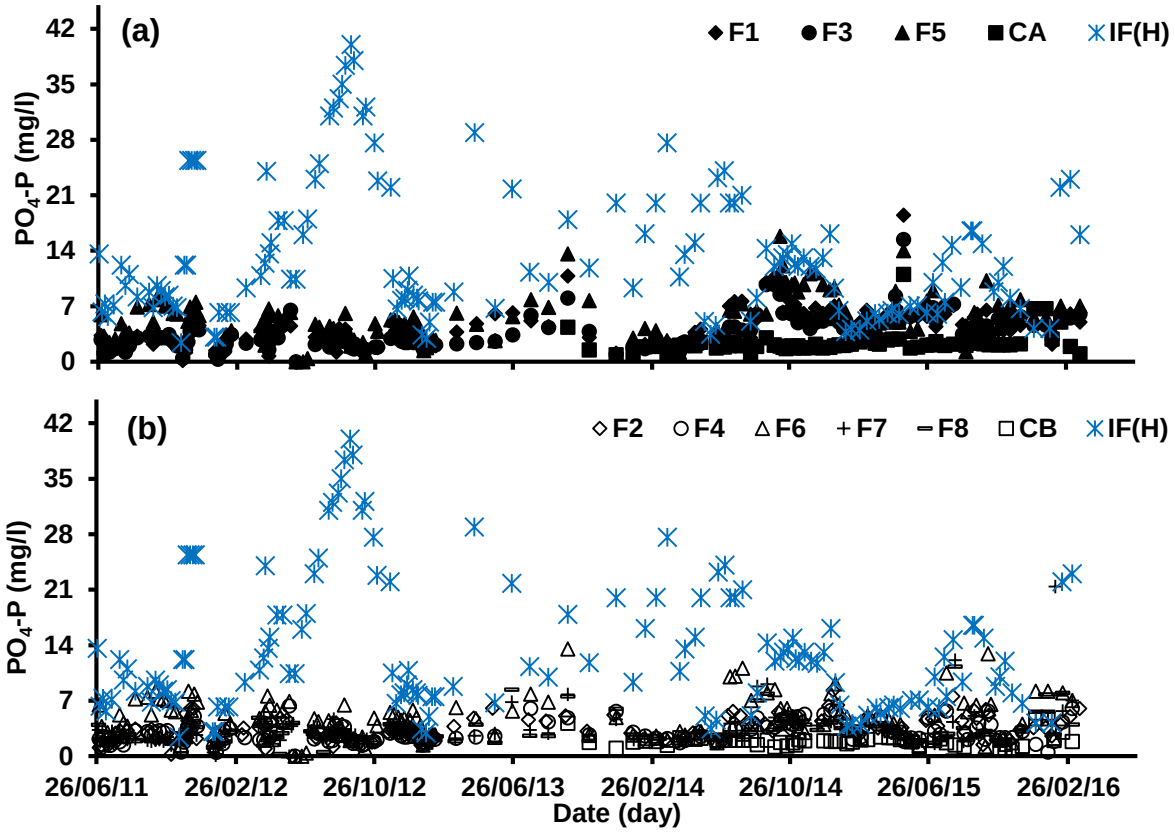


Figure 4.5: Overall variations in ortho-phosphate-phosphorus (PO_4-P) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

4.2.2.3 Comparison of particles

The primary mechanisms responsible for suspended solids elimination within constructed wetlands are: settling and sedimentation, microbial biodegradation, adsorption, aggregation and surface adhesion (ITRC, 2003; Garcia et al., 2010; Hua et al., 2013; Vymazal, 2014).

Findings show that the removal efficiency of SS for all experimental phases is generally relatively high (Tables 4.2 to 4.6), ranging from 67-97%. It has been suggested that the filter biomass improves with time (matured) and that the biodegradation rate is high (Scholz, 2003; De Biase et al., 2011). However, filters with hydrocarbon contamination showed elevated SS concentrations compared to those without hydrocarbons. This illustrates the effect of adding petroleum hydrocarbon compounds, as influent to the wetland system, which contain a combination of various organic particles. Depending on the stage of biodegradation over time, initially dying contaminated biomass, and later on, degraded diesel, contributed to elevated SS and turbidity values within the filters (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Moreover, some diesel components are water-insoluble, and when these gradually entered the wetland it led to an increase in the suspended solids concentrations within the filter bed (Sutton et al., 2013; Al-Isawi et al., 2015a). During the period of the second diesel spill (fifth phase, Table 4.6), SS removal efficiencies dropped for filters contaminated with diesel highlighting the effect of additional SS loads associated with high dosage of petroleum hydrocarbon.

Figures 4.6 and 4.7 provide an overview of the SS and TBD distribution over time. Most SS accumulated in the litter zone of all filters which resulted from long-term formation of dirt layers. The outflows were usually below the threshold value of 30 mg/l (Royal Commission on Sewage Disposal, 1915). These findings confirm results of previous studies (Hua et al., 2010; Sani et al., 2013b; Al-Isawi et al., 2015b; Scholz, 2015) noting the accumulation of SS in the top part of the litter zone within constructed wetlands, indicating that different aggregate-based substrates have little influence over SS detention within the filtration system, at least during the early stages of their operation. Furthermore, findings from this research showed high removal efficiency for SS for all

filters and the accumulation of SS in the wetland media did not impact negatively on the treatment performance of the wetlands system with no evidence to indicate clogging phenomena within the wetland bed even for filters contaminated with petroleum hydrocarbons (Al-Isawi et al., 2015a).

Generally, it has been noticed that the suspended solids concentrations of the effluent of the wetland system decreased with increasing age of the wetland. This is linked mostly to the development of biomass within the wetland system that provides an oxygen-rich environment, along with the improvement of the microbial community which supports a range of aerobic bacteria that leads to more degradation of the suspended solids across the bed of the wetland (Abou-Elela et al., 2014; Merriman & Hunt Iii, 2014; Paing et al., 2015a; Kim et al., 2016).

Table 4.7 presents the non-parametric Mann-Whitney U-test findings. There is a significant difference in performance for all filters related to SS depending on the operation stage of wetland system. Inflow COD load and aggregate size significantly ($p \leq 0.05$) impacted on the SS treatment performance in the earlier stage of wetland operation, while contact time impacted significantly ($p \leq 0.05$) after the wetlands system became mature. Turbidity performance results showed a significant difference ($p \leq 0.05$) in both contact time and rest time for long-term wetland system operation. This could be an indication that biodegradation and other reactions taking place in contaminated wetlands were responsible for turbidity differences observed. Furthermore, overall SS and TBD performances of wetland filters without diesel contamination were statistically different ($p \leq 0.05$) to the corresponding wetland filters operated with diesel contamination (Table 4.7).

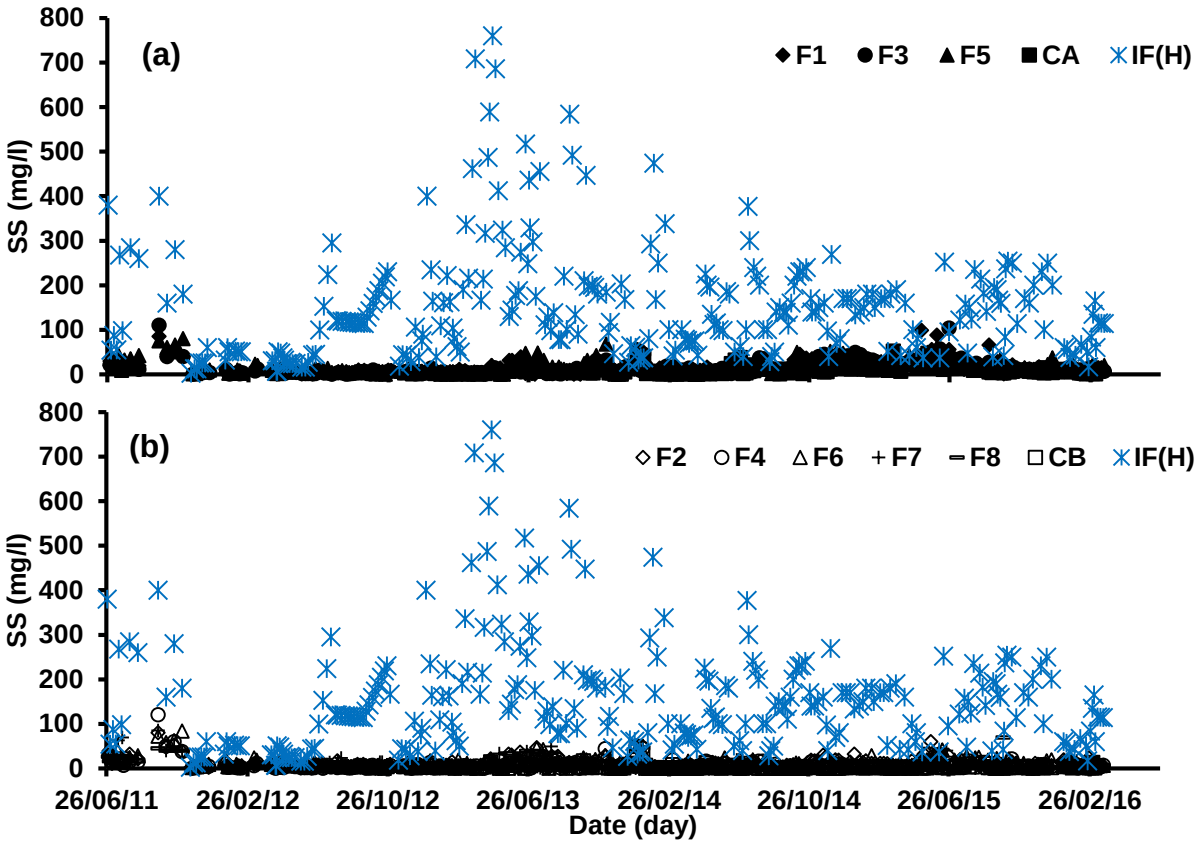


Figure 4.6: Overall variations in suspended solids (SS) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

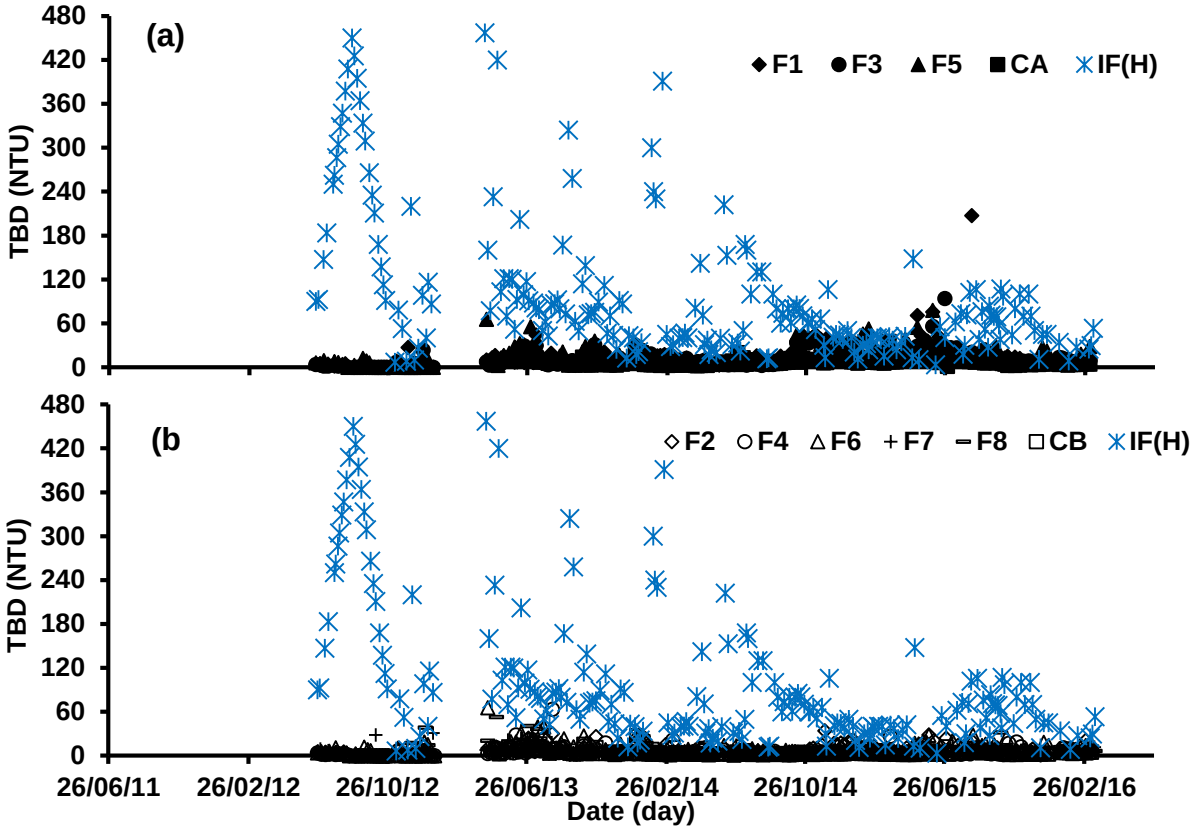


Figure 4.7: Overall variations in turbidity (TBD) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

4.2.2.4 Comparison of other water quality parameters (pH, redox potential, electrical conductivity, dissolved oxygen)

The pH conditions have a sensitive influence on the effluent quality including: COD, BOD, SS, and nutrients in constructed wetlands, as it is expected to have an influence on the ability of microbial populations to degrade pollutants (Eke & Scholz, 2008; Imfeld et al., 2009; Al-Baldawi et al., 2013b; Lavrova & Koumanova, 2013; Paing et al., 2015a).

In the current study, the pH values for raw wastewater (influent) were relatively variable and ranging from neutral to alkaline (Table 4.1), reflecting the use of real domestic wastewater. The overall pH values for effluent were ranging around the neutral zone for most of the experimental period (Tables 4.4 to 4.6), however, the values significantly ($p \leq 0.05$) decreased in wetland filters with diesel contamination (F1 and F5) during the period of pouring the diesel. This demonstrates that the high rate of nitrification process within these filters can lead to acidification of their environment (Scholz, 2010; Paing et al., 2015a). The preferable pH value range for most degraded bacteria is between 4 and 9.5, which is suitable for their survival (Kadlec & Wallace, 2009) and to maintain their activities (Xu et al., 2016). Figure 4.8 shows the distribution of pH values for all wetland filters during the whole experimental period. Statistical analysis between wetland filters indicates that aggregate size and inflow load had a significant impact on pH values ($p \leq 0.05$) regarding filters without petroleum hydrocarbon contamination (Table 4.7).

With regard to redox potential, electrical conductivity, and dissolved oxygen parameters used in this research, the collection of these data began during the periods after petroleum hydrocarbon application, and therefore assessment of the behaviour of wetland filters in terms of variations in these parameters before and after diesel contamination is not applicable. However, comparison between the similar filters (design and operational variables) with and without diesel contamination would be useful to assess their impact on treatment performance (Al-Isawi et al., 2015a). Findings related to these mentioned parameters during both fourth and fifth phases are shown in Tables 4.5 and 4.6. Statistical analysis showing comparison between wetland filters related to these variables for the period after diesel application is shown in Table 4.7.

Figure 4.9 shows the variation in redox potential values in all wetland filters for the period after petroleum hydrocarbon application. Monitoring the redox within the wetland filter can be useful to assess its role related to pollutants removal efficiency in the wetland system (Eke & Scholz, 2008; Al-Baldawi et al., 2013b). Generally, the variation in redox values was relatively small between wetland filters with and without diesel contamination (Tables 4.5 and 4.6). This might be due to the impact of the tidal-flow operation mode to provide a continuous oxygen availability within the wetland bed (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Moreover, the second period of diesel spill performance shows, surprisingly, no significant changes in wetland redox (Figure 4.9) as compared with other studies (Lin & Mendelssohn, 2009; Al-Baldawi et al., 2013b; Al-Baldawi et al., 2014a; Al-Baldawi et al., 2015a) which found increasing diesel concentration in contaminated wetland systems led to high reduction in the redox environment. This could be attributed to the high oxygen availability that impacted on wetland conditions observed (Figure 4.9). Findings of removal efficiency for water quality parameters (Tables 4.5 and 4.6) show that redox performance was constantly sufficient to produce a suitable treatment environment (Al-Isawi et al., 2015a). Statistical analysis of redox potential values did not show any difference which significantly ($p \geq 0.05$) impacted the treatment performance among the wetland filters (Table 4.7).

Regarding the electrical conductivity (EC) for wetland filters, Figure 4.10 presents the variation in EC values over the period of diesel spill application. Generally, overall conductivity performances were statistically compared between different wetland filters with and without diesel contamination and the results indicate that wetland filters with high inflow load (F5 and F6) were statistically different ($p \leq 0.05$) to those operated with low load rate (F3 and F4), reflecting the high organic matter associated with undiluted

inflow wastewater that resulted in an increase in the conductivity of treated wastewater in the wetland filter (Table 4.7), while conductivity results for other filters were statistically similar ($p \geq 0.05$).

With regard to dissolved oxygen in wetlands, dissolved oxygen is an essential parameter for metabolism of micro-organisms, and in order to optimize the performance of treatment processes, adequate dissolved oxygen concentration should be maintained in the wetland (Al-Baldawi et al., 2013b; De Biase et al., 2013; Hou et al., 2016). The primary pathways for oxygen transfer into constructed wetlands operated by the tidal-flow mode are: contact transfer at the interface of biofilm and atmosphere during drained times, wetland plants release via their roots, and DO associated with inflow wastewater (Wu et al., 2011b; Hou et al., 2016). In this study, Figure 4.11 presents the results of dissolved oxygen concentrations over time for all wetland filters. Generally, dissolved oxygen concentrations values showed an increase in with time for all wetland filters, highlighting the impact of the mature wetland system with tidal-flow mode operation that resulted in enhancement of the oxygen availability within the filter bed (Wu et al., 2011b; Wu et al., 2015e; Kim et al., 2016). However, wetland filters contaminated with diesel spills were associated with lower DO concentrations as compared with filters without diesel contamination (Tables 4.5 and 4.6). According to Lin and Mendelssohn (2009) and (Al-Baldawi et al., 2014a), petroleum hydrocarbon compounds in the constructed wetlands reduce the substrate's oxidation-reduction potential, indicating that the filter bed becomes more anaerobic which is the case in this study. Overall DO performances of wetland filters operated with low contact time (F7) were statistical significantly different ($p \leq 0.05$) from those operated with high contact time (F3 and F4) (Table 4.7). Moreover,

wetland filters with diesel contamination were statically different ($p \leq 0.05$) as compared with filters without diesel contamination.

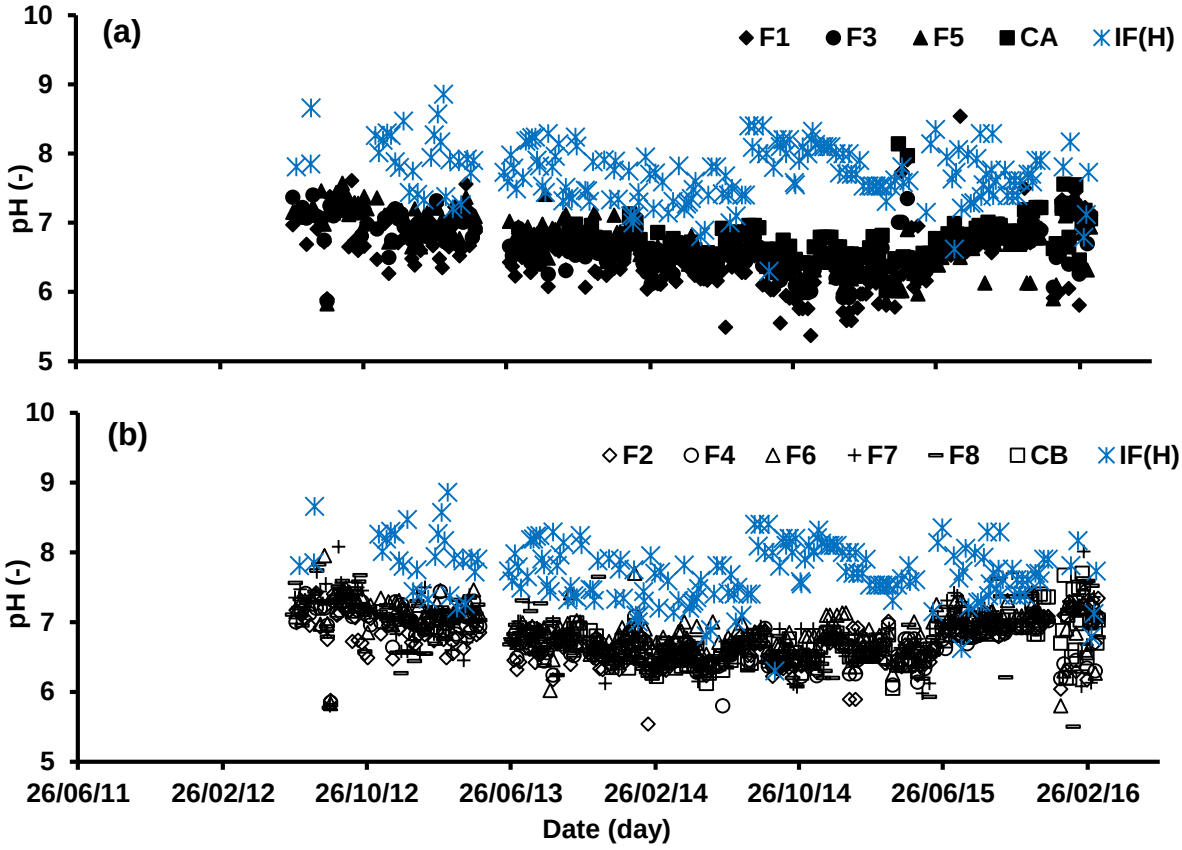


Figure 4.8: Overall variations in pH for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

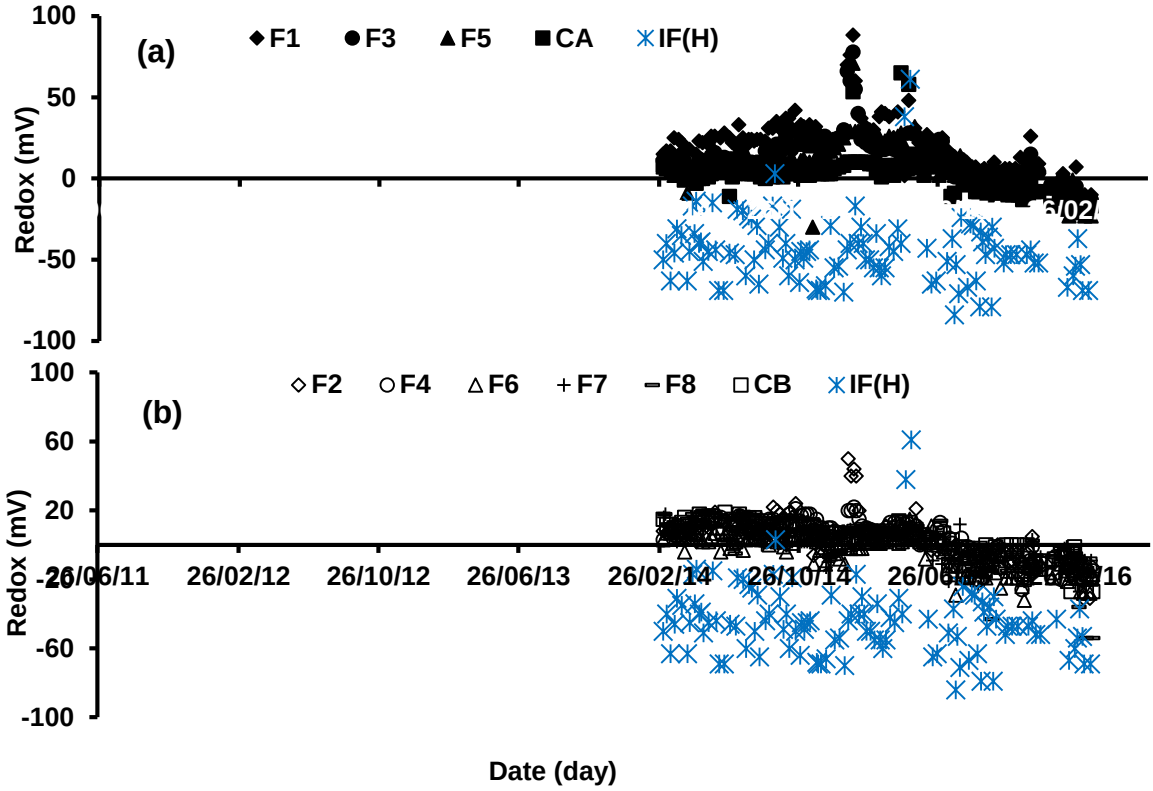


Figure 4.9: Overall variations in redox potential for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

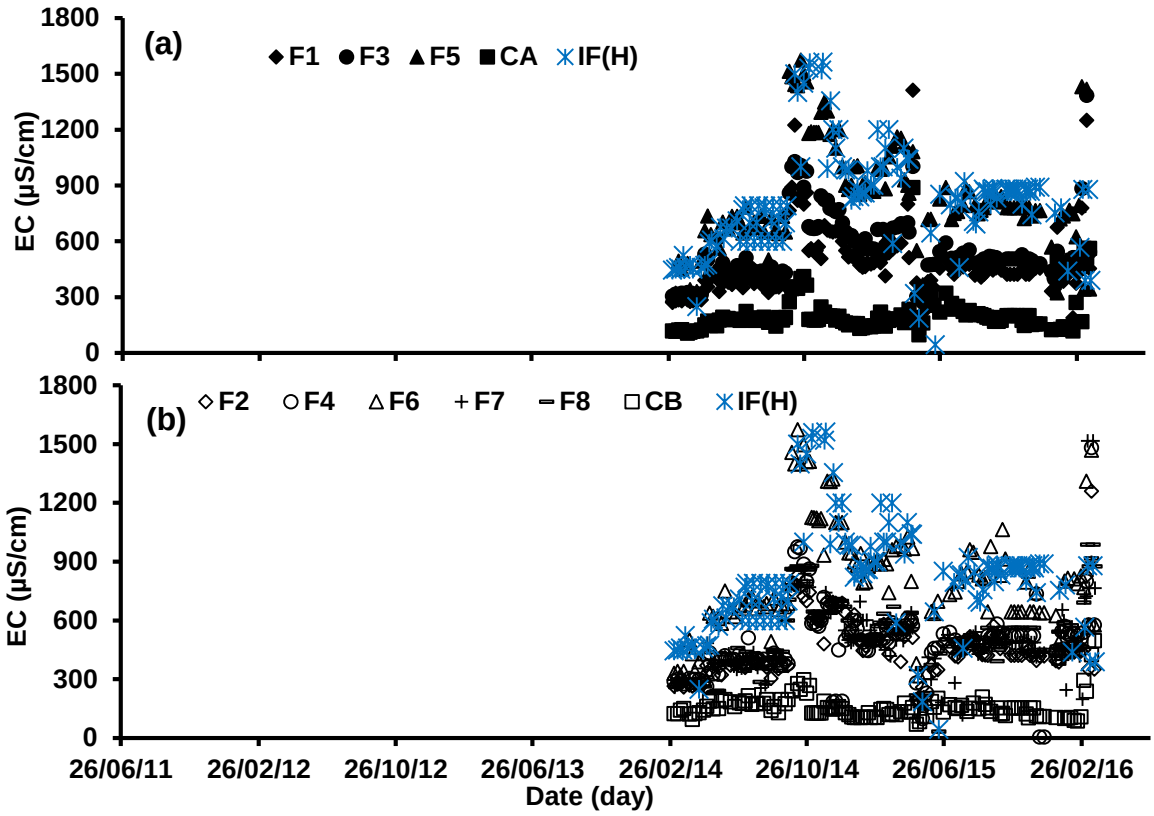


Figure 4.10: Overall variations in electrical conductivity (EC) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

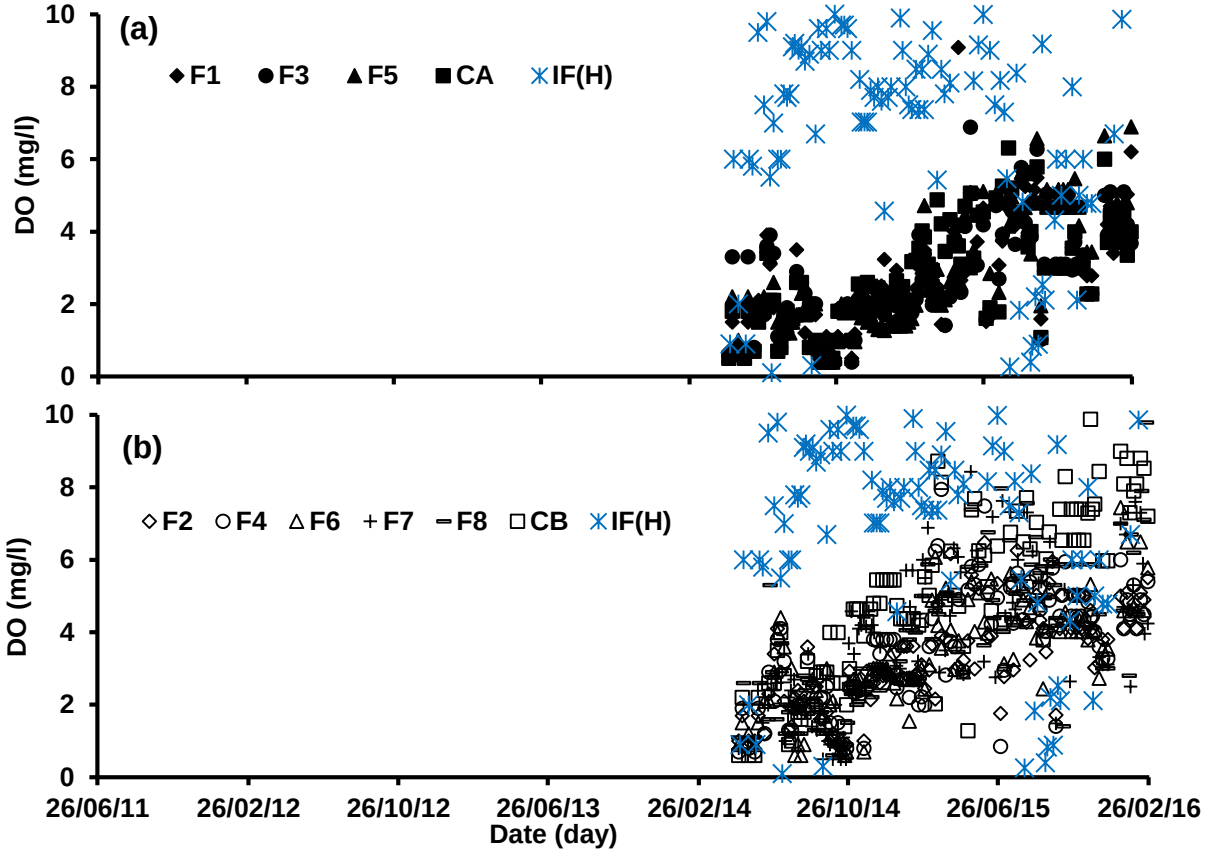


Figure 4.11: Overall variations in dissolved oxygen (DO) for the outflow of filters (a) with and (b) without diesel contamination. Note: IF(H), influent wastewater (before dilution); F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

4.3 Seasonal treatment performance of wetland filters

4.3.1 Seasonal inflow water quality

The seasonal performance of the inflow water quality for the selected parameters (COD, BOD, NH_4-N, NO_3-N, PO_4-P, and SS) data of over 55 months of wetland operation (June 2011 to March 2016) are shown in Tables 4.8 to 4.10. The inflow water quality data shows relatively high variability with season. This demonstrates the impact of using real wastewater as influent for the wetland system (Sani et al., 2013a; Al-Isawi et al., 2015b), which is usually subjected to seasonal and weather conditions. The mean inflow concentration values for COD, BOD, NH4-H, NO_3-N, PO_4-P, and SS, were relatively high and variable during the three periods: before diesel application (June 2011-September 2013), first diesel spill period (September 2013-September 2014), and second diesel spill period (September 2014-March 2016). The monitored data showed high variability and unexpected changes with seasons for most of the water quality parameters in addition to no clear trend in seasonal variability being observed.

Table 4.8: Seasonal inflow water quality parameters (value and sample number in brackets, and standard deviation) of domestic wastewater mixed with urban runoff before dilution for the period before diesel spill application (26/06/2011 to 21/09/2013).

Parameter	Unit	Summer 2011[a]	Autumn 2011[b]	Winter 2011/12[c]	Spring 2012[d]	Summer 2012[e]
Chemical oxygen demand	mg/l	407.0(10) ± 207.0	391.3(15) ±151.87	256.0(8) ±85.16	183.4(14) ±33.02	312.1(13) ±12.05
Biochemical oxygen demand	mg/l	nm[j]	nm[j]	nm[j]	nm[j]	101.0 (13) ±32.68
Ammonia-nitrogen	mg/l	20.4(8) ±8.8	21.2(10) ±5.82	27.9(7) ±10.45	49.1(12) ±13.6	71.5(11) ±7.53
Nitrate-nitrogen	mg/l	0.8(5) ±0.56	0.8(10) ±0.35	0.3(4) ±0.06	5.5(10) ±4.27	3.5(11) ±3.36
Ortho-phosphate-phosphorus	mg/l	9.0(9) ±2.77	12.9(15) ±8.15	5.0(6) ±2.34	14.7(9) ±4.53	29.9(11) ±8.20
Suspended solids	mg/l	185.8(8) ±126.2	145.3(11) ±132.9	49.1(6) ±9.32	27.5(16) ±12.90	132.0(16) ±55.54
Temperature	°C	15	7.8	4.1	9.2	21.6

Parameter		Autumn 2012[f]	Winter 2012/13[g]	Spring 2013[h]	Summer 2013[i]
Chemical oxygen demand	mg/l	261.0(14) ±96.75	230.3(11) ±91.94	186.0(2) ±2.83	244.7(3) ±110.73
Biochemical oxygen demand	mg/l	108.6(12) ±12.44	118.0(16) ±67.76	221.2(15) ±33.50	150.4(17) ±64.1
Ammonia-nitrogen	mg/l	65.0(14) ±13.5	46.0(12) ±21.99	69.4(2) ±4.81	79.07(3) ±46.4
Nitrate-nitrogen	mg/l	6.7(14) ±4.00	12.0(9) ±6.51	5.2(2) ±5.61	0.5(3) ±0.21
Ortho-phosphate-phosphorus	mg/l	18.71(9) ±10.52	7.18(11) ±2.43	17.81(2) ±15.68	14.36(3) ±6.48
Suspended solids	mg/l	125.7(14) ±77.28	158.5(17) ±100.83	379.9(18) ±206.44	232.9(18) ±162.11
Temperature	°C	11.8	9.0	17.9	24.1

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; and [j]not measured.

Table 4.9: Seasonal inflow water quality parameters (value and sample number in brackets, and standard deviation) of domestic wastewater mixed with urban runoff before dilution during the period of first diesel spill (22/09/2013 to 21/09/2014).

Parameter	Unit	Autumn 2013[a]	Winter 2013/14[b]	Spring 2014[c]	Summer 2014[d]
Chemical oxygen demand	mg/l	352.5(2) ±10.61	200.7(3) ±73.22	232.6(9) ±74.89	259.3(7) ±80.73
Biochemical oxygen demand	mg/l	167.1(14) ±110.00	104.3(12) ±72.56	95.7 (14) ±84.33	87.3 (15) ±64.96
Ammonia-nitrogen	mg/l	32.2(3) ±28.10	41.4(5) ±25.04	23.4(8) ±11.58	44.8(7) ±13.39
Nitrate-nitrogen	mg/l	0.8(2) ±0.12	5.7(5) ±5.48	1.8(8) ±1.27	1.0(7) ±0.52
Ortho-phosphate-phosphorus	mg/l	14.85(2) ±4.31	16.37(4) ±5.04	12.5(8) ±8.40	17.0(8) ±7.13
Suspended solids	mg/l	166.6(14) ±102.83	147.5(14) ±138.50	118.4(17) ±57.94	140.9(18) ±95.3
Temperature	°C	15	10.7	18.2	25.7

[a]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [b]21/12/14 to 19/03/14; [c]20/03/14 to 21/06/14; and [d]20/06/14 to 21/09/14.

Table 4.10: Seasonal inflow water quality parameters (value and sample number in brackets, and standard deviation) of domestic wastewater mixed with urban runoff before dilution during the period of second diesel spill (22/09/2014 to 19/03/2016).

Parameter	Unit	Autumn 2014[a]	Winter 2014/15[b]	Spring 2015[c]	Summer 2015[d]	Autumn 2015[e]
Chemical oxygen demand	mg/l	302.7(6) ±95.81	168.3(8) ±40.05	329.5(6) ±223.64	395.0(9) ±155.98	226.9(7) ±101.26
Biochemical oxygen demand	mg/l	195.1(18) ±65.19	215.0 (16) ±37.87	172.5(15) ±57.85	162.1(14) ±63.86	164.1(16) ±84.72
Ammonia-nitrogen	mg/l	49.6(8) ±8.59	21.2(6) ±8.08	6.9(8) ±3.06	22.3(9) ±3.77	21.5(7) ±6.29
Nitrate-nitrogen	mg/l	0.6(6) ±0.24	1.3(7) ±0.78	7.1(8) ±6.86	2.9(10) ±4.69	6.7(6) ±6.8
Ortho-phosphate-phosphorus	mg/l	12.7(10) ±1.05	8.3(7) ±4.88	6.3(7) ±0.61	11.0(9) ±4.28	10.1(6) ±3.06
Suspended solids	mg/l	162.2(16) ±67.01	154.9(13) ±34.97	111.9(10) ±78.07	147.1(14) ±57.88	173.1(13) ±71.27
Temperature	°C	13	7.1	14.1	27.2	13.7

Parameter	Unit	Winter 2015/16[f]
Chemical oxygen demand	mg/l	216.8(9) ±48.07
Biochemical oxygen demand	mg/l	145.6(10) ±92.31
Ammonia-nitrogen	mg/l	40.3(4) ±0.56
Nitrate-nitrogen	mg/l	18.5(6) ±2.78
Ortho-phosphate-phosphorus	mg/l	20.0(6) ±17.19
Suspended solids	mg/l	79.9(14) ±42.49
Temperature	°C	11.1

[a]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [b]21/12/14 to 19/03/2015; [c]20/03/15 to 20/06/2015; [d]21/06/15 to 21/09/15; [e]22/09/15 to 20/12/15; and [f]21/12/15 to 19/03/16.

4.3.2 Seasonal comparison of outflow water qualities

Figure 4.12a-f shows the overall seasonal comparison of the outflow water quality for all wetland filters. The figures demonstrate the results of the investigation into the relationship between various variables and hydrocarbon removal in constructed wetlands by assessing the roles played by seasonal changes. Note that data for Spring 2016 were not shown, since the data collection was stopped at 22/03/2016.

Generally, long-term operation of all wetland system filters (except for filters with diesel contamination) showed a high removal efficiency, particularly, in the period after the second diesel spill, of the major water quality parameters COD, BOD, NH_4-N, NO_3-N, and SS (Figure 4.12a,b,c,d,e,f). This could highlight the impact of full maturity of the wetland system, as a result of well-established microbial populations, vegetation and favourable operating conditions achieved over time (Scholz et al., 2002; Al-Isawi et al., 2015b; Scholz, 2015).

With the exception of the set-up period and the period after diesel spill application, all wetland filters showed a good seasonal COD treatment performance (Figure 4.12a) with clear seasonal trend of high COD concentrations in autumn and low COD concentrations in summer. The seasonal variations shown in water quality (COD) concentrations are probably due to temperature fluctuations and activity of micro-organisms (Al-Isawi et al., 2015b; Scholz, 2015). Seasonal variations have been also reported by several researchers, with the lower treatment performance occurring during the cold seasons (Song et al., 2006; Sani et al., 2013a). This lower concentration noted in cold weather could be as a result of slow activity of micro-organisms during the period which leads to low microbial contaminants attenuation. Several studies have shown that micro-organisms are not active and energetic in cold periods in wetland systems. For instance, Imfeld et al. (2009) stated

that wetland microbes are not efficient in organic compounds removal in cold period in wetland systems confirming the data of the current study. However, in this study, no significant differences in seasonal COD variations ($p \geq 0.05$) were noted among the filters in the last period of the experiment, highlighting the fact that the wetlands became fully matured (Figure 4.12a). This finding was also confirmed by (Vymazal, 2013c). This can be explained by the development of microbial activities that acclimatized over time leading to high pollutants degradation (Scholz, 2006; Sani et al., 2013a; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Scholz, 2015). Furthermore, BOD concentrations also showed a good treatment performance with a clear seasonal trend of high BOD concentrations in summer and low BOD concentrations in winter (Figure 4.12b). This trend was confirmed previously by (Scholz, 2010; Sani et al., 2013a; Al-Isawi et al., 2015b). The seasonal variations shown for BOD values could be possibly due to temperature fluctuations (Al-Isawi et al., 2015b; Scholz, 2015). In turn, the absence of plants in filters with diesel contamination indicates a direct effect of air temperature variations on removal rates. It has been shown that high temperatures during the summer season (more than 15°C), could have stimulated evaporation rates, and resulted in an increase in the BOD concentration values (Gikas & Tsihrintzis, 2012; Papaevangelou et al., 2012). The statistical analysis showed that a significant difference in seasonal variation ($p \leq 0.05$) for COD and BOD was noted when filters with high inflow load rates were compared with low inflow load rates. Furthermore, aggregate diameter, contact time, and resting time did not show significant differences on seasonal treatment performance. Regarding the filters with diesel contamination, it was difficult to find a clear seasonal trend as they are impacted by two dosages of diesel fuel each, poured in September 2013 and September 2014, respectively, which led to high changes in most of the water quality parameters with time (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b).

Figures 4.12c,d,e show the seasonal variation of ammonia-nitrogen, nitrate-nitrogen, and ortho-phosphate-phosphorus concentrations over time. With regard to the seasonal variation in NH_4-N concentrations, Figure 4.12c demonstrates no clear seasonal trend observed (Al-Isawi et al., 2015b). However, the lowest values, which are observed in spring, could be attributed to the high microbial activity which was elevated with increasing temperature in addition to increase in oxygen and carbon and as a result led to a high nitrification process within the wetland filter (Akratos & Tsihrintzis, 2007; Gikas & Tsihrintzis, 2012; Sani et al., 2013a). Moreover, this unclear seasonal variability trend observed for some water quality variables, especially in winter, might be due to soil microbes which may still have the capacity to decompose organic matter in winter, and low temperatures which enhance aerobic metabolism through the increase of dissolved oxygen saturation (Al-Isawi et al., 2015b). A typical standard set by environment agencies for NH_4-N removal concerning secondary wastewater treatment is 20 mg/l (Royal Commission on Sewage Disposal, 1915). However, NH_4-N concentrations for Filters 5 and 6 (subject to higher loading rate) were frequently above this threshold. However, the period during the fifth year of system operation showed an improvement in their treatment performance. This could be highlighting that the age of the wetland system led to improve treatment performance (Mustafa et al., 2009; Dong et al., 2012).

Regarding to NO_3-N concentration values, the result shows high values in winter and low values in summer (Figure 4.12d) confirming previous findings (Sani et al., 2013a; Al-Isawi et al., 2015b). This reflects the differentiated activities for bacterial species responsible for ammonification, nitrification, and denitrification during cold seasons as the activity levels of these enzymes are affected by variation in temperature and influent load (Xie et al., 2016). Moreover, the results showed a low water quality performance

during the winter. It is uncertain whether the poor winter performances were due to low temperatures alone or the combined effect of operating conditions and other variables. Furthermore, aggregate diameter, contact time, resting time and loading rate did not show any significant differences in seasonal nitrate-nitrogen treatment. Ortho-phosphate-phosphorus values (Figure 4.12e) were relatively high and variable, but no clear trends among filters were observed (Al-Isawi et al., 2015a). This demonstrates the long-term phosphorus accumulation as the wetland substrate begins the saturated stage. Moreover, PO_4-P treatment performance is considered temperature independent because most of the main treatment processes are physical and less are biological (Scholz, 2010; Sani et al., 2013a; Scholz, 2015), therefore it is difficult to find a clear variation trend with season.

Figure 4.12f shows the seasonal variation for suspended solids over time. There is no clear trend for seasonal variation of SS concentrations. This finding is in agreement with previous studies (Gikas & Tsihrintzis, 2012; Sani et al., 2013a). This demonstrates the high treatment performance of the wetland filters which provide a high infiltration removal process as well as development of high growth of micro-organisms and well-established wetland plants (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b).

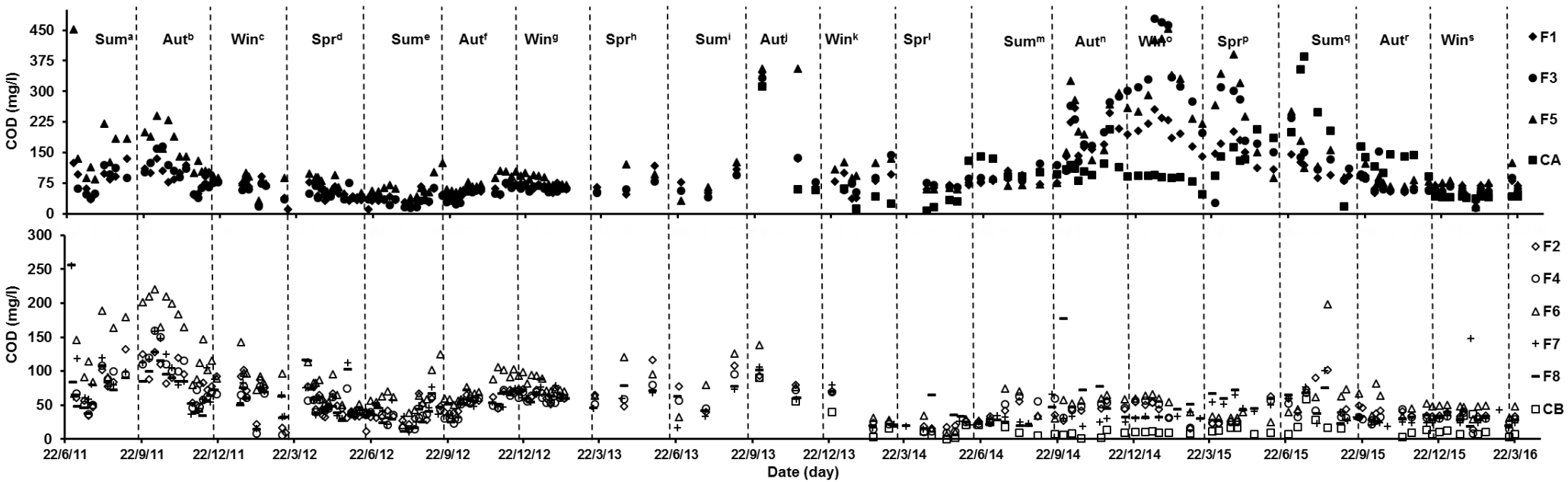


Figure 4.12a: Overall seasonal variations of chemical oxygen demand (COD) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; and [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

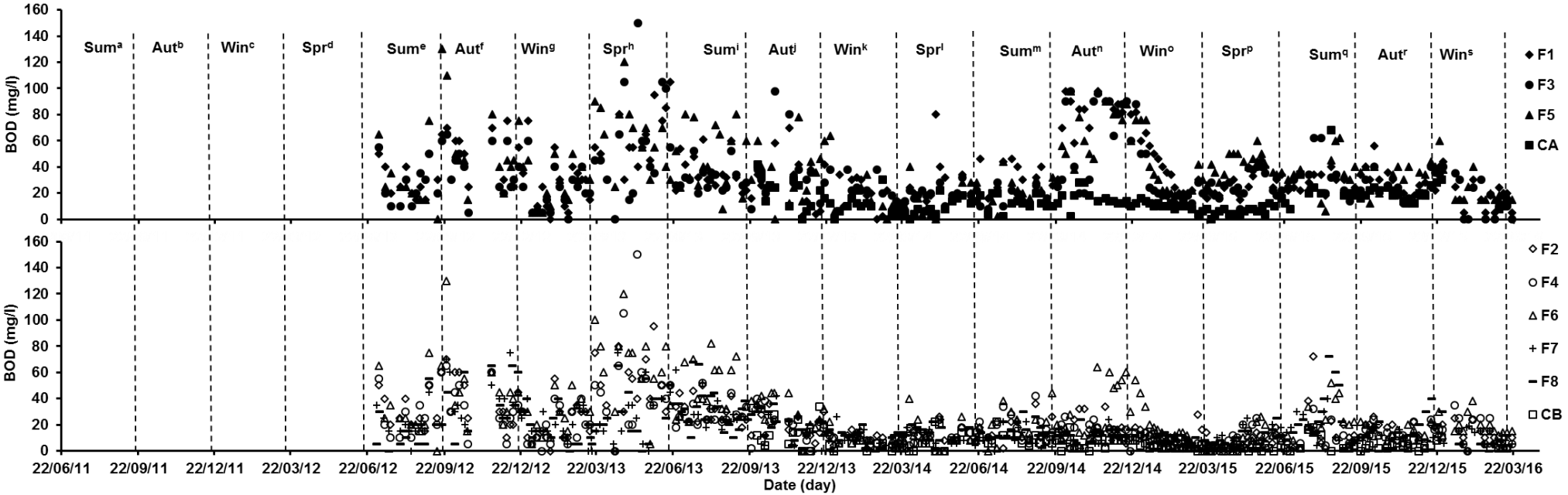


Figure 4.12b: Overall seasonal variations of biochemical oxygen demand (BOD) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

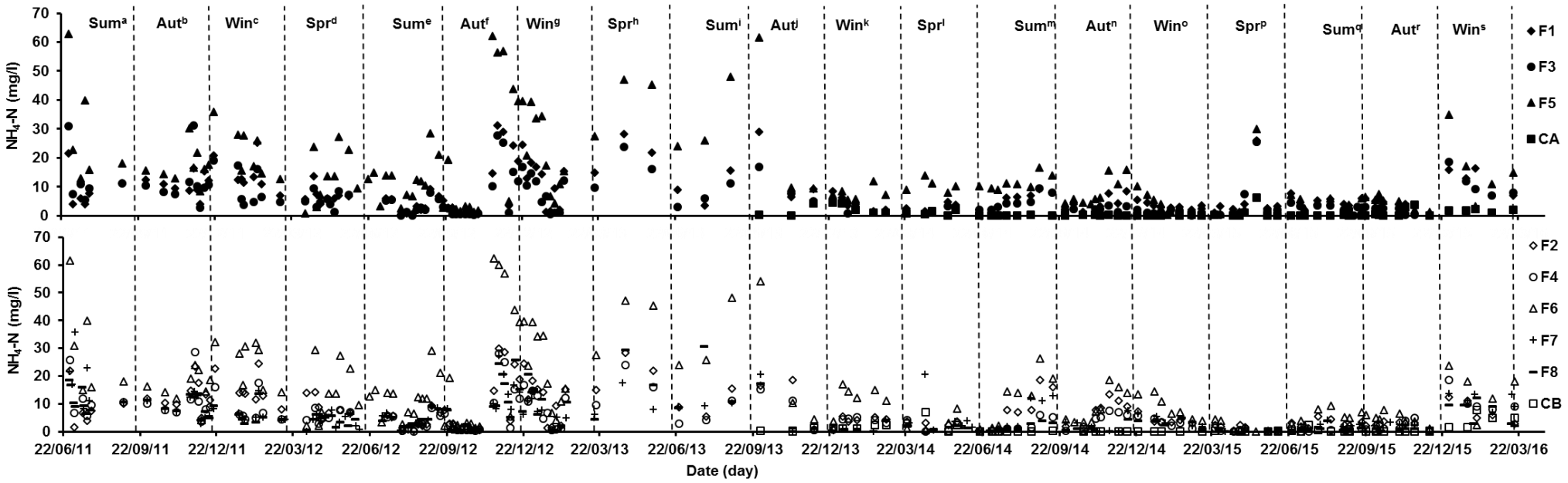


Figure 4.12c: Overall seasonal variations of ammonia-nitrogen (NH_4-N) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

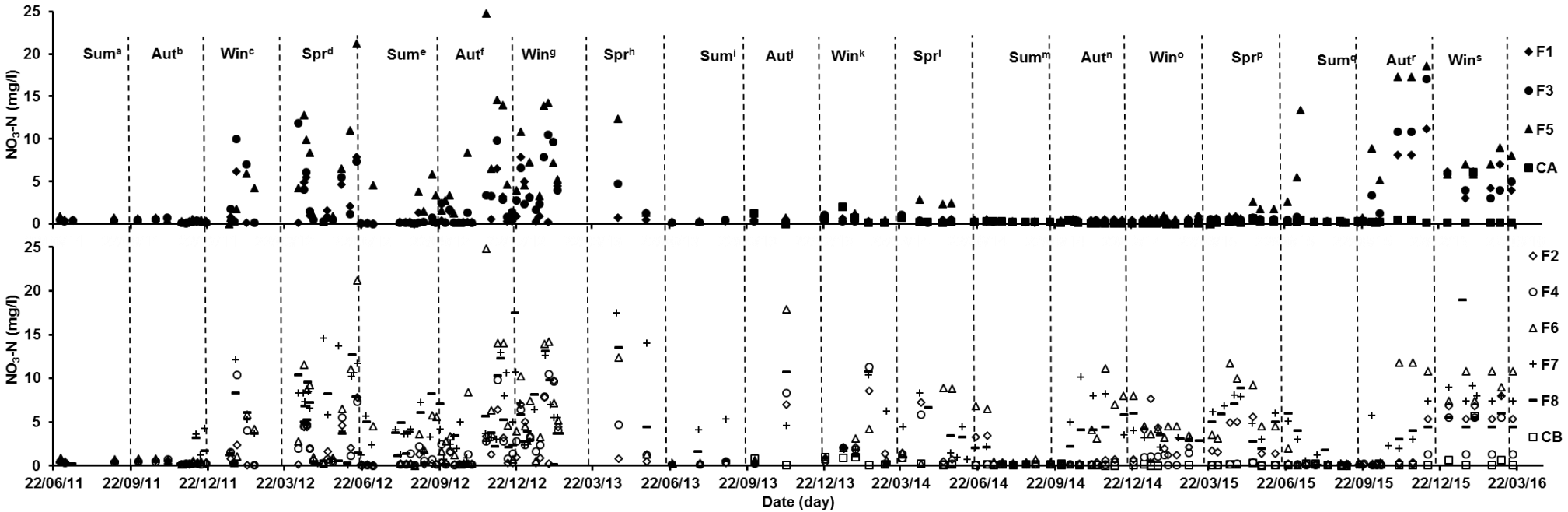


Figure 4.12d: Overall seasonal variations of nitrate-nitrogen (NO_3-N) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

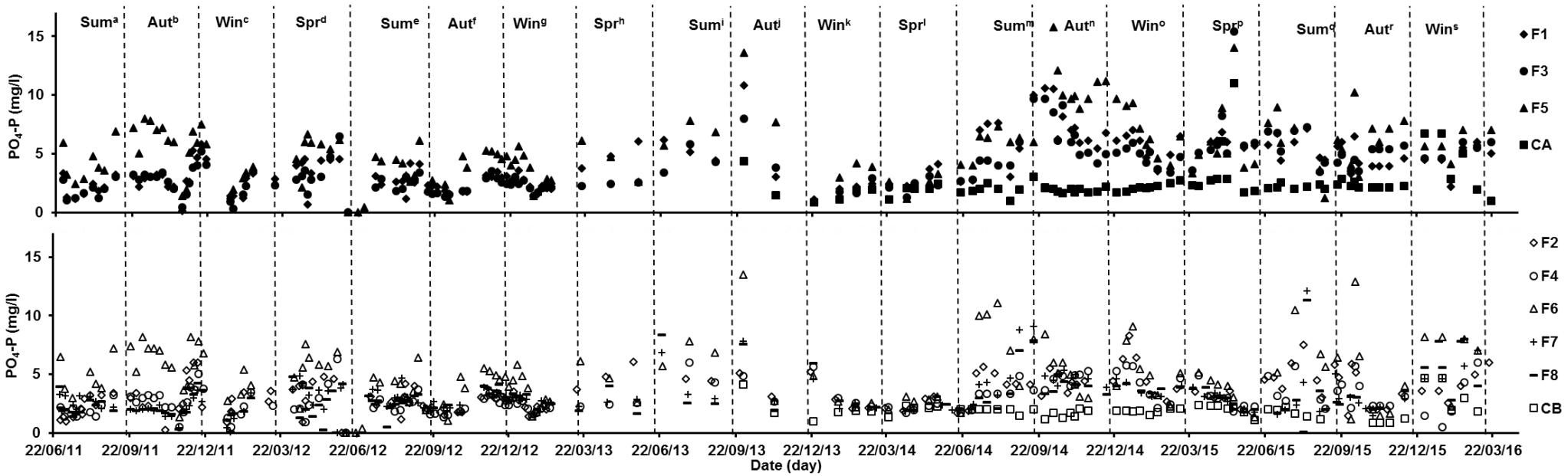


Figure 4.12e: Overall seasonal variations of ortho-phosphate-phosphorus (PO_4-P) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

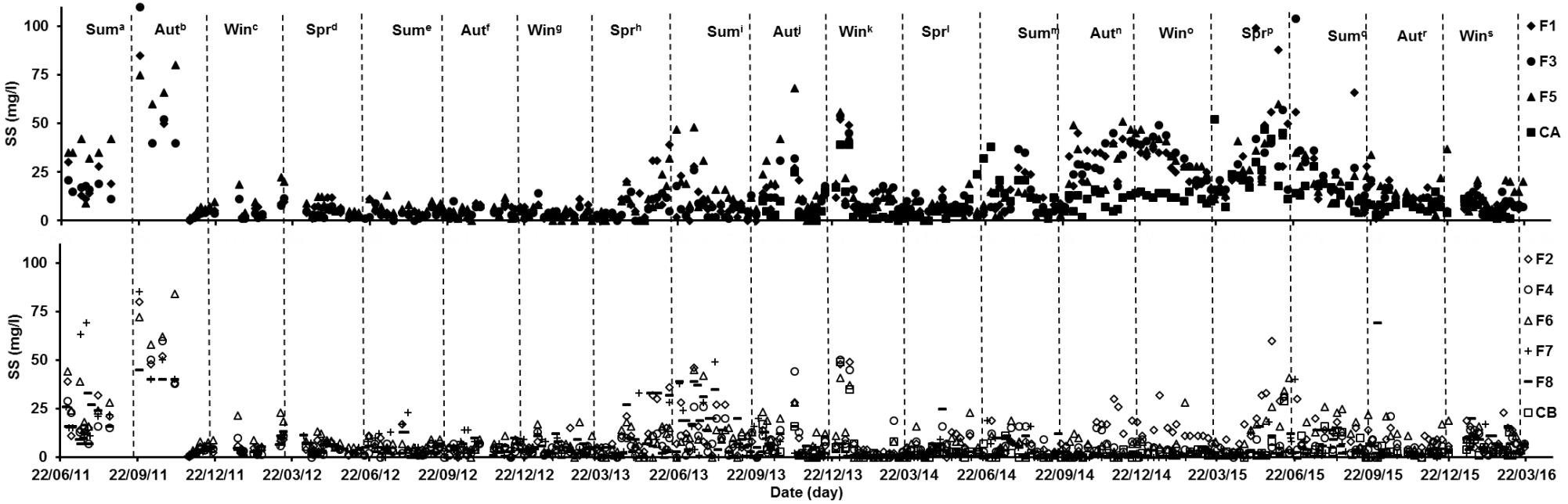


Figure 4.12f: Overall seasonal variations of suspended solids (SS) in filters (with/without) diesel contamination.

[a]21/06/11 to 22/09/11; [b]23/09/11 to 21/12/11; [c]22/12/11 to 19/03/12; [d]20/03/12 to 19/06/12; [e]20/06/12 to 21/09/12; [f]22/09/12 to 20/12/12; [g]21/12/12 to 19/03/13; [h]20/03/13 to 20/06/13; [i]21/06/13 to 21/09/13; [j]22/09/13 to 20/12/14 (first diesel dose poured on 26/09/2013); [k]21/12/14 to 19/03/14; [l]20/03/14 to 21/06/14; [m]20/06/14 to 21/09/14; [n]22/09/14 to 20/12/14 (second diesel dose poured on 26/09/2014); [o]21/12/14 to 19/03/2015; [p]20/03/15 to 20/06/2015; [q]21/06/15 to 21/09/15; [r]22/09/15 to 20/12/15; [s]21/12/15 to 19/03/16. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water).

4.4 Performance assessment of filter clogging within wetland filters

The results and discussions presented in this section have been published in the paper shown below:

Al-Isawi, R.H.K., Scholz, M., Wang, Y. & Sani, A. (2015). Clogging of vertical-flow constructed wetlands treating urban wastewater contaminated with a diesel spill. *Environmental, Science and Pollution Research*. 22, 12779–12803, doi:10.1007/s11356-014-3732-8.

4.4.1 Performance assessment for clogging processes within wetland filters based on water quality variables

Clogging of the porous media of SSF constructed wetlands results from the cumulative biological, chemical, and physical treatment processes within wetland systems (Knowles et al., 2011; Chang et al., 2012; Gikas & Tsihrintzis, 2012) and can be accompanied by a decrease in the treatment performance and pollutants removal of the wetland system (Fu et al., 2013; Huang et al., 2016). The main operational parameters related to clogging phenomena are solids loads and hydraulic conductivity rate (Knowles et al., 2010). Regarding this study, Table 4.11 provides an overview of the hydraulic conductivity measured as outflow volume during the five experimental phases (first experimental phase 27/06/11 to 25/09/11, second experimental phase 26/09/11 to 25/09/12; third experimental phase 26/09/12 to 25/09/13; fourth experimental phase 26/09/13 to 25/09/14; and fifth experimental phase 26/09/14 to 22/03/16). Hydraulic conductivity for all wetland filters in the first, second, third, and fourth experimental phases (Table 4.11), showed no significant differences in their values which meant that no clear indication of imminent clogging for any wetland filter was evident (Al-Isawi et al., 2015a). This

indicates that VF CW systems do not clog and prevent the hydraulic conductivity of the wetland filters and a small amount of diesel spill does not affect the operation performance of wetlands in the long term. While in the fifth experimental phase, there was a slight increase in the time required to drain wastewater from filters F5, F8, and CB as compared with the required time of draining the filters in the fourth experimental phase. This can be explained by a continuous accumulation of the particles and organic matter and development of a bio-film layer within the CW filter with long-term operation of the wetland system leading to reduced hydraulic conductivity (Song et al., 2015). Filter 5, which received a high inflow COD load, showed a significant decrease in hydraulic conductivity with time. This is explained by a large number of insoluble particles associated with the huge dosage of diesel (Al-Isawi et al., 2015a) applied to the affected filter in the fifth experimental phase (Al-Isawi et al., 2015a). Moreover, the inflow domestic wastewater contains organic matter in solid forms of different size and composition (Table 4.1) and accumulation of these particles with time can result in a decrease in the pores of the substrate media reducing the operational efficiency of the wetland system (Zhao et al., 2009).

Table 4.11 Hydraulic conductivity measured as the mean volume (l) of drained effluent per second.

Draining time (s)	60	120	180	240	300	360	420	480	540
Second experimental phase (26/09/2011 to 25/09/2012); n=5									
Filters 1 and 2 combined	2.31	1.88	1.34	1.30					
Filters 3 and 4 combined	2.07	1.76	1.37	0.84	0.40	0.40			
Filters 5 and 6 combined	1.60	1.41	1.24	0.93	0.83	0.40	0.28		
Filter 7	2.53	2.08	1.67	0.67					
Filter 8	2.32	2.07	1.76	1.81	0.06				
Filter A and B combined	2.04	1.93	1.62	0.63	0.54				
Third experimental phase (26/09/2012 to 25/09/2013); n=24									
Filters 1 and 2 combined	2.10	1.77	1.37	0.88	0.51	0.41	0.42	0.24	
Filters 3 and 4 combined	2.17	1.82	1.48	0.85	0.55	0.54	0.24	0.19	
Filters 5 and 6 combined	1.69	1.48	1.29	1.03	0.64	0.29	0.37	0.16	
Filter 7	2.32	2.05	1.56	0.83	0.41				
Filter 8	2.45	2.04	1.59	0.73	0.49	0.13			
Fourth experimental phase (26/09/2013 to 25/09/2014) (first diesel spill poured on 26/09/2013); n=38									
Filter 1	1.98	1.77	1.40	0.77	0.23				
Filter 2	2.58	2.11	1.46	0.44					
Filter 3	2.38	1.97	1.52	0.29					
Filter 4	2.04	1.83	1.45	0.91	0.55	0.18			
Filter 5	1.58	1.38	1.22	1.01	0.60	0.35			
Filter 6	2.19	1.74	1.53	0.87	0.91	0.22			
Filter 7	2.16	1.81	1.51	0.86	0.44				
Filter 8	2.14	1.86	1.38	0.89	0.55	0.36			
Control A	2.42	2.01	1.74	0.92	0.44				
Control B	1.85	1.53	1.36	1.17	0.69	0.32	0.33		
Fifth experimental phase (26/09/2014 to 22/03/2016) (second diesel spill poured on 26/09/2014); n=48									
Filter 1	1.88	1.67	1.37	0.73	0.22				
Filter 2	2.28	1.99	1.45	0.83	0.13				
Filter 3	2.14	1.92	1.12	0.71	0.36				
Filter 4	2.00	1.44	1.37	1.09	0.80	0.30			
Filter 5	1.36	1.18	1.12	0.90	0.60	0.45	0.28	0.18	
Filter 6	1.81	1.71	1.53	1.37	0.91	0.72			
Filter 7	2.06	1.81	1.51	0.86	0.44				
Filter 8	2.03	1.76	1.47	0.80	0.59	0.35	0.18		
Control A	2.12	1.91	1.84	0.97	0.54				
Control B	1.81	1.43	1.16	1.10	0.69	0.42	0.33		

The presentation of hydraulic conductivity within the cross-sectional area in the axial flow direction of each filter, with the application of Darcy's Law (eq. 2.1), demonstrates the hydraulic conductivity for the whole wetland filter without taking into account the

actual differences in porous media layers with depth and their impact on flowing the water (Platzer & Mauch, 1997; Nivala et al., 2012; Kim & Forquet, 2016). Therefore, the SS profile with wetland depth was measured to estimate where the flow restraint is likely to occur. Figures 4.13 and 4.14 indicate the suspended solids distribution within the wetland filters for the different depths at the five experimental phases (before and after diesel spills). None of the filters suffered from a breakthrough of solids, which would usually indicate that a filter is overloaded (Scholz et al., 2002; Scholz, 2006; Wu et al., 2015c).

The distribution of SS with depth (Figures 4.13 and 4.14) showed that the SS concentration decreased with an increase in filter depth. Due to adding the harvested above-ground biomass to the upper layer for each corresponding filter, an increase in the organic matter of the litter zone with time and in spring was noticed. Filters 5 and 6, which received wastewater without dilution, have SS concentrations higher than those of other filters (Figures 4.13c and 4.14), which received wastewater diluted with tap water. Due to the impact of low resting time of Filter 8 (Figure 4.13e), SS concentrations were higher than those of Filter 7 (Figure 4.13d). The fourth and fifth periods showed that SS concentrations for wetland filters were greater than those in the previous periods, highlighting two issues: firstly, the impact of the developing maturation stage of the wetland system leading to the continuous increase of a litter zone on the top layer of each wetland filter over the last two years of system operation and the subsequent effect on the wetland performance with time (Pedescoll et al., 2009; Pedescoll et al., 2011). The development of the litter zone was mainly from the strength of the organic matter and suspended solids associated with the influent wastewater and the dead wetland plants that were harvested and returned back to related wetland filters each winter. However, SS concentrations in the upper layer of wetland filters without hydrocarbon contamination

(F2, F4, and F6) in the fifth period were less than those in the fourth period which might reflect reaching the optimum stability stage of maturation of the wetland system, demonstrated by development of growth and activities of micro-organisms and the wetland plants system (Mustafa et al., 2009; Dong et al., 2012).

Secondly, filters subjected to the diesel spill showed SS concentrations higher than those without hydrocarbon contamination, particularly in the top layer of each filter. The effect of adding high dosage (150 g/l) petroleum hydrocarbon compounds to the selected wetland filters at the fifth period leads to an increase in accumulated matter within these filters (Figure 4.14). This observation confirms previous findings indicating that hydrocarbon compounds usually accumulate in the upper layers of wetland filters (Eke & Scholz, 2008). Moreover, the presence of mature wetland plants (Vymazal, 2014; Al-Isawi et al., 2015a) plays an indirect role in treatment of pollutants and prevention of clogging, by providing oxygen via the rhizosphere to oxygenate the surrounding area, and promote the growth of plant roots within filter media which in turn helps to increase decomposition of organic matter and prevents clogging by creating channels for the water to pass through (Stefanakis & Tsihrintzis, 2012; Wu et al., 2015f). Generally, the decrease in hydraulic conductivity values for the fifth experimental period and the continuous increasing of accumulated particles noticed in the upper layer of wetland filters, particularly filters with diesel contamination, did not present any severe negative impact in the treatment performance of the wetlands system (Tables 4.2 to 4.6) or decrease in hydraulic malfunctions, such as ponding of wastewater on the surface of the wetland filter.

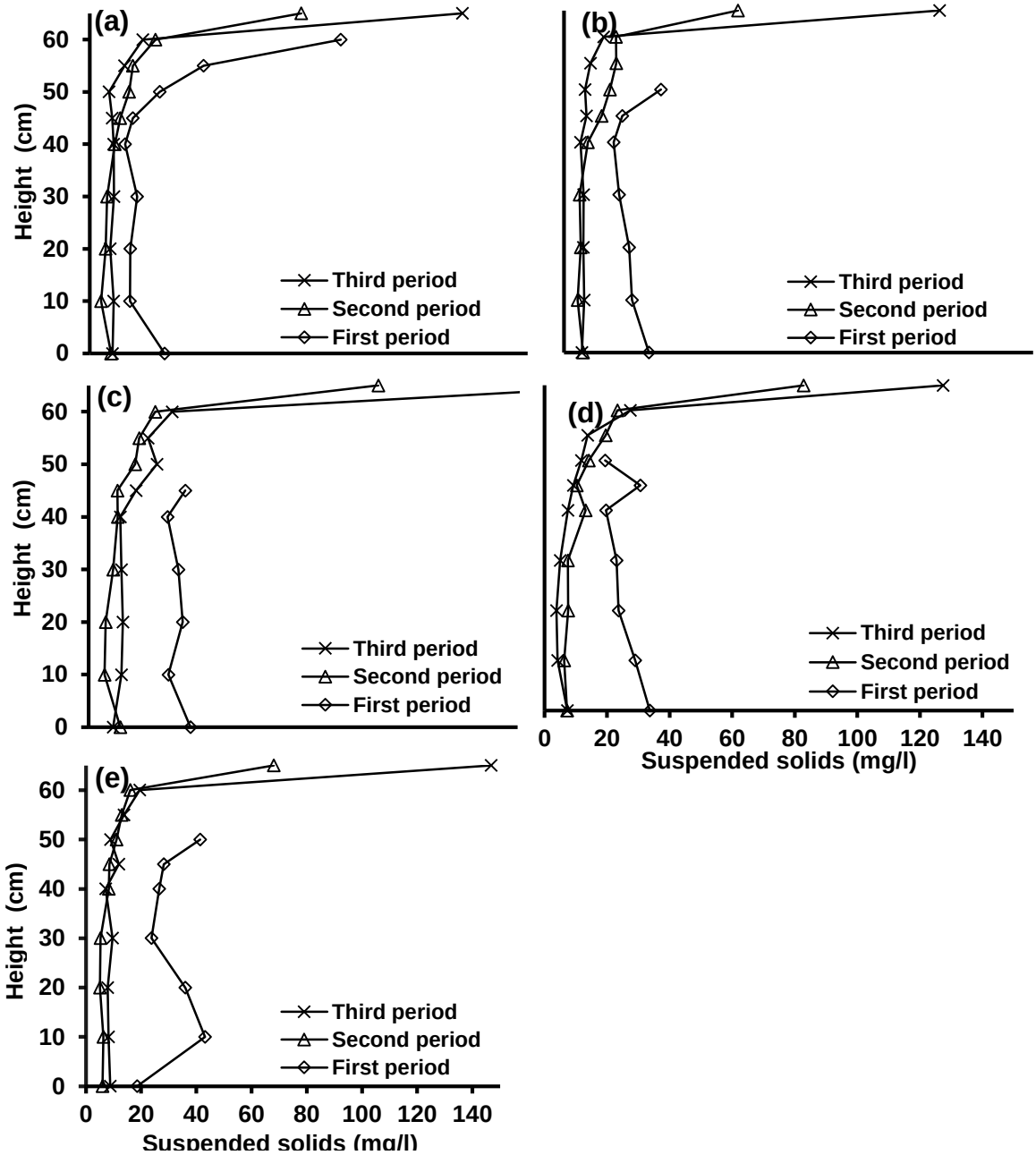


Figure 4.13: Comparison between suspended solids (SS) distributions for the first three experimental periods regarding (a) a combination of the data for Filters 1 and 2 for the first to the third periods, (b) a combination of the data for Filters 3 and 4 for the first to the third periods, (c) a combination of the data for Filters 5 and 6 for the first to the third periods, (d) Filter 7, and (e) Filter 8. Note that the sampling point in (c) 193 mg/l (third period) has not been displayed.

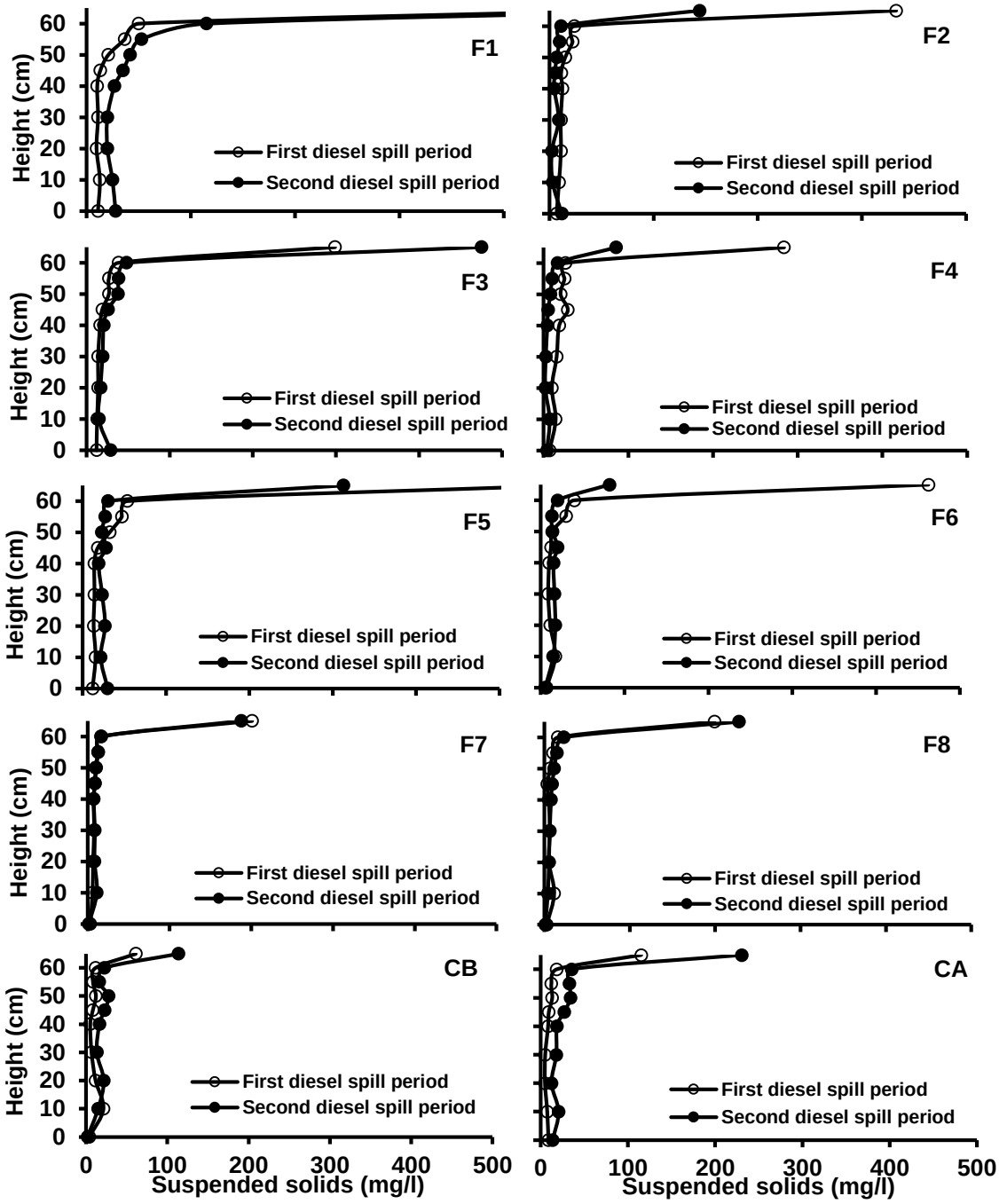


Figure 4.14: Comparison between suspended solids (SS) distributions for the two experimental spills periods. F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA, Control A (wetland filter receiving tap water); and CB, Control B (wetland filter receiving tap water). Note that the following sampling points have not been displayed: 612 mg/l (first diesel spill period) of F1, 622mg/l (second diesel spill period) of F1; 580 mg/l (first diesel spill period) of F5.

4.4.2 Performance assessment for clogging processes within wetland filters using simulation model

Figures 4.15 to 4.20 compare the experimental mean seasonal SS accumulation profiles with the modelled profiles for the first, second, third, fourth, and fifth experimental wetlands phases. No serious clogging was either observed or modelled for all phases. Modelling performance was rather poor for the set-up period, adequate for the first two years after the set-up period and variable after the diesel spills (Al-Isawi et al., 2015a).

The traditional UK standard for SS removal from secondary wastewater is 30 mg/l (Royal Commission on Sewage Disposal 1915). The removal efficiencies for SS were generally relatively high, particularly for the first three periods (Tables 4.2 to 4.4) before the diesel spills. However, some outflow values during the set-up phase, where the filter biomass was immature, were far above 30 mg/l due to the release of fines associated with the aggregates and the inability of the weak biofilm to retain solids originating from the wastewater. Tables 4.5 and 4.6 indicate clearly that filters with hydrocarbon contamination showed elevated SS concentrations compared to those without hydrocarbons. Depending on the stage of biodegradation over time, initially dying contaminated biomass and later on degraded diesel contributed to elevated SS values within the filters (Table 4.5 and 4.6).

The Wang-Scholz model was used to compare between measured and predicted suspended solids values. This clogging model was particularly suitable for Filters 1 to 6 after the set-up period (Figures 4.15 to 4.19) and before the introduction of diesel. However, the original model was not designed to deal with diesel spills resulting in unforeseen SS contributions in the first place. A modification has been carried out to the

Wang-Scholz model in the fifth experimental period to take into account the maturation stage of the wetland system by considering the impact of particles accumulation in the upper layer (litter zone) of the wetland filters as a source of suspended solids (Paing et al., 2015a; Samsó et al., 2015). An additional source of SS concentrations resulted from the decay of above-ground biomass (wetland plants) (F2, F4, F6, F7, F8, and CB) and/or petroleum hydrocarbon compounds within the contaminated filter (F1, F3, F5, and CA) (Al-Isawi et al., 2015a). Figure 4.20 presents a comparison between the experimental mean SS accumulation profiles with the modelled profiles for the fifth wetland phase.

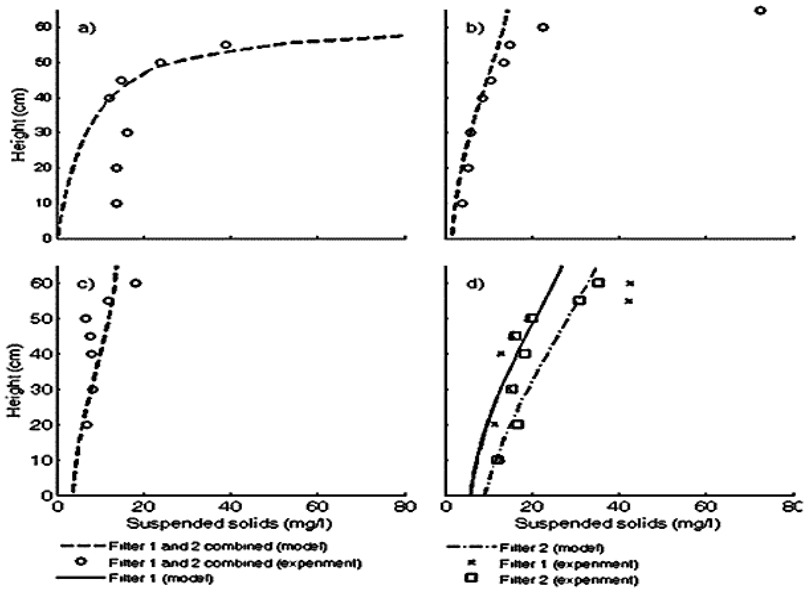


Figure 4.15: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within Filter 1 combined with Filter 2 after the (a) set-up period, (b) first year after the set-up period, and (c) second year after the set-up period, as well as at (d) fourth experiment period for Filters 1 and 2 separately.

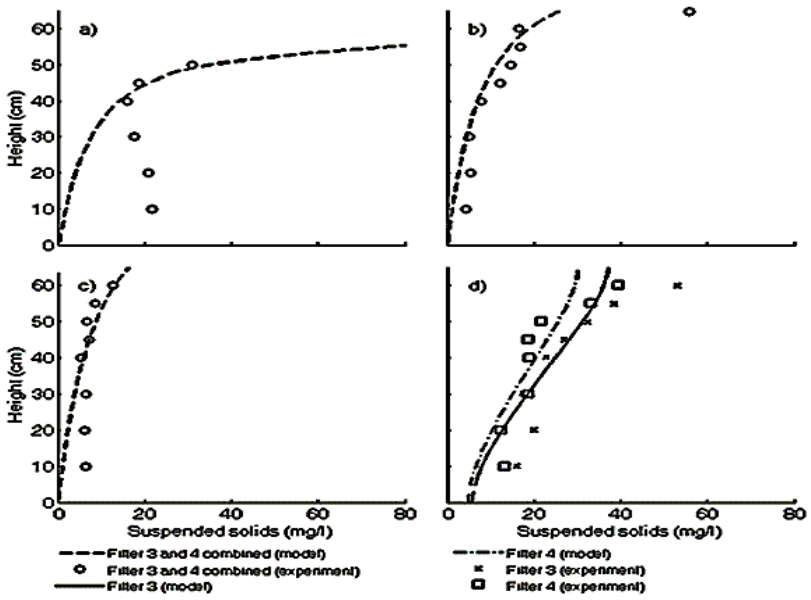


Figure 4.16: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within Filter 3 combined with Filter 4 after the (a) set-up period, (b) first year after the set-up period, and (c) second year after the set-up period, as well as at (d) fourth experiment period for Filters 3 and 4 separately.

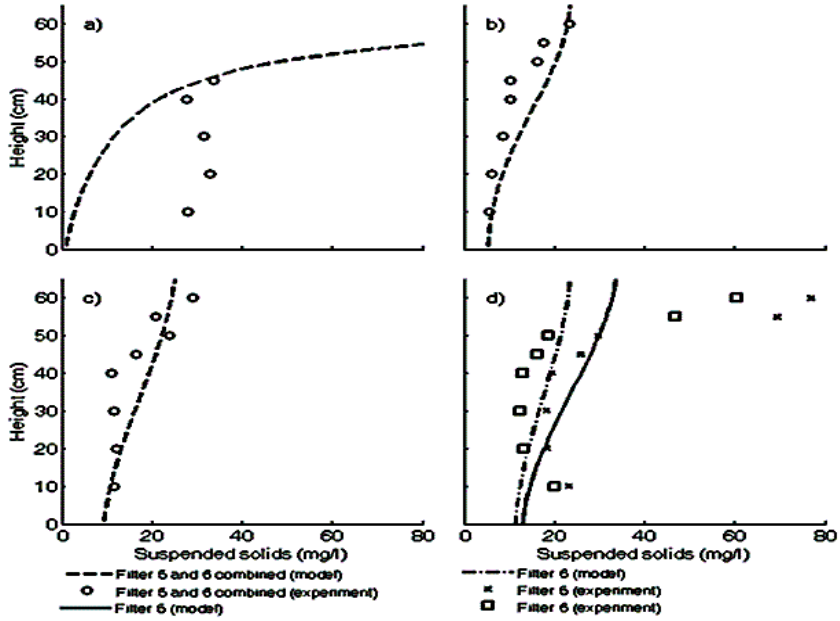


Figure 4.17: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within Filter 5 combined with Filter 6 after the (a) set-up period, (b) first year after the set-up period, and (c) second year after the set-up period, as well as at (d) fourth experiment period for Filters 5 and 6 separately.

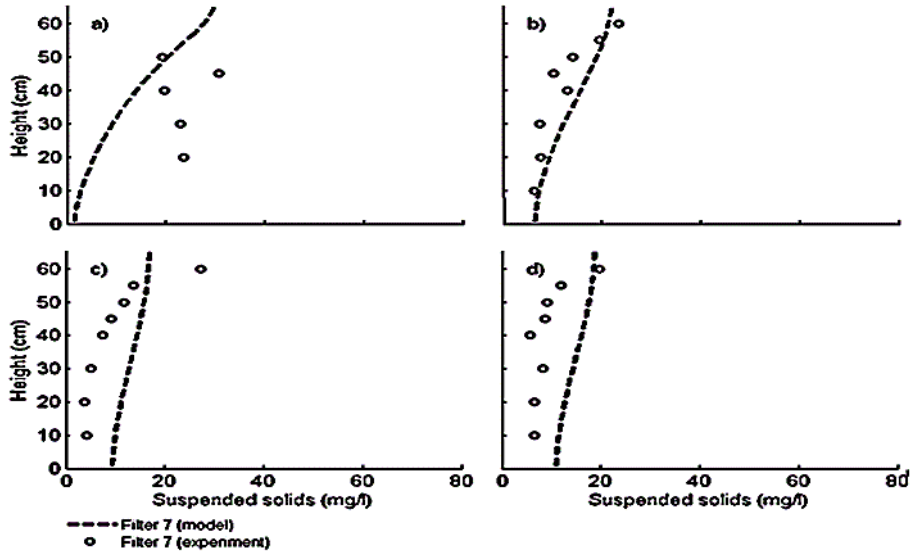


Figure 4.18: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within Filter 7 after the (a) set-up period, (b) first year after the set-up period, and (c) second year after the set-up period, as well as at (d) fourth experiment period.

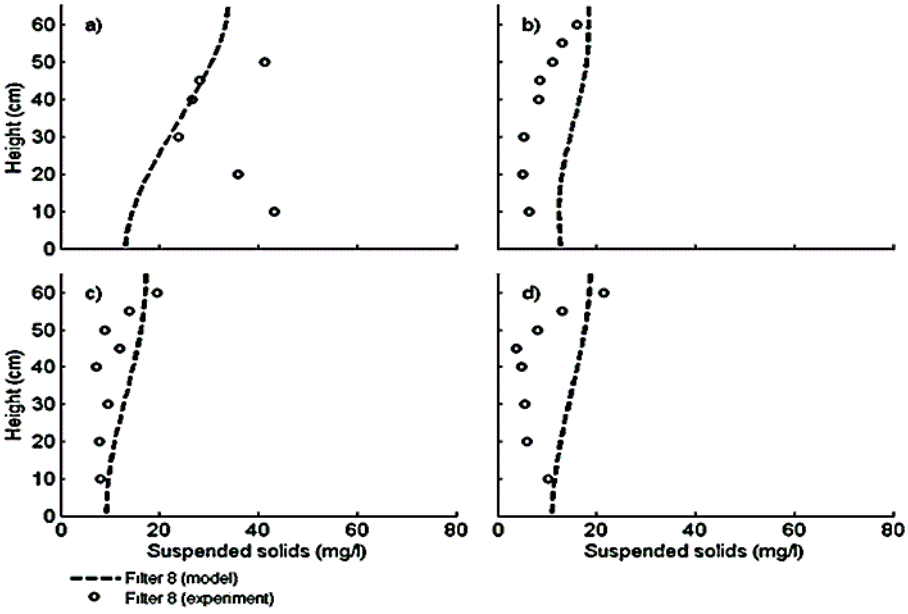


Figure 4.19: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within Filter 8 after the (a) set-up period, (b) first year after the set-up period, and (c) second year after the set-up period, as well as at (d) fourth experiment period.

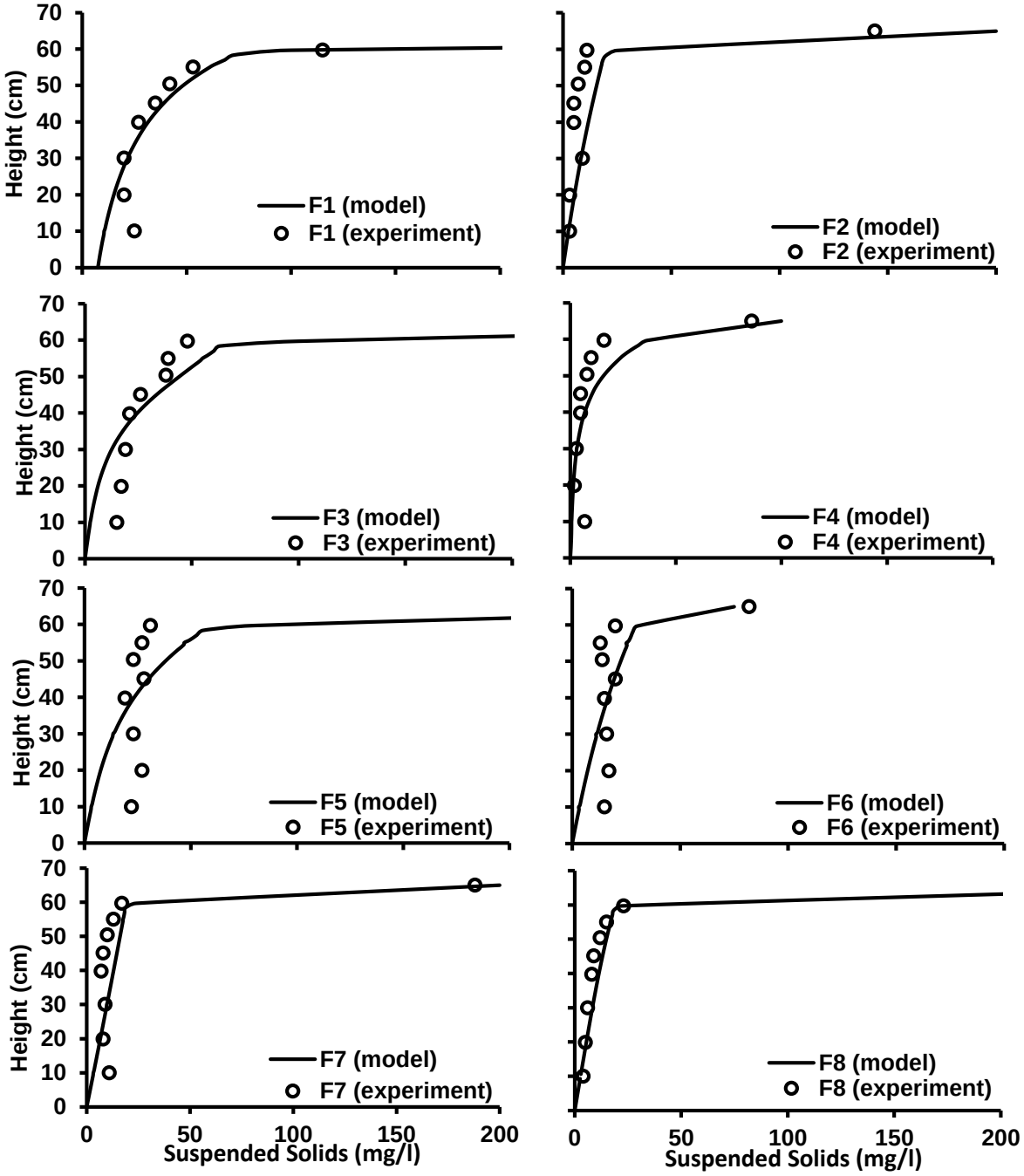


Figure 4.20: Comparison between the measured and modelled distribution of suspended solids (SS) with depth within all filters for fifth experiment period. F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; and F8, wetland filter 8.

4.5 Petroleum hydrocarbon treatment performance

The results and discussions presented in this section have been published in the paper shown below:

Al-Isawi, R.H.K., Sani, A., Almuktar, S., & Scholz, M. (2015). Vertical-flow constructed wetlands treating domestic wastewater contaminated by hydrocarbons. *Water Science and Technology* 71 (6), 938–946.

4.5.1 Inflow water quality

This section shows the overall inflow (raw wastewater without dilution) water quality parameters monitored in the wetland system during the period of about 30 months of petroleum hydrocarbon contamination poured into the selected wetland filters (F1, F3, F5, and CA). The raw domestic wastewater (preliminary treated wastewater) quality was monitored with time. Table 4.12 shows the overall inflow water quality before dilution with tap water for the period after pouring diesel fuel dosages into the wetland system to assess the wetlands treatment performance in removing petroleum hydrocarbon compounds and producing effluent in compliance with international standards. Natural background concentrations of diesel in the raw wastewater were low. The overall mean raw influent concentrations for total petroleum hydrocarbon, chemical oxygen demand, biochemical oxygen demand, ammonia-nitrogen, nitrate-nitrogen, ortho-phosphate-phosphorus, suspended solids, turbidity, pH, electrical conductivity, redox potential, and dissolved oxygen were 63.4 µg/l, 263 mg/l, 155 mg/l, 28 mg/l, 4 mg/l, 12 mg/l, 140 mg/l, 64 NTU, 7.7, 862 µS/cm, -44 mV, and 7.3 mg/l, respectively.

Table 4.12: Inflow water quality: (raw (i.e. before dilution) domestic wastewater mixed with urban runoff) from 26/09/13 to 22/03/2016 when selected wetland filters (F1, F3, F5, F7, and CA) were subjected to diesel spills.

Parameter	Unit	Number	Mean	Minimu-m	Maximu-m	Standard deviation
Total petroleum hydrocarbon	µg/l	14	63.4	0	780	206.8
Chemical oxygen demand	mg/l	66	263.2	100	660	85.33
Biochemical oxygen demand	mg/l	146	155.5	10	360	84.15
Ammonia-nitrogen	mg/l	66	28.6	0	70	17.37
Nitrate-nitrogen	mg/l	66	4.3	0.2	21	5.95
Ortho-phosphate-phosphorus	mg/l	66	12.1	3.4	50.5	7.48
Suspended solids	mg/l	145	140.1	17	474	82.31
Turbidity	NTU	133	64.6	3	391	55.97
pH	-	132	7.72	6.30	8.40	0.39
Electrical conductivity	µS/cm	130	869.9	185.8	2400	369.98
Redox potential	mV	107	-44.4	-84	61	20.74
Dissolved oxygen	mg/l	103	7.3	0.1	18.9	3.20

NTU, nephelometric turbidity unit

4.5.2 Comparison of outflow water quality

The wetland system comprises ten filters which vary in their design and operation, four of them (F1, F3, F5, and Control A) (Table 3.2) have been selected and used to assess the performance of the wetland system for offering the proper environment needed to remove the hydrocarbon contaminants in wastewater. Two diesel fuel dosages (low and high dose) were each poured into the selected filters on 26 September 2013 and on 26 September 2014, respectively, to assess the treatment performance of wetland filters in low and high diesel spill dosages. The concentrations of the two hydrocarbon dosages were 20 g/l and 150 g/l, respectively (Table 3.1). This subsection presents the water quality data which were monitored and analysed in these four contaminated filters over a period of 30 months (September 2013-March 2016). The results observed for water quality parameters are shown in Figures 4.21 to 4.31.

The changes in water quality parameters were compared according to two periods:

- First period of diesel spill (FPDS) (26/09/2013-25/09/2014, 20 g/l of one-off diesel fuel dose poured into (F1, F3, F5, and CA) on 26/09/2013), and;
- Second period of diesel spill (SPDS) (26/09/2014-22/03/2016, 150 g/l of one-off diesel fuel dose poured into (F1, F3, F5, and CA) on 26/09/2014).

Regarding the first period of diesel spill (20 g/l), all the water quality parameters of the wetland filters contaminated with hydrocarbon showed a reduction in their concentrations values, after about 5 months of pouring the first diesel spill dose (Al-Isawi et al., 2015b). This suggests that this period is sufficient for micro-organisms to acclimatize, grow, and improve their activities to establish a high level of treatment performance in the wetlands system, allowing the system to remove hydrocarbon contaminants effectively and produce effluent water within the permissible limits (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Moreover, the presence of good growth of mature wetland plants (reeds), provides habitat and support to microbial communities, which subsequently, can either directly biodegrade or catalyse chemical reactions and maintain the hydrocarbon biotransformation process (Ji et al., 2004; Ji et al., 2007; Das & Chandran, 2011; Al-Baldawi et al., 2013a; Hou et al., 2016). While in the second period of diesel spill, all the water quality parameters were relatively poor as compared with the first diesel spill period. This explains that urban wastewater contaminated with a high amount of different petroleum hydrocarbon compound spills adversely disturbs the water quality of constructed wetlands and affects their treatment efficiency (Tables 4.5 and 4.6) (Michel & Rutherford, 2013; Al-Isawi et al., 2015b; Yavari et al., 2015). However, 10 months after pouring the second dosage of diesel fuel (150 g/l) (Table 3.1), the performance of the wetlands system started to recover and the efficiency treatment of the wetland filters

gradually increased, being in relative compliance with standards limits for most of the water quality parameters (Figures 4.21-4.31).

Figures 4.21 and 4.22 present the temporal variation for COD and BOD concentrations during the first and second periods of diesel spills. In general, the concentration of COD varies in effluent wastewater depending on the nature of the inflow wastewater and the treatment processes occurring within the wetlands (Stefanakis et al., 2014; Yan & Xu, 2014). Petroleum hydrocarbon compounds in the environment, such as diesel spills, are associated with very high COD values (between about 100,000 and 1,000,000 mg/l; (Scholz, 2010)). The main treatment mechanisms for organic matter, which is represented by COD and BOD, in wetland systems are: aerobic, anaerobic, adsorption, filtration, and microbial metabolism (Carroll, 2005; Stefanakis et al., 2014; Xu et al., 2016). In this study, findings show that the wetland filters are poor in the key functions of COD and BOD removal efficiency (Tables 4.5 and 4.6), particularly at the initial stage of each diesel spill period, reflecting the impact of the high amount of different hydrocarbon compounds associated with the poured diesel spills (Al-Isawi et al., 2015b; Scholz, 2015). However, the calculated removal efficiency did not consider the additional COD associated with diesel spills. Thereafter, the treatment performance for these wetland filters gradually improved with time. Figures 4.21 and 4.22 show a noticeable decrease in COD and BOD concentrations gradually, during each period of diesel spill highlighting the rather rapid of hydrocarbon degradation and the ability of wetland filters to provide a suitable treatment environment for wastewater contaminated with hydrocarbon, leading to reduced diesel contaminants and improved water quality parameters with time (Al-Isawi et al., 2015b; Scholz, 2015). The treatment performance of F5 in terms of COD concentration values was less than that for F1 and F3 (Figure 4.21), reflecting the high

COD inflow load for F5 (Table 3.2). Moreover, the values of COD in the contaminated filters in the second period of diesel spill were about two times their values in the first period of diesel spill (Tables 4.5 and 4.6). This difference (in COD values) in all wetland filters was statistically significant ($p \leq 0.05$) between both periods of diesel spills, as shown in Table 4.13, which could possibly have resulted from the indirect artificial influence of the high amount of diesel compounds in the second period of diesel spill (150 g/l) that contributed to the increase in the COD in the inflow water, and led to increase the outflow COD values detected (Lohi et al., 2008; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Scholz, 2015). Regarding BOD concentration values, Figure 4.22 shows an improvement in BOD removal efficiencies over time. This improvement can be attributed to the development of microbial growth and their activities adjusted to the environmental conditions of the mature wetland system (Sani et al., 2013a; Al-Isawi et al., 2015a). Statistical analysis showed a significant difference ($p \leq 0.05$) related to BOD values of F3 between the first and second periods of diesel spill (Table 4.13). This may have resulted from the improvement of the surface area of the substrate media of F3 (small aggregate size) over time that led to provide a suitable environment for micro-organism growth.

Figures 4.23 to 4.25 present the temporal variations of ammonia-nitrogen, nitrate-nitrogen, and ortho-phosphate-phosphorus for the effluent of filters with diesel contamination during the two periods of diesel spills. In general, the main treatment mechanisms for nitrogen compounds in wetlands are: nitrification and denitrification processes that are achieved by micro-organisms (Vymazal, 2007a; Lee et al., 2009; Lavrova & Koumanova, 2013; Fan et al., 2016). Overall treatment performance of nitrogen compounds in wetland filters was relatively high (Figures 4.23 and 4.24). This finding proposes that nutrients (particularly, nitrate-nitrogen, Figure 4.24) have the

capacity to enhance hydrocarbon-adapted bacteria which are responsible for hydrocarbon degradation in the wetland filter. The presence of hydrocarbon in the wetland filters leads to a reduction in the nitrate concentration in their effluent (Liu et al., 2011; Al-Baldawi et al., 2015a). In general, biodegradation of diesel spills in F1, F3, and F5 led to a reduction of the availability of nutrients through these wetland filters. The addition of carbon (via diesel) stimulated the removal of nitrogen, which is needed by micro-organisms to decompose hydrocarbons (Liu et al., 2011; Al-Isawi et al., 2015a). Furthermore, the high performance of wetland filters to remove of NH_4-N (Figure 4.23) in this study could be attributed to the high aeration provided by the tidal-flow mode strategy (Choudhary et al., 2011; Fan et al., 2013b; Chen & Vymazal, 2015; Wu et al., 2016a) applied in the operation of the wetlands which enhances the growth of ammonia-oxidizing bacteria, achieving high ammonia nitrification (Stefanakis & Tsihrintzis, 2012; Murphy et al., 2016). Statistical comparison of nutrients (nitrate-nitrogen, and ammonia-nitrogen) between the two diesel spill periods shows the contaminated wetlands (F1, F3, and F5) to be statistically similar ($p \geq 0.05$) regarding NH_4-N and NO_3-N, with the exception of wetland Filter 5 which was significantly different ($p \leq 0.05$) in NO_3-N between the two spill periods (Table 4.13). Phosphorus removal in constructed wetlands is a complex process. It happens through a combination of numerous processes: plant uptake, adsorption, microbial growth, and precipitation within substrates (Vymazal, 2011b; Lavrova & Koumanova, 2013; Ge et al., 2016a). Vertical-flow constructed wetlands are normally not efficient in treating PO_4-P compounds (Vymazal, 2007a, 2010; Scholz, 2015; Valipour & Ahn, 2016), especially when the system reaches maturation (Mustafa et al., 2009). In this study, there is a noticeable decline in the PO_4-P treatment performance in all contaminated filters (F1, F3, F5, and CA) (Figure 4.25). This decrease in phosphorous treatment efficiency can be explained by firstly, a lack of wetland plants

in these contaminated filters due to the toxic impact of the high diesel dosage, such that there is no treatment via plant uptake, and secondly, the maturity of wetland filters resulting in the wetland media reaching the saturation stage. This finding is in agreement with the study of Wu et al. (2012), who found a decline in phosphorus removal efficiency from 90% to 10% after 6 years of wetland system operation. Statistical analysis of PO_4-P effluent indicates that the first period of diesel spill was significantly different ($p \leq 0.05$) from second period for all wetland filters (Table 4.13).

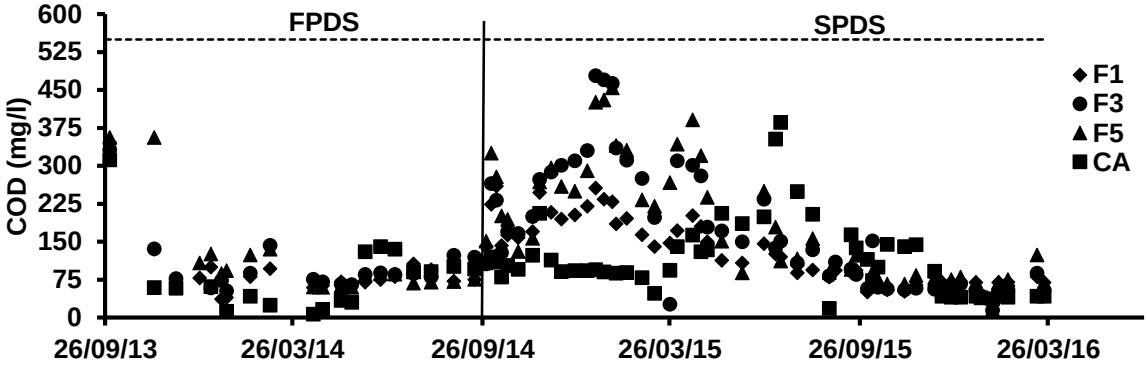


Figure 4.21: Temporal variations of chemical oxygen (COD) demand for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

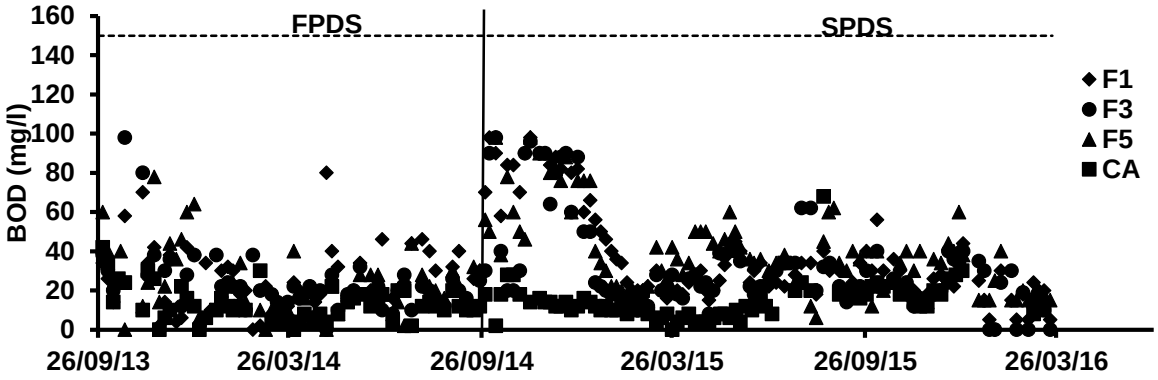


Figure 4.22: Temporal variations of biochemical oxygen demand (BOD) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

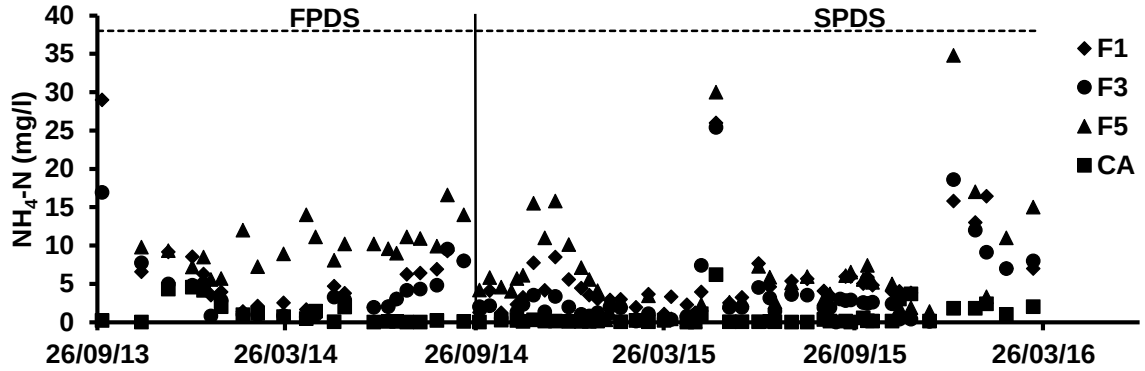


Figure 4.23: Temporal variations of ammonia-nitrogen (NH_4-N) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 6/09/2013 and 26/09/2014 respectively.

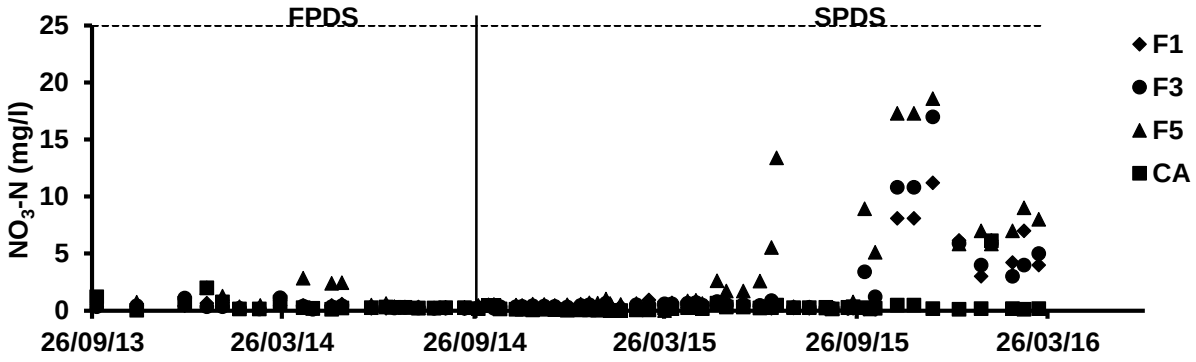


Figure 4.24: Temporal variations of nitrate-nitrogen (NO_3-N) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014, respectively.

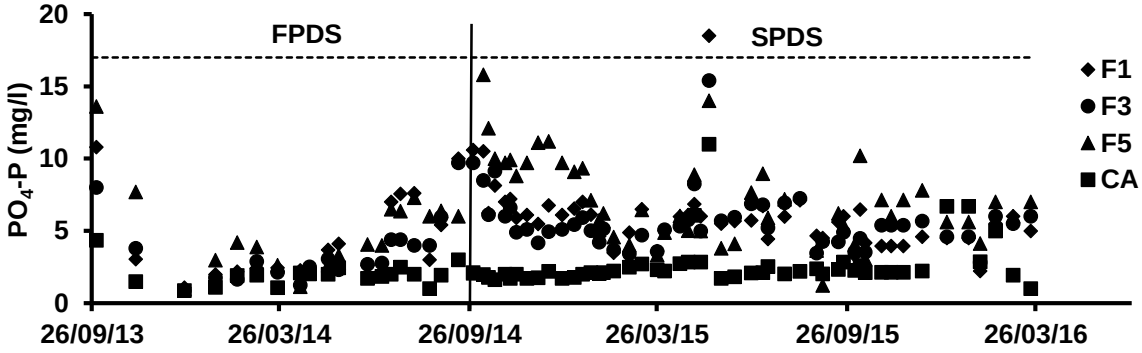


Figure 4.25: Temporal variations of ortho-phosphate-phosphorus (PO_4-P) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

Figures 4.26 and 4.27 show the variations in suspended solids and turbidity during the two diesel spill periods. The main removal mechanisms of solids in constructed wetlands are: settling and sedimentation, microbial degradation, and sorption (Kadlec & Wallace, 2009; Hua et al., 2013; Stefanakis et al., 2014; Weedon et al., 2016). Generally, suspended

solids and turbidity values showed the same pattern in the variation of their concentration values during the two periods of diesel spills.

The SS concentrations varied during the two periods of diesel spills depending on the stage of hydrocarbon treatment within each wetland filter (Figure 4.26). Initially, the elevated SS concentrations in the filters contaminated with diesel spills, resulted from the impact of the high dosage of hydrocarbon compounds subjected to the filters that led to dying above-ground wetland plants (*P. australis*) and decaying biomass which contributed to increased SS and turbidity as by-products of the degradation process (De Biase et al., 2011; Al-Isawi et al., 2015b; Scholz, 2015; Xie et al., 2016), as discussed before in section 4.4. Later on, the high values of SS in the contaminated filters (Al-Isawi et al., 2015a) highlight the additional particles load produced from the diesel biodegradation process within the wetland filter (Stefanakis et al., 2014; Al-Isawi et al., 2015a). The degradation of hydrocarbon compounds led to reduced availability of nutrients for micro-organisms which in turn affected the degradation process of SS in the wetland filter and resulted in an increase in SS values (spatially, in the litter zone layer) (Eke, 2008). With the continued degradation of hydrocarbon compounds with time, the SS concentrations decreased. This could be linked with a well-established microbial population, which might improve efficiency with time. The presence of a low amount of hydrocarbon can lead to improved growth of micro-organisms which in turn, contribute to degradation of SS in wetland filters (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). The performance of the wetland filters to treat SS was better (about double) in the first period of diesel spill as compared with second period reflecting the high second diesel dosage impact (Figure 4.26). The statistical analysis showed a significant difference ($p \leq 0.05$) for SS effluents of F3 and F5 between first and second periods of diesel spills (Table 4.13).

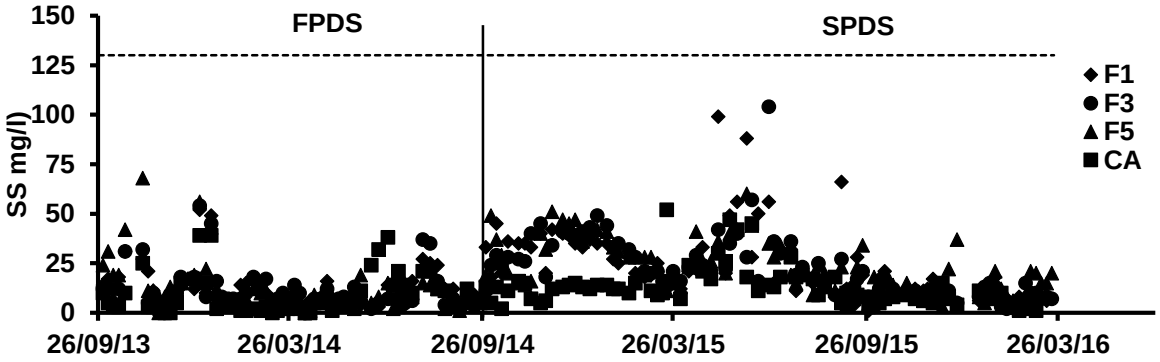


Figure 4.26: Temporal variations of suspended solids (SS) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

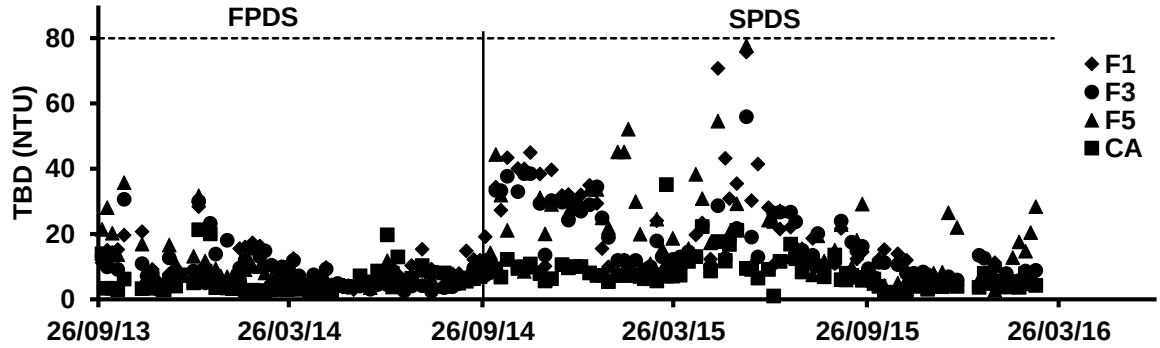


Figure 4.27: Temporal variations of turbidity (TBD) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

Figure 4.28 presents the variations in outflow pH values for wetland filters contaminated with hydrocarbon (F1, F3, F5, and CA). The pH conditions are important for provision of a suitable environment for microbial growth and survival within the hydrocarbon contamination area (Lin & Mendelssohn, 2009; Xu et al., 2016). In this study, overall pH

values were within the allowable range between 4 and 9.5 which is suitable for the survival of most bacteria (Kadlec & Wallace, 2009). The second period of diesel spill showed slight acidic pH values as compared with the first diesel spill period (Figure 4.28). This is probably due to the presence of a high amount of decomposed material resulting from the degradation of hydrocarbon compounds (Kadlec & Wallace, 2009) trapped within the mature plants root system and old plant material (Al-Isawi et al., 2015b). The analysis of effluent pH (Table 4.13) indicates that pH values of the wetland filters in the period of the first diesel spill were statistically similar ($p>0.05$) to those in the period of the second diesel spill.

Figure 4.29 shows the temporal variation in redox values for the two periods of diesel spills. The aerobic and anaerobic treatment conditions of the wetland system can be distinguished partially by dissolved oxygen and redox measurements (Ong et al., 2010) as they are considered the main factors that determine the treatment pattern (Imfeld et al., 2009) within a contaminated area. According to Lin and Mendelssohn (2009), diesel fuel affected the treatment environment around the rhizosphere of wetland plants which resulted in a decrease in the redox values. By increasing the diesel fuel dosage, the wetland condition would become more anaerobic (Al-Baldawi et al., 2013a). In this research, the redox potential monitored data show that initially, the first diesel dosage impacted on the rhizosphere and caused a slight decrease in the redox potential, reflecting the impact of the shock hydrocarbon dosage on the treatment environment within the wetland bed (Al-Isawi et al., 2015a). However, the high hydrocarbon compounds during the period of the second diesel spill, surprisingly, did not result in a high change in redox potential values. This can be explained by the effect of maturation of the system, that resulted in acclimatization of large microbial communities, which in turn can survive

under extreme hydrocarbon conditions. Moreover, the application of the tidal-flow operation mode contributes to providing high aeration in the wetland filters (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). The statistical analysis results (Table 4.13) show that there is no significant difference in redox potential values of F1, F3, and F5 between the two diesel spill periods ($p \geq 0.05$).

Electrical conductivity is an important indicator, necessary for assessment of the wetland filters performance among other water quality variables. It was monitored to evaluate its impact on petroleum hydrocarbon treatment within each filter. Figure 4.30 shows the EC values for the two periods of diesel spills. With the exception of control A, the values of EC during the period of the first diesel spill were relatively lower than those in the period of the second diesel spill (Table 4.6), highlighting the impact of high hydrocarbon compounds poured in the period of the second spill (Lin & Mendelssohn, 2009). Moreover, wetland filters F1 and F3 show higher performance in EC values as compared with F5 (Figure 4.30, Tables 4.5 and 4.6). This demonstrates the effect of the high inflow load subjected to filter F5 associated with a high amount of organic matter (undiluted inflow wastewater) that led to an increase in conductivity conditions within the filter (Eke, 2008; Al-Isawi et al., 2015b). The statistical analysis of EC effluent (Table 4.13) indicates that EC values of the wetland filters in the period of the first diesel spill were significantly different ($p \leq 0.05$) to those in the period of the second diesel spill.

Figure 4.31 shows the temporal variations in dissolved oxygen concentrations during both periods of diesel spills. A sufficient amount of dissolved oxygen is required to ensure better abundance and metabolism of microbial communities which in turn leads to achieve optimum hydrocarbon treatment within wetland filters (Imfeld et al., 2009; Tang et al., 2009). Eke (2008), noted that 1-2 mg/l dissolved oxygen concentration is sufficient

to maintain a suitable petroleum hydrocarbon treatment in a wetland system. Moreover, Vymazal (2010), revealed that an effective degradation process through microbial communities can be achieved under anoxic/anaerobic conditions as the concentration of dissolved oxygen in the lower layers of wetland beds is limited. In this study, monitoring DO data showed a very low DO concentration in all contaminated filters during the first weeks of pouring the first diesel spill dosage (Table 4.5). This may have resulted from the high shocked amount of diesel spill applied to the wetland environment that led to disturb the micro-organism activities (Li et al., 2012; Norton, 2014). The statistical analysis shows no significant difference in DO values ($p \geq 0.05$) between the two periods of diesel spills. This can be explained, again, by the high aeration achieved by the application of the intermittent mode used in wetland operation.

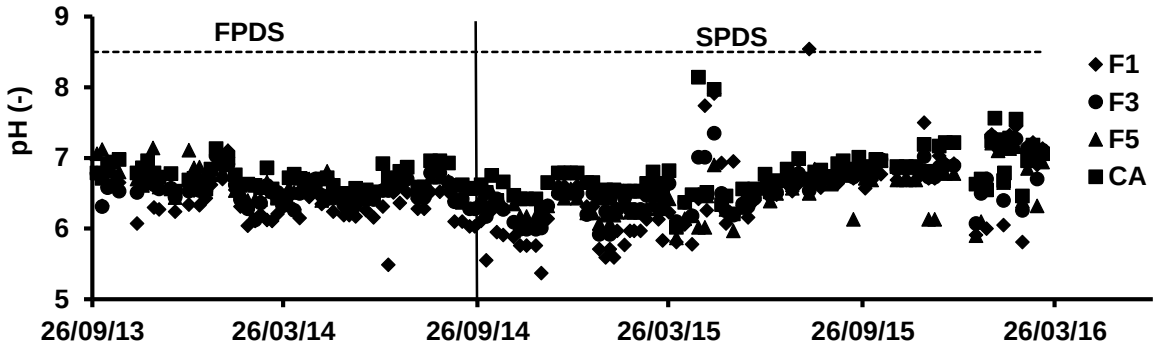


Figure 4.28: Temporal variations of pH for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

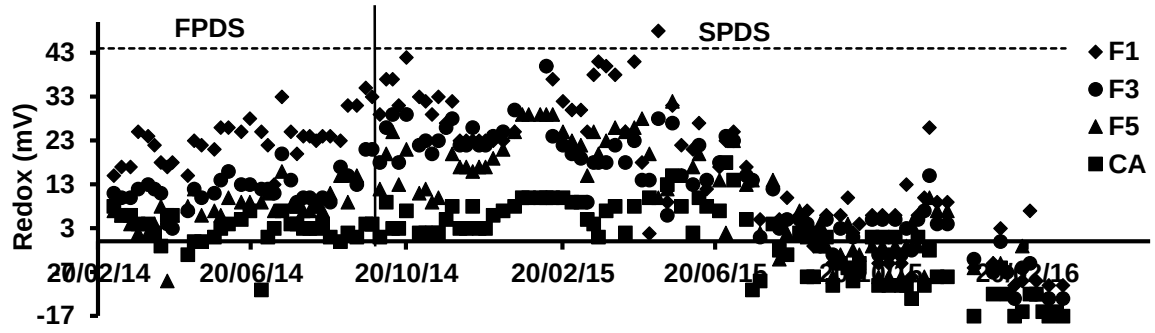


Figure 4.29: Temporal variations of redox potential for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

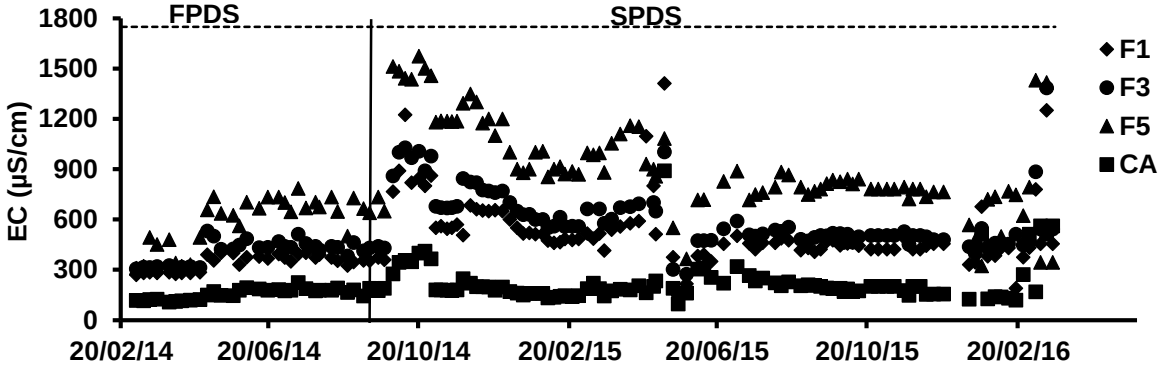


Figure 4.30: Temporal variations of electrical conductivity (EC) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

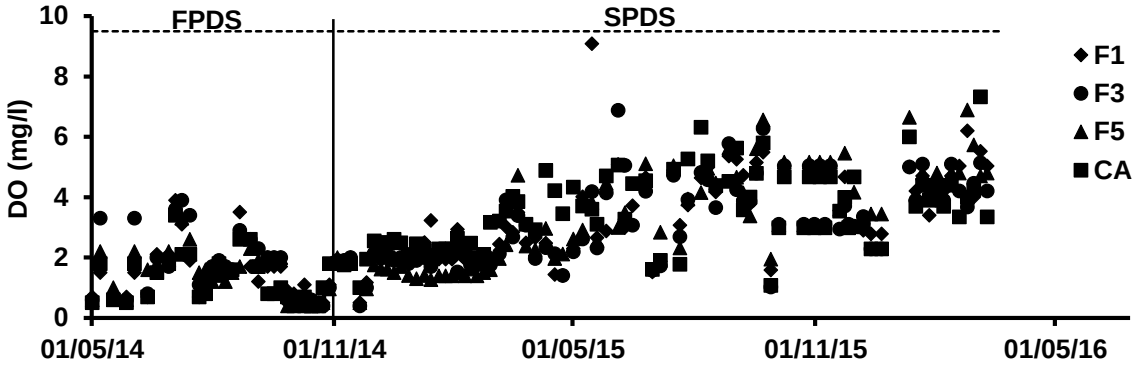


Figure 4.31: Temporal variations of dissolved oxygen (DO) for the effluent of filters with diesel contamination. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water); FPDS, first period of diesel spill; and SPDS, second period of diesel spill. The diesel fuel was poured into the filters on 26/09/2013 and 26/09/2014 respectively.

Table 4.13: Overview of the statistically significant differences between p-values regarding outflow water quality variables (mg/l) of different wetland filters using the non-parametric Mann-Whitney U-test (26/09/13 to 22/03/16).

Parameter	Hydrocarbon influence[a]	Hydrocarbon influence[b]	Hydrocarbon influence[c]
Chemical oxygen demand	0.014	0.001	0.024
Biochemical oxygen demand	0.054	0.047	0.067
Ammonia-nitrogen	0.346	0.511	0.566
Nitrate-nitrogen	0.320	0.072	0.013
Ortho-phosphate-phosphorus	0.004	0.001	0.006
Suspended solids	0.050	0.000	0.017
pH	0.386	0.441	0.666
Redox potential	0.333	0.104	0.420
Electrical conductivity	0.004	0.001	0.001
Dissolved oxygen	0.103	0.090	0.203

[a]Comparison between Filter 1 first and second hydrocarbon dosage; [b]Comparison between Filter 3 first and second hydrocarbon dosage; [c]Comparison between Filter 5 first and second hydrocarbon dosage. Note: A *p-value* is the probability of obtaining a test statistic at least as extreme as the one that was actually observed. Filters are statistically significantly different only if the *p-value*<0.05 for the corresponding water quality parameter.

4.5.3 Petroleum hydrocarbon components removal mechanism in the wetland filters

This section documents analysis dedicated to testing the sustainability of the constructed wetlands by assessing their ability to treat a high dosage of petroleum hydrocarbon such as a diesel fuel spill mixed with urban wastewater, which are associated with considerable human health and environmental concerns. One of the main challenges in the design and operation of wetland filters is ensuring their ability to provide a suitable environment for the preferred microorganism community to treat and remove high strength toxic hydrocarbon pollutants in wastewater. Some of the hydrocarbon components such as diesel are more complex and their removal mechanism within constructed wetlands is not yet entirely known.

Generally, vertical-flow constructed wetlands have shown their ability to treat various types of petroleum hydrocarbon compounds via different processes including: volatilization, biodegradation, adsorption, and aeration (De Biase et al., 2011; De Biase et al., 2013; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Guittonny-Philippe et al., 2015a). In this study, diesel fuel has been chosen as a model for petroleum hydrocarbon compounds to assess the ability of different wetland filters to treat and remove a high dosage of diesel fuel with its components from urban wastewater. Two dosages of diesel fuel were added each to the selected wetland system (F1, F3, F5, and CA) on 26 September 2013 and on 26 September 2014, respectively. The concentrations of the two diesel dosages were 20 g/l and 150 g/l, respectively (Table 3.1). The measured hydrocarbon components in all wetland filters were: aliphatic, aromatic, total petroleum hydrocarbon (TPH), methyl tertiary butyl ether (MTBE), benzene, toluene, ethylene, xylene (BTEX), o-xylene, m-p xylene and volatile petroleum hydrocarbon (VPH) over a

period of 34 months (September 2013-July 2016). Table 4.14 shows the hydrocarbon concentration for each component in raw diesel fuel.

Natural background concentrations of diesel in the raw urban wastewater were low and, in this study, relatively variable for most months during the year, based on the nature of the real wastewater collected. It is assumed that diesel contamination mostly occurred as urban non-point source pollution accompanied with rainfall. It can be noticed however, that a small amount of petroleum hydrocarbon was frequently detected in the inflow wastewater. All wetland filters without diesel contamination (F2, F4, F6, F7, F8, and CB) showed very high treatment efficiency in removing this hydrocarbon during the experimental period (diesel compounds were found to be at less than the 10 µg/l detection limit in their outflow concentrations). This indicates the high ability of these wetland filters to remove completely, such a small amount of petroleum hydrocarbon pollutants that could be found in wastewater (Al-Isawi et al., 2015b).

Table 4.14: Overview of the hydrocarbon concentration for raw diesel fuel, sample analysed in March 2014.

Analyte (µg/l)	Method	Diesel
Aliphatic EC5-7	AN15-1	71900
Aliphatic >EC7-8	AN15-1	538000
Aliphatic >EC8-10	SOP05	19465
Aliphatic >EC10-12	SOP05	1180882
Aliphatic >EC12-16	SOP05	273642
Aliphatic >EC16-35	SOP05	246575
Aliphatic >EC35-44	SOP05	419
Total Aliphatics (TALPHA) EC5-44 (I)	SOP05	2330883
Aromatic EC5-7	AN15-1	366000
Aromatic >EC7-8	AN15-1	63000
Aromatic >EC8-10	SOP05	572
Aromatic >EC10-12	SOP05	3296
Aromatic >EC12-16	SOP05	8672
Aromatic >EC16-21	SOP05	6672
Aromatic >E21-35	SOP05	7866
Aromatic >EC35-44	SOP05	36

Table 4.14 (cont.)

Total Aromatics (TAROM) EC5-44 (II)	SOP05	456114
Total TPH[a] (=I+II)	SOP05	2786997
MTBE[b] (III)	AN15a	<10
Benzene (IV)	AN15a	64120
Toluene (V)	AN15a	302300
Ethylbenzene (VI)	AN15a	9405
m,p-xylene (VII)	AN15a	34890
o-xylene (VIII)	AN15a	17570
Other VPH[c] (IX)	AN15a	1038900
Total VPH[d] (=III+IV+V+VI+VII+VIII+IX)	AN15	1467185

The equivalent carbon number index is indicated by EC. [a]total petroleum hydrocarbon, [b]methyl tertiary butyl ether, [c]volatile petroleum hydrocarbon, [d]total volatile petroleum hydrocarbon.

Figures 4.32 to 4.35 present an overview of the total petroleum hydrocarbons (TPH), total aliphatic (TALPHA), and total aromatic (TAROM) concentration results observed in the outflow from the four wetland filters (F1, F3, F5, and Control A) contaminated with diesel over a period of 34 months (September 2013-July 2016). Generally, the observed petroleum hydrocarbon concentration values for the selected filters during the two periods of diesel spills were relatively low as compared with the huge amounts of the two diesel dosages that were applied with influent to the selected filters. This highlights the effect of the maturity of wetland filters to establish a large quantity of wetland plants (above and below ground biomass), an accumulated litter zone over about five years, a strong bio-film layer, and high growth of microbial populations (Tanner et al., 1998; Scholz, 2003; Lee & Scholz, 2007; Dong et al., 2012; Scholz, 2015). It is suggested that the poured diesel hydrocarbon compounds were, initially, accumulated in the upper layers of mature wetland filters, thereafter, an amount of hydrocarbon was subjected to a series of treatment processes that led to treat, transform, and reduce its concentration in the wetland filter. The released hydrocarbon concentration was gradually reduced with time. The reduction in petroleum hydrocarbon concentrations depends on the efficiency of the

treatment processes which occurred in each of the selected wetland filters and their corresponding interactions with the surrounding environments (Eke & Scholz, 2008; Wallace et al., 2011a; Stefanakis et al., 2014; Al-Isawi et al., 2015a; Stefanakis et al., 2016). The total petroleum hydrocarbon (TPH) concentrations for the effluent of selected filters (F1, F3, F5, and CA) during the first diesel spill period were less than those in the second diesel spill period reflecting the effect of the higher diesel dosage inflow (150 g/l) (Table 3.1) applied in the second spill period (Figures 4.32 to 4.35).

During the first diesel spill period, findings showed very low effluent concentration values for (TPH, TAROM, and TALPHA) for all filters due to the very high amount of hydrocarbon which was extracted during this period. This suggests that the wetland filters are effective and had good treatment performance to treat a diesel spill dosage of 20 g/l from urban wastewater (Al-Isawi et al., 2015b). This can be explained by the presence of sufficient nutrients and the regular presence of aerobic conditions (i.e. tidal flow mode and *P. australis* enriching the substrate with oxygen via their root zone, stimulating and speeding-up biodegradation and volatilization) within the filter (Scholz, 2006, 2015). The treatment efficiencies are high for all filters. This observation confirms previous studies by Al-Baldawi et al. (2014), explaining that diesel removal was high in their wetland systems as a result of increased availability of the oxygen in the rhizosphere, which led to high degradation rates of hydrocarbon. Table 4.15 provides an overview of the petroleum hydrocarbon results (an approximately 10-month period of time after the first diesel dosage was poured). Traces of total aliphatics and total aromatics were recorded, particularly for those filters treating wastewater contaminated by the diesel spill (Table 4.15). Volatile petroleum hydrocarbons were virtually absent (Table 4.15). For TPH, the results showed very low concentration values for all filters. After the diesel has been

drawn into the filters during water exchange, phytoremediation is considered to increase oil attenuation by *P. australis* taking in small molecular hydrocarbons.

The degradation of petroleum hydrocarbon is a function of nutrient (specifically, nitrogen and phosphorus) availability. Natural attenuation for petroleum hydrocarbons biodegradation can be achieved where nutrients are available in sufficient concentrations (Eke & Scholz, 2008; Yan & Xu, 2014; Xu et al., 2016). Biodegradation processes of diesel spills in filters F1, F3 and F5 (Table 4.15) reduced the availability of nutrients to micro-organisms and *P. australis*. However, as the biodegradation of diesel improves with time, small amounts of residual petroleum hydrocarbon stimulate the growth of some micro-organisms, and lead to an increase in the degradation rate. The diesel was removed well by all contaminated filters (Table 4.15) due to biodegradation. The addition of carbon (via diesel) also promoted the removal of nitrogen, which is required by micro-organisms to degrade hydrocarbons (Table 4.5; (Scholz, 2010, 2015)). An optimal ratio of food, nutrients and trace elements is required to avoid the unnecessary release of elements, present in excess, from wetland sediments (Dong et al., 2013; Tao & Yu, 2013; Dzakpasu et al., 2015). Hutchinson et al. (2001) proposed an optimal ratio of 100:2:0.2 for the carbon/nitrogen/phosphorus ratio regarding greenhouse experiments based on phytoremediation of petroleum hydrocarbons. The corresponding ratio of COD/ammonia-nitrogen + nitrate-nitrogen/ortho-phosphate-phosphorus for the present study was 246:36:16 (or 19,880:288:0.2). It follows that nitrogen was present in abundance, supporting hydrocarbon degradation.

Wei et al. (2014) observed that diesel-degrading bacteria became more active with increasing diesel concentration in the rhizosphere of wetland plants, which explains the self-cleaning effect also observed in the present study. Furthermore, the passive aeration

of the aggregates to increase biodegradation provides root exudates for microbial co-metabolization of oil components and other molecules (Lin & Mendelssohn, 2009). Co-metabolism by micro-organisms in this study can be defined as the simultaneous degradation of two compounds, in which the degradation of the second compound (root exudates) depends on the presence of the first compound (diesel).

Results observed of petroleum hydrocarbon compounds effluent in the contaminated wetland filters indicated that the wetland filter with small aggregate media (F3) was better in terms of treatment performance as compared with the wetland filter with large aggregate size (F1) (Table 4.15). This finding suggests that filter media of wetland F3 provide an adequate surface area for biofilm establishment (more favourable thriving atmosphere for microbes to biodegrade pollutants) (Brix & Arias, 2005; Meng et al., 2014). Wetland filter F5 (concentrated inflow load) showed high hydrocarbon compounds in its effluent as compared with wetland filter F3 (diluted inflow load) highlighting the impact of the high inflow load of F5 that resulted in additional influent hydrocarbon with inflow wastewater (Al-Isawi et al., 2015b). Control A, which lacks mature biomass, showed the highest TPH concentration values compared with those for other filters (Table 4.15). Moreover, *P. australis* had a delayed and reduced growth during the post-hydrocarbon period. This can be explained by diesel toxicity to micro-organisms (Truu et al., 2015; Hou et al., 2016), which formed a weak biofilm due to the absence of sufficient nutrients in the tap water. Although Filter 8 lacked diesel contamination, the TPH concentration was 76 µg/l (Table 4.15). This can be explained by the elevated loading rate for this filter, resulting in the accumulation of hydrocarbon originating from the petroleum background concentration in wastewater (Al-Isawi et al., 2015b).

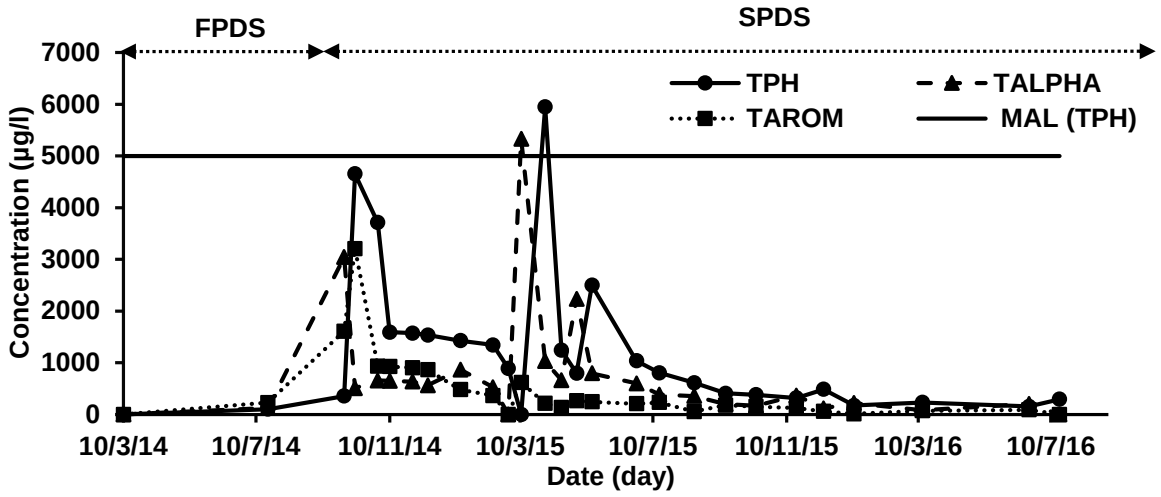


Figure 4.32: Overview of the total petroleum hydrocarbon (TPH), total aliphatic (TALPHA), and total aromatic (TAROM) concentrations observed in the effluent filter F1. Note that: MAL, maximum allowable limit; FPDS, first period of diesel spill; SPDS, second period of diesel spill. Data collection started in March 2014 and stopped on 18/07/16.

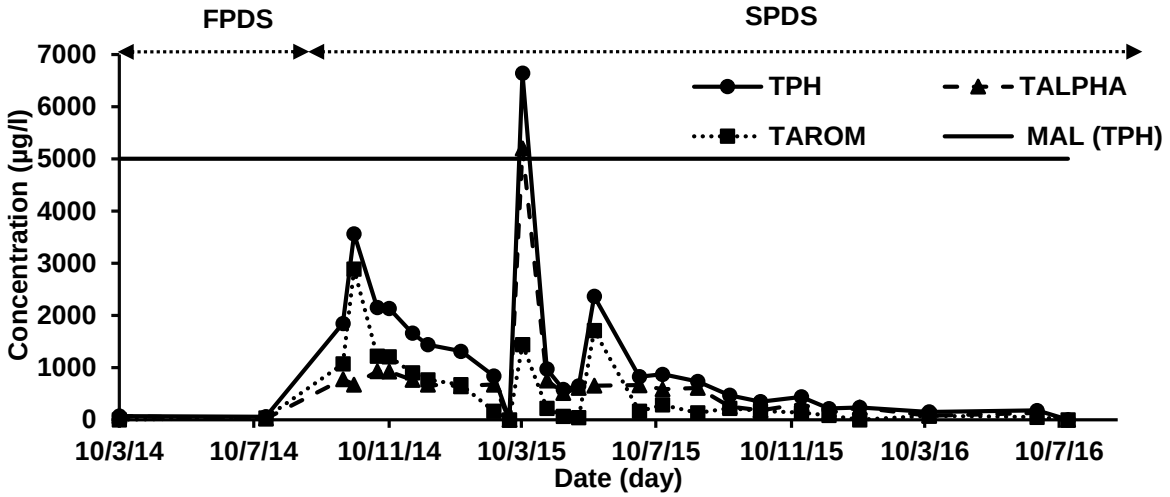


Figure 4.33: Overview of the total petroleum hydrocarbon (TPH), total aliphatic (TALPHA), and total aromatic (TAROM) concentrations observed in the effluent filter F3. Note that: MAL, maximum allowable limit; FPDS, first period of diesel spill; SPDS, second period of diesel spill. Data collection started in March 2014 and stopped on 18/07/16.

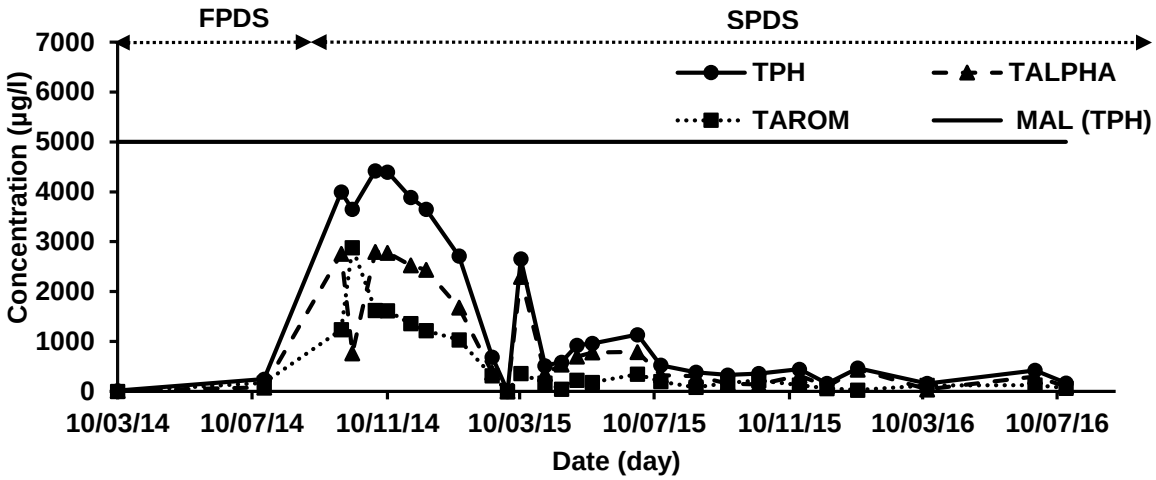


Figure 4.34: Overview of the total petroleum hydrocarbon (TPH), total aliphatic (TALPHA), and total aromatic (TAROM) concentrations observed in the effluent filter F5. Note that: MAL, maximum allowable limit; FPDS, first period of diesel spill; SPDS, second period of diesel spill. Data collection started in March 2014 and stopped on 18/07/16.

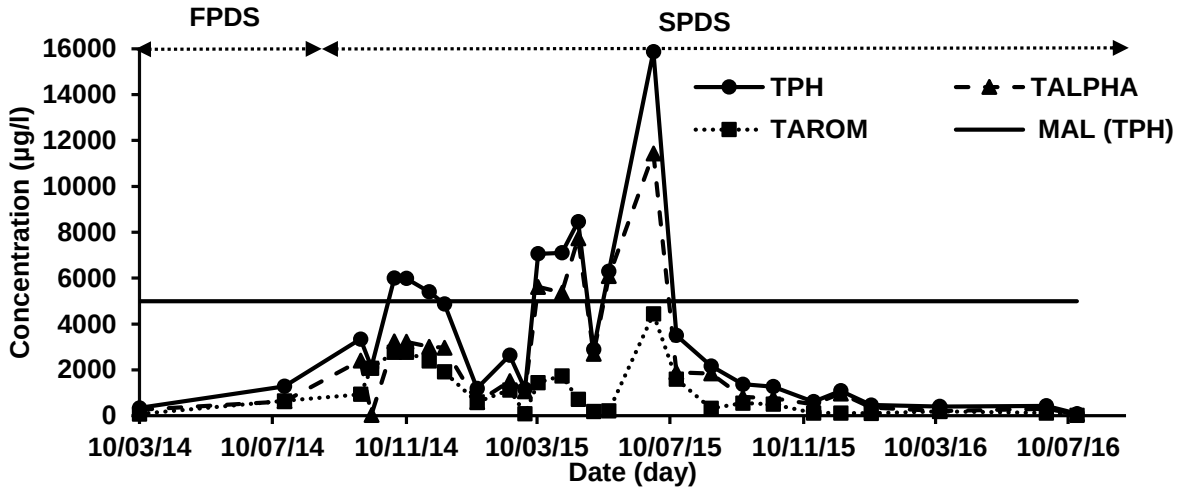


Figure 4.35: Overview of the total petroleum hydrocarbon (TPH), total aliphatic (TALPHA), and total aromatic (TAROM) concentrations observed in effluent of Control CA. Note that: MAL, maximum allowable limit; FPDS, first period of diesel spill; SPDS, second period of diesel spill. Data collection started in March 2014 and stopped on 18/07/16.

Table 4.15: Overview of the hydrocarbon analysis for 21 July 2014. Filters F1, F3, F5 and Control CA were contaminated with diesel.

Analyte (µg/l)	Method	Filter F1	Filter F2	Filter F3	Filter F4	Filter F5	Filter F6	Filter F7	Filter F8	Control CA	Control CB	Inflow
Aliphatic EC5-7	AN15-1	20	<10	20	<10	21	<10	<10	60	10	<10	≤1*
Aliphatic >EC7-8	AN15-1	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	≤1*
Aliphatic >EC8-10	SOP05	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	185
Aliphatic >EC10-12	SOP05	<1*	<10	<1*	<10	<1*	<10	<10	<1*	73	<10	76
Aliphatic >EC12-16	SOP05	32	<10	<1*	<10	17	<10	<10	16	207	<10	16
Aliphatic >EC16-35	SOP05	72	<10	<1*	<10	34	<10	<10	<1*	414	<10	31
Aliphatic >EC35-44	SOP05	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	≤1*
Total Aliphatics (TALPHA) EC5-44 (I)	SOP05	124	<10	20	<10	72	<10	<10	76	631	<10	309
Aromatic EC5-7	AN15-1	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	≤1*
Aromatic >EC7-8	AN15-1	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	≤1*
Aromatic >EC8-10	SOP05	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	19
Aromatic >EC10-12	SOP05	<1*	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	54
Aromatic >EC12-16	SOP05	<1*	<10	<1*	<10	26	<10	<10	<1*	232	<10	215
Aromatic >EC16-21	SOP05	56	<10	27	<10	106	<10	<10	<1*	304	<10	157
Aromatic >E21-35	SOP05	115	<10	10	<10	35	<10	<10	<1*	117	<10	27
Aromatic >EC35-44	SOP05	58	<10	<1*	<10	<1*	<10	<10	<1*	<1*	<10	≤1*
Total Aromatics (TAROM) EC5-44 (II)	SOP05	229	<10	37	<10	167	<10	<10	<1*	653	<10	473
Total TPH[a] (=I+II)	SOP05	353	<10	57	<10	240	<10	<10	76	1284	<10	782
MTBE[b] (III)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Benzene (IV)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Toluene (V)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Ethylbenzene (VI)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
m,p-xylene (VII)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
o-xylene (VIII)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Other VPH[c] (IX)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Total VPH[d] (=III+IV+V+VI+VII+VIII+IX)	AN15	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

Note: The detection limit was 10 µg/l. Figures indicated by a * were less than the detection limit. The equivalent carbon number index is indicated by EC. [a]total petroleum hydrocarbon, [b]methyl tertiary butyl ether, [c]volatile petroleum hydrocarbon, [d]total volatile petroleum hydrocarbon.

The results also, showed a high treatment efficiency for benzene, toluene, ethylbenzene and xylene-volatile aromatic compounds and methyl tertiary butyl ether (Table 4.15). It is suggested that volatilization and phytovolatilization are the likely main removal mechanisms during the first period of diesel application (Imfeld et al., 2009). Thereafter, hydrocarbon contaminants are likely to migrate further into the cover layer, which increases the efficiency of this layer as a diffusive bioreactive barrier. De Biase et al. (2011) showed that hydrocarbons are still subject to biodegradation even after entering the gas phase. In the cover layer, the development of equilibrium between the gas phase and the residual water phase allows the contaminants to re-enter the water phase, where they can be biodegraded by the microbial community. The total volatile petroleum hydrocarbon compounds were virtually completely removed from all wetland filters (Table 4.15), supported by the operation regime allowing air to be drawn into the filters (Scholz, 2015; Pan et al., 2016).

Table 4.16 shows the experimental result of evaporation of diesel concentration trend in the greenhouse conditions for the two diesel application periods. Based on the evaporation experiments, about 30% of the diesel had evaporated within the first month of diesel application. No further evaporation was noticed on visual inspection thereafter which means the concentration of the diesel remained constant with time.

Table 4.16: Evaporation trend of inflow raw diesel used in greenhouse experimental vertical-flow constructed wetlands.

Raw diesel fuel volume (ml)			
Date	First diesel spill period	Date	Second diesel spill period
26/09/2013	500	26/09/2014	500
29/09/2013	463	29/09/2014	475
01/10/2013	443	02/10/2014	450
04/10/2013	420	04/10/2014	450
09/10/2013	370	09/10/2014	425
15/10/2013	355	12/10/2014	420

Table 4.16 (cont.)

19/10/2013	354	16/10/2014	410
22/10/2013	353	20/10/2014	370
26/10/2013	353	24/10/2014	355
27/10/2013	350	26/10/2014	355
29/10/2013	350	28/10/2014	355

Based on the results obtained during the period of the first diesel dose, which showed a high performance treatment for all petroleum hydrocarbon components, this period has been considered as an acclimatization stage for the wetland filters (F1, F3, F5, and CA) to apply a higher diesel dosage. According to the studies of Mills et al. (2003), Das and Chandran (2011), Wang et al. (2011b), and Patil et al. (2012), the micro-organisms that have prior exposure to petroleum hydrocarbon and have adapted to survive in a hydrocarbon contaminated area, have better performance capabilities to grow, thrive, and degrade hydrocarbon compounds rapidly, as compared with those from previously uncontaminated conditions.

On 26th September 2014, (150 g/l) of diesel fuel was added to the same wetland filters (F1, F3, F5, and CA) to assess the response of the wetland system when a higher concentration is added in addition to the previously applied one (though much of the initial one was already removed). EPA (2005), set 5000 µg/l for a TPH concentration in effluent wastewater as a maximum allowable value for discharging into water courses. Wetland filter F1 and F3 (Figures 4.32 and 4.33) showed more variations than F5 (Figure 4.34) in the observed hydrocarbon concentration values during the 6-months after pouring the diesel dosage into these wetland filters, exceeding the maximum permissible concentration value limits of TPH in March 2015. Thereafter, the hydrocarbon concentrations (TPH, TAROM , and TALPHA) showed a gradual decrease in all selected filters with time (Figures 4.32 to 4.35) indicating the natural adaptation of micro-organisms to survive in the presence of high diesel fuel dosage. This indicates the ability

of the selected wetland filters including both high (F5) and low (F1 and F3) loading rate ones to accommodate and treat such a high petroleum hydrocarbon compounds dosage. This has been confirmed recently by a number of researchers (Al-Baldawi et al., 2013e; Al-Baldawi et al., 2014a; Al-Baldawi et al., 2015a; Guittonny-Philippe et al., 2015b) and (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b) who stated high treatment efficiency of numerous organic compounds, including TPH, from their wetland systems. The authors concluded that the high treatment could be attributed to aeration in their systems in the former, and wetland maturity, sufficient nutrient supplied, and high intermittent aeration achieved over time in the latter, which might have elevated the microorganism activity, hence resulting in high microbial biodegradation of hydrocarbon components. Control A was poor in hydrocarbon degradation efficiency (Figure 4.35) highlighting low microbes resulting from a lack of nutrients in the tap water received by this affected filter (CA).

In the case of a lack of wetland plants, petroleum hydrocarbon compounds can be degraded by volatilization, eluviation and photolysis (Peng et al., 2009) in addition to degradation by micro-organisms (Liu et al., 2011; Yavari et al., 2015; Hou et al., 2016). The results from this study suggest that the relationship between microbial community, wetland plants and hydrocarbon degradation activity in constructed wetland can be complex and environment dependant. However, the role of macrophytes in treatment wetlands has been controversial. Some researchers have documented that macrophytes can improve hydrocarbon contaminants removal (Omari et al., 2003; Wang et al., 2011a; Al-Sbani et al., 2016). Alternatively, others did not detect any significant difference in treatment performance between planted and unplanted systems (Scholz & Xu, 2002; Eke & Scholz, 2008). In this study, the lack of macrophytes in the contaminated filters during the second period of diesel spill, with the resultant toxicity impact of the high diesel fuel

dosage, had a minor effect on the petroleum hydrocarbon treatment performance. It is suggested that a mature wetland system elevates the microbial population and promotes their activity to degrade pollutants, additionally these microbes were adapted (from the first diesel dosage) to survive in the presence of the second, high diesel fuel dosage.

Table 4.17 provides an overview of the petroleum hydrocarbon results (July 2016). Traces of total aliphatics were recorded for all filters treating wastewater with/without diesel contamination. Total aromatics (apart from F5 and CA) and volatile petroleum hydrocarbons were virtually absent (Table 4.17). It is suggested that the high removal efficiencies in the effluents from all filters are consistent with the increased availability of oxygen in these areas and its subsequent decrease in concentration with depth (Al-Isawi et al., 2015b). Wetland filter F5 showed less fluctuations in effluent petroleum hydrocarbon concentrations as compared with those in other filters (Figure 4.34), additionally, TPH values of F5 were below the permissible allowable limits during the experimental period. Findings of the second period of diesel spill suggested that the diesel treatment performance in wetland with small filter media (aggregates) and high inflow load (F5) was better than that in other wetland filters. This might be due to the high capability of the wetland filter to provide a suitable habitat for hydrocarbon-degrading microbes (Tang et al., 2009; Al-Baldawi et al., 2015a). This demonstrates that the impact of the continuous supply of nutrients associated with influent to F5 can maintain sufficient microbial activity and subsequently relatively high hydrocarbon treatment efficiencies (Al-Isawi et al., 2015b).

Table 4.17: Overview of the hydrocarbon analysis for 18 July 2016. Filters F1, F3, F5 and Control CA were contaminated with diesel.

Analyte (µg/l)	Method	Filter F1	Filter F2	Filter F3	Filter F4	Filter F5	Filter F6	Filter F7	Filter F8	Control CA	Control CB	Inflow
Aliphatic EC5-7	AN15-1	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	≤1*
Aliphatic >EC7-8	AN15-1	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	≤1*
Aliphatic >EC8-10	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	10
Aliphatic >EC10-12	SOP05	<10	<10	<10	<10	15	<10	<10	<10	24	<10	20
Aliphatic >EC12-16	SOP05	<10	<10	<10	<10	60	<10	<10	<10	33	<10	72
Aliphatic >EC16-35	SOP05	<10	<10	<10	<10	27	<10	<10	<10	12	<10	220
Aliphatic >EC35-44	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	31
Total Aliphatics (TALPHA) EC5-44 (I)	SOP05	<10	<10	<10	<10	102	<10	<10	<10	69	<10	353
Aromatic EC5-7	AN15-1	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	≤1*
Aromatic >EC7-8	AN15-1	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	≤1*
Aromatic >EC8-10	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	19
Aromatic >EC10-12	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	20
Aromatic >EC12-16	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	10
Aromatic >EC16-21	SOP05	<10	<10	<10	<10	40	<10	<10	<10	21	<10	60
Aromatic >E21-35	SOP05	<10	<10	<10	<10	25	<10	<10	<10	<1*	<10	107
Aromatic >EC35-44	SOP05	<10	<10	<10	<10	<1*	<10	<10	<10	<1*	<10	54
Total Aromatics (TAROM) EC5-44 (II)	SOP05	<10	<10	<10	<10	65	<10	<10	<10	21	<10	270
Total TPH[a] (=I+II)	SOP05	<10	<10	<10	<10	167	<10	<10	<10	90	<10	623
MTBE[b] (III)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Benzene (IV)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Toluene (V)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Ethylbenzene (VI)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
m,p-xylene (VII)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
o-xylene (VIII)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Other VPH[c] (IX)	AN15a	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Total VPH[c] (=III+IV+V+VI+VII+VIII+IX)	AN15	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

Note: The detection limit was 10 µg/l. Figures indicated by a * were less than the detection limit. The equivalent carbon number index is indicated by EC. [a]total petroleum hydrocarbon, [b]methyl *tertiary* butyl ether, [c]volatile petroleum hydrocarbon, [d]total volatile petroleum hydrocarbon.

Figures 4.36 and 4.37 present the average mean concentrations of each petroleum hydrocarbon fraction (C5 to C44) of aliphatic and aromatic hydrocarbons respectively, in the outflow wastewater of the selected filters (F1, F3, F5, and CA) for both diesel spill periods. In the two periods of diesel spills, the treatment efficiencies of both aliphatic and aromatic (i.e., all hydrocarbon fractions; C5-C44) were high for all filters as compared with the huge amounts of both inflow diesel fuel dosages. Moreover, all wetlands without hydrocarbon contamination (F2, F4, F6, F7, F8, and Control B) had very high treatment efficiencies for all hydrocarbon fractions (data not shown). Aliphatic hydrocarbons showed a good degradation effect with the range of fractions C8 to C12. This may be due to the strong performance of micro-organisms found in the wetland system in the degradation of aliphatic hydrocarbons with these carbon ranges of diesel. Findings of aliphatic hydrocarbons also showed that the highest concentration values were observed between C16 to C35 (Figure 4.36). Generally, aliphatic hydrocarbon compounds are described to be more resistant to degradation by microbes when the molecular weight is increased (Venosa & Zhu, 2003; Greenwood et al., 2008; Liu et al., 2011; Yavari et al., 2015). Control A showed the highest hydrocarbon concentration values for the most aliphatic fractions, highlighting low biodegradation occurring within the filter bed resulting from low microbial community due to a lack of the essential nutrient needed for microbe growth (Al-Isawi et al., 2015b).

Findings showed high efficiency of wetland filters in removing aromatic hydrocarbons within the range C5-C7 (Figure 4.37). This is because aromatic hydrocarbons with low molecular weight have greater ability to dissolve into the water and rapidly become degraded (Venosa & Zhu, 2003; Liu et al., 2011). The wetland system also showed a high treatment performance for aromatic hydrocarbon within the range C21-C35. This could

be attributed to the good degradation effect of the consortium of bacteria found in wetland filters to degrade this range of aromatic fractions. Furthermore, the low hydrocarbon concentration values observed in C35-C44 effluent of both aliphatic and aromatic fractions are due to their presence in low concentration values in the raw diesel fuel (Table 4.14).

Generally, aromatic hydrocarbons are reported to be highly soluble in water and to be more degradable than aliphatic hydrocarbons (Yavari et al., 2015; Hou et al., 2016). In this research, aromatic compounds were relatively lower than aliphatic compounds in wetland filter F5 (Figure 4.34), highlighting the impact of the high filter F5 inflow load in providing sufficient nutrient to elevate the growth of the microbial population with time, which in turn are capable of increasing the degradation rate. This finding is in agreement with Mills et al. (2003) who found that elevated nutrient levels from influent wastewater possibly provided a nutrient rich environment to rapidly biodegrade aromatic hydrocarbons.

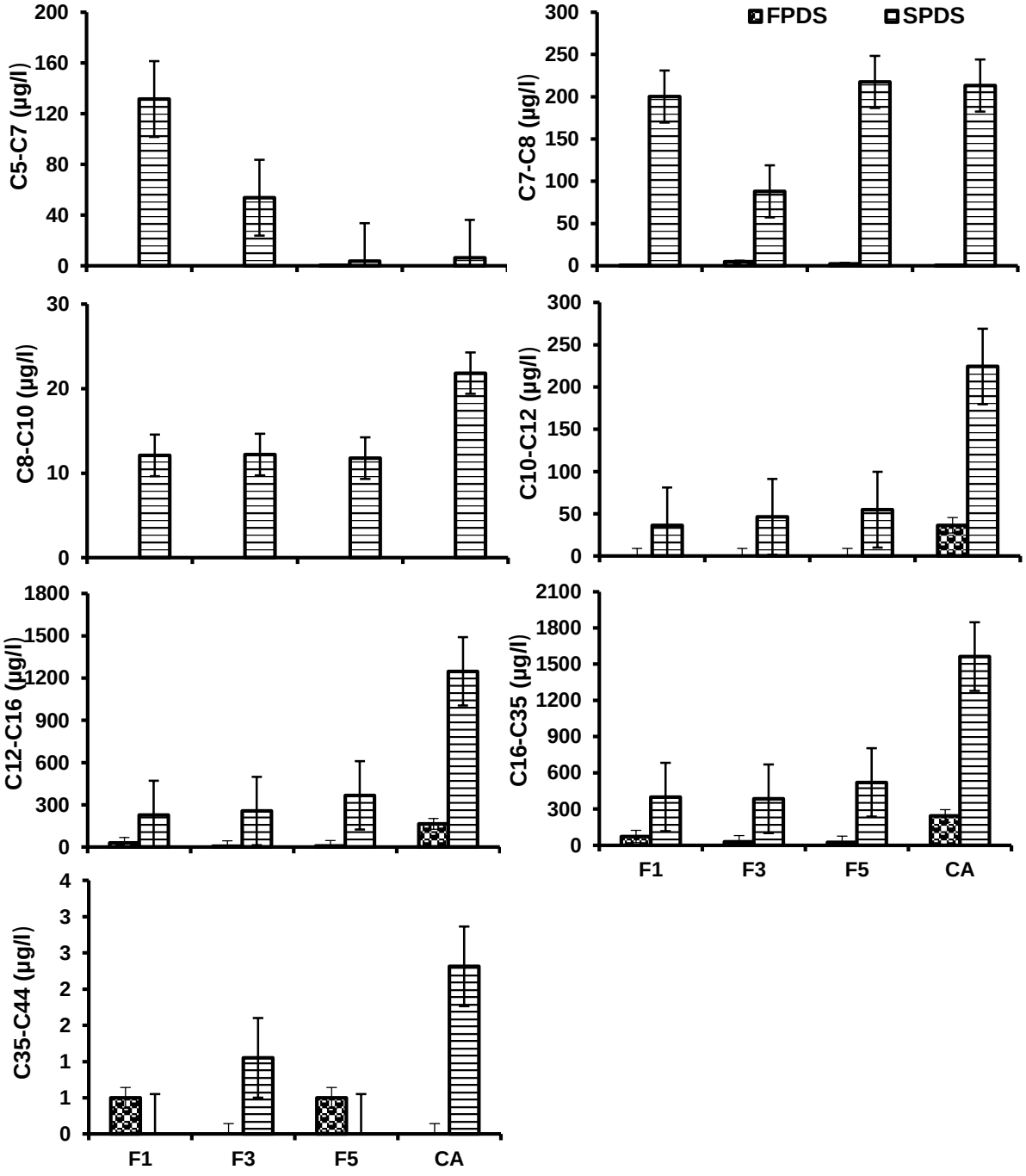


Figure 4.36: Comparison between hydrocarbon components (aliphatics) for the effluents of wetland filters (F1, F3, F5 and CA). Note C, carbon number index; FPDS, first period of diesel spill; and SPDS, second period of diesel spill.

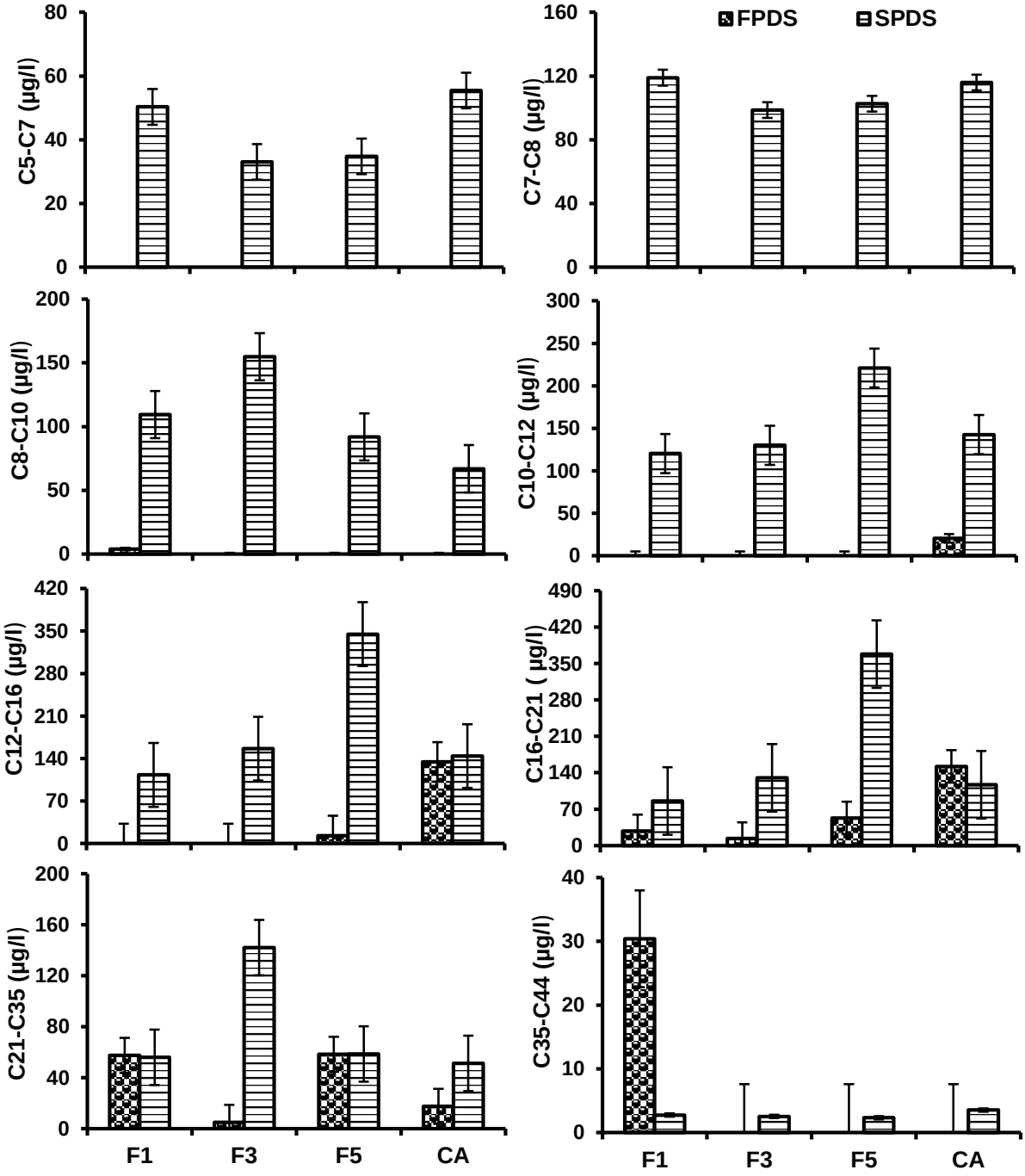


Figure 4.37: Comparison between hydrocarbon components (aromatics) for the effluents of wetland filters (F1, F3, F5 and CA). Note C, carbon number index; FPDS, first period of diesel spill; and SPDS, second period of diesel spill.

Figure 4.38 presents the average concentration values for volatile hydrocarbons in the contaminated wetland filters for both periods of diesel spills. Findings from this research showed a high treatment efficiency for benzene, toluene, ethylbenzene and xylene-volatile aromatic compounds (BTEX) and methyl tertiary butyl ether hydrocarbons (MTBE). The BTEX and MTBE compounds are considered of great concern as they are characterized by their high solubility and mobility in water. Owing to their related health risks, concentration limits have been restricted for both (5 µg/l, respectively) in drinking water (USEPA, 2009). In this research, comparison of the observed results of the volatile hydrocarbon components in the effluent with those in the raw diesel fuel (Table 4.14), showed that the wetland filters had high ability to treat volatile hydrocarbons, and toluene showed the highest treatment when comparing its concentration in the effluent with that in raw diesel fuel. The volatile hydrocarbons are reported to have high solubility in water and be readily to degrade by micro-organisms or evaporate to atmosphere (Yavari et al., 2015; Stefanakis et al., 2016). Results of nutrients (particularly effluent nitrate concentration, Figure 4.24), showed that nutrients were supportive of BTEX degradation and this is in agreement with findings of (Eke & Scholz, 2008) who found that presence of nitrate was the most supportive for the biodegradation process. The general treatment efficiency order (from low to high) of volatile hydrocarbons in the selected wetland filters was: MTBE, benzene, ethylbenzene, toluene, m,p-xylene, and o-xylene. The concentration of MTBE was very tiny in the effluent indicating that there is no MTBE hydrocarbon in the raw diesel fuel (Table 4.14).

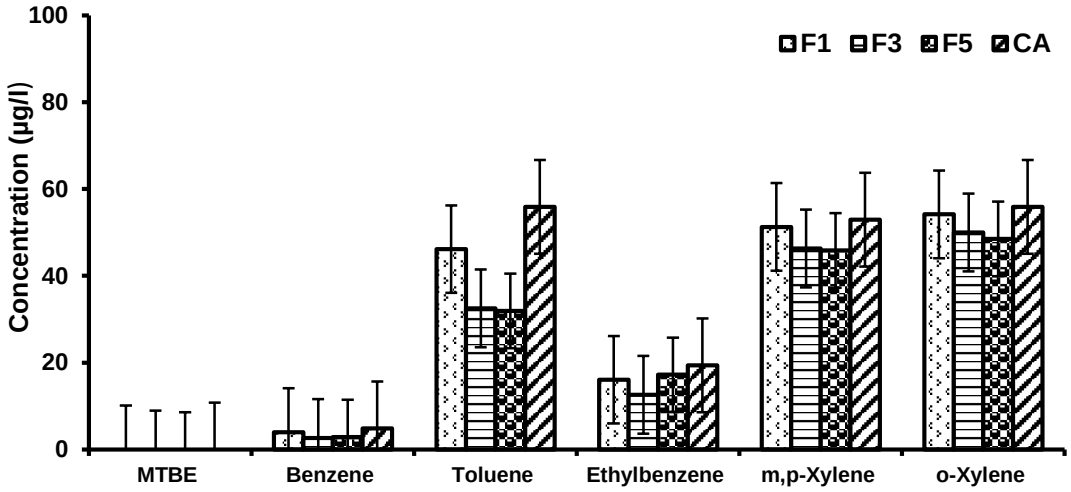


Figure 4.38: Comparison between hydrocarbon components (volatile hydrocarbons) for the effluents of wetland filters (F1, F3, F5 and CA) (contaminated with diesel).

Table 4.18 presents the previous studies dealing with treatment wetlands treating urban wastewater contaminated with petroleum hydrocarbons compounds, such as aromatic, aliphatic and total petroleum hydrocarbons. Comparison of these results with the present findings of this research showed that the effluent from all contaminated filters in the first period of diesel spill were within the recommended secondary wastewater treatment standards, except most of the values for Control A. Regarding the second period of diesel spill, the effluent hydrocarbon concentrations of the contaminated filters were initially high and fluctuated as a result of the high dosage of hydrocarbon compounds applied to the system. However, all the contaminated filters showed an improvement and a reduction in their concentration values with time, and to be compliant with outflow values recommended for secondary wastewater treatment standards, reflecting the impact of the fully mature wetland system, with prior exposure to hydrocarbon contaminants and sufficient nutrient provided with influent and elevated micro-organisms, to achieve high hydrocarbon treatment performance.

Table 4.18: Overview of references summarizing typical hydrocarbon concentrations in wetlands and associated standard thresholds measured in µg/l.

Analyte	Secondary wastewater treatment standards for hydrocarbon	Typical outflow of wetlands treating specifically petroleum hydrocarbons	Typical outflow of wetlands treating domestic wastewater
Aliphatic >EC5-7	–	–	–
Aliphatic >EC7-8	–	–	–
Aliphatic >EC8-10	300[a]	25[d]	–
Aliphatic >EC10-12	300[a]	55[d]	–
Aliphatic >EC12-16	300[a]	210[d]	–
Aliphatic >EC16-35	300[a]	73[d]	–
Total Aliphatics EC5-44	–	101[d]	–
Aromatic >EC5-7	–	–	–
Aromatic >EC7-8	–	–	–
Aromatic >EC8-10	20[b]	0.6[e]	–
Aromatic >EC10-12	100[a]	0.5[e]	–
Aromatic >EC12-16	100[a]	ND[f]	ND[k]
Aromatic >EC16-21	–	–	–
Aromatic >EC21-35	–	–	–
Total Aromatics EC5-44	300[a]	0.17[g]	0.17[g]
Total TPH	5000[c]	0.12-0.28[h,i,j]	25000[f]

EC, equivalent carbon number index; TPH, total petroleum hydrocarbons; [a]WHO (2005); [b]Scottish Environmental Protection Agency (2004); [c]EPA (2005); [d]Bergier (2011); [e]Wallace et al. (2011a); [f]Al-Baldawi et al. (2013f); [g]Fountoulakis et al. (2009); [h]Kadlec and Knight (1996); [i]Moshiri (1993); [j]Tchobanoglous and Burton (1991); [k]Giraud et al. (2001)); and ND, not detected.

4.5.4 Impact of hydrocarbon on wetland plant growth

This subsection documents the result of the findings concerning the impact of petroleum hydrocarbon compounds on the growth of wetland plants (macrophytes) specifically treating urban wastewater in constructed wetlands. The results information regarding the effects of petroleum hydrocarbons on the wetland plants are presented in a manner that will help guide researchers and designers to improve spill dose-response efficiency (Pezeshki et al., 2000; Ji et al., 2007; Al-Baldawi et al., 2015a; Hou et al., 2016).

Phytoremediation of diesel by wetland plants is promising (Cao et al., 2012; Al-Baldawi et al., 2014a; Truu et al., 2015). However, Armstrong et al. (2009) reported that oil infiltrates the gas space system of *P. australis* via its nodal and leaf sheath stomata (minute openings for gas exchange), reducing oxygen diffusion and convective flows into the rhizome system. Oxygenation of the above-ground portions of plants and the narrow region of soil close to the roots is also decreased. Furthermore, gas exchange via gas films are impeded in the saturated aggregate zone. Plants can also be weakened by diesel fuel-induced failure of emerging buds, which is a considerable risk during the growing season (Zhang et al., 2013; Hou et al., 2016). It follows that wetland plant growth characteristics and colour changes can be used as indicators for the effectiveness of remediation of petroleum-based hazardous pollution. The growth response of reeds depends upon the concentrations of diesel fuel (Zhang et al., 2013; Yavari et al., 2015; Hou et al., 2016).

Figure 4.39 shows a comparison of plant growth between wetland filters contaminated with hydrocarbon and wetlands filters without hydrocarbon contamination during first and second periods of diesel spills. The growth response of *P. australis* depended upon the concentrations of diesel fuel. In the period of the first diesel dosage, visible toxic responses to diesel were observed in the growth of *P. australis* in all filters contaminated

with diesel (F1, F3, F5, and Control A) in the period between September 2013-June 2014). Afterward, filters with hydrocarbon contamination showed a gradual improvement in their plant growth except Control A which exhibited a delay in its plant growth as compared with other filters and in July 2014, *P. australis* started to grow in this filter. This is due to the lack of essential nutrients for plant growth within the tap water received as influent to this filter (Al-Isawi et al., 2015a).

Phragmites australis plants may also assist in enhancing the removal efficiencies in all wetland filters, due to their ability to transport oxygen from the atmosphere to the rhizosphere (Omari et al., 2003; Al Mahruki et al., 2006; Vymazal, 2013a; Zheng et al., 2016) and derive organic carbon, which acts as an electron donor in the removal process (Chen et al., 2012). The microbial density, activity, and diversity are enhanced in the plant rhizosphere (Kadlec & Wallace, 2009; Truu et al., 2015; Hou et al., 2016), so it is suggested that the roots of the plants serve as a substrate for microbial attachment. The presence of diesel may encourage reeds to absorb the contaminants as a material required to synthesize enzymes (Wang et al., 2011a). This assessment can be justified by the presence of sufficient nutrients and regular aerobic conditions (De Biase et al., 2011) within the filter. Both the tidal flow mode and *P. australis* contributed to enriching the substrate with oxygen via the root zone, thus stimulating and speeding-up biodegradation and volatilization of contaminants within the filter bed (Vymazal, 2013a; Al-Isawi et al., 2015a; Zhi et al., 2015).

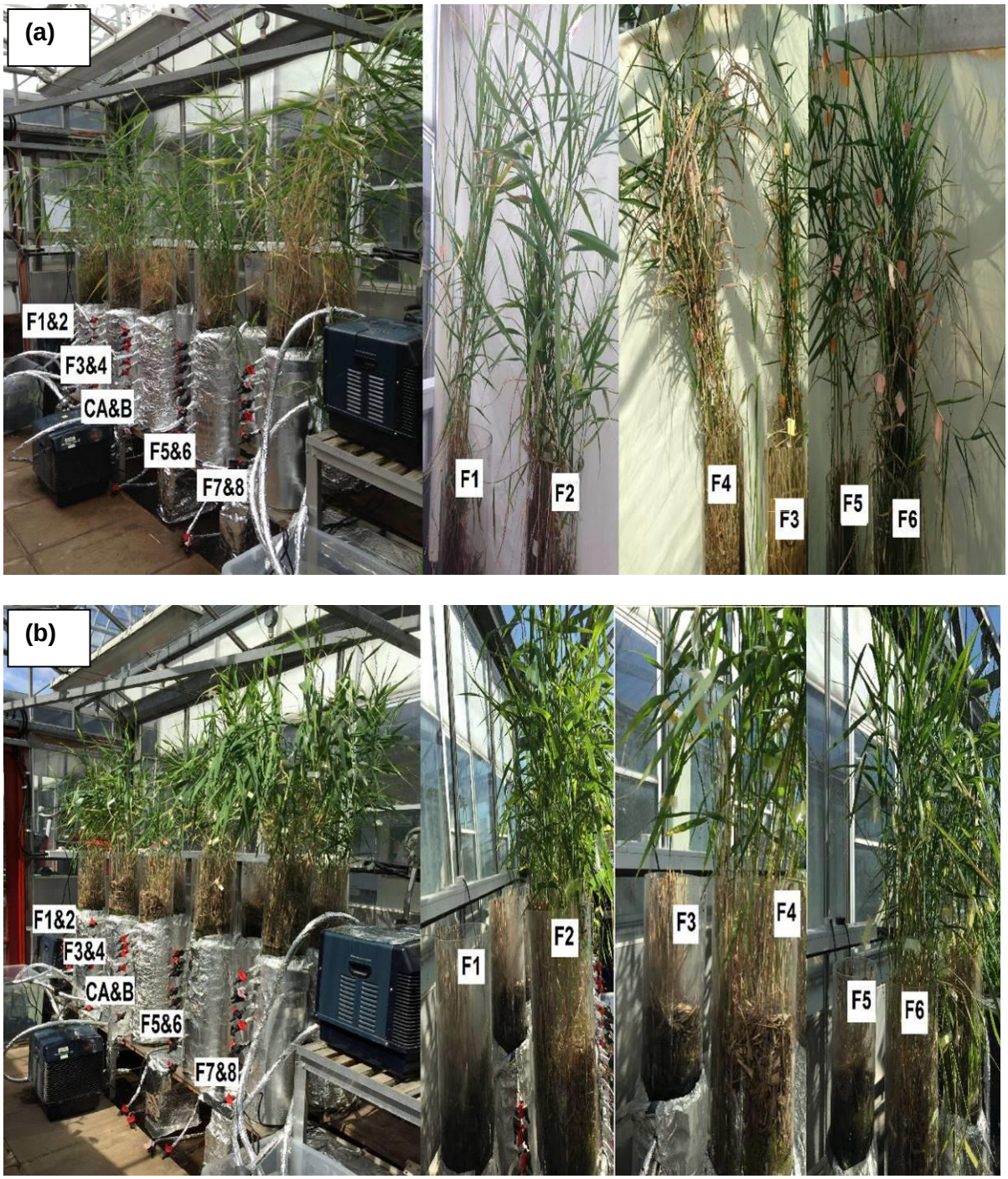


Figure 4.39: Comparison of wetland plants (reeds) growth between (a) the filters in the first diesel spill period (photo taken in August 2014), and (b) the filters in the second diesel spill period (photo taken in August 2015). Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; CA, Control A (wetland filter receiving tap water).

Table 4.19 indicates key wetland plant growth characteristics for the first period of diesel spill. A reduction in growth is apparent for filters subjected to diesel contamination. Findings of the health indices of *P. australis* (Cav.) Trin. ex. Steud. (Common Reed), including: number of stems with their branches in each wetland filter in addition to the length and diameter of each individual stem of reeds, are shown for the periods before and after the first diesel spill. The results of the above-ground biomass in the period after the first diesel spill dosage showed that the above-ground biomass of the wetland plants in filters without diesel contamination (F2, F4, F6, F7, F8, and CB) was relatively higher than that in filters with diesel contamination. This suggests that higher concentrations of diesel would restrain the synthesis of chlorophyll enzyme, thereby reducing the plants' chlorophyll content and photosynthesis, and inhibiting the growth of plants, while diesel in low concentrations might serve as nutrition to the plant's growth. The increment of reed growth under lower concentrations of diesel indicated that low concentrations of diesel could enhance plants' ability to absorb the material required to synthesize certain necessary enzymes (Ghobrial, 2008; Wang et al., 2011a). Regarding the period of the second diesel spill, and as a result of the high diesel spill dosage (975 g of diesel fuel), the wetland plants died in all filters contaminated with hydrocarbon after two months from the date of adding the second dose (Figure 4.39b) reflecting the high toxic impact of such high diesel compounds on reed growth.

Table 4.19: Growth characteristics of *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed) for the periods before and after the first diesel spill.

Parameter	Unit	Minimum	Mean	Maximum	Standard deviation
First to third experimental phase (27/06/2011 to 25/09/2013)					
Filter 1 (30 stems; 26 branches; 620 leaves)					
Length of stem	mm	760.0	1187.0	1840.0	28.34
Diameter of stem	mm	1.1	2.5	4.0	0.70
Filter 2 (32 stems; 1 branch; 437 leaves)					
Length of stem	mm	605.0	835.0	1250.0	16.50
Diameter of stem	mm	1.1	2.2	3.0	0.40

Table 4.19 (cont.)

Filter 3 (24 stems; 21 branches; 414 leaves)					
Length of stem	mm	620.0	1341.0	2275.0	54.10
Diameter of stem	mm	1.9	3.2	5.1	1.00
Filter 4 (26 stems; 2 branches; 493 leaves)					
Length of stem	mm	750.0	996.0	1250.0	16.60
Diameter of stem	mm	1.8	3.0	4.4	0.70
Filter 5 (31 stems; 22 branches; 633 leaves)					
Length of stem	mm	600.0	1168.0	2310.0	41.40
Diameter of stem	mm	1.0	2.7	4.1	0.70
Filter 6 (26 stems; no branches; 440 leaves)					
Length of stem	mm	685.0	818.0	1070.0	10.60
Diameter of stem	mm	1.2	2.0	2.9	0.60
Filter 7 (24 stems; 1 branch; 358 leaves)					
Length of stem	mm	640.0	974.2	1400.0	16.20
Diameter of stem	mm	1.2	2.6	4.2	0.70
Filter 8 (33 stems; no branches; 448 leaves)					
Length of stem	mm	655.0	1089.0	1550.0	25.70
Diameter of stem	mm	1.6	2.5	4.6	0.80
Control A (17 stems; 2 branches; 151 leaves)					
Length of stem	mm	700.0	1095.0	1640.0	26.20
Diameter of stem	mm	1.5	2.2	3.3	0.60
Control B (19 stems; no branches; 178 leaves)					
Length of stem	mm	810.0	1143.0	1610.0	22.6
Diameter of stem	mm	1.7	2.3	3.0	0.40
Fourth experimental phase (26/09/2013 to 30/04/2014)					
Filter 1 (7 stems; no branches; 34 leaves)					
Length of stem	mm	630.0	908.0	1270.0	24.33
Diameter of stem	mm	1.6	2.6	3.7	0.92
Filter 2 (13 stems; no branches; 53 leaves)					
Length of stem	mm	630.0	735.0	880.0	7.17
Diameter of stem	mm	1.7	2.5	2.7	0.26
Filter 3 (9 stems; no branches; 56 leaves)					
Length of stem	mm	780.0	980.0	1350.0	18.42
Diameter of stem	mm	2.2	3.2	3.6	0.45
Filter 4 (16 stems; no branches; 84 leaves)					
Length of stem	mm	710.0	887.1	980.0	8.74
Diameter of stem	mm	1.6	3.2	3.7	0.67
Filter 5 (2 stems; no branches; 14 leaves)					
Length of stem	mm	930	940.0	950.0	1.41
Diameter of stem	mm	2.2	2.6	3.1	0.66
Filter 6 (17 stems; no branches; 96 leaves)					
Length of stem	mm	710.0	891.3	1035.0	9.38
Diameter of stem	mm	1.8	2.8	3.3	0.46
Filter 7 (21 stems; no branches; 139 leaves)					
Length of stem	mm	720.0	913.0	1070.0	11.62
Diameter of stem	mm	2	3.2	3.9	0.50
Filter 8 (30 stems; no branches; 224 leaves)					
Length of stem	mm	640.0	1010.7	1280.0	15.48
Diameter of stem	mm	2.0	3.1	3.9	0.52
Control A (no stems; no branches; no leaves)					
Length of stem	mm	0.0	0.0	0.0	0.00
Diameter of stem	mm	0.0	0.0	0.0	0.00
Control B (5 stems; no branches; 16 leaves)					
Length of stem	mm	630.0	686.0	820.0	8.44
Diameter of stem	mm	1.8	2.4	3.1	0.52

Figure 4.40 indicates differences in plant leaf colour. The results showed that *P. australis* in wetland filters F6, F7, and F8 associated with darker green, 7.5GY and 2.5G, leaves as compared with those in other wetland filters reflecting the impact of high nutrient loads that applied with inflow wastewater to these filters. Moreover, the plants in wetland filters contaminated with diesel (F3, F5, and F7) associated with light green leaves as compared with those in filters without hydrocarbon contamination. Control A, received tab water as inflow, showed no growth in wetland plants during this period highlighting the lack of nutrient associated with the influent tab water. Findings showed that hydrocarbon contamination led to relatively minor changes in the leaf colour (Al-Isawi et al., 2015a). Elevated diesel concentrations are associated with low chlorophyll concentrations. Green pigments can be found in the leaves of *P. australis*. The leaf colour analysis for Filter 1 showed more leaves of light green colours 5Y and 2.5GY (Munsell, 1977) compared to leaves of Filter 2. This suggests that diesel restrains the synthesis of chlorophyll enzymes, thereby reducing the plant chlorophyll content and photosynthesis, and inhibiting the growth of plants (Wang et al., 2011a; Truu et al., 2015). Photosynthesis is the process used by *P. australis* and to convert light energy from the sun into chemical energy, which is later released to fuel activities such as growth and the release of oxygen (Ghobrial, 2008; Wang et al., 2011b; Cao et al., 2012).

5Y[a]				2.5GY					5GY			7.5GY				2.5G			
6\2[b]	6\4	6\6	6\8	6\2	6\4	6\6	6\8		6\4	6\6	6\8	6\2	6\4	6\6	6\8	6\2	6\4	6\6	6\8
5\2	5\4	5\6		5\2	5\4	5\6	5\8		5\4	5\6	5\8	5\2	5\4	5\6	5\8	5\2	5\4	5\6	5\8
									4\4	4\6	4\8	4\2	4\4	4\6		4\2	4\4	4\6	
									3\4			3\2	3\4			3\2	3\4		
Filter 1:																			
0	0	2	1	0	0	0	3		0	3	1	0	0	1	0	0	0	0	0
0	0	0		0	0	1	5		3	12	0	0	1	0	0	0	0	0	0
									0	0	0	0	1	0		0	0	0	
									0			0	0			0	0		
Filter 2:																			
0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	0
0	0	0		1	0	0	0		0	0	2	0	13	2	0	0	0	0	0
									3	3	0	5	16	0		2	1	0	
									0			0	5			0	0		
Filter 3:																			
0	0	0	1	0	0	0	4		3	1	0	0	0	0	0	0	0	0	0
0	0	0		0	0	1	0		1	0	0	0	1	1	0	0	0	0	0
									2	10	9	0	12	8		0	0	0	
									0			0	1			0	0		
Filter 4:																			
0	0	0	0	0	2	2	5		4	3	0	1	0	0	0	0	0	0	0
0	0	0		1	0	0	0		1	2	0	0	1	12	0	2	2	0	0
									10	2	0	1	20	12		1	2	0	
									0			0	0			0	0		
Filter 5:																			
0	0	0	0	0	0	0	0		1	0	0	0	1	0	0	0	0	0	0
0	0	0		0	0	0	0		0	1	2	0	2	1	0	0	0	0	0
									2	0	1	1	0	1		0	0	0	
									1			0	0			0	0		
Filter 6:																			
0	0	0	0	0	0	0	1		0	0	0	0	1	0	1	0	0	0	0
0	0	1		0	0	0	2		1	2	0	1	2	0	0	15	0	0	0
									4	1	0	0	19	6		1	18	0	
									0			0	15			0	5		
Filter 7:																			
0	0	0	1	0	0	0	0		0	1	0	0	2	2	0	0	0	0	0
0	0	0		0	0	1	0		1	0	1	0	12	13	13	2	0	0	0
									1	1	3	0	14	12		8	12	5	
									0			8	17			0	11		
Filter 8:																			
0	0	0	0	0	0	1	0		0	1	0	0	0	0	0	0	0	0	0
0	0	2		0	0	0	2		5	4	0	0	5	0	0	0	0	0	0
									3	2	8	19	27	33		5	32	0	
									2			31	4			17	21		
Control A																			

0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
									0	0	0	0	0	0		0	0	0	
									0			0	0			0	0		
Control B:																			
0	0	0	0	0	0	0	0		1	0	0	0	0	0	0	0	0	0	0
0	0	0		0	0	0	0		0	0	1	0	3	0	0	0	0	0	0
									0	3	0	1	2	3		0	0	0	
									0			1	1			0	0		

Figure 4.40: Leaf colour determinations (Munsell, 1977) for all leaves for the fourth experimental phase (first period of diesel spill). [a]Hue; and [b]Value (lightness and darkness of a colour)/Chroma (degree of strength or saturation).

4.6 Comparative performance between the mature vertical-flow constructed wetlands and artificial ponds

The results and discussions presented in this section have been published in the journal paper shown below:

Al-Isawi, R.H.K., Sanak, R., & Scholz, M. (2016). Comparative study of domestic wastewater treatment by mature vertical-flow constructed wetlands and artificial ponds. Ecological Engineering, 100, (8-18). DOI: org/10.1016/j.ecoleng.2016.12.017.

4.6.1 Inflow water quality

The characterization of the preliminary treated raw domestic wastewater (five years of wetland system operation) taken from the treatment plant is presented in Table 4.20, which illustrates the general inflow water quality for the whole experiment. Seven parameters were used to assess the treatment performance of the wetland system. The mean water quality parameter concentrations of the undiluted influent for COD, BOD, NH_4-N, NO_3-N, PO_4-P, SS, and pH were as follows: 281.3 mg/l, 151.8 mg/l, 39.6 mg/l, 4.1 mg/l, 13.3 mg/l and 157.6 mg/l and 7.72, respectively. Throughout the monitoring period of the wetland system, the water quality analysis for the undiluted influent (preliminary treated real domestic wastewater) showed a high variation of all water quality parameters except for pH (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). The data variability was relatively high (Table 4.20 and Figure 4.41), reflecting the nature of real domestic wastewater subject to changing consumer behaviour. Variability of the influent can also be linked to shock loads to the sewers, weather conditions, seasonal variation, and dilution of the wastewater by precipitation.

Wetland effluent concentration variations often mirrored influent concentration variability (Figure 4.41). The undiluted mean influent (real domestic wastewater) concentrations during the comparative study period for COD, BOD, NH_4-N, NO_3-N, PO_4-P, SS and pH were as follows: 404.8 mg/l, 260.0 mg/l, 20.7 mg/l, 0.4 mg/l, 13.1 mg/l, 176.8 mg/l and 7.85, respectively.

Generally, the average pollutant concentrations of the preliminary treated domestic wastewater were similar to those reported in literature (Stefanakis et al., 2014; Scholz, 2015). Moreover, the COD to BOD ratio of the influent (preliminarily treated domestic wastewater) was about 1.85, which is slightly higher than 1.14 as reported in the literature (Stefanakis et al., 2014). This indicates that a substantial part of the organic matter will be easy-to-degrade biologically. Therefore, it may be concluded that the influent has a high biodegradability and can be classified as rather low-strength wastewater.

Table 4.20: Overview of the inflow water quality for mature wetland systems without dilution (preliminarily treated domestic wastewater) for the period from 27 June 2011 to 31 December 2015.

Parameter	Unit	Number	Mean	Standard deviation	Minimum	Maximum
Chemical oxygen demand	mg/l	147	281.3	130.96	100.0	660.0
Biochemical oxygen demand[a]	mg/l	211	151.8	77.61	10.0	360.0
Ammonia-nitrogen	mg/l	143	39.6	23.78	0.0	131.8
Nitrate-nitrogen	mg/l	129	4.1	4.96	0.2	20.9
Ortho-phosphate-phosphorus	mg/l	137	13.3	8.38	2.4	40.0
Suspended solids	mg/l	253	157.6	126.79	2.4	760.0
pH[b]	-	171	7.72	0.40	6.30	8.86

[a] start of measurement on 2 July 2012; and [b] start of measurement on 22 June 2012.

4.6.2 Comparison of outflow water quality

This subsection focuses on the long-term treatment behaviour of the wetland system. During the sampling period (June 2011 to December 2015), samples were collected from the four filters of the wetland system and analysed for each of the assessed parameters.

Table 4.21 summarizes the overall outflow water quality. For each of the water quality parameters, the removal percentages of the wetlands were calculated and are shown in Table 4.21.

Findings demonstrated that the wetland system could efficiently reduce SS (91.3-92.4%), BOD (74.9-81.3%), NH_4-N (62.079.2%), PO_4-P (59.8-64.7%) and COD (58.6-70.8%). The removals of NO_3-N, however, were often negative (source rather than sink of pollution). After treatment, the average corresponding effluent concentrations were between 1.5 and 4.1 mg/l, resulting in reduction efficiencies of between -107.6 and 23.7%.

The relatively high COD and BOD removal by the mature constructed wetlands was achieved by physical and microbial processes. The porosity of the wetland media reduced over time. Even fine solids are trapped during filtration for a rather long time, therefore, allowing hydrolysis of organic solids and subsequent biodegradation to proceed rapidly (Ruiz et al., 2010).

Furthermore, the tidal-flow operation strategy is beneficial in overcoming poor water distribution challenges and improving the oxygen mass transfer and diffusion from the open air into the wetlands (Stefanakis et al., 2014). Anoxic conditions were promoted in the filter bed due to the low porosity of the wetland media, enhancing anaerobic biodegradation pathways.

Table 4.21: Comparison of outflow water quality variables for the mature wetland systems between 27 June 2011 and 31 December 2015.

Parameter	Unit	Number	Mean	Removal (%)	Minimum	Maximum	Standard deviation
Filter 4							
Chemical oxygen demand	mg/l	135	51.9	62.8	6.0	160.0	26.98

Table 4.21 (cont.)

Biochemical oxygen demand	mg/l	205	19.1	74.9	0	150.0	18.24
Ammonia-nitrogen	mg/l	131	5.4	79.6	0	28.6	6.07
Nitrate-nitrogen	mg/l	125	1.5	23.7	0	11.3	2.48
Ortho-phosphate-phosphorus	mg/l	129	2.9	59.8	0	6.3	1.25
Suspended solids	mg/l	247	6.9	91.3	0	120	11.16
pH	-	195	6.7	n/a[a]	5.8	7.4	0.30
Filter 6							
Chemical oxygen demand	mg/l	136	82.15	70.8	6.53	452.0	55.16
Biochemical oxygen demand	mg/l	207	28.3	81.3	0	245.0	27.51
Ammonia-nitrogen	mg/l	138	15.0	62.0	0.1	62.2	14.29
Nitrate-nitrogen	mg/l	124	4.1	1.2	0	24.8	4.83
Ortho-phosphate-phosphorus	mg/l	128	4.7	64.7	0	13.5	2.49
Suspended solids	mg/l	243	9.7	93.8	0	84.0	11.68
pH	-	193	6.9	n/a[a]	5.8	7.9	0.29
Filter 7							
Chemical oxygen demand	mg/l	142	54.4	60.9	10.9	255.0	32.82
Biochemical oxygen demand	mg/l	230	16.6	78.2	0	75.0	13.87
Ammonia-nitrogen	mg/l	143	5.7	78.6	0	35.3	6.11
Nitrate-nitrogen	mg/l	134	4.1	-109.6	0	17.5	3.78
Ortho-phosphate-phosphorus	mg/l	138	3.0	58.5	0	12.1	1.57
Suspended solids	mg/l	277	6.0	92.4	0	85.0	10.58
pH	-	220	6.8	n/a[a]	5.8	8.0	0.36
Filter 8							
Chemical oxygen demand	mg/l	147	57.7	58.6	11.8	360.0	39.59
Biochemical oxygen demand	mg/l	245	18.6	75.6	0	72.0	14.19
Ammonia-nitrogen	mg/l	142	5.5	79.2	0	30.6	6.28
Nitrate-nitrogen	mg/l	131	3.4	-74.9	0	17.5	3.56
Ortho-phosphate-phosphorus	mg/l	137	2.9	60.8	0	11.3	1.52
Suspended solids	mg/l	322	6.0	92.4	0	69.0	8.93
pH	-	268	6.79	n/a[a]	5.7	7.8	0.38
Control B							
Chemical oxygen demand	mg/l	58	15.66	nm[b]	0.23	90.3	16.90
Biochemical oxygen demand	mg/l	129	6.0	nm[b]	0.0	34.0	6.15
Ammonia-nitrogen	mg/l	62	0.5	nm[b]	0.0	6.9	1.13
Nitrate-nitrogen	mg/l	61	0.2	nm[b]	0.0	1.0	0.27
Ortho-phosphate-phosphorus	mg/l	63	1.8	nm[b]	0.9	4.2	0.53
Suspended solids	mg/l	126	3.4	nm[b]	0.0	49.0	6.52
pH	-	128	6.64	n/a[a]	6.05	7.40	0.25
Air temperature	°C	974	15.7	n/a[a]	2.0	34.0	6.2

[a] not applicable; [b]not measured and NTU, nephelometric turbidity unit.

The annual results of the wetland filter studies between 2011 and 2015 are presented in Figure 4.41. Figures 4.41a and 4.41b show the COD and BOD influent and effluent concentrations of the vertical-flow constructed wetlands, respectively. The effluent concentrations were influenced by the fluctuations of the influent COD and BOD. Generally, the effluent concentration values were acceptable (excluding set-up period; COD values ≤87.5 mg/l and BOD values ≤44.6 mg/l), if compared with influent concentrations values. It is usually difficult to reduce the COD concentrations below 50 mg/l after secondary treatment (Korkusuz et al., 2005). Moreover, the wetland filter (F6) that received concentrated wastewater (without dilution) was significantly ($p<0.05$) different to the wetland (F4) in terms of COD and BOD that received diluted wastewater during the first three years of wetland operation. Later on, there was no difference between them, while all wetlands that received diluted wastewater as influent did not show a significant ($p>0.05$) difference between each other in terms of COD and BOD. This can be explained by the rather low organic content of the (diluted) wastewater transferred to the wetland systems, which in turn is reflected by the absence of clogging phenomena in the pores of the filter substrates as reported by Al-Isawi et al. (2015a). The treatment efficiencies of the experimental wetlands for the removal of organics are generally highly dependent on the oxygen available in the bed. It is suggested that sufficient oxygen diffusion into all wetlands could also be responsible for the similar trend of COD and BOD values between wetland filters (Jia et al., 2010; Wu et al., 2015b).

The NH_4-N and NO_3-N effluent concentrations of the wetlands (Figures. 4.41c and 4.41d) varied between 1.2 and 25.2 mg/l and between 0.3 and 6.0 mg/l, respectively). The wetland systems showed an improvement in NH_4-N concentration reduction over time. The relatively high nitrification capacities of the tidal-flow mode operated vertical-flow

wetland systems can be attributed to greater oxygen transfer from the atmosphere to the wetlands (Saeed & Sun, 2012; Wu et al., 2015b). Moreover, it is known that vegetation could have increased nitrification through the oxygenation of the substrate. As the wetland filters matured, the growth of the root system might have supported the establishment of a rich and productive community of attached nitrifying micro-organisms by providing greater surface areas (Meng et al., 2014; Murphy et al., 2016). Lower effluent ammonium concentrations and higher effluent nitrate concentrations can be explained by the mature biofilm that developed over time as the surface area increased and aerobic nitrification enhanced and anoxic denitrification processes reduced (Korkusuz et al., 2005). Moreover, temperature and pH (Table 4.21) were within the range that could support both nitrification and denitrification processes (Wang & Li, 2015; Xie et al., 2016).

Annual inflow and outflow PO_4-P concentrations for all wetlands are shown in Figure 4.41e. During the monitoring period, the influent PO_4-P values were variable reflecting changes in water and detergent usage characteristics. Generally, PO_4-P retention in the wetlands is a function of the effluent quality, loading rate and substrate (Li et al., 2015; Valipour & Ahn, 2016). During the start-up period and the first year of wetland system operation, the values of PO_4-P concentrations ranged between 2.2 mg/l and 5.6 mg/l, and the corresponding PO_4-P removal efficiencies were relatively high as the aggregates were new, reflecting their high absorption ability. For the remaining period, the effluent PO_4-P concentrations increased over time. Even when the influent PO_4-P was low, there was a significant increase ($p< 0.05$) in the outflow PO_4-P concentrations as compared with those concentrations in the earlier periods of wetlands system operation, which indicates that the wetland media might have become saturated by phosphate accumulation. The

removal of PO_4-P depends on the availability of the calcium, aluminium and iron concentrations of the substrate (Li et al., 2015). Since the material used as aggregates in sub-surface constructed wetland systems is siliceous (minimum of 30%) pea gravel, which usually does not contain high concentrations of these elements, the PO_4-P removal is rather low among wetlands.

Figure 4.41f indicates the annual variations of SS. During the wetland start-up period, the effluent SS concentrations were relatively high (16.9 to 28.2 mg/l). Over time, SS effluent values decreased to between 3.9 mg/l and 10.8 mg/l. These low SS concentrations were due to the physical retention of solids at the surface of the wetland filter. This layer of dirt (often called schmutzdecke) is considered a key factor for improving overall treatment efficiency (Scholz, 2015). Moreover, a five-year-old constructed wetland planted with emergent plants having a complex and strong root system enhanced SS reduction by providing a larger surface area, reducing water velocity and reinforcing settling and filtration in the rhizosphere (Korkusuz et al., 2005; Paing et al., 2015a).

The pH values were within the allowable range between 4 and 9.5, which is suitable for the survival of most bacteria (Kadlec & Wallace, 2009) and varied between slightly alkaline values for the inlet (7.72±0.40) and slightly acidic ones for the outlets (6.72±0.30, 6.92±0.29, 6.83±0.36 and 6.79±0.38 for F4, F6, F7 and F8, respectively). The acidic outflow pH values of the wetlands planted with reeds compared to the alkaline influent was probably due to the decomposition of organic wastewater components (Kadlec & Wallace, 2009) trapped within the mature plants root system and old plant material (Al-Isawi et al., 2015a).

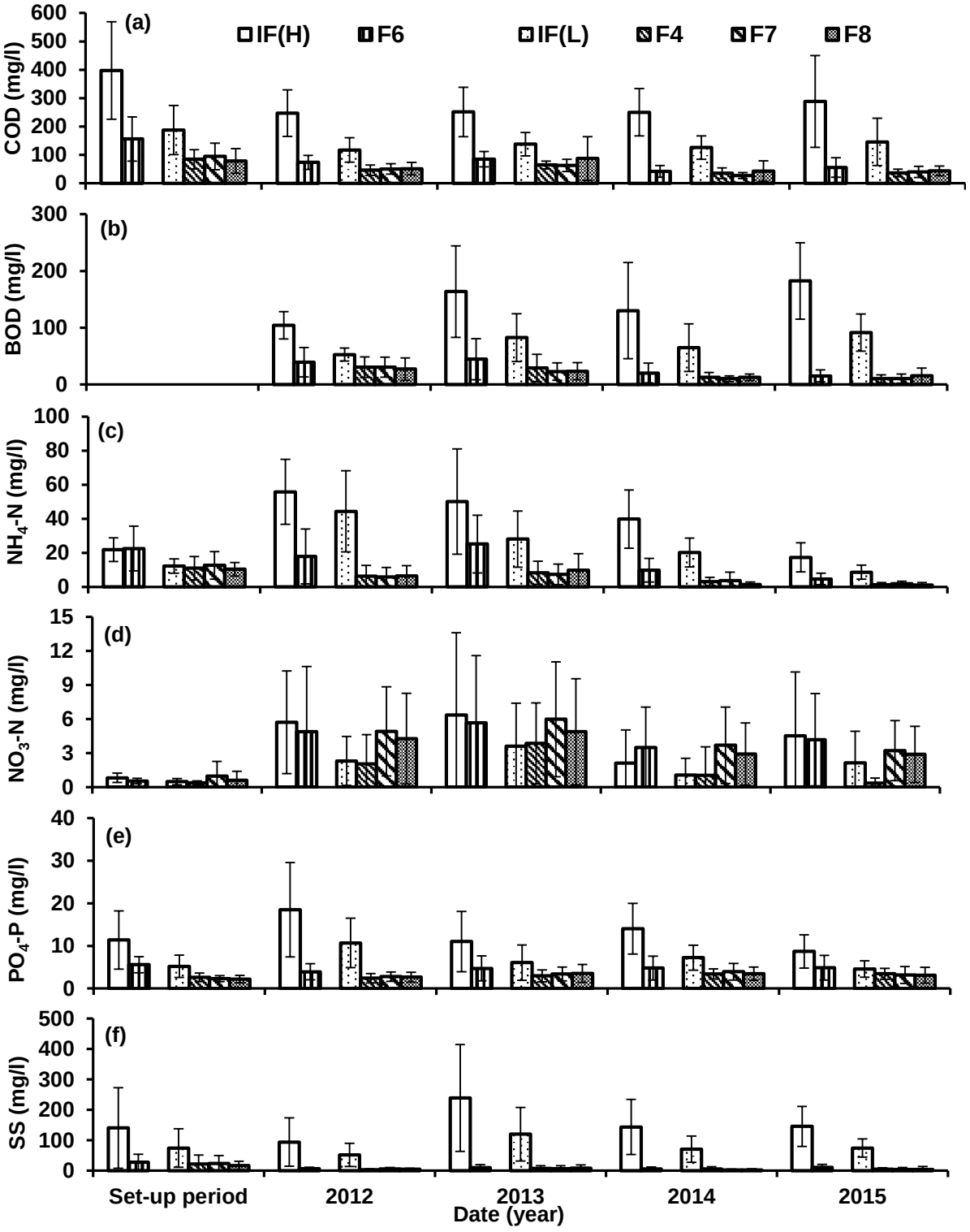


Figure 4.41: Annual variations of water quality parameters for the wetland system: (a) chemical oxygen demand (COD); (b) biochemical oxygen demand (BOD); (c) ammonia-nitrogen (NH_4-N); (d) nitrate-nitrogen (NO_3-N); (e) ortho-phosphate-phosphorus (PO_4-P); and (f) suspended solids (SS); IF(H), wastewater influent

without dilution (high rate); IF(L), diluted wastewater influent (low rate); F4, Wetland filter 4 effluent; F6, wetland filter 6 effluent; F7, wetland filter 7 effluent; and F8, wetland filter 8 effluent.

4.6.2.1 Comparison of chemical oxygen demand for various systems

Figures 4.42 to 4.47 show an overview of the mean (±standard deviation) inflow and outflow concentrations of various pollutants treated by four different operational filter sets (high contact time, high loading rate, low contact time, and low resting time):

- Set 1 (with high contact time) includes F4 (wetland planted with reeds), P1 (pond operated without reeds), P2 (pond planted with reeds) and P3 (pond planted with reeds and aerated).
- Set 2 (with high loading rate in terms of COD) includes F6 (wetland planted with reeds), P4 (pond operated without reeds), P5 (pond planted with reeds) and P6 (pond planted with reeds and aerated).
- Set 3 (with low contact time) includes F7 (wetland planted with reeds), P7 (pond operated without reeds), P8 (pond planted with reeds) and P9 (pond planted with reeds and aerated).
- Set 4 (with low resting time) includes F8 (wetland planted with reeds), P10 (pond operated without reeds), P11 (pond planted with reeds) and P12 (pond planted with reeds and aerated).

Note that the inflow water quality is different for all four filters (see Table 3.3). Table 4.22 shows p-values calculated by the non-parametric Mann-Whitney U-test for outflow water quality variables regarding different wetlands and ponds (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b).

The COD has been removed relatively well from all four treatment sets (Figure 4.42a to 4.42d). Previous studies (Von Sperling et al., 2010; Mburu et al., 2013) showed that sub-surface constructed wetlands were better than ponds in COD removal, which is in agreement with current findings. However, results also indicated that COD is treated more efficiently in mature wetlands compared to aerated ponds. Furthermore, similar reduction trends for COD concentrations were observed for Figures 4.42a, 4.42c and 4.42d. This indicates that the contact time and resting time do not have any corresponding significant ($p>0.05$) role during treatment.

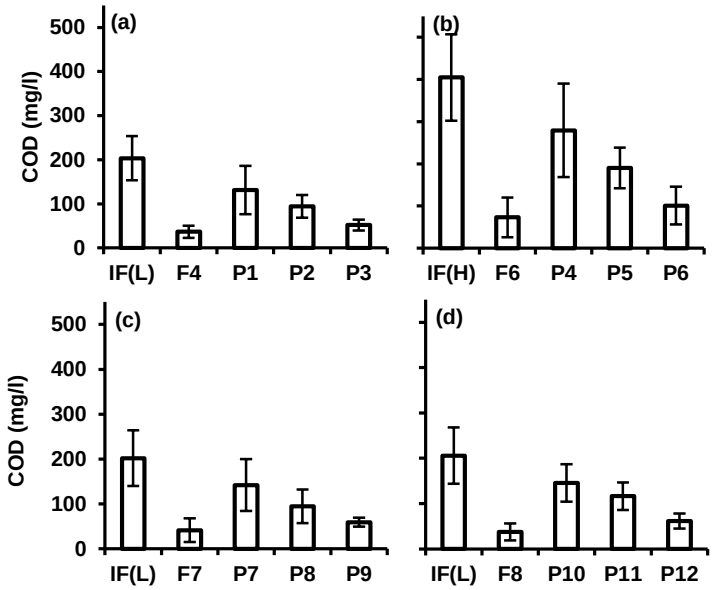


Figure 4.42: Chemical oxygen demand (COD) comparison between the wetland and pond systems for the inflow and outflow for sets with (a) high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

The Urban Waste Water Treatment (England and Wales) Regulations (UK Government, 1994) implementing the Council Directive 91/271/EEC Concerning Urban Waste-water

Treatment (European Community, 1991) set a threshold of 125 mg/l for secondary wastewater treatment. The mean COD values for the wetland system indicate that all wetlands had relatively good COD removal, while all unplanted ponds did not comply with this threshold. A maximum reduction in COD concentration was observed for F4 (36.86 mg/l) followed by P3 (51.8 mg/l), P2 (94.2 mg/l) and P1 (131.5 mg/l), which is also shown graphically in Figure 4.42a. The good COD reduction concentration values of F4 could be linked to the aerobic conditions created due to the tidal mode of wetland operation with additional aeration facilitating aerobic microbial growth on the mature biofilm layer and boosting the biodegradation of organic matter (Vymazal, 2011b).

In set 1, there is a significant difference between F4 and P2, which indicates that the presence of substrate in wetlands with a high surface area enhances microorganism development and leads to an increase in the ability to degrade pollutants (Kadlec & Wallace, 2009). No significant ($p>0.05$) difference was noted between P1 and P2, which may indicate that plants were unimportant in organic carbon retention. In all filter sets, there is a significant difference ($p<0.05$) between ponds planted with reeds and aerated ponds planted with reeds, reflecting the role of aeration in COD removal. This high COD removal rate for aerated ponds (P3, P6, P9 and P12) as compared with the remaining ponds could be due to biodegradation of organic matter by aerobic micro-organisms that grow. The aerobic degradation of soluble organic matter is performed by aerobic heterotrophic bacteria (Korkusuz et al., 2005; Tomova et al., 2013).

A high loading rate (set 2) showed higher COD removal efficiency rates than other sets. This indicates that both systems (wetlands and ponds) which received high loading rates with inflow COD concentrations equal to 404.8 mg/l (set 2; Figure 4.42b) were more efficient as compared with sets 1, 3 and 4 (in removing COD). Moreover, a significant

difference ($p<0.05$) was recorded between P4 (279.4 mg/l) and P5 (190.0 mg/l). This indicates that for a high loading rate (concentrated wastewater), the COD removal efficiency was higher for ponds planted with reeds than for the ones without plants. It is suggested by (Korboulewsky et al., 2012) that domestic wastewater with high nutrients supports the growth of reeds and results in planted wetlands which outperform unplanted ones, mainly due to the rhizosphere stimulating microbial community density and activity by providing roots with high surface area for microbial growth, a supply of carbon compounds through root exudates and a micro-aerobic environment via the release of root oxygen.

Table 4.22: Overview of the statistically significant differences (indicated by p-value and h) between outflow water quality variables of different wetlands and ponds systems using the non-parametric Mann-Whitney U-test for data collected between 13 July 2015 and 13 October 2015.

Parameter	COD[a]	BOD[b]	NH_4-N[c]	NO_3-N[d]	PO_4-P[e]	SS[f]
Unit	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Design comparison between wetlands and ponds (presence of media (aggregate))						
Wetland 4 and Pond 2[g]	0.001 (1)	0.004 (1)	0.001 (1)	0.093 (0)	0.159 (0)	0.001 (1)
Wetland 6 and Pond 5[h]	0.001 (1)	0.430 (0)	0.001 (1)	0.105 (0)	0.149 (0)	0.001 (1)
Wetland 7 and Pond 8[i]	0.002 (1)	0.432 (0)	0.001 (1)	0.011 (1)	0.123 (0)	0.001 (1)
Wetland 8 and Pond 11[j]	0.001 (1)	0.737 (0)	0.001 (1)	0.128 (0)	0.007 (1)	0.001 (1)
Design comparison between ponds						
Presence of *Phragmites australis* (Common Reed)						
Ponds 1 and 2[g]	0.063 (0)	0.317 (0)	0.023 (1)	0.353 (0)	0.353 (0)	0.315 (0)
Ponds 4 and 5[h]	0.011 (1)	0.879 (0)	0.184 (0)	0.072 (0)	0.566 (0)	0.698 (0)
Ponds 7 and 8[i]	0.012 (1)	0.868 (0)	0.015 (1)	0.225 (0)	0.171 (0)	0.041 (1)
Ponds 10 and 11[j]	0.123 (0)	0.515 (0)	0.315 (0)	0.143 (0)	0.926 (0)	0.436 (0)
Availability of aeration						
Ponds 2 and 3[g]	0.001 (1)	0.670 (0)	0.001 (1)	0.001 (1)	0.019 (1)	0.001 (1)
Ponds 5 and 6[h]	0.001 (1)	0.926 (0)	0.001 (1)	0.001 (1)	0.019 (1)	0.002 (1)
Ponds 8 and 9[i]	0.009 (1)	0.196 (0)	0.001 (1)	0.001 (1)	0.089 (0)	0.012 (1)
Ponds 11 and 12[j]	0.001 (1)	0.745 (0)	0.001 (1)	0.001 (1)	0.002 (1)	0.005 (1)

Note: P-value, probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true; h, response indicator; if h=1, units are statistically significantly different (*P-value*<0.05) for the corresponding water quality parameter; if h=0, the difference is not significant. [a]chemical oxygen demand; [b]biochemical oxygen demand; [c]ammonia-nitrogen; [d]nitrate-nitrogen; [e]ortho-phosphate-phosphorus; [f]suspended solids; [g]same high

contact time (Table 3.3); [h]same high load (Table 3.3); [i]same low contact time (Table 3.3); and [j]same low resting time (Table 3.3).

4.6.2.2 Comparison of biochemical oxygen demand for various systems

The traditional UK standard for BOD removal from secondary wastewater is 20 and 25 mg/l for sensitive and less sensitive (e.g., many coastal discharges) areas, respectively (Royal Commission on Sewage Disposal, 1915). More recently, the Urban Waste Water Treatment (England and Wales) Regulations (UK Government, 1994) define a threshold of 25 mg/l for secondary wastewater treatment. In set no.1, maximum BOD reduction was obtained for F4 (13.7 mg/l) followed by P1 (19.5 mg/l), P2 (30.0 mg/l) and P3 (28.8 mg/l). A significant difference ($p<0.05$) was noted between F4 and P2 designs (Figure 4.43a), and this is attributed to the difference in the nature of the treatment (i.e. biofilm fastened to the gravel media in the wetlands) (Mburu et al., 2013) combined with a high contact time, which is sufficient for organic degradation.

No significant ($p>0.05$) differences were observed between different treatment sets (Table 4.22; Figures 4.43b to 4.43d). moreover, it has been noted that no significant ($p>0.05$) differences were observed between planted ponds (P2, P5, P8 and P11) when compared with the ones without planting (P1, P4, P7 and P10), and the ones with aeration (P3, P6, P9 and P12) ,which indicates that the plant and/or aeration presence in the ponds did not improve the removal of BOD. The BOD removal efficiency was greater for all four filters of set 2 as compared with the other three sets. This indicates the good capability of both wetland and pond systems to reduce high BOD inflow loads (260 mg/l; concentrated domestic wastewater). Maximum BOD removals were obtained for F6 and F7, if compared to the other designs for both sets (Figures 4.43b and 4.43c). This may also be attributed to the maturity of the wetlands, which created enough biofilm within

the substrate to support a good growth of selected micro-organisms (aerobic degrading bacteria), which have the capability for organic pollutant degradation (Nurk et al., 2005).

The maximum BOD reduction was observed for the second design P10 (13.2 mg/l) as compared with F8 (24.0 mg/l), P11 (17.6 mg/l) and P12 (18.0 mg/l) as shown in Figure 4.43d. This high organic load decrease is achieved through settling of organic SS within the pond and subsequent degradation by micro-organisms. However, the BOD removal in set 4 (all four designs) was higher, if compared with sets 1 and 3. This shows that the resting time played a vital role during BOD removal. Moreover, a low resting time limits the generation of BOD from algae in the pond systems (Mburu et al., 2013). This will lead to a decrease of BOD values in ponds of set 4.

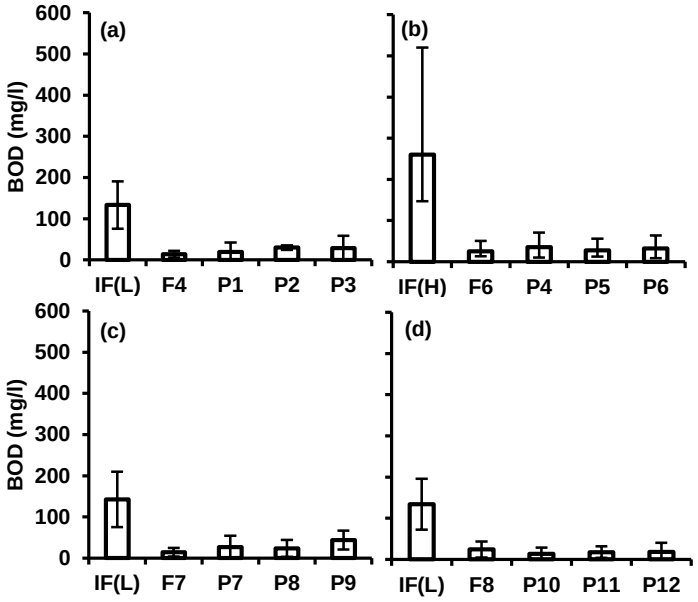


Figure 4.43: Biochemical oxygen demand (BOD) comparison between the wetland and pond systems for the inflow and outflow for sets with (a) high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

4.6.2.3 Comparison of ammonia-nitrogen for various systems

Findings indicate that NH_4-N has been removed well with respect to all four designs (Figure 4.44a). For the treatment system discussed in this research, the regulations (UK Government, 1994) set no threshold for ammonia-nitrogen. However, a potential guideline threshold for ammonia-nitrogen in the context of this experiment would be 20 mg/l. With the exception of aerated ponds, wetland filters in all sets were better than ponds in NH_4-N removal. This observation contradicts previous studies (Von Sperling et al., 2010; Mburu et al., 2013). The difference can be explained by the role of mature wetland filters highlighting the effect of both mature reeds and biomass within wetland filters. A significant ($p<0.05$) difference was recorded between F4 and P2, P1 and P2, and P2 and P3 designs. Maximum NH_4-N reduction efficiencies were noted for P3 (0.24 mg/l) followed by F4 (0.56 mg/l), P2 (5.3 mg/l) and P1 (7.7 mg/l). This shows that aeration is important in ammonia oxidation during the treatment procedure. Ponds with high contact time and planted with reeds (P2) showed a higher treatment performance in terms of NH_4-N as compared with ponds without plants (P1) and this may indicate the ability of reeds to remove NH_4-N (Kadlec & Wallace, 2009). However, in the first design (F4), the reeds along with the substrate and microbial biofilm were essential in ammonia-nitrogen removal.

Significant ($p<0.05$) differences were observed between F6 and P5, and between P5 and P6 designs (Figure 4.44b). No significant ($p>0.05$) difference was observed between P4 and P5 designs (Table 4.22). Here, in ponds with high loading rate, reeds do not play a vital role in NH_4-N removal. Again, aeration is essential in ammonia-nitrogen removal. The high COD load of the inflow does not affect the NH_4-N removal efficiency in the

aeration ponds planted with reeds. Moreover, the same NH_4-N removal trends were observed in set 2, when compared with set 1.

For set 1 (high contact time), a significant difference ($p<0.05$) was detected between P2 (with reeds) and P1 (without reeds) as indicated in Figure 4.44c. For set 3 (low contact time), a significant ($p<0.05$) difference was observed between P8 (with reeds) and P7 (without reeds), which might be due to the alterations in contact time and this may indicate the ability of reeds in NH_4-N removal (Kadlec & Wallace, 2009; Scholz, 2015) (see Table 3.3). Similar trends for NH_4-N removal were observed (Figure 4.44d). Good removal of NH_4-N was recorded for the first (F8) and fourth (P12) designs. No significant ($p>0.05$) difference was detected between the second (P10) and the third (P11) designs, which may be due to a low resting time.

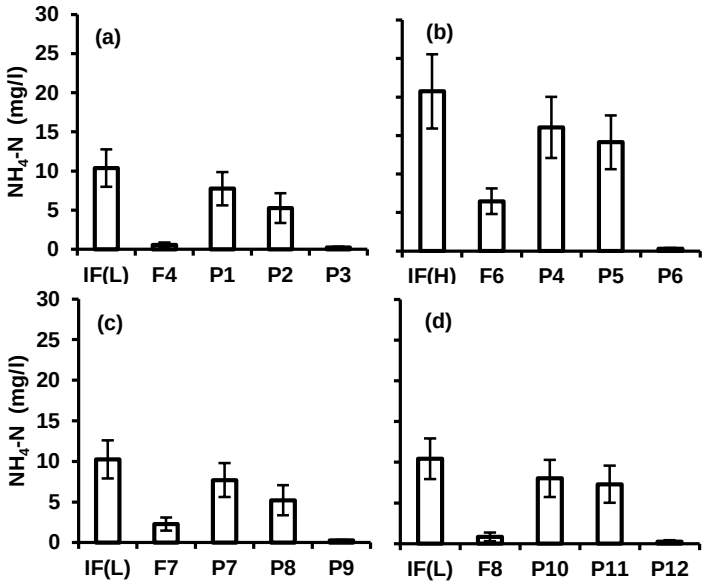


Figure 4.44: Ammonia-nitrogen (NH_4-N) comparison between the wetland and pond systems for the inflow and outflow for sets with (a) high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some

wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

4.6.2.4 Comparison of nitrate-nitrogen concentrations treated by various systems

The regulations (UK Government, 1994) also set no threshold for nitrate-nitrogen of relevance for the treatment system discussed in this research. Nevertheless, a realistic guideline threshold value for nitrate-nitrogen in the context of this experiment could be 50 mg/l. The NO_3-N concentrations in the inflow are ≤0.5mg/l (Figure 4.45). For filters with high contact times (set 1; Figure 4.45a), no significant ($p>0.05$) difference was observed between F4 (0.08 mg/l) and P2 (0.23 mg/l) designs. For set 3 with low contact time as shown in Figure 4.45c, there is a significant difference ($p<0.05$) in NO_3-N reduction between F7 (0.42 mg/l) and P8 (0.10 mg/l). No statistically significant ($p>0.05$) difference was observed between ponds with reeds (P2, P5, P8 and P11) and those without reeds (P1, P4, P7 and P10), which indicates the lack of effect of plant presence in pond systems on the treatment performance. On the other hand, significant differences ($p<0.05$) were observed between the ponds planted with reeds (P2, P5, P8 and P11) and ponds planted with reeds and subject to aeration (P3, P6, P9 and P12) for all sets (Table 4.22). Here, the aerated ponds containing reeds were linked to higher nitrate-nitrogen concentrations reflecting the oxygen availability for the nitrification process (Vymazal, 2007a).

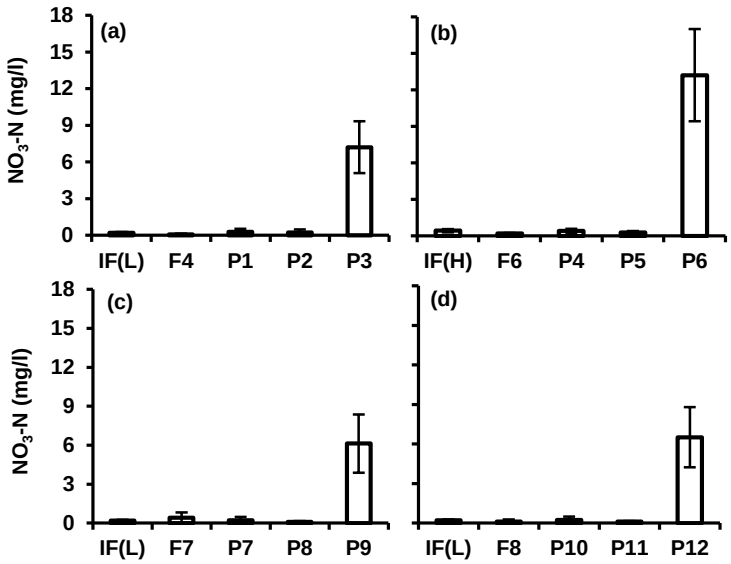


Figure 4.45: Nitrate-nitrogen (NO_3-N) comparison between the wetland and pond systems for the inflow and outflow for sets with (a)high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

4.6.2.5 Comparison of ortho-phosphate-phosphorous for various systems

Relatively good PO_4-P removal was observed between the mean inflow and outflows from the fourth filter design regarding all sets (Figure 4.46). The regulations (UK Government, 1994) set a threshold of 2 mg/l for total phosphorus for communities between 10,000 and 100,000 inhabitants. However, a threshold for ortho-phosphate-phosphorus that would relate to the treatment system discussed in the context of this research does not exist. However, a realistic guide concentration for ortho-phosphate-phosphorus is 1 mg/l. Maximum PO_4-P removal efficiency was observed for the aerated ponds (fourth design) followed by the wetlands (first design). No significant ($p>0.05$)

difference was recorded between F4 and P2, and P1 and P2 designs (Figure 4.46a). The results indicate that the presence of plants in pond systems did not play a significant role ($p>0.05$) in treatment performance as compared with ponds without plants (Table 4.22). However, for low resting times (set 4), a significant ($p<0.05$) differences was noted between F8 (2.9 mg/l) and P11 (5.7 mg/l) designs; there is an accumulation in PO_4-P due to the high frequency of the loading rates associated with these filters (Li et al., 2015). Moreover, the PO_4-P removal in the aeration ponds comprising reeds was more efficient than those in the other designs.

A significant ($p<0.05$) difference was witnessed between the third (P5; 9.42 mg/l) and fourth (P6; 4.22 mg/l) designs (Figure 4.46b). The maximum PO_4-P removal efficiency was noted for the fourth design followed by the first, third and second designs. However, set 2 with high COD load in the inflow did not have any impact on the PO_4-P removal efficiency when compared to filters in set 1. There is a similar trend when compared to filters in sets1 and 3 (Figure 4.46c). Here, less contact time did not make a significant ($p>0.05$) difference in terms of PO_4-P reduction.

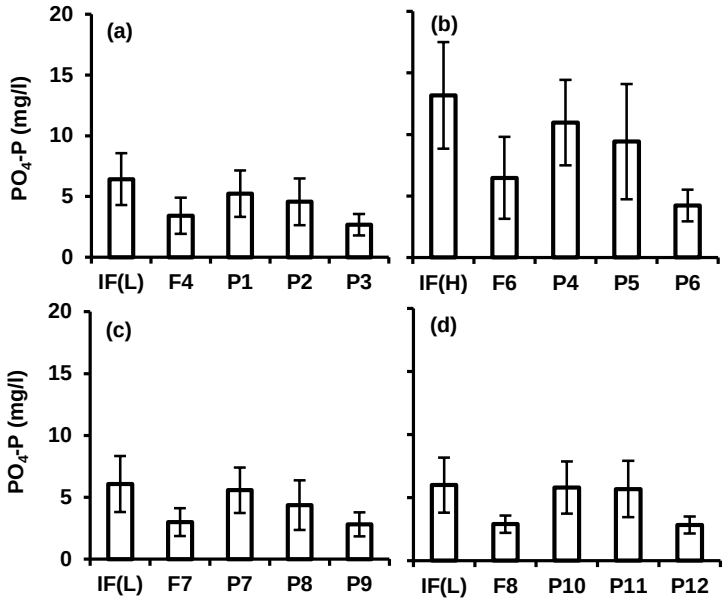


Figure 4.46: Ortho-phosphate-phosphorus (PO_4-P) comparison between the wetland and pond systems for the inflow and outflow for sets with (a) high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

4.6.2.6 Comparison of particles treated by various systems

The traditional UK standard for SS outflow from secondary wastewater is 30 mg/l (Royal Commission on Sewage Disposal, 1915). The same reduction trend of SS for the four sets was noticed (Figure 4.47). Maximum SS removal efficiency was observed for wetlands followed by ponds planted with reeds and subject to aeration, ponds planted with reeds, and finally ponds without plants. A significant ($p<0.05$) difference was noted between wetlands (F4, F6, F7 and F8) and ponds planted with reeds (P2, P5, P8 and P11) (Table 4.22). Five-year-old constructed wetlands planted with reeds and having an extensive rhizome and root system could be the reason for the enhanced SS removal efficiency by the provision of a larger surface area, reducing the water velocity and reinforcing filtration

and settling in the vertical-flow wetland bed (Al-Isawi et al., 2015a). Moreover, it is clear that for the planted ponds (P2, P5, P8 and P11), the SS concentration drastically dropped with a significant ($p<0.05$) effect in terms of performance when compared with aerated ponds P3, P6, P9 and P12. The aerobic conditions at these ponds with additional aeration improved the growth of aerobic micro-organisms and enhanced biodegradation processes of organic SS (Meng et al., 2014). For all filter sets excluding low contact time, there is no significant ($p>0.05$) difference between ponds without plants (P1, P4, and P10) as compared with those with plants (P2, P5, and P11). In the case of low contact time, a significant ($p<0.05$) difference was observed between P7 and P8 designs (Figure 4.47c), indicating that there is insufficient contact time for a micro-aerobic root environment to release oxygen, which subsequently increases the uptake of SS (Brix & Arias, 2005).

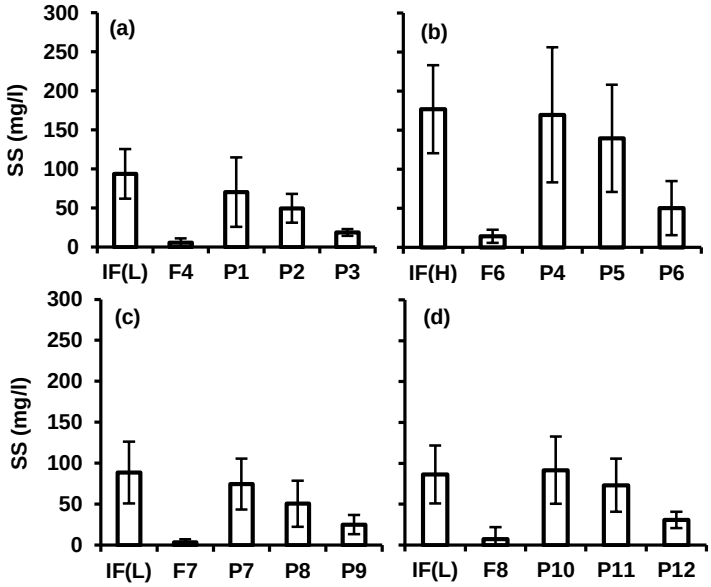


Figure 4.47: Suspended solids (SS) comparison between the wetland and pond systems for the inflow and outflow for sets with (a) high contact time; (b) high loading rate (in terms of COD); (c) low contact time; and (d) low resting time for the period between 13 July 2015 and 13 October 2015. IF(H), wastewater influent without dilution (high rate); IF(L), diluted wastewater influent (low rate); some

wetlands were planted with *Phragmites australis*. P1, 4, 7 and 10 were without plants; P2, 5, 8 and 11 were planted; and P3, 6, 9 and 12 were planted and aerated.

4.7 Recycling performance: assessment of chilli yields production

The results and discussions presented in this section have been published in the following four papers shown below:

Al-Isawi, R.H.K., Scholz, M., & Al-Faraj, F.A.M. (2016). Assessment of diesel-contaminated domestic wastewater treated by constructed wetlands for irrigation of chillies grown in a greenhouse. *Environmental, Science and Pollution Research*, 1-21. doi: 10.1007/s11356-016-7706-x.

Al-Isawi, R.H.K., Almuktar, S.A.A.-A.N., & Scholz, M. (2016). Recycling of river, rain, gully pot and grey waters for irrigating Chillies. *Environmental Monitoring and Assessment,* (2016) 188:287, doi:10.1007/s10661-016-5285-4.

Almuktar, S.A.A.-A.N., Scholz, M., Al-Isawi, R.H.K., & Sani A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for irrigating Chillies and Sweet Peppers. *Agricultural Water Management* 149, 1-22. doi.org/10.1016/j.agwat.2014.10.025.

Almuktar, S.A.A.-A.N., Scholz, M., Al-Isawi, R.H.K., & Sani A. (2015). Recycling of domestic wastewater treated by vertical-flow wetlands for watering of vegetables. *Water Practice & Technology*, 01/2015; 10(2):1-20. doi:10.2166/wpt.2015.052.

4.7.1 Irrigated water quality analysis for greenhouse experiment

4.7.1.1 Overview

The wetland effluent was used as the influent for the chillies. Figures 4.48 to 4.50 indicate the variations of water quality parameters of the irrigation water. The changes in water quality parameters were compared according to three planting phases:

- Phase 1 (planting period before fruiting);
- Phase 2 (planting period after fruiting) and;
- Phase 3 (planting period after fruiting and after the second diesel dosage).

The water quality parameters of particular focus are COD, NH_4-N, NO_3-N, PO_4-P, pH, redox and electrical conductivity. The irrigation water was grouped into four sets: set 1 for filters subject to contamination with diesel (Filters 1, 3 and 5 as well as Control A); set 2 for filters without diesel contamination (Filters 2, 4, 6, 7 and 8 as well as Control B); and set 3 and 4 for comparison purposes (preliminarily treated wastewater, preliminarily treated wastewater (one part) mixed with tap water (four parts), tap water, deionized water and tap water with fertilizer (0.7 ml/l), river water, rain water, gully pot, real and artificial grey waters). Both tap water and deionized water types indicated no notable changes over the period of the experiment and thus are not presented in Figure 4.50. For more details regarding mean, standard deviation and sample number values for all water quality parameters over the three phases, refer to Table 4.23.

Table 4.23: Comparison of the water quality of the inflow waters received by the chilli pots (value, sample number (in brackets) and standard deviation, if applicable).

Parameter	Unit	Overall[a]	RPBF[b]	RPAF[c]	RPAFD[d]
Filter 1 (outflow)					
Total petroleum hydrocarbons	µg/l	1986(6)±1829.72	100(1)±nm	332(1)±nm	2872(4)±1560.5
Chemical oxygen demand	mg/l	133.8(22)±66.50	61.3(2)±3.04	78.9(9)±13.29	191.9(11)±40.79
Biochemical oxygen demand	mg/l	51.5(40)±30.20	38.0(4)±30.24	28.0(20)±11.09	84.1(16)±10.57
Ammonia-nitrogen	mg/l	4.5(21)±2.73	1.4(2)±0.39	5.2(10)±2.53	4.5(9)±2.89
Nitrate-nitrogen	mg/l	0.3(22)±0.10	0.3(2)±0.17	0.3(10)±0.13	0.3(10)±0.05
Ortho-phosphate-phosphorus	mg/l	6.0(23)±2.51	2.4(2)±0.13	5.4(10)±2.56	7.3(11)±1.78
Suspended solids	mg/l	17.3(41)±14.09	6.0(6)±5.55	9.7(21)±7.53	33.6(14)±8.49
Turbidity	NTU[e]	15.9(39)±13.93	6.1(6)±2.38	7.4(19)±3.94	31.5(14)±11.55
pH	–	6.2(40)±0.32	6.4(6)±0.15	6.2(20)±0.24	6.0(14)±0.38
Redox potential	mV	26.2(43)±6.22	18.2(5)±2.95	25.4(22)±4.99	29.8(16)±5.91
Conductivity	µS/cm	503.2(44)±208.83	318.1(5)±51.25	369.6(21)±22.23	710.4(18)±177.46
Dissolved oxygen	mg/l	1.5(40)±0.78	1.1(2)±0.57	1.7(21)±0.88	1.3(17)±0.64
Filter 2 (outflow)					
Total petroleum hydrocarbons	µg/l	<10	nm	<10	<10
Chemical oxygen demand	mg/l	35.5(18)±13.24	17.3(2)±1.63	32.2(9)±11.48	44.8(7)±9.59
Biochemical oxygen demand	mg/l	15.6(40)±8.50	16.0(4)±6.93	14.1(20)±8.17	17.3(16)±9.38
Ammonia-nitrogen	mg/l	5.2(21)±5.40	3.1(2)±1.21	6.5(10)±6.39	7.0(9)±4.64
Nitrate-nitrogen	mg/l	1(19)±1.80	7.2(2)±0.20	0.9(10)±1.33	0.4(8)±0.28
Ortho-phosphate-phosphorus	mg/l	3.8(20)±1.23	2.0(2)±0.15	3.8(10)±1.34	4.3(8)±0.77
Suspended solids	mg/l	7.9(41)±7.51	2.3(6)±2.25	6.3(21)±4.72	12.7(14)±9.74
Turbidity	NTU[e]	6.5(38)±6.27	2.8(6)±0.70	4.0(18)±1.48	11.2(14)±8.37
pH	–	6.5(40)±0.18	6.6(6)±0.12	6.5(20)±0.18	6.5(14)±0.20
Redox potential	mV	10.1(43)±6.16	6.6(5)±3.36	11.5(22)±5.01	9.3(16)±7.78
Conductivity	µS/cm	491.7(44)±171.61	307.3(5)±46.71	372.0(21)±24.84	682.6(18)±83.58
Dissolved oxygen	mg/l	2.0(40)±0.85	1.4(2)±0.49	2.1(21)±0.84	1.9(17)±0.87
Filter 3 (outflow)					
Total petroleum hydrocarbons	µg/l	1554(6)±1340.93	69(1)±nm	37(1)±nm	2305(4)±861.8
Chemical oxygen demand	mg/l	153.8(22)±86	73.2(2)±3.61	88.2(9)±20.28	221.7(11)±71.77
Biochemical oxygen demand	mg/l	37.4(41)±30.68	17.7(6)±9.42	18.5(19)±6.67	67.3(16)±29.44

Table 4.23 (cont.)

Ammonia-nitrogen	mg/l	3.1(21)±2.24	0.8(2)±0.21	4.4(10)±2.51	2.3(9)±1.16
Nitrate-nitrogen	mg/l	0.3(22)±0.09	0.3(2)±0.20	0.3(10)±0.08	0.3(10)±0.07
Ortho-phosphate-phosphorus	mg/l	5.1(23)±2.38	1.9(2)±0.88	4.3(10)±2.18	6.4(11)±1.89
Suspended solids	mg/l	16.3(41)±13.65	5.5(6)±4.23	10.0(21)±9.45	30.4(14)±9.80
Turbidity	NTU[e]	13.4(38)±12.73	6.0(6)±2.25	4.9(18)±1.95	27.6(14)±10.53
pH	–	6.4(40)±0.21	6.6(6)±0.13	6.5(20)±0.15	6.2(14)±0.20
Redox potential	mV	16.3(43)±6.91	7.4(5)±4.04	13.1(22)±3.85	23.5(16)±3.50
Conductivity	µS/cm	593.3(44)±216.48	396.2(5)±109.90	439.9(21)±26.76	827.1(18)±128.37
Dissolved oxygen	mg/l	1.7(40)±0.96	2.0(2)±1.91	2.0(21)±0.95	1.3(17)±0.73
Filter 4 (outflow)					
Total petroleum hydrocarbons	µg/l	<10	nm	<10	<10
Chemical oxygen demand	mg/l	37.5(18)±19.03	10.9(2)±0.64	35.9(9)±21.30	47.2(7)±8.45
Biochemical oxygen demand	mg/l	14.4(41)±9.27	11.2(5)±8.67	16.3(20)±9.89	13.0(16)±8.64
Ammonia-nitrogen	mg/l	2.8(21)±2.81	0.1(2)±0.11	1.8(10)±2.14	4.1(9)±3.07
Nitrate-nitrogen	mg/l	0.5(22)±1.30	5.8(2)±1.87	0.1(10)±0.05	0.2(8)±0.16
Ortho-phosphate-phosphorus	mg/l	3.6(20)±1.24	1.8(2)±0.19	3.0(10)±0.93	4.7(8)±0.58
Suspended solids	mg/l	6.0(41)±4.95	6.3(6)±5.20	6.0(21)±5.18	5.7(14)±4.86
Turbidity	NTU[e]	4.3(38)±2.64	7.3(6)±3.10	4.0(18)±2.82	3.5(14)±0.94
pH	–	6.5(40)±0.19	6.5(6)±0.12	6.5(20)±0.23	6.5(14)±0.18
Redox potential	mV	12.1(43)±4.33	9.0(5)±2.74	12.2(22)±3.46	13.0(16)±5.44
Conductivity	µS/cm	500.4(44)±209.31	317.9(5)±67.12	398.5(21)±37.61	670.1(18)±234.52
Dissolved oxygen	mg/l	2.1(40)±0.99	1.3(2)±0.85	2.2(21)±0.90	2.2(17)±1.12
Filter 5 (outflow)					
Total petroleum hydrocarbons	µg/l	2698(6)±2016.75	14(1)±nm	218(1)±nm	3989(4)±324.2
Chemical oxygen demand	mg/l	149.8(22)±92.10	60.3(2)±0.35	73.9(9)±9.95	228.2(11)±64
Biochemical oxygen demand	mg/l	38.7(42)±29.70	9.0(6)±8.37	21.5(20)±9.86	71.3(16)±19.68
Ammonia-nitrogen	mg/l	10.1(22)±3.99	12.6(2)±2.05	11.0(10)±2.53	8.9(10)±5.15
Nitrate-nitrogen	mg/l	0.7(22)±0.77	1.5(2)±1.87	0.8(10)±0.89	0.5(10)±0.05
Ortho-phosphate-phosphorus	mg/l	7.2(23)±3.77	1.8(2)±0.89	5.3(10)±1.60	10.0(11)±3.25
Suspended solids	mg/l	15.8(41)±15.33	6.0(6)±4.34	7.2(21)±5.21	32.9(14)±13.97
Turbidity	NTU[e]	11.9(38)±10.67	4.7(6)±0.87	5.8(18)±2.19	22.7(14)±10.81
pH	–	6.5(39)±0.16	6.6(6)±0.14	6.5(19)±0.10	6.4(14)±0.14

Table 4.23 (cont.)

Redox potential	mV	9.9(43)±8.51	5.0(5)±8.12	8.8(22)±3.24	12.9(16)±12.27
Conductivity	µS/cm	918.3(44)±361.21	507.1(5)±187.64	672.7(21)±63.86	1319.1(18)±150.56
Dissolved oxygen	mg/l	1.5(40)±0.77	1.4(2)±1.13	1.9(21)±0.71	1.1(17)±0.63
Filter 6 (outflow)					
Total petroleum hydrocarbons	µg/l	<10	nm	<10	<10
Chemical oxygen demand	mg/l	43.1(18)±21.25	24.9(2)±13.29	34.9(9)±23.52	58.8(7)±3.67
Biochemical oxygen demand	mg/l	21.2(42)±17.64	9.3(6)±8.91	16.9(20)±12.05	31.0(16)±21.35
Ammonia-nitrogen	mg/l	9.2(22)±7.21	0.5(2)±0.11	10.2(10)±8.58	9.1(10)±5.69
Nitrate-nitrogen	mg/l	3.8(21)±3.88	0.2(2)±1.87	3.4(10)±3.85	4.7(9)±4.05
Ortho-phosphate-phosphorus	mg/l	5.3(21)±2.83	2.9(2)±0.32	6.0(10)±3.66	5.0(9)±1.70
Suspended solids	mg/l	5.6(41)±4.77	4.2(6)±2.32	5.9(21)±5.93	5.8(14)±3.57
Turbidity	NTU[e]	4.4(38)±2.55	3.5(6)±1.57	4.9(18)±3.26	4.2(14)±1.69
pH	–	6.8(40)±0.17	6.8(6)±0.14	6.7(20)±0.16	6.8(14)±0.20
Redox potential	mV	2.0(43)±4.92	4.6(5)±5.27	2.6(22)±3.05	0.4(16)±6.48
Conductivity	µS/cm	882.7(44)±355.35	423.4(5)±126.88	661.4(21)±54.94	1268.6(18)±178.53
Dissolved oxygen	mg/l	2.0(40)±0.03	1.2(2)±0.42	2.1(21)±1.12	2.1(17)±0.95
Filter 7 (outflow)					
Total petroleum hydrocarbons	µg/l	<10	nm	<10	<10
Chemical oxygen demand	mg/l	27.8(18)±11.35	14.1(2)±0.07	31.0(9)±14.01	27.5(7)±4.97
Biochemical oxygen demand	mg/l	10.2(46)±5.59	15.0(6)±5.76	9.8(23)±5.72	8.9(17)±4.70
Ammonia-nitrogen	mg/l	4.2(23)±5.37	10.6(2)±14.32	4.3(12)±4.81	2.6(9)±2.96
Nitrate-nitrogen	mg/l	3.3(19)±3.33	8.3(2)±0.32	1.3(10)±0.36	5.6(7)±3.42
Ortho-phosphate-phosphorus	mg/l	4.2(20)±1.99	1.9(2)±0.13	4.3(10)±2.62	4.6(8)±0.49
Suspended solids	mg/l	2.7(52)±4.08	2.7(7)±2.98	4.0(27)±5.06	0.6(18)±0.50
Turbidity	NTU[e]	3.4(49)±2.61	2.7(7)±0.69	4.3(24)±3.30	2.4(18)±1.43
pH	–	6.6(54)±0.19	6.5(7)±0.21	6.6(26)±0.16	6.6(21)±0.23
Redox potential	mV	4.9(54)±5.79	7.4(7)±4.50	7.3(27)±4.08	0.9(20)±6.07
Conductivity	µS/cm	511.6(51)±179.00	337.0(5)±100.39	399.3(26)±99.31	701.1(20)±78.35
Dissolved oxygen	mg/l	2.2(49)±1.24	1.4(2)±0.85	1.8(26)±0.74	2.8(21)±1.52
Filter 8 (outflow)					
Total petroleum hydrocarbons	µg/l	1463(2)±2069.0	nm	2926(1)±nm	<10
Chemical oxygen demand	mg/l	48.0(18)±37.12	64.3(2)±0.42	28.1(9)±9.08	68.8(7)±51.03

Table 4.23 (cont.)

Biochemical oxygen demand	mg/l	13.4(53)±5.64	14.3(8)±6.36	13.9(24)±6.58	12.5(21)±4.14
Ammonia-nitrogen	mg/l	1.4(21)±1.59	0.7(2)±0.12	1.5(10)±1.40	1.4(9)±1.93
Nitrate-nitrogen	mg/l	2.9(16)±2.22	6.6(2)±1.90	1.5(8)±1.43	3.9(7)±1.94
Ortho-phosphate-phosphorus	mg/l	3.6(19)±1.60	1.9(2)±0.10	3.6(10)±2.11	4.0(8)±0.58
Suspended solids	mg/l	2.9(54)±4.38	6.0(9)±7.55	3.0(29)±3.81	1.1(16)±1.00
Turbidity	NTU[e]	3.3(51)±2.99	5.4(9)±4.82	3.2(26)±2.68	2.4(16)±1.38
pH	–	6.5(56)±0.17	6.5(9)±0.10	6.5(29)±0.18	6.4(18)±0.18
Redox potential	mV	11.6(57)±6.65	14.5(8)±3.78	13.5(30)±5.92	7.4(19)±6.85
Conductivity	µS/cm	532.8(60)±214.52	315.3(7)±62.82	394.5(29)±99.60	763.3(24)±104.97
Dissolved oxygen	mg/l	2.0(55)±0.87	1.9(2)±1.06	2.0(28)±0.93	2.1(25)±0.83
Control A (outflow)					
Total petroleum hydrocarbons	µg/l	3081(6)±2269.2	345(1)±nm	1270(1)±nm	4218(4)±1808.1
Chemical oxygen demand	mg/l	96.1(22)±44.54	11.5(2)±6.58	94.3(9)±40.19	113.0(11)±33.50
Biochemical oxygen demand	mg/l	13.4(39)±6.05	9.6(5)±7.13	12.9(20)±4.96	15.6(14)±6.66
Ammonia-nitrogen	mg/l	0.3(21)±0.50	1.0(2)±0.67	0.3(10)±0.61	0.1(9)±0.11
Nitrate-nitrogen	mg/l	0.2(22)±0.11	0.2(2)±0.03	0.2(10)±0.05	0.2(10)±0.16
Ortho-phosphate-phosphorus	mg/l	2.0(23)±0.37	2.1(2)±0.04	2.0(10)±0.53	1.9(11)±0.19
Suspended solids	mg/l	10.9(41)±8.17	2.8(6)±2.79	13.4(21)±9.59	10.5(14)±4.55
Turbidity	NTU[e]	7.6(38)±3.51	3.0(6)±0.90	7.8(18)±3.71	9.3(14)±1.90
pH	–	6.6(40)±0.16	6.6(6)±0.08	6.7(20)±0.16	6.6(14)±0.17
Redox potential	mV	2.9(43)±3.33	1.6(5)±4.16	2.4(22)±3.55	4.0(16)±2.56
Conductivity	µS/cm	205.3(44)±72.73	135.3(5)±23.88	177.7(21)±17.81	256.8(18)±87.63
Dissolved oxygen	mg/l	1.5(40)±0.78	1.2(2)±0.92	1.5(21)±0.77	1.6(17)±0.83
Control B (outflow)					
Total petroleum hydrocarbons	µg/l	<10	nm	<10	<10
Chemical oxygen demand	mg/l	9.9(18)±7.28	8.3(2)±4.14	12.2(9)±9.13	7.4()±
Biochemical oxygen demand	mg/l	8.4(40)±5.53	11.0(6)±7.77	9.4(20)±4.77	5.9(14)±4.87
Ammonia-nitrogen	mg/l	0.5(21)±1.59	6.9(2)±0.12	0.3(10)±0.72	0.0(9)±0.03
Nitrate-nitrogen	mg/l	0.1(19)±0.05	0.2(2)±0.12	0.1(10)±0.02	0.0(7)±0.03
Ortho-phosphate-phosphorus	mg/l	1.9(20)±0.32	2.2(2)±0.15	2.0(10)±0.20	1.6(8)±0.34
Suspended solids	mg/l	1.7(40)±2.19	1.5(6)±1.22	2.0(21)±2.70	1.3(13)±1.58
Turbidity	NTU[e]	2.6(38)±0.79	2.4(6)±0.16	2.5(18)±0.88	2.8(14)±0.83
pH	–	6.5(39)±0.18	6.5(6)±0.13	6.5(20)±0.17	6.6(13)±0.20

Table 4.23 (cont.)

Redox potential	mV	10.8(42)±4.18	13.4(5)±3.44	12.8(22)±3.30	7.1(15)±2.76
Conductivity	µS/cm	176.7(43)±42.64	149.6(5)±26.40	175.9(21)±21.34	185.6(17)±60.96
Dissolved oxygen	mg/l	2.9(40)±1.38	1.4(2)±1.13	2.2(21)±0.94	3.9(17)±1.19
Deionized water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	0.0(4) ±0	0.0(1)	0.0(1)	0.0(2)±0
Biochemical oxygen demand	mg/l	0.4(16)±0.63	0.0(4)±0	0.6(8)±0.74	0.5(4)±0.44
Ammonia-nitrogen	mg/l	0.0(2)±0	0.0(1)±nm	0.0(1)±nm	nm
Nitrate-nitrogen	mg/l	0.0(2)±0	0.0(1)±nm	0.0(1)±nm	nm
Ortho-phosphate-phosphorus	mg/l	0.0(2)±0	0.0(1)±nm	nm	0.0(1)±nm
Suspended solids	mg/l	0.5(25)±0.59	0.3(6)±0.82	0.7(12)±0.43	0.4(7)±0.76
Turbidity	NTU[e]	0.3(25)±0.40	0.2(5)±0.34	0.3(13)±0.37	0.5(7)±0.51
pH	–	6.0(24)±0.76	6.1(7)±1.08	5.8(10)±0.69	6.3(7)±0.29
Redox potential	mV	27.3(11)±34.08	57.2(3)±54.48	17.7(6)±19.69	11.5(2)±2.12
Conductivity	µS/cm	4.1(11)±2.44	3.5(4)±3.17	6.1(4)±0.06	2.2(3)±0.97
Dissolved oxygen	mg/l	8.0(9)±0.94	8.3(1)±nm	7.9(4)±0.85	7.9(4)±0.85
Tap water (100%)					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	2.3(6)±0.08	2.3(2)±0.07	2.3(2)±0.14	2.3(2)±0.07
Biochemical oxygen demand	mg/l	2.6(18)±1.37	5.0(2)±1.41	2.3(10)±0.82	2.5(6)±1.52
Ammonia-nitrogen	mg/l	0.2(8)±0.06	0.1(2)±0.05	0.2(2)±0.07	0.2(4)±0.09
Nitrate-nitrogen	mg/l	0.2(9)±0.10	0.4(2)±0.08	0.1(3)±nm	0.2(4)±0.17
Ortho-phosphate-phosphorus	mg/l	0.6(7)±0.20	0.5(3)±0.05	nm	0.7(4)±0.30
Suspended solids	mg/l	0.9(25)±1.09	0.7(6)±1.63	0.8(12)±0.97	1.3(7)±0.76
Turbidity	NTU[e]	1.3(25)±1.09	1.1(5)±0.79	2.0(13)±0.73	0.8(7)±0.42
pH	–	6.3(24)±0.70	5.9(7)±0.96	6.4(10)±0.66	6.4(7)±0.32
Redox potential	mV	23.9(15)±12.12	27.7(3)±6.47	23.9(10)±14.30	18.0(2)±4.24
Conductivity	µS/cm	77.2(11)±9.59	75.2(4)±10.61	85.0(4)±5.77	69.4(3)±4.61
Dissolved oxygen	mg/l	8.9(9)±0.53	9.4(1)±nm	9.1(4)±0.27	8.5(4)±0.58
Tap water with fertilizer (0.7 ml/l)					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	2.5(6)±0.10	2.5(2)±0	2.6(2)±0.07	2.4(2)±0.14

Table 4.23 (cont.)

Biochemical oxygen demand	mg/l	15.1(17)±11.67	8.7(3)±1.53	17.6(10)±13.36	13.5(4)±11.12
Ammonia-nitrogen	mg/l	2.4(7)±0.07	2.4(2)±0.07	2.5(2)±0.07	2.4(3)±0.06
Nitrate-nitrogen	mg/l	5.6(3)±0.13	5.6(1)±nm	5.6(2)±0.18	nm
Ortho-phosphate-phosphorus	mg/l	4.4(2)±0.07	4.3(1)±nm	nm	4.4(1)±nm
Suspended solids	mg/l	2.2(25)±1.29	1.3(6)±0.82	2.3(12)±1.56	2.7(7)±0.76
Turbidity	NTU[e]	3.1(25)±1.53	3.0(5)±0.56	3.7(13)±1.54	2.3(7)±1.73
pH	–	6.3(23)±0.25	6.2(6)±0.20	6.2(10)±0.22	6.5(7)±0.25
Redox potential	mV	27.6(9)±18.79	56.1(1)±nm	24.5(6)±18.93	22.5(2)±10.61
Conductivity	µS/cm	185.9(7)±67.88	211.0(2)±57.98	175.0(4)±86.60	179.0(1)±nm
Dissolved oxygen	mg/l	7.2(9)±0.99	8.1(1)±nm	7.3(4)±0.98	7.0(4)±1.15
Wastewater (20%); tap water (80%)					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	48.4(22)±19.98	41.2(2)±26.59	48.6(12)±14.62	49.91(12)±27.25
Biochemical oxygen demand	mg/l	28.4(42)±20.36	12.7(3)±11.02	17.5(22)±14.25	45.4(17)±16.26
Ammonia-nitrogen	mg/l	8.0(23)±2.96	4.1(2)±4.92	7.5(12)±2.78	9.7(9)±1.77
Nitrate-nitrogen	mg/l	0.2(23)±0.19	0.5(2)±0.49	0.3(12)±0.16	0.1(9)±0.07
Ortho-phosphate-phosphorus	mg/l	3.0(24)±0.87	2.6(2)±0.14	3.4(12)±1.11	2.68(10)±0.25
Suspended solids	mg/l	49.0(25)±23.44	26.0(6)±26.89	55.1(12)±12.30	58.1(7)±24.84
Turbidity	NTU[e]	18.5(25)±11.83	9.1(5)±3.55	17.7(13)±12.96	26.8(7)±7.68
pH	–	7.1(23)±0.28	7.0(6)±0.22	7.1(10)±0.22	7.1(7)±0.43
Redox potential	mV	-4.7(39)±10.41	-7.2(7)±3.35	-3.8(22)±11.08	-4.8(10)±12.53
Conductivity	µS/cm	182.1(47)±105.19	84.4(7)±51.65	139.6(24)±15.43	288.6(16)±114.26
Dissolved oxygen	mg/l	8.2(41)±2.63	3.8(2)±3.18	7.3(23)±2.49	10.0(16)±1.03
Wastewater (100%)					
Total petroleum hydrocarbons	µg/l	271(2)±318.20	nm	496(1)	46(1)
Chemical oxygen demand	mg/l	257.0(22)±85.56	206.0(2)±132.94	249.7(13)±74.04	285.2(7)±99.02
Biochemical oxygen demand	mg/l	124.9(43)±84.58	48.0(5)±32.71	84.3(21)±60.05	197.7(17)±66.10
Ammonia-nitrogen	mg/l	40.2(23)±14.80	20.5(2)±24.61	37.3(12)±13.88	48.3(9)±8.84
Nitrate-nitrogen	mg/l	1.1(23)±0.93	2.4(2)±2.43	1.2(12)±0.80	0.6(9)±0.16
Ortho-phosphate-phosphorus	mg/l	13.4(26)±5.54	12.1(2)±1.98	14.1(13)±7.85	12.8(11)±0.93
Suspended solids	mg/l	146.2(48)±76.17	145.7(6)±74.06	136.0(26)±82.68	162.8(16)±66.98
Turbidity	NTU[e]	70.7(43)±47.52	63.3(6)±45.59	81.0(21)±60.12	59.9(16)±22.76

Table 4.23 (cont.)

pH	–	7.7(44)±0.48	7.4(6)±0.22	7.6(22)±0.55	8.0(16)±0.21
Redox potential	mV	-42.4(43)±18.39	-36.0(7)±16.75	-38.3(21)±19.48	-51.1(15)±15.05
Conductivity	µS/cm	965.4(52)±503.52	448.6(6)±110.66	689.9(28)±79.81	1566.7(18)±380.48
Dissolved oxygen	mg/l	7.4(43)±2.81	3.5(2)±3.61	6.5(23)±2.99	9.0(18)±1.22
River water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	6.3(21)±8.56	2.3(2)±12.94	6.3(12)±7.04	6.3(7)±99.02
Biochemical oxygen demand	mg/l	5.6(43)± 4.58	2.4(5)±32.71	5.2(21)±6.05	6.0(17)±66.10
Ammonia-nitrogen	mg/l	8.1(23)± 4.80	1.1(2)±24.61	8.1(12)±13.88	8.1(9)±8.84
Nitrate-nitrogen	mg/l	20.1(25)±0.93	3.4(4)±3.43	20.1(12)±0.80	20.1(9)±0.16
Ortho-phosphate-phosphorus	mg/l	5.6(26)±5.24	1.5(2)±1.98	5.6(13)±5.85	5.6(11)±0.93
Suspended solids	mg/l	3.7(48)±7.17	0.9(6)±64.06	3.3(26)±8.68	4.1(16)±6.98
Turbidity	NTU[e]	2.9(43)±4.52	2.1(6)±45.59	2.8(21)±3.12	2.9(16)± 2.76
pH	–	7.3(45)±0.58	6.9(7)±0.22	7.3(22)±0.55	7.2(16)±0.21
Redox potential	mV	130.4(43)±18.39	32.4(7)±16.75	120.3(21)±15.48	140.5(15)±15.05
Conductivity	µS/cm	200.4(52)±53.52	85.0(6)±10.66	201.3(28)±6.81	199.6(18)±30.48
Dissolved oxygen	mg/l	8.2(43)±2.01	9.9(2)±6.61	8.5(23)±1.97	7.9(18)±1.20
Rain water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	15.9(7)±0.48	2.3(3)±0.67	15.9(2)±3.14	15.9(2)±0.97
Biochemical oxygen demand	mg/l	9.9(18)±1.37	2.4(2)±1.41	10.0(10)±0.82	9.8(6)±1.52
Ammonia-nitrogen	mg/l	0.0(8)±0.36	1.1(2)±0.55	0.0(2)±0.07	0.0(4)±0.09
Nitrate-nitrogen	mg/l	0.7(9)±0.10	3.4(2)±0.08	0.7(3)±2.3	0.7(4)±0.57
Ortho-phosphate-phosphorus	mg/l	1.7(8)±0.40	1.5(5)±0.05	1.7(1)±nm	1.7(4)±0.30
Suspended solids	mg/l	5.0(25)±1.09	0.9(6)±1.63	3.4(12)±0.87	6.6(7)±0.26
Turbidity	NTU[e]	4.8(25)±1.09	2.1(5)±0.79	4.0(13)±0.73	5.6(7)±0.42
pH	–	6.6(24)±0.70	6.9(7)±0.36	6.2(10)±0.66	6.9(7)±0.32
Redox potential	mV	98.6(16)±12.15	32.4(4)±6.47	98.4(10)±14.30	99.4(2)±4.24
Conductivity	µS/cm	74.6(11)±9.59	85.0(4)±20.61	61.6(4)±5.77	87.6(3)±4.61
Dissolved oxygen	mg/l	7.2(12)±0.53	9.9(3)±0.55	8.1(4)±0.27	6.2(4)±0.58
Gully pot water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm

Table 4.23 (cont.)

Chemical oxygen demand	mg/l	17.7(18)±7.28	2.3(2)±4.14	17.7(9)±9.13	17.7(5)±4.88
Biochemical oxygen demand	mg/l	64.8(40)±5.53	2.4(6)±7.77	72.5(20)±4.77	57.0(14)±4.87
Ammonia-nitrogen	mg/l	11.1(21)±1.59	10.1(2)±0.12	11.1(10)±0.72	11.1(9)±0.03
Nitrate-nitrogen	mg/l	17.8(19)±0.05	3.4(2)±0.12	17.8(10)±0.02	17.8(7)±0.03
Ortho-phosphate-phosphorus	mg/l	14.2(20)±0.32	1.5(2)±0.15	14.2(10)±0.20	14.2(8)±0.34
Suspended solids	mg/l	106.3(40)±2.19	0.9(6)±1.22	112.4(21)±2.70	100.2(13)±1.58
Turbidity	NTU[e]	88.1(38)±0.79	2.1(6)±0.16	97.6(18)±0.88	78.6(14)±0.83
pH	–	7.2(39)±0.18	6.9(6)±0.13	6.9(20)±0.17	7.4(13)±0.20
Redox potential	mV	41.5(42)±4.18	32.4(5)±3.44	94.0(22)±3.30	-11.8(15)±2.76
Conductivity	µS/cm	897.9(43)±42.64	85.0(5)±26.40	1008(21)±21.34	787.8(17)±60.96
Dissolved oxygen	mg/l	5.9(40)±1.38	9.9(2)±1.13	8.1(21)±0.94	3.7(17)±1.19
Real grey water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	301.0 (6)±0.88	2.3(2)±0.77	301.0(2)±0.14	301.0(2)±0.07
Biochemical oxygen demand	mg/l	64.3(18)±1.17	2.4(2)±1.47	63.0(10)±0.82	65.6(6)±1.52
Ammonia-nitrogen	mg/l	2.3(8)±0.06	1.1(2)±0.95	2.3(2)±0.07	2.3(4)±0.09
Nitrate-nitrogen	mg/l	1.2(9)±0.19	3.4(2)±0.58	1.2(3)±5.43	1.2(4)±0.67
Ortho-phosphate-phosphorus	mg/l	12.0(7)±0.20	1.5(3)±0.95	12.0(4)±0.97	12.0(4)±0.80
Suspended solids	mg/l	449.9(25)±1.09	0.9(6)±1.63	329.1(12)±0.97	570.6(7)±0.96
Turbidity	NTU[e]	249.5(25)±1.09	2.1(5)±0.79	239.7(13)±0.73	259.2(7)±0.49
pH	–	7.2(24)±2.70	6.9(7)±0.96	7.3(10)±0.66	7.0(7)±0.32
Redox potential	mV	129.4(15)±11.12	32.4(3)±6.47	156.0(10)±14.30	102.8(2)±4.24
Conductivity	µS/cm	509.4(11)±9.59	85.0(4)±10.61	531.1(4)±5.77	487.6(3)±4.61
Dissolved oxygen	mg/l	6.5(9)±0.63	9.9(1)±nm	7.5(4)±0.27	5.4(4)±0.58
Artificial grey water					
Total petroleum hydrocarbons	µg/l	nm	nm	nm	nm
Chemical oxygen demand	mg/l	87.5 (18)±5.24	2.3(2)±1.33	87.5(9)±8.41	87.5(7)±9.59
Biochemical oxygen demand	mg/l	29.8(40)±8.50	2.4(4)±7.93	14.0(20)±9.17	15.8(16)±9.38
Ammonia-nitrogen	mg/l	1.3(21)±2.40	1.1(2)±1.21	1.3(10)±6.59	1.3(9)±4.64
Nitrate-nitrogen	mg/l	0.9(19)±1.80	3.4(2)±2.20	0.9(10)±2.33	0.9(8)±8.27
Ortho-phosphate-phosphorus	mg/l	9.0(20)±1.21	1.5(2)±6.15	9.0(10)±1.36	9.0(8)±6.77
Suspended solids	mg/l	54.0(41)±7.51	0.9 (6)±2.25	52.7(21)±4.72	55.4(14)±7.74

Table 4.23 (cont.)

Turbidity	NTU[e]	24.8(38)±6.27	2.1(6)±8.70	23.3(18)±1.48	26.2(14)±8.37
pH	–	8.0(40)±0.18	6.96(6)±0.12	7.9(20)±0.11	8.0(14)±0.20
Redox potential	mV	-36.5(43)±6.16	32.4(5)±3.36	-11.0(22)±5.01	-62.8(16)±7.78
Conductivity	µS/cm	1447.5(44)±171.61	85.0(5)±46.71	1440.0(21)±24.84	1455.3(18)±13.18
Dissolved oxygen	mg/l	6.8(40)±0.95	9.9(2)±0.49	6.91(21)±0.84	6.6(17)±0.87

[a]Overall period: 08/04/14 to 24/12/14; [b]Replanting period before fruiting: 08/04/14 to 11/05/14; [c]Replanting period after fruiting: 12/05/14 to 25/09/14; [d]Replanting period after fruiting (second diesel spill on 26/09/14): 26/09/14 to 24/12/14; [e]nephelometric turbidity unit; and Note: nm, not measured.

4.7.1.2 Water quality of wetland filters with diesel contamination (set 1)

Planting Phase 3 showed a notable increase in COD concentration values compared with those in both Phases 1 and 2 (Figure 4.48a). This could mainly be attributed to the effect of the application of the second dosage of diesel fuel. The COD can be used as an indication for organic pollutants that may induce lipid peroxidation and toxicity to plants. Hydrocarbon compounds such as diesel are generally linked to high COD values (Scholz, 2010, 2015). The lowest value of COD for Filter 1 can be assigned to the presence of a substrate of larger aggregate diameter (20 mm), which has been shown to enhance oxygen supply, and better wastewater distribution provides an opportunity to develop a strong layer of biofilm within the voids between aggregates (Sani et al., 2013a). This layer improved with time, as the system started to mature (microbial acclimatization). An active biofilm increased the biodegradation process during the three periods (Harvey et al., 2002).

Concerning NH_4-N concentrations (Figure 4.48b), a slight upward trend was observed for Filter 1, whereas Control A showed a modest downward trend. The overall mean concentrations of NH_4-N from low to high followed this order: Filter 3<Filter 1<Filter 5. The corresponding values were 3.1 mg/l, 4.5 mg/l and 10.1 mg/l, respectively. The overall mean concentration of Control A was estimated at 0.3 mg/l. A significant difference ($p<0.05$) was noted between NH_4-N of Filter 3 (low inflow load) that of Filter 5 (high inflow load). Significant differences between filters are summarized in Table 4.24. The NH_4-N concentrations for sample water of Filter 5 exceeded the corresponding threshold of 5 mg/l (Food and Agricultural Organization (FAO, 2003)). This can be attributed to the effect of a high loading rate (concentrated inflow without dilution) as discussed, previously in section 4.5 (Al-Isawi et al., 2015b).

As far as NO_3-N (Figure 4.48c) is concerned, the concentrations for Filter 3 compared to those of Filter 5 were significantly ($p<0.05$) different from each other. This indicates the impact of the inflow loading rate of wetlands systems on outflow water NO_3-N concentrations (Table 3.2) as indicated previously (Vymazal, 2010; Gajewska et al., 2015; Scholz, 2015). The overall mean concentrations followed this order: Filter 1 (0.3 mg/l)=Filter 3 (0.3 mg/l)<Filter 5 (0.7 mg/l). The NO_3-N mean value for Control A was estimated at 0.2 mg/l. The results reveal that NO_3-N concentrations for all examined wetland outflow waters are less than the maximum threshold, which is 30 mg/l (FAO, 2003). According to Ayers and Westcot (1985), crops are relatively unaffected until nitrogen in the irrigation water exceeds 30 mg/1. The presence of hydrocarbon in the wetland filters results in a reduction of the nitrate concentration in the outflow water (Liu et al., 2011; Al-Baldawi et al., 2015b). In general, biodegradation of diesel spills in Filters 1, 3 and 5 led to a reduction of the availability of nutrients through these wetland filters. The addition of carbon (via diesel) stimulated the removal of nitrogen, which is needed by micro-organisms to decompose hydrocarbons (Liu et al., 2011; Al-Isawi et al., 2015b).

Phosphorus is essential and often limited in freshwater; it plays a significant role in many ecosystems due to its impact on eutrophication (Withers & Haygarth, 2007; Scholz, 2010). Concerning PO_4-P concentrations (Figure 4.48d), despite relatively high fluctuations, in particular during Phase 3, an increase in PO_4-P values can clearly be seen over time for Filters 1, 3 and 5, whereas Control A indicates a slight downward decrease. The filters followed the following order for PO_4-P (Figure 4.48d) from low to high: Filter 3<Filter 1<Filter 5. The corresponding overall mean concentrations were 5.1 mg/l, 6.0 mg/l, and 7.2 mg/l, respectively. The overall mean value of Control A was 2.0 mg/l. A threshold value of 2 mg/l has been proposed by (FAO, 2003), and is considered as a limit

for the concentration of ortho-phosphate-phosphorus. In general, vertical-flow constructed wetlands are poor in removing PO_4-P compounds (Vymazal, 2007a; Scholz, 2010; Vymazal, 2010; Scholz, 2015), especially when the system reaches maturation (Scholz, 2015). Findings revealed that Filters 1, 3 and 5 were not able to efficiently remove PO_4-P. The positive trend refers to the accumulation of PO_4-P over time (Scholz, 2015). The statistical analysis indicated that aggregate diameter as well as contact and rest times did not show any significant ($p>0.05$) differences between outflow water for these wetland filters.

Regarding pH values (Figure 4.48e), the average values from low to high followed this order: Filter 1 (6.2)<Filter 3 (6.4)<Filter 5 (6.5). A slightly higher pH value (6.6) was obtained for Control A. For irrigating purposes, pH values for these types of irrigation water were within the normal range between 6.0 and 8.5 (FAO, 2008; Scholz, 2010).

Regarding the redox potential values (Figure 4.48f), the overall mean redox potential values followed this order: Filter 5 (9.9 mV)<Filter 3 (16.3 mV)<Filter 1 (26.2 mV). Control A had a redox potential value of 2.9 mV. The presence of shocked diesel initially impacted on the rhizosphere and caused a slight decrease in the redox potential, indicating that the environment was becoming more anaerobic with the increase in diesel (Lin & Mendelssohn, 2009; Liu et al., 2011). However, improvement in petroleum hydrocarbon removal over time and due to the tidal-flow mode that was applied for wetland filters operation, an increase was noticed in the redox environment within the wetlands bed (Al-Isawi et al., 2015a).

Electrical conductivity is the most important measure of salinity, which poses a great hazard to crops (FAO, 2012) and determines the suitability of water for irrigation. Conductivity plays an important role in the suitability of water for irrigation. High levels

of electrical conductivity in water create saline soil. According to SEPA State Environmental Protection Administration (2005), salts negatively impact on the growth of plants, and the corresponding soil structure and permeability, indirectly affecting plant development as well. However, the electrical conductivity values of all outflow waters were below the maximum threshold of 3000 µS/cm (FAO, 2003). A notable increase was recorded for the electrical conductivity values of Filter 5, particularly during Phase 3. Filters 1 and 3 showed a moderate electric conductivity increase over time (Figure 4.48g). The overall mean values from low to high were in this order: Filter 1 (503 µS/cm)<Filter 3 (593 µS/cm)<Filter 5 (918.3 µS/cm). For Control A, the value was 205 µS/cm.

Despite the fluctuations and changes observed for all water quality parameters (except for pH) over the three stages, Phase 3 showed the most notable changes with significant ($p<0.05$) increases for most water quality parameters: COD, NO_3-N, NH_4-N and total petroleum hydrocarbon (TPH). The corresponding p-values were 0.000, 0.029, 0.032 and 0.037 in this order. This could mainly be attributed to the impact of the second high dosage of diesel fuel spill (Lin & Mendelssohn, 2009; Liu et al., 2011). For more details about mean, standard deviation and sample number values of water quality parameters over the three phases, see Table 4.23. The statistical analysis to identify potentially significant differences between filters is summarized in Table 4.24.

Table 4.24: Overview of the statistically significant differences (indicated by p-value (h)) between outflow water quality variables of different wetland filters using the non-parametric Mann-Whitney U-test for data collected between 8 April 2014 and 24 December 2014.

Parameter	COD[a]	NH_4-N[b]	NO_3-N[c]	PO_4-P[d]	Redox[e]	EC[f]
Unit	mg/l	mg/l	mg/l	mg/l	mV	µS/cm
Potential differences between the diesel-contaminated wetland filters (set 1)						
Filter 1 and 3[g]	0.331 (0)	0.131 (0)	0.970 (0)	0.163 (0)	0.000 (1)	0.004 (1)
Filter 3 and 5[h]	0.490 (0)	0.000 (1)	0.002 (1)	0.044 (1)	0.000 (1)	0.010 (1)
Potential differences between wetland filters without diesel contamination (set 2)						
Filter 2 and 4[g]	0.650 (0)	0.006 (1)	0.012 (1)	0.547 (0)	0.000 (1)	0.491 (0)
Filter 4 and 6[h]	0.293 (0)	0.001 (1)	0.000 (1)	0.102 (0)	0.000 (1)	0.000 (1)
Filter 4 and 7[i]	0.226 (0)	0.488 (0)	0.000 (1)	0.495 (0)	0.000 (1)	0.556 (0)
Filter 7 and 8[j]	0.029 (1)	0.144 (0)	0.757 (0)	0.296 (0)	0.000 (1)	0.710 (0)

Note: *p*-value, probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true; *h*, response indicator (shown in brackets); if $h=1$, units are statistically significantly different (p-value<0.05) for the corresponding water quality parameter; if $h=0$, the difference is not statistically significantly different (p-value>0.05). [a]chemical oxygen demand; [b]ammonia-nitrogen; [c]nitrate-nitrogen; [d]ortho-phosphate-phosphorus; [e]redox potential; [f]electrical conductivity; [g]influence of aggregate diameter (Table 3.2); [h]influence of inflow COD load (Table 3.2); [i]influence of contact time (Table 3.2); and [j]influence of resting time (Table 3.2).

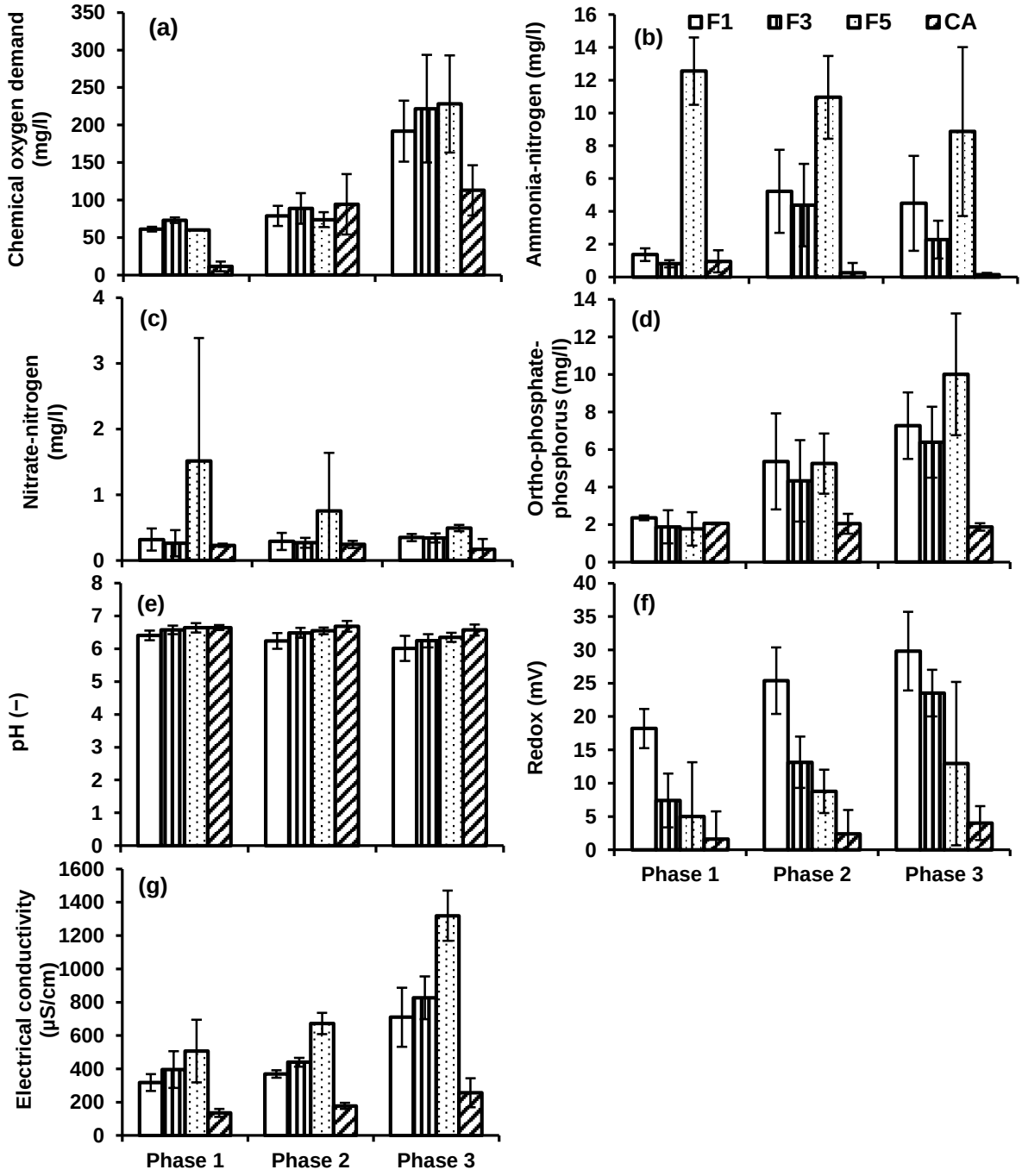


Figure 4.48: Mean and standard deviation of water quality parameters of irrigation water obtained from wetland filters contaminated with diesel: (a) chemical oxygen demand; (b) ammonia-nitrogen; (c) nitrate-nitrogen; (d) ortho-phosphate-phosphorus; (e) pH; (f) redox (potential); and (g) electrical conductivity. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; and CA, Control A (wetland filter receiving tap water).

4.7.1.3 Irrigation water from wetland filters without diesel contamination (set 2)

The findings revealed high differences in COD concentrations between the three phases (Figure 4.49a). This is due to the seasonal treatment changes of the wetland filters (Tang et al., 2009; Sani et al., 2013a). The overall mean concentrations from low to high were in this order: Filter 7<Filter 2<Filter 4<Filter 6<Filter 8. The corresponding values are 27.8 mg/l, 35.5 mg/l, 37.5 mg/l, 43.1 mg/l and 48.0 mg/l, respectively. Generally, the COD concentrations were remarkably low, if compared to the high inflow COD value (285.3 mg/l) for the wetland system, which can be a result of biofilm maturation within the wetland system as micro-organisms responsible for biodegradation acclimatize (Scholz, 2010; Liu et al., 2011; Sutton et al., 2013). Furthermore, the high COD values of Filter 8, if compared with those of the other filters, reflect the impact of low resting time on the treatment performance (Table 3.2). The importance of a long resting time is to aerate the filter substrate and subsequently enhance biodegradation (Scholz, 2010; Vymazal, 2010, 2011b; Scholz, 2015). The data analysis showed no significant ($p>0.05$) difference in COD values of Filter 2 if compared to those for Filter 4, indicating no effect of aggregate size on treatment performance (Table 3.2). However, there is a significant ($p<0.05$) difference between Filters 7 and 8, reflecting the impact of resting time. In comparison, the lowest COD value was recorded for Control B (no diesel contamination).

The NH_4-N concentration data are widely scattered for all three phases (Figure 4.49b). This can be explained by the seasonal variations in water quality treatment performance of the wetland system (Tang et al., 2009; Sani et al., 2013a; Scholz, 2015). The overall mean concentrations from low to high were obtained in this order: Filter 8<Filter 4<Filter 7<Filter 2<Filter 6. The corresponding NH_4-N concentrations were 1.4 mg/l, 2.8 mg/l, 4.2 mg/l, 5.2 mg/l and 9.2 mg/l, respectively. Control B had a value of 0.5 mg/l. Generally,

the outflow NH_4-N concentration values were low if compared to the inflow value of 40.2 mg/l. Table 4.23 highlights the high ability of the wetland system to treat NH_4-N. All NH_4-N values (except for Filter 6) were within the permissible value for crop irrigation of 5 mg/l (FAO, 2003).

With respect to NO_3-N concentrations (Figure 4.49c), a decline was observed for Filters 2 and 4 (high contact time), whereas Control B showed steady concentrations (originally the inflow water without nutrients) over the three examined phases. This could be attributed to a high contact time that resulted in the provision of more time for treatment processes to remove pollutants (Vymazal, 2011b; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b; Scholz, 2015). The average NO_3-N concentrations from low to high were obtained in this order: Filter 4<Filter 2<Filter 8<Filter 7<Filter 6. The corresponding concentrations were 0.5 mg/l, 1.0 mg/l, 2.9 mg/l, 3.3 mg/l, and 3.8 mg/l, respectively. The concentration of Control B was 0.1 mg/l. Statistically, there is a significant ($p<0.05$) difference of outflow NO_3-N values for Filters 4 and 7 highlighting the impact of low contact time on the NO_3-N treatment performance of the wetland system (Table 3.2). Moreover, a significant ($p<0.05$) difference between Filter 4 and Filter 6, in terms of outflow NO_3-N values, was also noted. This indicates the impact of inflow COD load (Table 3.2) on the treatment performance of NO_3-N within the wetland filters (Vymazal, 2010; Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). In general, all NO_3-N outflow values were very low (Figure 4.49c), being below the threshold of 30 mg/l (FAO, 2003).

With regard to PO_4-P (Figure 4.49d), all filters showed upward tendencies, whereas Control B was linked to a slight drop. The overall mean concentrations from low to high followed this order: Filter 4=Filter 8<Filter 2<Filter 7<Filter 6. The corresponding values were 3.6 mg/l, 3.6 mg/l, 3.8 mg/l, 4.2 mg/l, and 5.3 mg/l, respectively. Furthermore, the

concentration of Control B was 1.9 mg/l. The PO_4-P concentrations (except for Control B) were higher than the threshold limit (2 mg/l) for irrigation use (FOA, 2003). This is because of the difficulty in removing accumulated phosphorus particles by constructed wetlands (Vymazal, 2010; Scholz, 2015).

As for pH (Figure 4.49e), despite some fluctuations over the three periods, the majority of the data were around 6.5. The pH values were within the normal range of 6.0 to 8.5 (FAO, 2003). Concerning the redox potential values (Figure 4.49f), some negative values were measured over the three stages, in particular during Phase 3. Filters 2 and 4 remained unchanged while a decline was noted for the remaining filters.

A remarkable change was recorded for electrical conductivity (Figure 4.49g). Phase 3 indicated a significant ($p<0.05$) increase compared to Phases 1 and 2. Filter 6, which received a high loading rate compared to those of other filters, was linked to sharp trend reversals. Filters 2, 4, 7 and 8 had similar data trends, whereas Control B remained unchanged. However, the electrical conductivity for all wetland outflows complied with the threshold of 3000 μS/cm (FAO, 2003). For more details concerning mean, standard deviation and sample number values for all water quality parameters over the three phases, see Table 4.23. Moreover, significant differences between filters are summarized in Table 4.24.

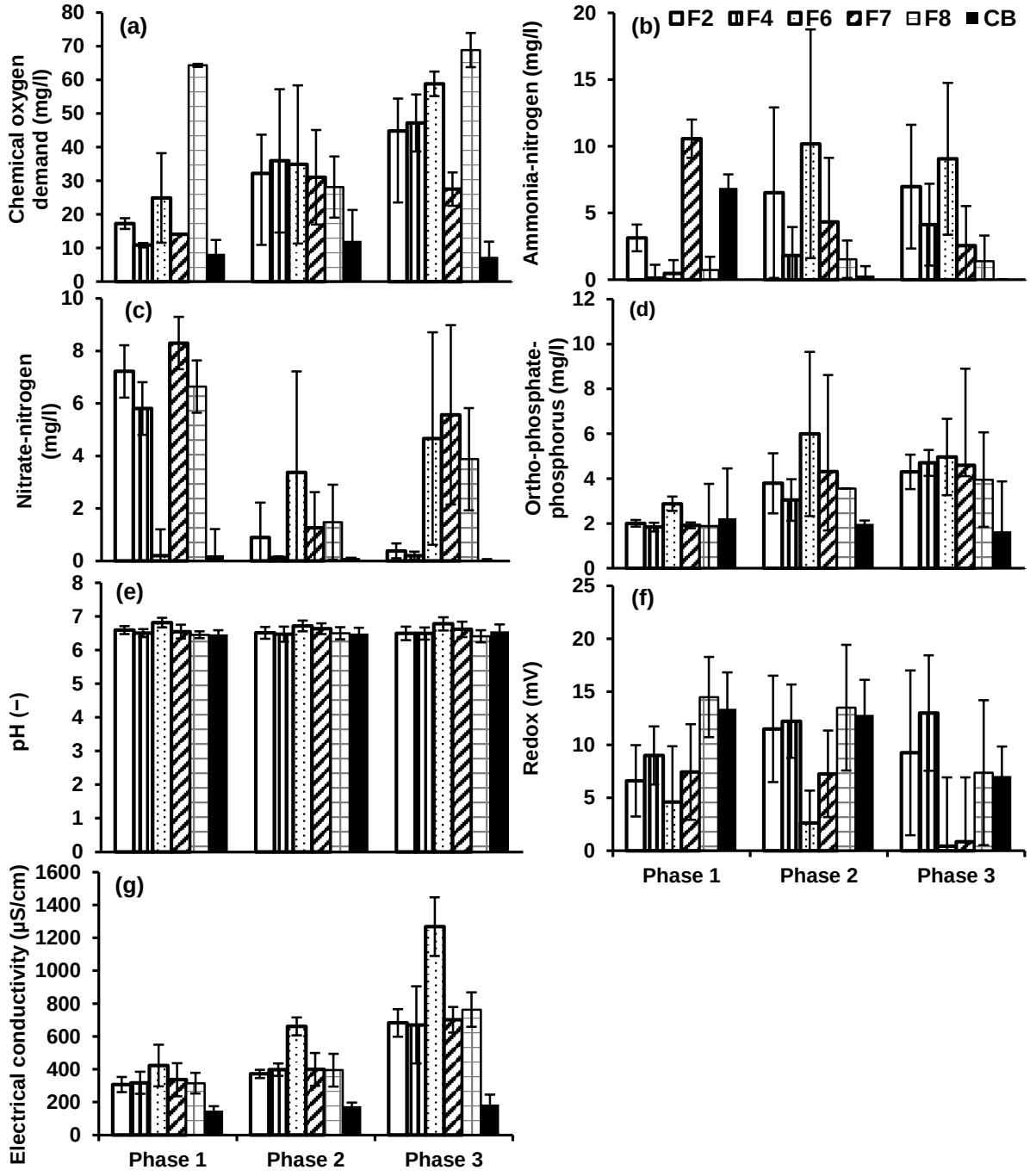


Figure 4.49: Mean and standard deviation of water quality parameters for irrigation water obtained from wetland filters without diesel contamination: (a) chemical oxygen demand; (b) ammonia-nitrogen; (c) nitrate-nitrogen; (d) ortho-phosphate-phosphorus; (e) pH; (f) redox (potential); and (g) electrical conductivity. Note: F2, wetland filter 2; F4, wetland filter 4; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; and CB, Control B (wetland filter receiving tap water).

4.7.1.4 Standard water types for comparison purposes (set 3 and set 4)

The water quality variability for preliminarily treated wastewater (raw wastewater) (Figure 4.50) was rather high, indicating the use of highly variable domestic wastewater (Chu et al., 2004; Scholz, 2010, 2015). The COD concentration values were the highest for real grey water (301.0 mg/l), then preliminary treated wastewater (257.0 mg/l) followed by wastewater diluted with tap water (48.4 mg/l). In comparison, the lowest values were measured for river water (6.3 mg/l). Pots receiving rain and gully pot water had similar COD concentrations (15.9 and 17.7 mg/l, respectively). Artificial grey water had lower COD values (87.5 mg/l) than real grey water (301.0 mg/l). Figure 4.50a presents the mean COD values for both set 3 and set 4 wastewater. Considering NH_4-N concentrations, the order of overall mean values from low to high was as follows: deionized water (0.0 mg/l)<tap water (0.2 mg/l)<tap water with fertilizer (2.4 mg/l)<wastewater diluted by tap water (8.0 mg/l)< river water (9.9 mg/l)<gully pot (11.1 mg/l)< wastewater (40.2 mg/l). Preliminarily treated wastewater and wastewater diluted with tap water, gully pot and river water (Figure 4.50b) showed elevated NH_4-N concentrations exceeding the threshold, according to FAO (2003), of 5 mg/l.

With regard to NO_3-N, low to high overall mean values followed this order: deionized water (0.0 mg/l)<both tap water and wastewater diluted by tap water (0.2 mg/l)<wastewater (1.1 mg/l)<tap water with fertilizer (5.6 mg/l). Here, also NO_3-N concentration values for all types of irrigation water were less than the permissible values of 30 mg/l (FAO, 2008). However, there are minor concerns for both river and gully pot water, because moderate restrictions exist for values between 5 and 30 mg/l. Nitrate is very soluble in water and can easily move through soil (Deffeyes, 2006; Fangli et al., 2011; Singh et al., 2012). The pH values showed the following order from low to high:

deionized water (6.0)<tap water (6.3)<tap water with fertilizer (6.3)<wastewater diluted by tap water (7.1)<wastewater (7.7). Overall mean values for both preliminary treated wastewater and diluted wastewater are shown in Figure 4.50e. Although the results indicated that all pH values were within the permissible range (6.5-8.5) according to FAO (2008), the preliminary treated wastewater values were slightly alkaline. Many micronutrients are less available when the water is alkaline according to the World Health Organization (World Health Organization WHO, 2006). The overall mean PO_4-P values were as follows: wastewater (13.4 mg/l), tap water spiked with fertilizer (4.4 mg/l), and wastewater diluted with tap water (3.0 mg/l). With the exception of rain water (1.7 mg/l), all types of wastewater exceeded the permissible value of 2 mg/l for irrigation (FAO, 2003). The overall mean electrical conductivity concentrations from low to high were in this order: deionized water (4.1 µS/cm)<tap water (77.21 µS/cm)<wastewater diluted by tap water (182.1 µS/cm)<tap water with fertilizer (185.9µS/cm)<wastewater (965.4 µS/cm). The conductivity was high for artificial grey water (1447.5 µS/cm), which considerably increased the salinity of the irrigated soil, subsequently affecting plant growth negatively (Al-Hamaiedeh & Bino, 2010). However, a low value of conductivity was observed for rain water (74.6 µS/cm). If the experiment had been continued over winter, it is likely that the conductivity of gully pot water would have been the highest due to salting of roads in the UK (Scholz, 2003). However, the experiment was stopped before road salting was necessary.

The electrical conductivity values for all types of irrigation waters were below the threshold of 3000 µS/cm (FAO, 2003). Overall mean values for both set 3 and set 4 are shown in Figure 4.50g. For more details regarding mean, standard deviation, and sample

number values of water quality parameters over the three phases, the reader may refer to Table 4.23.

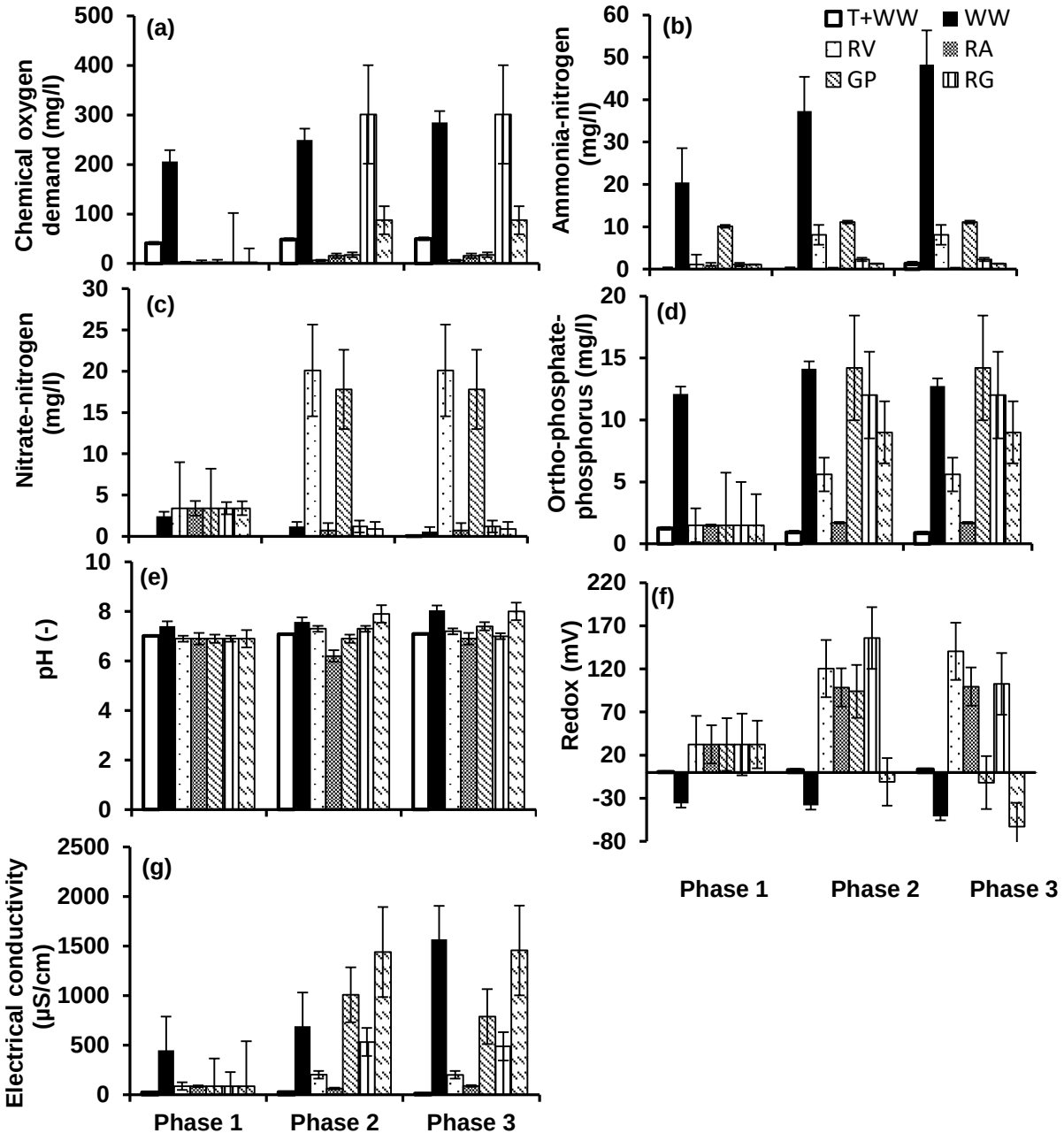


Figure 4.50: Mean and standard deviation of water quality parameters for various irrigation water types for comparison purposes. Note: WW+T, one part wastewater mixed with four parts tap water; WW, preliminarily treated wastewater; RV, river water; RA, rain water; GP, gully pot water; RG, real grey water; AG, artificial grey water.

4.7.1.5 Biochemical oxygen demand, suspended solids, turbidity and dissolved oxygen

The highest mean BOD value (Table 4.23) was for preliminarily treated wastewater (124.9 mg/l) followed by gully pot water and real grey water (64.8 and 64.3 mg/l, respectively), Filter 1 (51.5 mg/l), Filter 3 (37.4 mg/l), Filter 5 (38.7 mg/l) and diluted wastewater (28.4 mg/l). In contrast, the lowest five-day BOD was recorded for river water (5.6 mg/l). The higher BOD values observed for Filter 1 explain the effect of large aggregate diameter (Table 3.2). These findings are consistent with those reported by Al-Isawi et al. (2015b) and Al-Isawi et al. (2015a).

The highest mean value for SS (Table 4.23) was recorded for real grey water (449.9 mg/l) followed by that for preliminarily treated wastewater (146.2 mg/l), gully pot water (106.3 mg/l), preliminarily treated wastewater diluted with tap water (49.0 mg/l) and wetlands contaminated with diesel: Filter 1<Filter 3< Filter 5. In comparison, the lowest mean value was noted for river water (3.7 mg/l). The values of SS for filters contaminated with hydrocarbon are relatively high, if compared with those for filters without hydrocarbon contamination. Filters subjected to diesel spills showed elevated SS concentrations. Initially, dying above-ground *P. australis* plants and decaying biomass contributed to SS and turbidity as by-products of the biodegradation process (De Biase et al., 2011; Scholz, 2015). Thereafter, degraded diesel led to additional SS loads as discussed in section 4.4 and a related paper on modelling filter clogging by SS (Al-Isawi et al., 2015a).

Similar trends have been noted for turbidity (Table 4.23). High values of SS and turbidity enhance the development of hydrophobicity in the soils and, thereafter, impact on plant growth (Chu et al., 2004; Travis et al., 2010).

Dissolved oxygen is an important parameter for growing crops. High DO concentrations in irrigation water used for greenhouses can pay huge dividends for growers. Nutrient absorption occurs in the root zone of plants, and it cannot occur unless oxygen is present (Tchobanoglous et al., 2007). The benefits of dissolved oxygen go beyond mere root growth. Augmented oxygen can lessen root problems such as those associated with pythium and phytophera, and can decrease secondary infections (World Health Organization WHO, 2006). Higher DO values were generally observed for wetland filters without diesel contamination (2.0-2.9 mg/l) (Table 4.23) as compared with those for diesel-contaminated filters (1.5-1.7 mg/l). The reduction of the amount of available DO in the diesel-contaminated filters was linked with an improvement in the hydrocarbon removal efficiencies as micro-organisms, which are responsible for biodegradation, acclimatized (Sutton et al., 2013). Reduction in DO concentration values resulted from the microbial transformation and mineralization of organic matter and nutrients (in which micro-organisms play an important role) within wetland filters. However, Eke (2008) showed that DO of 1-2 mg/l is enough to effectively achieve hydrocarbon removal within wetland filters. For more details about mean, standard deviation and sample number values of water quality parameters for the three phases, readers may refer to Table 4.23.

4.7.1.6 Findings regarding trace elements

Figure 4.51 provides an overview of the ICP–OES (Inductively Coupled Plasma–Optical Emission Spectrometer) results for selected trace elements measured in the irrigation water. The sodium adsorption ratio (SAR) is defined as the tendency of water to lead to a replacement of calcium (Ca) and magnesium (Mg) ions adsorbed to the soil minerals with sodium (Na) ions (APHA, 2005). This indicator is applied to determine the sodium hazard of irrigation water. The findings of the analysed water samples showed that all

types of irrigation water have low SAR values between 0.2 and 3.2 me/l (Figure 4.51a), which presents no irrigation challenge as the standard range is between 0 and 15 me/l. The pre-treated wastewater is therefore suitable for irrigation of edible crops (FAO, 2012; Fredj et al., 2014; Tsado et al., 2014).

Considering the FAO (2003) threshold of 2 mg/l for potassium (FAO, 2003), the outflows from all wetland filters (except for Controls A and B), preliminary treated wastewater, diluted wastewater, river water and gully water were linked to relatively high potassium concentrations (Figure 4.51b). With regard to manganese (Figure 4.51d), the FAO (2003) threshold is 0.2 mg/l. The diesel-contaminated Filters 1, 3 and 5 showed high manganese concentrations (Figure 4.51d). Real grey water is, also high (0.26 mg/l) in manganese compared to other water types. Manganese represents an essential trace element for growing of crops (SEPA State Environmental Protection Administration, 2005; Almuktar et al., 2015b). However, high manganese concentrations are often toxic. Manganese phytotoxicity causes a reduction of biomass and photosynthesis, as well as biochemical challenges including oxidative stress (Millaleo et al., 2010). Regarding iron (Figure 4.51c), diesel-contaminated filters were generally relatively high in iron concentrations if compared with the corresponding uncontaminated filters, explaining the impact of diesel contamination on iron concentrations of the outflow waters. However, iron concentrations in all types of irrigated water (with the exception of preliminarily treated wastewater) were below the permissible limit of 5 mg/l (FAO, 2003; Norton-Brandão et al., 2013). Cadmium concentrations were only detected in both gully and artificial waters with values (0.13 and 0.16 mg/l, respectively) which considerably exceeded the threshold of 0.01 mg/l for irrigation water (FAO, 2003). This heavy metal is toxic to most organisms. Some crops take up cadmium from soil or contaminated irrigation water, and may enrich

it in their corresponding roots and shoots. Cadmium-induced effects include oxidative stress and geno-toxicity, as well as inhibition of the photosynthetic process and root metabolism (Andresen & Küpper, 2013). Moreover, findings indicate that artificial grey water is linked to a high copper concentration of 0.27 mg/l. This value is above the threshold of 0.2 mg/l set by (FAO, 2012). According to Panou-Filotheou et al. (2001), the impact of copper toxicity on plants may result in significant structural alterations, which result in reduced metabolic activity and subsequently negatively affect plant growth. No significant effect of metals in terms of plant growth, density of plants and the growth of chilli fruits was noted for the first eight months of the experiment. Dalahmeh (2013) suggested that soil and bark adsorb metals and other pollutants associated with wastewater to reduce pollutants to below their corresponding guideline values. It follows that the top soil layer and the bark (on top of the soil to reduce evaporation) used in the current experiment may be responsible for some of the reduction in pollutants.

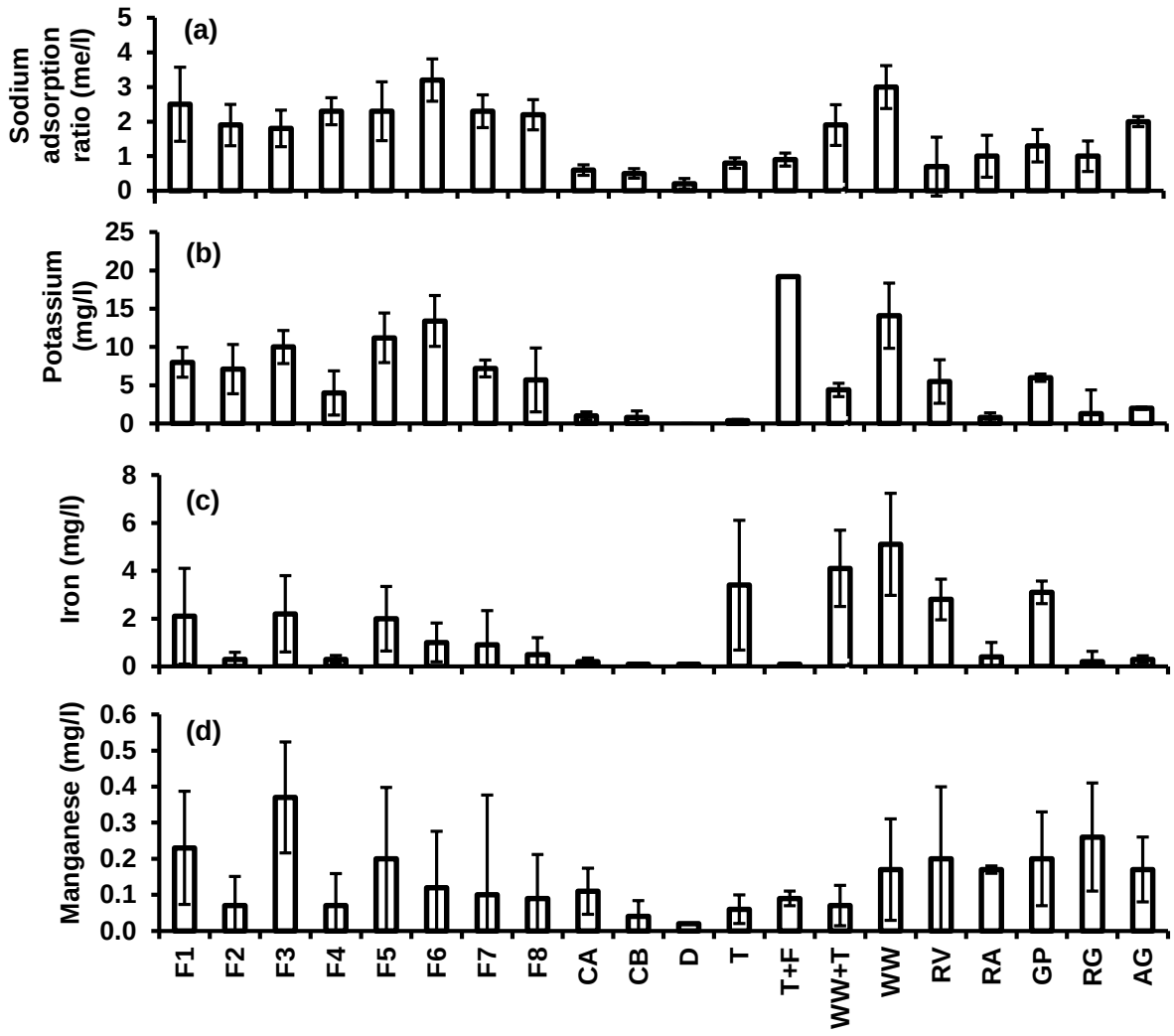


Figure 4.51: Overview of the Inductively Coupled Plasma (ICP) Optical Emission Spectrometer findings for selected elements of the inflow waters received by the chilli plants: (a) sodium adsorption ratio (sodium/((calcium+magnesium)/2)0.5); (b) potassium; (c) iron; and (d) manganese. Note: Elements not shown were not detected; sample number was 15 for data entries; F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA and CB, Controls A & B (wetland filters receiving tap water); D, deionized water; T, tap water; T+F, tap water mixed with fertilizer (0.7 ml/l); WW+T, one part wastewater mixed with four parts tap water; WW, preliminarily treated wastewater; RV, river water; RA, rain water; GP, gully pot water; RG, real grey water; and AG, artificial grey water.

Figure 4.52 shows the element concentrations detected in chilli fruits. Arsenic, boron, barium, bismuth, cadmium, cobalt, chromium, copper, lithium, nickel, lead, strontium and titanium were either below or close to the detection limits. Overall, potassium, calcium and magnesium concentrations in all analysed fruits were higher than those reported by Ciju (2013). Each 100 g of dried chillies contained 1870 mg potassium, 45 mg calcium and 88 mg magnesium. These minerals are important for humans to maintain bone structure, muscle and nerve function control, and blood stream. Concentrations for the other metals were below recommended thresholds: 50 mg/kg for zinc, 500 mg/kg for manganese and 425 mg/kg for iron (FAO/WHO, 2001).

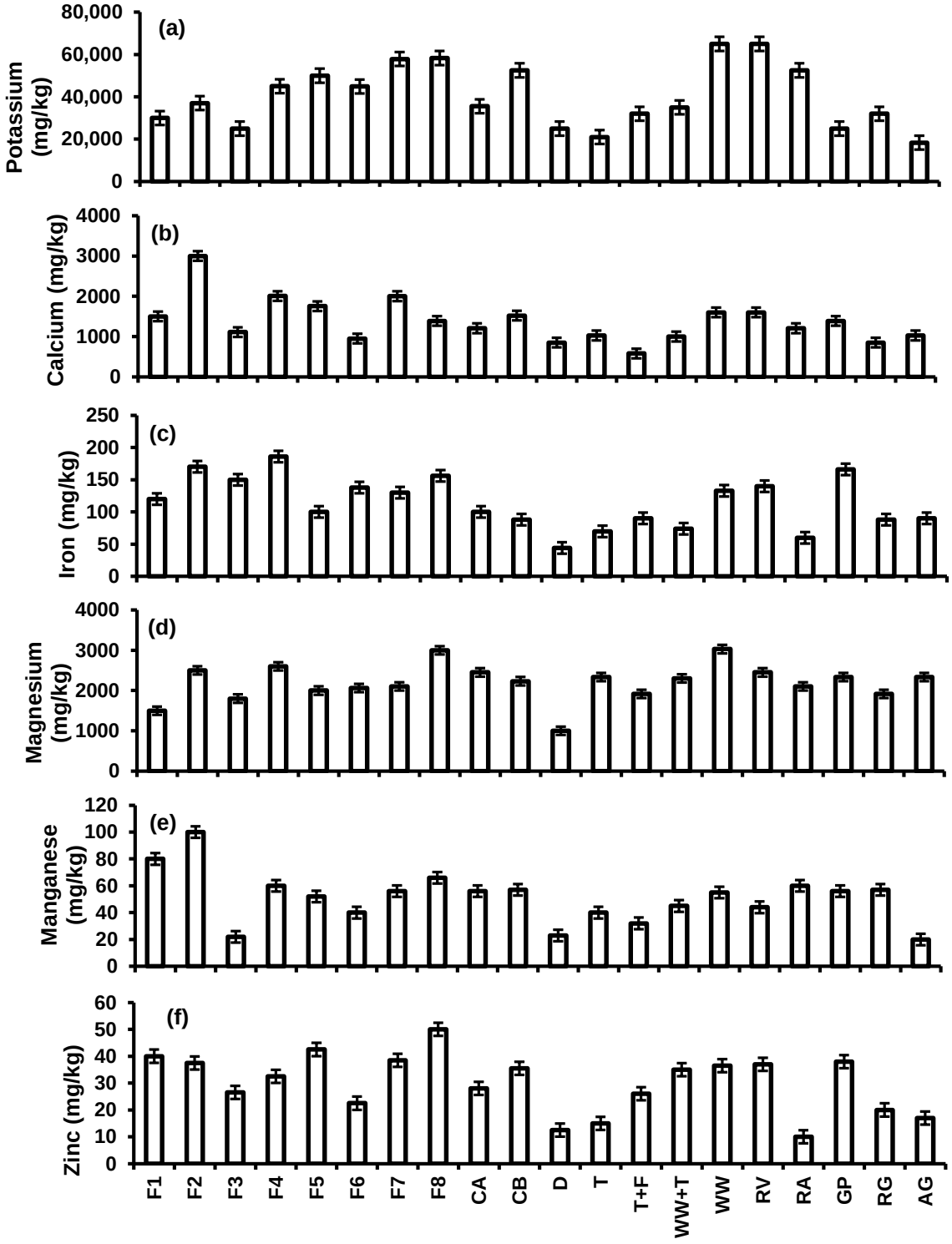


Figure 4.52: Inductively Coupled Plasma (ICP) Optical Emission Spectrometer analysis for selected elements in chilli fruits. Note: Twelve fruit samples per type of irrigation water were analysed. Arsenic, boron, barium, bismuth, cadmium, cobalt, chromium, copper, lithium, nickel, lead, strontium and titanium were either below or close to the detection limits. F1, wetland filter 1; F2, wetland filter 2; F3, wetland

filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA and CB, Controls A & B (wetland filters receiving tap water); D, deionized water; T, tap water; T+F, tap water mixed with fertilizer (0.7 ml/l); WW+T, one part wastewater mixed with four parts tap water; WW, preliminarily treated wastewater; RV, river water; RA, rain water; GP, gully pot water; RG, real grey water; and AG, artificial grey water.

4.7.1.7 Hydrocarbon analysis findings

Total petroleum hydrocarbon (TPH) was used to measure the overall hydrocarbon compounds in water samples. During the period of wetlands system operation, particularly the initial period after adding the second dosage of diesel, there was a continuous release of hydrocarbon concentration associated with the outflow water from wetland filters contaminated with diesel, and this is due to the huge dosage amount of hydrocarbon subjected to the system meaning the wetland filters could not purify the effluent completely. In order not to add extra pressure on water resources by throwing this effluent directly to them, it is better to find a way to recycle this water in irrigation purposes to minimize its impact on the environment.

Figure 4.53 provides an overview of TPH values for set 1 wetland filters (contaminated with diesel). Diesel background concentrations were low in the raw wastewater. In the three planting phases there was a notable reduction in TPH values compared with the high amount of the two inflow diesel spills (20 and 150 g/l). However, variations in TPH concentration values were recorded in the outflow water of Filters 1, 3 and 5 as well as Control A over the three planting phases. Regarding the first and second planting phases (Phase 1 and 2), which were during the first diesel spill (20 g/l) period, the TPH concentrations for the outflows from all wetlands except for Control A were in compliance, for example, with the Chinese standard for irrigation water quality (SEPA

State Environmental Protection Administration, 2005) of chillies, setting a maximum allowable value of 1 mg/l. The Chinese standards have been referenced here, considering that China is estimated to produce more than 50% of peppers globally. In the third planting phase (during the second diesel spill of 150 mg/l), all wetland filters showed relatively high TPH concentrations in their outflow waters. The TPH concentrations from high to low followed this order: Control A>Filter 5>Filter 1>Filter 3.

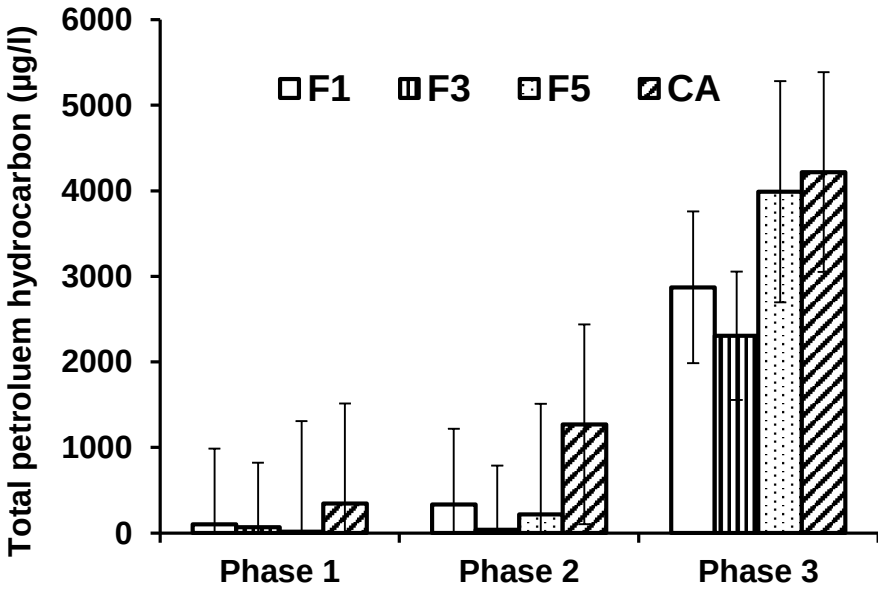


Figure 4.53: Variation of hydrocarbon concentrations for Filters 1, 3 and 5 as well as Control A for the three planting phases. Note: F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; and CA, Control A (wetland filter receiving tap water).

The hydrocarbon reduction in the outflow waters obtained from wetlands subjected to diesel spills is consistent with the increased availability of oxygen (due to tidal-flow mode) in the upper filter location and the subsequent decrease in concentration with depth. Biodegradation of diesel in Filters 1, 3 and 5 reduced nutrients. This has been noted for the NH_4-N and NO_3-N outflow concentrations (Figures 4.48b,c). However, as biodegradation of diesel progressed, it can be assumed that small amounts of remaining hydrocarbon actually enhanced the growth of some micro-organisms increasing the degradation rate (Liu et al., 2011).

The indirect aeration of aggregates to enhance biodegradation provides root exudates for microbial co-metabolization of oil (Lin & Mendelssohn, 2009). Co-metabolism by micro-organisms in the context of this study can be defined as the simultaneous degradation of two compounds, in which the degradation of root exudates depends on the presence of diesel.

Control A (lacking mature biomass as discussed in section 4.5) exhibited a high TPH concentration over time. Furthermore, *P. australis* (wetland plant) had a delayed and reduced growth rate during the second diesel spill. This is due to diesel toxicity to organisms and macrophytes (Vymazal, 2010; Liu et al., 2011; Vymazal, 2014; Scholz, 2015).

4.7.2 Growth comparisons of chilli plants

4.7.2.1 Boundary conditions and water consumption

During the three planting phases, the light intensity measurements inside the greenhouse were within the suggested allowable range from roughly 8600 lux to 17200 lux (Deli & Tiessen, 1969). Flower inhibition and/or abscission (here the natural detachment of flowers) as well as plant growth disorders can be caused if insufficient light intensity is applied during the growth of plants. These findings are in agreement with what was previously presented in the study (Almuktar et al., 2015b). Table 4.25 summarizes the environmental conditions for all planting periods. According to Nickels (2012), temperatures were within the preferred ranges for various chilli plant growth stages. However, for this experiment and during the period of fruiting (during summer), the temperature records were relatively high during some days, between 20°C and 29°C. These temperature records complied with the values linked to the highest photosynthesis rate, which takes place between 24°C and 29°C (Bhatt & Srinivasa Rao, 1989). Relative humidity measurements within the range of 60 to 90% had little impact on plants. Less than 50% relative humidity could negatively impact the pollination of flowers and the fruit development (Nickels, 2012).

Table 4.25: Overview of environmental boundary conditions associated with the planted chillies. Note that the number of observations for temperature and humidity is given in brackets.

Parameter	Unit	Overall[a]	FPGP[b]	FPAGP[c]	SRPBF[d]	SRPAF[e]	SRPAFD[f]
Temperature (one-off record during greenhouse visit)	°C	18.5 ±5.35 (188)	19.8 ±1.92 (19)	26.9 ±1.30 (18)	18.3 ±3.03 (19)	20.3 ±2.81 (85)	11.5 ±3.49 (47)
Temperature (minimum within a 24-hour period)	°C	15.0 ±4.63 (186)	16.5 ±2.37(19)	15.7 ±3.28(16)	14.7 ±1.86 (19)	17.8 ±2.80 (85)	9.9 ±3.57 (47)
Temperature (maximum within a 24-hour period)	°C	19.7 ±5.69 (186)	20.2 ±4.93(16)	29.7 ±3.46 (17)	23.1 ±4.69 (19)	22.7 ±2.96 (85)	13.0 ±3.71 (47)
Relative humidity (one-off record during greenhouse visit)	%	77 ±9.3 (178)	79 ±5.5(13)	75 ±6.7(14)	70 ±6.7 (22)	76 ±7.4 (82)	84 ±9.6 (47)
Relative humidity (minimum within a 24-hour period)	%	64 ±15.4 (185)	63 ±12.1(19)	68 ±10.7(15)	52 ±15.5 (22)	62 ±10.9 (82)	73 ±17.3 (47)
Relative humidity (maximum within a 24-hour period)	%	85 ±7.5 (185)	84 ±6.3(18)	87 ±4.6(16)	77 ±5.6 (22)	83 ±4.9 (82)	92 ±6.7 (47)
Temperature (one-off record outside the greenhouse)	°C	14.5 ±4.53 (137)	14.9 ±3.45 (11)	15.3 ±2.78 (17)	17.2 ±1.16 (7)	17.4 ±2.37 (55)	10.7 ±3.88 (47)
Relative humidity (one-off record outside the greenhouse)	%	53 ±10.7 (136)	54 ±11.2(15)	55 ±12.4(12)	54 ±10.0 (7)	48 ±10.9 (55)	56 ±13.5 (47)

[a]12/02/14 to 24/12/14;
[b]First planting (germination period): 12/02/14 to 09/03/14;
[c]First replanting (after germination period): 10/03/14 to 07/04/14;
[d]Second replanting period before fruiting: 08/04/14 to 11/05/14;
[e]Second replanting period after fruiting (i.e. development of first fruit): 12/05/14 to 25/09/14; and
[f]Second replanting period after fruiting (second diesel spill on 26/09/14): 26/09/14 to 24/12/14

Table 4.26 shows the total water volumes for all plants for various planting stages. The germination period was excluded, as all plants during this period were sprayed with tap water. The results show that the productivity of plants was independent of water consumption.

Table 4.26: Overview of the total water volumes for chilli plants for different planting periods.

Inflow source	Total irrigation water volume[a] (l)				Standard deviation (l)			
	FRP[b]	SRPBF[c]	SRPAF[d]	SRPAFD[e]	FRP[b]	SRPBF[c]	SRPAF[d]	SRPAFD[e]
Filter 1 outflow	0.25	2.35	14.74	5.57	0.05	0.26	0.27	0.24
Filter 2 outflow	0.25	2.55	17.34	6.48	0.05	0.17	0.54	0.28
Filter 3 outflow	0.25	2.40	16.33	5.51	0.05	0.39	0.35	0.34
Filter 4 outflow	0.24	2.45	17.44	6.28	0.04	0.03	0.27	0.14
Filter 5 outflow	0.25	2.68	17.49	6.33	0.04	0.15	0.40	0.28
Filter 6 outflow	0.26	2.75	18.02	6.56	0.07	0.26	0.83	0.64
Filter 7 outflow	0.26	2.63	18.04	6.59	0.07	0.25	0.30	0.34
Filter 8 outflow	0.25	2.71	17.16	6.86	0.05	0.31	0.69	0.24
Control A outflow	0.25	2.37	15.05	5.80	0.05	0.40	0.33	0.14
Control B outflow	0.25	2.22	16.21	6.23	0.08	0.25	0.90	0.05
Deionized water	0.31	2.60	16.12	5.55	0.08	0.10	0.32	0.11
Tap water (100%)	0.31	2.60	17.12	6.38	0.07	0.12	0.41	0.28
Tap water with fertilizer (0.7 ml/l)	0.30	3.02	17.60	6.62	0.07	0.04	0.57	0.11
Wastewater (20%); tap water (80%)	0.32	2.79	17.83	7.73	0.08	0.25	0.40	0.22
Wastewater (100%)	0.31	2.80	17.91	7.23	0.07	0.10	0.27	0.18
River water	0.26	2.75	18.52	6.86	0.07	0.36	0.93	0.64
Rain water	0.25	2.37	16.05	5.55	0.05	0.48	0.38	0.14
Gully pots water	0.25	2.55	16.34	6.78	0.05	0.67	0.54	0.28
Real grey water	0.30	3.05	17.60	6.52	0.05	0.04	0.57	0.11
Artificial grey water	0.32	2.75	17.23	7.43	0.08	0.15	0.30	0.22

[a]Each value represents the means of the total water volume based on six replicates; [b]First replanting period: 12/02/14 to 07/04/14; [c]Second replanting period before fruiting: 08/04/14 to 11/05/14; [d]Second replanting period after fruiting:12/05/14 to 25/09/14; and [e]Second replanting period after fruiting (second diesel spill on 26/09/14): 26/09/14 to 24/12/14.

4.7.2.2 Wetland design and operation variable impacts on chillies

The impact of wetland design and operation variables on chilli growth is shown in Figure 4.54. In general, the quantity of diesel compounds determines the toxicity to plants, and

hampers their growth (Singh et al., 2012). Furthermore, accumulated compounds of hydrocarbon in soil media hinder air diffusion through the pores, which causes anaerobic conditions, and subsequently permeability reductions of the soil environment, negatively affecting the diversity of micro-organisms (Sutton et al., 2013), and thus preventing chilli plants from achieving uptake of nutrients. In the first and second planting phases (period of first diesel dosage), findings showed (Table 4.27) no significant ($p>0.05$) difference in terms of the impact of irrigation water on plant growth between filters with and without diesel contamination. This suggests that small amounts of hydrocarbon would not affect the growth of plants (Al-Baldawi et al., 2013b; Al-Baldawi et al., 2014a; Al-Baldawi et al., 2015a). Moreover, the composition of compost was still fresh without contaminants accumulating at the beginning of the bud development period. After that, with passing time, plant buds were varied highlighting the effect of hydrocarbon accumulation on those pots receiving irrigation water contaminated with diesel (Figure 4.54a). Moreover, most flowers were lost in plants irrigated with diesel-contaminated filters illustrating the effect of toxic hydrocarbon compounds (Figure 4.54b); particularly during the third planting phase after the second diesel spill (i.e. three weeks after application). With regard to the fruits during the third phase, they either fell off or showed reductions in their growth due to the toxicity of chemicals associated with high levels of hydrocarbon compounds (Liu et al., 2011; García-Delgado et al., 2012). Table 4.28 shows the number of buds, flowers and fruits associated with each chilli plant. Statistically, plants irrigated with outflow from diesel-contaminated filters exhibited significantly ($p<0.05$) fewer fruit numbers than those for plants irrigated with outflows from filters without diesel contamination. For diesel-contaminated filters, small aggregate sizes (Filter 3 and 5) performed significantly ($p< 0.05$) better compared to larger sizes (Filter 1). Small aggregate diameters correlated

positively with large surface areas, allowing more micro-organisms to degrade hydrocarbon pollutants (Scholz, 2015).

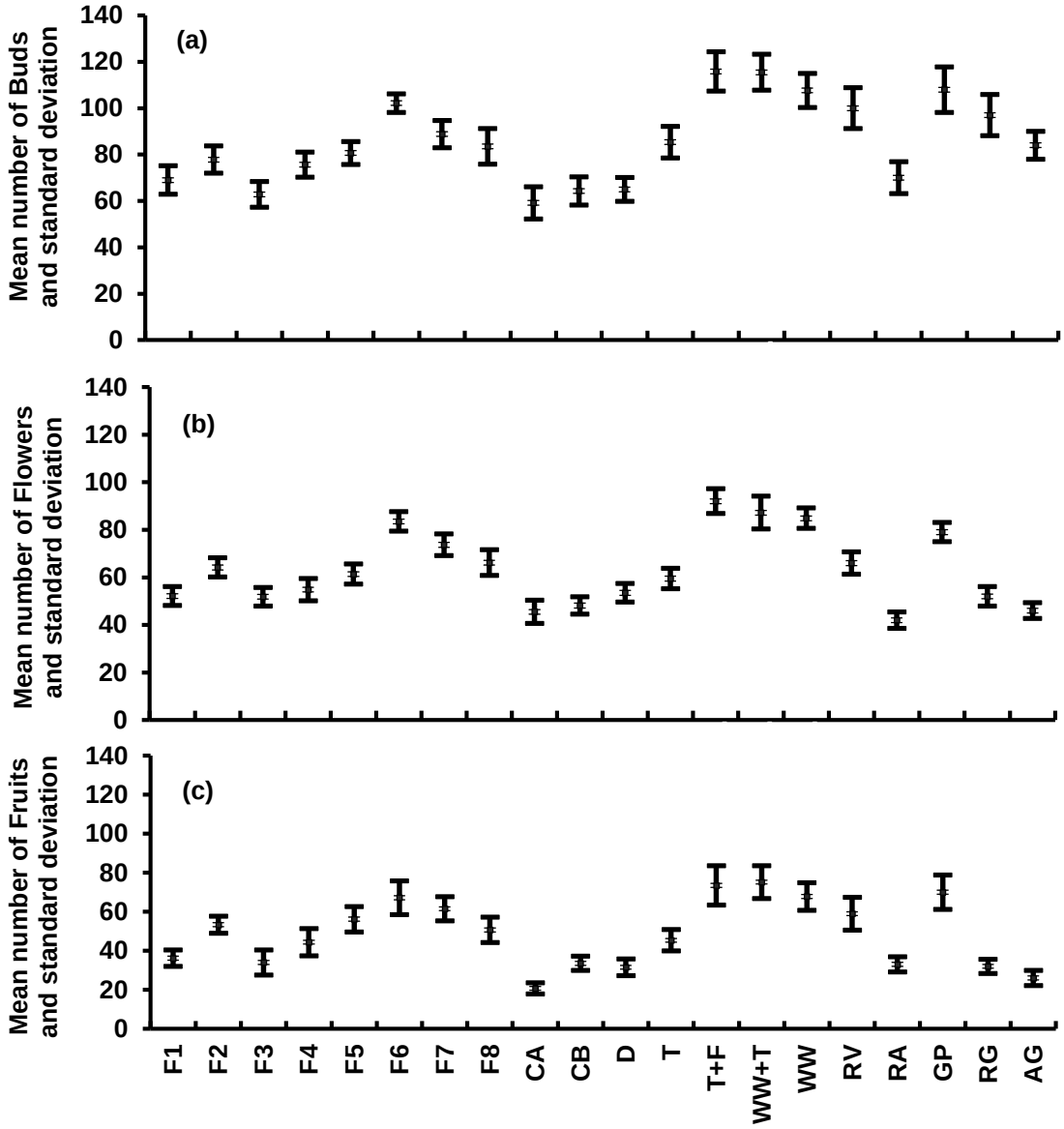


Figure 4.54: Mean and standard deviation of (a) bud, (b) flower, and (c) fruit developments for chilli plants. Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA and CB, Controls A and B (wetland filters receiving tap water); D, deionized water; T, tap water; T+F, tap water mixed with fertilizer (0.7 ml/l); WW+T, one part wastewater mixed with four parts tap water;

WW, preliminarily treated wastewater; RV, river water; RA, rain water; GP, gully pot water; RG, real grey water; and AG, artificial grey water.

The wetland filter with a high loading rate (Filter 5) released more nutrients associated with its effluent compared to Filters 1 and 3. Filter 5 received a high inflow load containing high amounts of nutrients (treatment efficiency decreased with increasing nutrient load) compared to Filter 3 receiving fewer nutrients, because the influent wastewater was diluted with tap water (Al-Isawi et al., 2015a; Al-Isawi et al., 2015b). Filters 1 and 3 suffered from a deficiency in nutrients in the outflow water. This was a result of the impact of hydrocarbon compound degradation processes by micro-organisms, which used these nutrients during hydrocarbon biodegradation in the wetland system (De Biase et al., 2011; Liu et al., 2011). The NO_3-N values were lower for filters contaminated with diesel compared to those without diesel. The addition of diesel-related carbon enhanced the nitrogen removal by micro-organisms (Liu et al., 2011).

The analysis indicated a significant ($p<0.037$) difference in the fruit numbers between Filters 3 and 5; it can clearly be seen that the productivity of fruits associated with Filter 5 (56) is better than that associated with Filters 1 and 3 (36 and 34, respectively) as shown in Figure 4.54c. This can be explained by the continuous supply of nutrients associated with the treated water from this filter, as it receives concentrated domestic wastewater without dilution (Becerra-Castro et al., 2015). Control A (contaminated with diesel) showed the least fruit numbers if compared to other filters. This is due to the lack of nutrients associated with its effluent (Aiello et al., 2007). Table 4.27 summarizes the statistical analysis (non-parametric Mann-Whitney U-test) showing the differences in chilli fruits due to various types of irrigation water.

Table 4.27: Overview of the statistically significant differences between chilli fruit variables of different wetland filters and five types of irrigated water using the non-parametric Mann-Whitney U-test (08/04/14-24/12/14).

Parameter	Unit	Statistic	Aggregate diameter[a]	Contact time[b]	Resting time[c]	Inflow chemical oxygen demand load[d]
For filters without hydrocarbon						
Weight	g	*P-value*	0.657	0.005	<0.000	0.220
		h	0	1	1	0
Length	mm	*P-value*	0.206	<0.000	<0.000	<0.000
		h	0	1	1	1
Width	mm	*P-value*	0.605	<0.000	<0.000	0.004
		h	0	1	1	1
Bending	-	*P-value*	0.268	0.001	<0.000	0.311
		h	0	1	1	0
No. Buds	-	*P-value*	0.810	0.200	0.749	0.025
		h	0	0	0	1
No. Flowers	-	*P-value*	0.337	0.109	0.251	0.037
		h	0	0	0	1
No. Fruits	-	*P-value*	0.199	0.109	0.262	0.078
		h	0	0	0	0
Total price	£	*P-value*	0.902	0.773	0.688	0.695
		h	0	0	0	0
Parameter	Unit	Statistics	Aggregate diameter[e]	Contact time[f]	Resting time[g]	Inflow chemical oxygen demand load[h]
For filters with hydrocarbon						
Weight	g	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
Length	mm	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
Width	mm	*P-value*	0.070	<0.000	<0.000	<0.000
		h	0	1	1	1
Bending	-	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
No. Buds	-	*P-value*	0.470	0.078	0.749	0.229
		h	0	0	0	0
No. Flowers	-	*P-value*	1.000	0.109	0.251	0.521
		h	0	0	0	0
No. Fruits	-	*P-value*	0.629	0.045	0.262	0.037
		h	0	0	0	1
Total price	£	*P-value*	0.312	0.523	0.688	0.031
		h	0	0	0	1
Parameter	Unit	Statistics	Filters 1 and 2	Filters 3 and 4	Filters 5 and 6	Control A and B
Comparison between filters with and without hydrocarbon						

Table 4.27 (cont.)

Weight	g	*P-value*	<0.000	<0.000	<0.000	<0.001
		h	1	1	1	1
Length	mm	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
Width	mm	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
Bending	l	*P-value*	<0.000	0.037	<0.000	<0.000
		h	1	1	1	1
No. Buds	-	*P-value*	0.630	0.297	0.109	0.522
		h	0	0	0	0
No. Flowers	-	*P-value*	0.228	0.810	0.037	0.574
		h	0	0	1	0
No. Fruits	-	*P-value*	0.055	0.108	0.261	0.053
		h	0	0	0	0
Total price	£	*P-value*	0.097	0.108	0.053	0.062
		h	0	0	0	0

Parameter	Unit	Statistics	Lack of nutrients[i]	Fertilizer[j]	Diluted tap water[k]	Diluted wastewater[l]
Weight	g	*P-value*	<0.000	0.011	<0.000	<0.000
		h	1	1	1	1
Length	mm	*P-value*	<0.000	0.029	<0.000	<0.000
		h	1	1	1	1
Width	mm	*P-value*	<0.000	0.453	0.002	<0.000
		h	1	0	1	1
Bending	-	*P-value*	<0.000	<0.000	<0.000	<0.000
		h	1	1	1	1
No. Buds	-	*P-value*	0.013	0.037	0.150	0.262
		h	1	1	0	0
No. Flowers	-	*P-value*	0.378	0.006	0.010	0.810
		h	0	1	1	0
No. Fruits	-	*P-value*	0.004	0.013	0.008	0.575
		h	1	1	1	0
Total price	£	*P-value*	0.353	0.332	0.288	0.829
		h	0	0	0	0

[a]Comparison between the mean daily values of Filter 2, and the mean daily values of Filter 4;
[b]Comparison between the mean daily values of Filter 4, and Filter 7;
[c]Comparison between Filters 7 and 8;
[d]Comparison between mean daily values of Filter 4, and mean daily values of Filter 6;
[e]Comparison between the mean daily values of Filter 1, and the mean daily values of Filter 3;
[f]Comparison between the mean daily values of Filter 3, and Filter 7;
[g]Comparison between Filters 7 and 8;
[h]Comparison between mean daily values of Filter 3, and mean daily values of Filter 5;
[i]Comparison between the mean daily values of deionized water and the mean daily values of tap water;
[j]Comparison between the mean daily values of tap water (100%) and tap water with fertilizer (0.7ml/l);
[k]Comparison between tap water (100%) and tap water (80%) with wastewater (20%); and
[l]Comparison between mean daily values of tap water (80%) with wastewater (20%) and mean daily values of wastewater (100%).
Note: *P-value*, probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true; h, response indicator; if *h*=1, filters are statistically

significantly different (*P-value*< 0.05) for the corresponding water quality parameter; if *h*=0, the difference is not significant

The time for filling and emptying the filters made a significant ($p<0.05$) difference on chilli growth in terms of the length, width and weight of fruits. Filters with a short contact time (Filter 7) performed better for most water quality parameters, resulting in a good harvest of chilli fruits (62) compared to those with longer contact times (Filter 4 linked to 44 fruits). Furthermore, results indicated that there is a significant ($p<0.05$) difference in terms of resting time (Filters 7 and 8). A low resting time means a high frequency of loading the wetland (Filter 8), which increases the pollutants associated with this filter (Al-Isawi et al., 2015b; Belhaj et al., 2015). Findings also revealed that good productivity of fruit numbers was associated with a high loading rate (Filter 6). This is due to the good performance of water quality outflow parameters and sufficient nutrients for plant growth (Nickels, 2012; Becerra-Castro et al., 2015).

The chilli fruit number linked to tap water (45) was less than the one for plants linked to diluted wastewater (75) as shown in Figure 4.54c. As the compost became depleted of nutrients, the harvest increased for plants receiving pre-treated wastewater compared to those plants which only depend on nutrients received from the compost. Furthermore, findings designate that nutrients obtained by chillies due to a combination of wastewater and tap water were sufficient to result in a profitable harvest (Norton-Brandão et al., 2013) as compared with those linked to preliminary wastewater (66). The high amount of turbidity and SS associated with preliminarily treated wastewater enhanced the development of hydrophobicity in soils, which subsequently affected the growth of plants (Travis et al., 2010; Becerra-Castro et al., 2015).

Table 4.28: Overview of total number of buds, flowers and fruits for chilli (C) plants until 24 December 2014.

Inflow source	Total bud number	Total flower number	Total fruit number before harvest	Total fruit number after harvest
Filter 1	C1(91);C2(83);C3(87); C4(58);C5(70);C6(25)	C1(69);C2(50);C3(67); C4(50);C5(57);C6(20)	C1(53);C2(33);C3(36); C4(39);C5(41);C6(15)	C1(46);C2(30);C3(31); C4(34);C5(40);C6(15)
Filter 2	C7(83);C8(99);C9(79); C10(80);C11(87);C12(39)	C7(74);C8(64);C9(67); C10(66);C11(49);C12(33)	C7(72);C8(73);C9(56); C10(46);C11(55);C12(18)	C7(70);C8(71);C9(53); C10(43);C11(49);C12(18)
Filter 3	C13(86);C14(50);C15(83); C16(77);C17(55);C18(26)	C13(79);C14(42);C15(71); C16(51);C17(44);C18(20)	C13(38);C14(36);C15(47); C16(36);C17(24);C18(23)	C13(34);C14(33);C15(42); C16(35);C17(21);C18(23)
Filter 4	C19(90);C20(83);C21(56); C22(97);C23(75);C24(53)	C19(70);C20(54);C21(47); C22(53);C23(39);C24(49)	C19(54);C20(64);C21(40); C22(40);C23(31);C24(37)	C19(52);C20(60);C21(40); C22(39);C23(29);C24(36)
Filter 5	C25(63);C26(82);C27(61); C28(60);C29(55);C30(34)	C25(54);C26(50);C27(43); C28(54);C29(47);C30(25)	C25(17);C26(21);C27(30); C28(19);C29(20);C30(17)	C25(16);C26(19);C27(29); C28(17);C29(19);C30(15)
Filter 6	C31(72);C32(88);C33(73); C34(74);C35(54);C36(25)	C31(40);C32(59);C33(57); C34(66);C35(47);C36(20)	C31(39);C32(39);C33(32); C34(44);C35(32);C36(15)	C31(34);C32(39);C33(30); C34(43);C35(30);C36(15)
Filter 7	C37(78);C38(113);C39(83); C40(91);C41(79);C42(33)	C37(68);C38(65);C39(69); C40(61);C41(61);C42(26)	C37(66);C38(63);C39(72); C40(58);C41(46);C42(32)	C37(60);C38(61);C39(70); C40(54);C41(44);C42(29)
Filter 8	C43(115);C44(122);C45(98); C46(111);C47(103);C48(64)	C43(109);C44(86);C45(90); C46(98);C47(61);C48(43)	C43(83);C44(58);C45(80); C46(86);C47(57);C48(33)	C43(79);C44(57);C45(78); C46(84);C47(56);C48(31)
Control A	C49(117);C50(79);C51(111); C52(102);C53(91);C54(53)	C49(98);C50(69);C51(75); C52(89);C53(82);C54(29)	C49(92);C50(55);C51(70); C52(73);C53(61);C54(18)	C49(91);C50(53);C51(69); C52(71);C53(60);C54(16)
Control B	C55(86);C56(112);C57(104); C58(110);C59(64);C60(25)	C55(71);C56(94);C57(84); C58(71);C59(58);C60(19)	C55(48);C56(84);C57(51); C58(62);C59(45);C60(14)	C55(48);C56(82);C57(50); C58(61);C59(40);C60(14)
Deionized water	C61(74);C62(67);C63(68); C64(58);C65(69);C66(54)	C61(58);C62(52);C63(59); C64(49);C65(62);C66(41)	C61(33);C62(36);C63(30); C64(27);C65(32);C66(31)	C61(29);C62(32);C63(29); C64(25);C65(29);C66(27)
Tap water	C67(99);C68(88);C69(68); C70(89);C71(82);C72(86)	C67(76);C68(52);C69(46); C70(69);C71(60);C72(54)	C67(66);C68(47);C69(38); C70(40);C71(40);C72(41)	C67(60);C68(43);C69(34); C70(40);C71(39);C72(40)
Tap water/fertilizer	C73(92);C74(131);C75(134); C76(133);C77(84);C78(121)	C73(82);C74(121);C75(87); C76(73);C77(77);C78(112)	C73(70);C74(103);C75(66); C76(61);C77(49);C78(92)	C73(70);C74(102);C75(66); C76(59);C77(47);C78(90)
Wastewater/tap	C79(128);C80(120);C81(116); C82(122);C83(93);C84(114)	C79(96);C80(72);C81(105); C82(87);C83(74);C84(89)	C79(78);C80(56);C81(100); C82(66);C83(67);C84(84)	C79(75);C80(53);C81(98); C82(64);C83(65);C84(81)
Wastewater	C85(115);C86(130);C87(109); C88(91);C89(104);C90(97)	C85(105);C86(103);C87(62); C88(49);C89(81);C90(82)	C85(86);C86(74);C87(56); C88(43);C89(71);C90(77)	C85(82);C86(71);C87(54); C88(41);C89(70);C90(73)

Table 4.28 (cont.)

River water	C91(118);C92(73);C93(154); C94(71);C95(97);C96(84)	C91(81);C92(39);C93(59); C94(85);C95(68);C96(62)	C91(65);C92(32);C93(58); C94(78);C95(61);C96(60)	C91(63);C92(32);C93(56); C94(71);C95(60);C96(53)
Rain water	C97(41);C98(40);C99(81); C100(126);C101(93);C102(41)	C97(28);C98(44);C99(36); C100(45);C101(62);C102(36)	C97(21);C98(37);C99(28); C100(39);C101(41);C102(33)	C97(19);C98(33);C99(23); C100(37);C101(38);C102(31)
Gully pot water	C103(99);C104(126);C105(95); C106(72);C107(106);C108(149)	C103(87);C104(89);C105(80); C106(65);C107(82);C108(71)	C103(79);C104(81);C105(71); C106(58);C107(75);C108(53)	C103(77);C104(79);C105(67); C106(55);C107(71);C108(51)
Real grey water	C109(75);C110(114);C111(130); C112(124);C113(77);C114(60)	C109(47);C110(53);C111(47); C112(81);C113(45);C114(38)	C109(34);C110(34);C111(33); C112(30);C113(30);C114(31)	C109(33);C110(29);C111(28); C112(27);C113(25);C114(30)
Artificial grey water	C115(131);C116(69);C117(63); C118(84);C119(75);C120(82)	C115(91);C116(32);C117(32); C118(50);C119(39);C120(29)	C115(29);C116(30);C117(27); C118(19);C119(34);C120(16)	C115(27);C116(28);C117(23); C118(15);C119(31);C120(11)

The number of buds, flowers, and fruits with associated classes obtained in the greenhouse (Al-Isawi et al., 2016b) markedly differed from those in a related study (Almuktar et al., 2015), indicating that greenhouse conditions benefit chilli plant growth over laboratory environments supported by artificial growth light. Both high temperature and sun intensity during summer resulted in an increase of the chilli yield in the greenhouse environment.

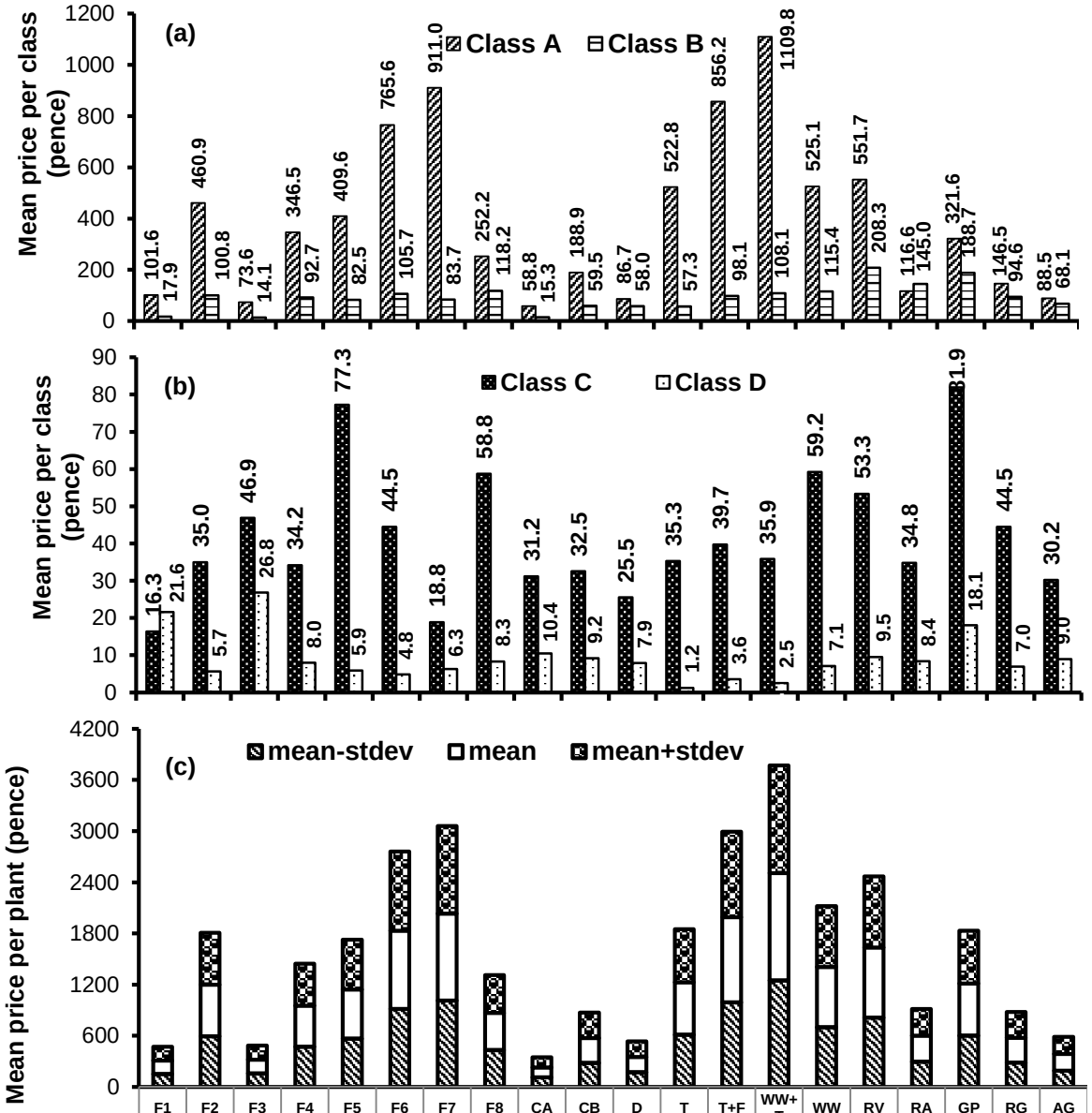
4.7.3 Cost-benefit analysis for fruits

The classification scheme used for laboratory-gown chillies (Almuktar et al., 2015b, 2015a) was applied for the greenhouse environment (Al-Isawi et al., 2016c; Al-Isawi et al., 2016b) (Table 4.29), which, should therefore be of more international interest. Note that only the variables length, width, weight and bending (Almuktar et al., 2015b) were used for classifying the harvested fruits. The economic value of the harvest of chilli plants was estimated according to the mean national prices on the UK market between January and June 2015.

Table 4.29: Chilli (C) harvest classification scheme (Almuktar et al., 2015b; Scholz, 2015; Al-Isawi et al., 2016a))

Variable	Class A	Class B	Class C	Class D	Class E
Quality class	Outstanding	Good	Good	Satisfactory	Unsatisfactory
Mean price pence (Sterling)/gram	C: 2.00	C: 1.00	C: 0.50	C: 0.25	C: 0.00
Length (L, mm)	Very long (L≥80)	Long (60≤ L<80)	Medium (40≤ L<60)	Short (20≤L<40)	Very short (L<20)
Width (W, mm)	Very wide(W≥20)	Wide (16≤W<20)	Medium (12≤W<16)	Slim (8≤W<12)	Very slim (W<8)
Weight (w, g)	Very Large(w≥9)	Large (7≤w<9)	Medium (5≤w≤7)	Small (3≤w<5)	Very Small (w<3)
Bending	Characteristically bend; L/W≥3.5	Characteristically bend; L/W≥3.5	Characteristically bend; L/W≥3.5	Uncharacteristically bend; L/W<3.5	Uncharacteristically bend; L/W<3.5

Figure 4.55 indicates the fiscal value of the harvest. Figures 4.55a and 4.55b show the average price of fruits linked to Classes A, B, C and D for each type of irrigation water. However, Class E (representing essentially organic waste) has been excluded because no monetary value for fruits is linked to this category. Figure 4.55c shows the total price for each plant irrigated with one type of water. Table 4.30 presents more details about the price associated with each chilli plant. The highest average price of harvested fruits, which is estimated at 1256 pence, and the greatest number of fruits of Class A were obtained from chillies watered with tap water diluted by wastewater.



	F1	F2	F3	F4	F5	F6	F7	F8	CA	CB	D	T	T+F	WW+T	WW	RV	RA	GP	RG	AG
mean+stdev	162.8	609.6	166.6	490.8	584.2	929.0	1027.8	444.6	118.9	299.6	183.3	622.8	1004.0	1263.1	713.6	834.5	315.5	619.2	300.7	203.0
mean	157.4	602.3	161.4	481.4	575.2	920.6	1019.8	437.5	115.7	290.1	178.1	616.6	997.6	1256.3	706.9	822.8	304.8	610.2	292.6	195.8
mean-stdev	152.0	595.0	156.2	472.0	566.2	912.2	1011.8	430.4	112.5	280.7	173.0	610.4	991.2	1249.5	700.2	811.1	294.1	601.2	284.5	188.6

Figure 4.55: Economic return for varied classes of harvested chilli fruits. Note: no financial return for Class E. F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA and CB, Controls A and B (wetland filters receiving tap water); D, deionized water; T, tap water; T+F, tap water mixed with fertilizer (0.7 ml/l); WW+T, one part wastewater mixed with four parts tap water; WW,

preliminary treated wastewater; RV, river water; RA, rain water; GP, gully pot water; RG, real grey water; and AG, artificial grey water.

The findings concerning the economic return from chilli fruits are not in agreement with those by Almuktar et al., (2015b), indicating that greenhouse conditions are better than artificial light growth environments. The average yield price per plant obtained from the greenhouse (Table 4.30) ranged from 300 to 4000 pence, which is significantly ($p<0.05$) (Al-Isawi et al., 2016b) higher than the range from 0 to 150 pence linked to the yield per plant in the study of Almuktar et al., (2015b).

Table 4.30: Overview of the outcome of the chilli (C) harvest (before or on 24 December 2014) classification scheme (greenhouse environment).

Inflow source	Class A	Class B	Class C	Class D	Class E	Mean pence per plant
Filter 1	C1(217.3);C2(0.0); C3(181.2);C4(62.6); C5(111.2);C6(37.2)	C1(21.2);C2(4.8); C3(5.2);C4(32.5); C5(27.7);C6(15.8)	C1(14.9);C2(13.2); C3(28.9);C4(14.7); C5(26.3);C6(0.0)	C1(57.6);C2(12.9); C3(10.8);C4(17.5); C5(18.2);C6(12.4)	C1(0);C2(0); C3(0);C4(0); C5(0);C6(0)	157.4
Filter 2	C7(614.4);C8(536.9); C9(501.6);C10(507.7); C11(507.1);C12(97.5)	C7(155.2);C8(140.4); C9(148.0);C10(74.8); C11(62.2);C12(24.0)	C7(57.1);C8(57.5); C9(12.9);C10(22.7); C11(33.8);C12(25.9)	C7(9.2);C8(9.6); C9(2.9);C10(4.2); C11(3.3);C12(4.7)	C7(0);C8(0); C9(0);C10(0); C11(0);C12(0)	602.3
Filter 3	C13(152.2);C14(40.0); C15(127.1);C16(92.3); C17(29.7);C18(0.0)	C13(20.8);C14(9.4); C15(9.7);C16(30.4); C17(10.4);C18(3.9)	C13(44.5);C14(57.9); C15(49.6);C16(54.6); C17(45.9);C18(29.0)	C13(19.5);C14(17.9); C15(69.2);C16(16.9); C17(6.5);C18(31.1)	C13(0);C14(0); C15(0);C16(0); C17(0);C18(0)	161.4
Filter 4	C19(305.4);C20(359.6); C21(525.2);C22(319.7); C23(297.4);C24(271.6)	C19(104.5);C20(134.1); C21(59.8);C22(87.2); C23(56.7);C24(114.0)	C19(51.3);C20(40.2); C21(11.6);C22(62.4); C23(22.5);C24(17.2)	C19(7.3);C20(15.1); C21(15.7);C22(3.2); C23(2.2);C24(4.3)	C19(0);C20(0); C21(0);C22(0); C23(0);C24(0)	481.36
Filter 5	C25(316.1);C26(228.3); C27(791.5);C28(550.0); C29(265.2);C30(306.6)	C25(52.2);C26(75.4); C27(116.5);C28(77.5); C29(115.2);C30(57.9)	C25(122.4);C26(134.5); C27(77.9);C28(65.9); C29(42.9);C30(20.0)	C25(4.3);C26(1.0); C27(5.6);C28(0.0); C29(3.7);C30(20.5)	C25(0);C26(0); C27(0);C28(0); C29(0);C30(0)	575.2
Filter 6	C31(1071.9);C32(704.4); C33(1041.2);C34(946.9); C35(324.4);C36(504.6)	C31(140.1);C32(92.5); C33(108.5);C34(94.4); C35(151.0);C36(47.8)	C31(54.9);C32(26.6); C33(49.1);C34(61.3); C35(60.4);C36(14.7)	C31(3.3);C32(3.4); C33(8.2);C34(14.0); C35(0.0);C36(0.0)	C31(0);C32(0); C33(0);C34(0); C35(0);C36(0)	920.6
Filter 7	C37(1171.9);C38(883.9); C39(766.8);C40(1138.1); C41(1086.5);C42(418.6)	C37(98.4);C38(69.7); C39(160.5);C40(110.5); C41(51.9);C42(11.2)	C37(37.0);C38(14.4); C39(30.4);C40(19.7); C41(7.1);C42(4.4)	C37(13.9);C38(3.7); C39(1.0);C40(16.0); C41(3.3);C42(0.0)	C37(0);C38(0); C39(0);C40(0); C41(0);C42(0)	1019.8
Filter 8	C43(120.5);C44(453.9); C45(283.9);C46(303.7); C47(241.1);C48(110.4)	C43(108.0);C44(157.6); C45(160.3);C46(166.4); C47(92.2);C48(24.8)	C43(40.3);C44(112.3); C45(49.3);C46(72.1); C47(54.6);C48(23.9)	C43(38.0);C44(5.8); C45(0.0);C46(6.1); C47(0.0);C48(0.0)	C43(0);C44(0); C45(0);C46(0); C47(0);C48(0)	437.5
Control A	C49(66.9);C50(89.3); C51(112.6);C52(34.2); C53(31.8);C54(18.0)	C49(10.0);C50(19.7); C51(17.8);C52(12.7); C53(12.7);C54(18.7)	C49(31.0);C50(15.0); C51(54.9);C52(17.0); C53(36.0);C54(33.3)	C49(6.0);C50(14.1); C51(10.6);C52(19.6); C53(10.0);C54(2.3)	C49(0);C50(0); C51(0);C52(0); C53(0);C54(0)	115.7
Control B	C55(121.7);C56(234.3); C57(162.0);C58(166.0); C59(389.3);C60(60.0)	C55(67.2);C56(75.6); C57(86.3);C58(72.2); C59(43.8);C60(11.8)	C55(46.2);C56(31.1); C57(15.2);C58(42.3); C59(19.7);C60(40.8)	C55(22.2);C56(7.2); C57(5.5);C58(8.0); C59(11.0);C60(1.2)	C55(0);C56(0); C57(0);C58(0); C59(0);C60(0)	290.1

Table 4.30 (cont.)

Deionized water	C61(184.6);C62(88.6); C63(0.0);C64(77.5); C65(169.4);C66(0.0)	C61(26.5);C62(64.2); C63(70.9);C64(57.4); C65(50.8);C66(78.3)	C61(36.1);C62(40.2); C63(29.0);C64(14.4); C65(11.9);C66(21.4)	C61(9.3);C62(7.9); C63(6.9);C64(4.7); C65(12.0);C66(6.3)	C61(0);C62(0); C63(0);C64(0); C65(0);C66(0)	178.1
Tap water	C67(579.7);C68(562.2); C69(474.1);C70(624.3); C71(393.2);C72(503.3)	C67(115.4);C68(41.3); C69(37.4);C70(31.2); C71(62.1);C72(56.4)	C67(42.8);C68(46.3); C69(29.7);C70(30.5); C71(31.9);C72(30.7)	C67(3.5);C68(0.0); C69(0.0);C70(1.0); C71(1.2);C72(1.6)	C67(0);C68(0); C69(0);C70(0); C71(0);C72(0)	616.6
Tap water/fertilizer	C73(867.3);C74(1340.4); C75(540.3);C76(850.1); C77(561.2);C78(977.9)	C73(80.0);C74(118.4); C75(91.8);C76(85.4); C77(74.1);C78(138.7)	C73(28.5);C74(62.0); C75(61.7);C76(22.8); C77(7.4);C78(56.0)	C73(2.0);C74(3.4); C75(3.0);C76(0.9); C77(10.1);C78(2.1)	C73(0);C74(0); C75(0);C76(0); C77(0);C78(0)	997.6
Wastewater/tap	C79(1050.8);C80(632.9); C81(1504.1);C82(1238.8); C83(812.0);C84(1420.6)	C79(108.7);C80(79.7); C81(163.9);C82(101.6); C83(122.7);C84(72.1)	C79(54.5);C80(40.0); C81(33.2);C82(10.1); C83(35.4);C84(42.0)	C79(0.0);C80(4.5); C81(1.2);C82(0.0); C83(4.2);C84(5.0)	C79(0);C80(0); C81(0);C82(0); C83(0);C84(0)	1256.3
Wastewater	C85(345.5);C86(421.7); C87(603.2);C88(424.4); C89(657.8);C90(698.2)	C85(121.2);C86(146.6); C87(106.8);C88(93.3); C89(127.9);C90(96.6)	C85(115.6);C86(58.6); C87(48.3);C88(15.3); C89(46.7);C90(70.8)	C85(8.7);C86(19.2); C87(1.7);C88(0.0); C89(1.2);C90(11.8)	C85(0);C86(0); C87(0);C88(0); C89(0);C90(0)	706.9
River water	C91(854.9);C92(506.7); C93(459.8);C94(294.9); C95(583.9);C96(610.1)	C91(222.6);C92(76.9); C93(239.6);C94(338.4); C95(131.9);C96(240.1)	C91(44.4);C92(18.7); C93(64.9);C94(82.4); C95(59.3);C96(49.6)	C91(1.7);C92(2.9); C93(5.5);C94(17.1); C95(15.3);C96(14.3)	C91(0);C92(0); C93(0);C94(0); C95(0);C96(0)	822.8
Rain water	C97(0);C98(130.6); C99(91.0);C100(126.0); C101(219.0);C102(132.9)	C97(115.6);C98(71.2); C99(129.6);C100(208.3); C101(142.1);C102(202.8)	C97(11.4);C98(52.3); C99(22.7);C100(58.3); C101(48.0);C102(16.0)	C97(12.0);C98(8.1); C99(0.8);C100(4.9); C101(12.5);C102(11.5)	C97(0);C98(0); C99(0);C100(0); C101(0);C102(0)	304.8
Gully pots water	C103(466.4);C104(377.2); C105(443.9);C106(85.2); C107(285.1);C108(271.4)	C103(180.6);C104(250.2); C105(148.4);C106(167.1); C107(193.8);C108(191.9)	C103(84.1);C104(90.3); C105(71.8);C106(111.4); C107(88.1);C108(45.4)	C103(20.4);C104(26.1); C105(30.2);C106(10.6); C107(12.3);C108(8.5)	C103(0);C104(0); C105(0);C106(0); C107(0);C108(0)	610.2
Real grey water	C109(289.5);C110(169.0); C111(235.9);C112(27.9); C113(133.9);C114(22.6)	C109(46.2);C110(111.8); C111(116.2);C112(107.0); C113(113.2);C11472.9)	C109(58.5);C110(28.4); C111(40.0);C112(59.5); C113(27.9);C114(52.5)	C109(11.4);C110(9.6); C111(0);C112(1.7); C113(7.9);C114(11.0)	C109(0);C110(0); C111(0);C112(0); C113(0);C114(0)	292.6
Artificial grey water	C115(0);C116(138.4); C117(28.8);C118(87.1); C119(198.8);C120(77.7)	C115(83.2);C116(64.7); C117(23.6);C118(84.5); C119(123.9);C120(28.8)	C115(28.5);C116(38.2); C117(42.6);C118(18.3); C119(33.9);C120(19.3)	C115(10.3);C116(10.0); C117(12.0);C118(17.5); C119(2.6);C120(1.2)	C115(0);C116(0); C117(0);C118(0); C119(0);C120(0)	195.8

Note that the lowest variable class entry for any individual fruit assessment will determine the final class. However, only the following numerical and objective variables were used to classify fruits for the purpose of this study: length, width, weight and bending. Values shown per plant represent pence (Sterling).

For all wetland-based experiments, Filter 7 is associated with the greatest yield in terms of its overall economic value. Furthermore, Filter 7 provides the highest financial return linked to Class A. This can be explained by a combination of small aggregate size, low contact time and high resting time. This interpretation is concordant with what was indicated by chillies grown in lab conditions. Despite that, the irrigation with wastewater diluted by tap water resulted in a higher overall yield (Figure 4.55c). Generally, all fruits harvested from diesel-contaminated filters (Filters 1, 3 and 5 as well as Control A) were weak, indicating the negative impact of diesel contamination on chilli plants. However, Filter 5 had the highest number of fruits linked to Class A. This is possibly due to the balanced presence of minerals and nutrients that were needed for plant growth due to a high loading rate. Figure 4.56 shows the growth comparison for the selected fruits harvested from Filter 7 (without diesel contamination) and diesel-contaminated wetlands (Filters 1, 3 and 5).

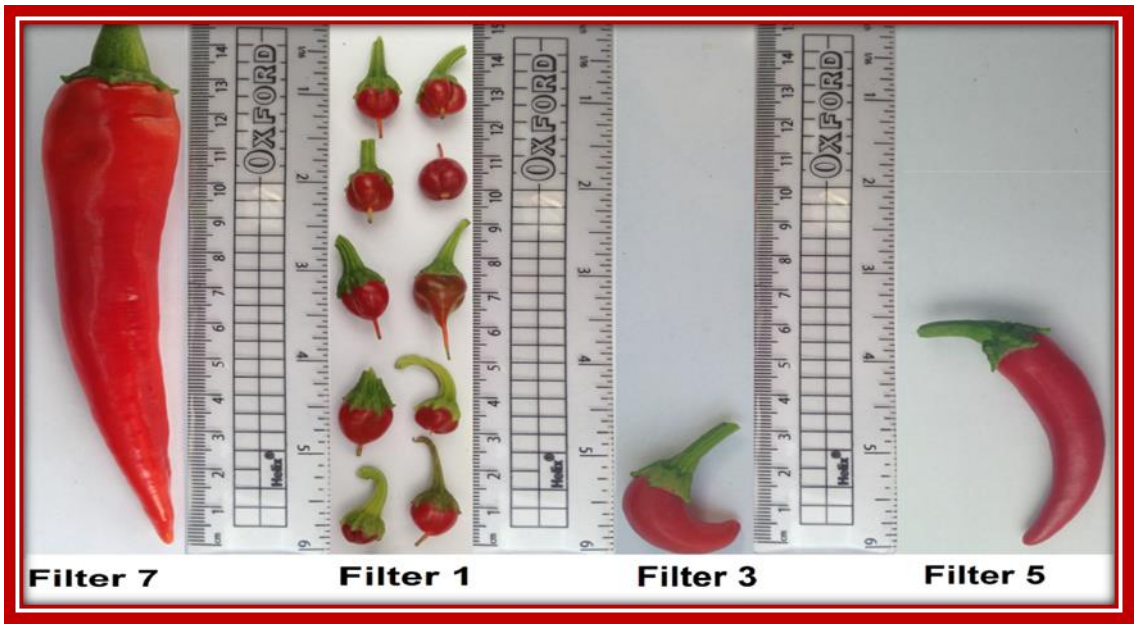


Figure 4.56: Photographs of example chilli harvests linked to Filter 7 (without diesel contamination) and Filters 1, 3 and 5 (diesel-contaminated). Note: Unhealthy fruits were associated with outflow waters from Filters 1 and 3; F1, wetland filter 1; F3, wetland filter 3; F5, wetland filter 5; and F7, wetland filter 7.

The lowest price, estimated at 157 pence, was associated with Filter 1. The fruits linked to Filter 3 were slightly better than those associated with Filter 1. Most fruits were categorized as Classes D and E, which can be explained by the acidic nature of the outflow water from this filter, resulting in a lack of trace elements essential for plant growth (FAO, 2016). The plants that were watered with deionized water and Control B outflow exhibited a decline in their productivity over time. This could be assigned to nutrient depletion over time (Nickels, 2012). For filters without diesel contamination, a high value of fruits was associated with a low contact time (Filter 7; 1020 pence) as shown in Figure 4.55c. However, the plants associated with Filter 6 show a high overall fruit price (921 pence). A significant ($p<0.05$) number of fruits were linked to Class C. The impact of the presence of hydrocarbon on the treatments in terms of price associated with yields was

statistically insignificant. However, marketable yields were higher for filters without hydrocarbon contamination. River water and gully pot water were associated with the high number of fruits categorized as Class A. Concerning rain water, most fruits belonged to Class B. Overall yields of chillies irrigated by grey water were low, indicating potential problems with salinity as discussed by (Al-Hamaiedeh & Bino, 2010).

The outweigh for filters without hydrocarbon compared to those subject to hydrocarbon influence in terms of mean price per plant (%) was obtained using Equation 4.1 as shown below:

$$W(\%) = [1 - \left(\frac{F_{i(with\ hydrocarbon)}}{F_{i(without\ hydrocarbon)}}\right)] \times 100 \quad \text{.....(4.1)}$$

where W represents the weight of filters without hydrocarbon to those linked with hydrocarbon in terms of mean price per plant (%). The W(%) values for the ratios Filter 1/Filter 2, Filter 3/Filter 4, Filter 5/Filter 6, and Control A/Control B were 74%, 67%, 38%, and 60%, respectively. The overarching performance of filters lacking hydrocarbon compared to those associated with hydrocarbon is estimated at about 60%. Results also show that the W value for Filter 5/Filter 6 was the lowest among the others. It follows that even with a notable adverse impact of diesel contamination, Filter 5 performed slightly better than would have been expected. This result is comparable to that published by (Singh et al., 2012). However, such results need further investigation in order to achieve a higher level of performance for filters with, compared to those without, the influence of hydrocarbon.

5

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Experimental vertical-flow constructed wetland filters were used to examine the internal processes and effectiveness of different mature vertical-flow constructed wetland systems, (some subjected to shock loads of diesel spill contamination) in treating urban wastewater and to compare the impact of different design and operational variables on the treatment efficiency and clogging processes within each wetland bed. Furthermore, the experiment also assessed the potential for re-using the treated wastewater from diverse mature wetlands (with/without diesel contamination) in the irrigation of chillies. The overall results show that the vertical-flow wetlands with different design and operational variables are highly efficient for the treatment of petroleum hydrocarbon and other water quality variables. Clogging phenomena were not observed in any of the laboratory scale vertical-flow constructed wetlands after about five years of operation. The experiment also shows that chillies can be grown successfully using wastewater treated by constructed wetlands.

The key conclusions resulting from this research are summarized as follows:

1- All wetland systems had relatively high removal efficiencies for the main water quality parameters regardless of filter set-up and the period of diesel spill, which impeded plant development and led to poor water quality (except for nitrate-nitrogen (NO_3-N) used partly for biodegradation of diesel). The first experimental phase (start-up period) showed relatively high removal efficiencies of chemical oxygen demand (COD), ortho-phosphate-phosphorus (PO_4-P), and suspended solids (SS) in all wetland filters. The second experimental phase indicated highest removal efficiencies of COD, biochemical oxygen demand (BOD), ammonia-nitrogen (NH_4-N), PO_4-P, turbidity (TBD) and SS regardless of operational and design parameters. Findings in the third experimental phase showed compliance with secondary wastewater treatment standards was achieved by all wetlands regarding NH_4-N, NO_3-N and suspended solids, and non-compliance with those standards for BOD and PO_4-P. Higher COD inflow concentrations had a significantly positive impact on the treatment performance for COD, PO_4-P and SS. The wetland with the largest aggregate size had the lowest mean NO_3-N outflow concentration. Regarding the period after diesel spills, the filter with the highest COD loading but no diesel contamination performed the best in terms of COD and BOD removal. Filters contaminated by diesel performed worse in terms of COD and BOD, but considerably better regarding nitrate-nitrogen removal. Nitrate-nitrogen in some wetland filters (with/without diesel contamination) recorded negative values in their outflow concentration highlighting that the filters served as a source for NO_3-N.

2- Findings of seasonal treatment performance showed that COD, and NO_3-N have a seasonal trend with high removal in summer compared to other seasons, while BOD treatment was high in winter compared to summer. However, no clear seasonal trend of NH_4-N, PO_4-P, and SS treatment was observed. With regard to the period after diesel spills, it is difficult to estimate a clear seasonal treatment trend between contaminated filters. However, a clear reduction in pollutant concentration values was observed over time that reflected the mature vertical-flow wetlands with tidal-flow mode had well-established microbial population growth that can treat effectively even under cold seasons.

3- Serious clogging phenomena impacting negatively on the treatment performance and the hydraulic conductivity were not observed, which is surprising considering that the wastewater load was high and the filters can be regarded as mature. This reflects the high performance of VF CWs operated with intermittent mode in treating various contaminants (organic and inert) effectively over about five years without clogging. The proposed Wang-Scholz model to assess wetland filter clogging is simple, transparent and delivers good estimations for less complex filter operations. Modelling results were generally poor for the set-up period, adequate for the first two years after the set-up period and variable after the diesel spills. The model was never designed to deal with diesel spills, biological growth, and decaying of plant materials. However, modification was considered to include the impact of petroleum hydrocarbon compounds and maturity wetlands on increasing SS accumulated on the top of each wetland filter. Observed results were confirmed with modelled ones and indicated that wetland filters with small aggregate size, high contact time, and low inflow COD load were more efficient

in reducing SS accumulation within the filter depth. The Wang-Scholz model performed well for less complex operations.

4- The present study is the first to investigate vertical-flow constructed wetlands for the treatment of a high dosage of petroleum hydrocarbon compounds. Overall results showed that petroleum hydrocarbon compounds were highly degraded during the two periods of diesel spills and the concentration values (TPH, TAROM and TALPHA) were reduced with time in all contaminated filters. This indicates that vertical-flow constructed wetlands are an effective remediation technology for urban wastewater contaminated with a high dosage of diesel spills. The very high removal of the hydrocarbon contaminants at the wetland filter where aerobic conditions (by intermittent flow mode) prevailed, creating favourable conditions for microbial growth, implies biodegradation is a dominant removal process. Additionally, the high activity and degradation capability of microbial community results from prior exposure of the wetland filter to petroleum hydrocarbon contaminants. This confirms that during the initial time frame (first diesel spill period) the microbial community adapted, before the second, high diesel compounds dosage was applied. These factors provided an environment conducive for the rapid bioremediation of the petroleum hydrocarbon in the contaminated wetlands system. Furthermore, findings of the first diesel spill period showed that the diesel dose of 20 g/l led to cause temporal small toxic effects to wetland plant health. However, the plants recovered in all contaminated filters with time, highlighting that reed is tolerant to diesel contamination at low concentration levels (20 g/l), and can be considered as a potential plant which can be used for restoring the diesel-contaminated area. The second diesel spill (150 g/l) led to the death of all the plants in the selected filters,

reflecting the high toxicity impact of hydrocarbon compounds. However, the lack of wetland plants in the mature wetlands contaminated with diesel in the second period spill was found to not affect the long-term system treatment performance. The high treatment efficiency with the absence of plants in the selected wetland filters indicates that plant uptake provides a minor contribution to the observed hydrocarbon contaminants removal. Findings suggest that volatilization, and biodegradation are likely to be the main petroleum hydrocarbon removal mechanisms in the vertical-flow wetlands system.

5- Regarding comparison assessment between two treatment systems (mature VF CWs without diesel contamination and new artificial ponds) operated in parallel to treat urban wastewater, mature vertical-flow constructed wetlands were proven to be a successful treatment option for urban wastewater. The results of a five-year wetland monitoring campaign showed that mature systems greatly improved the outflow water quality for COD, BOD, NH_4-N and SS over time. Findings indicate the likely presence of both mature reeds and mature nitrification and denitrification communities within each wetland filter. After four years of operation, PO_4-P concentrations started to increase, which is an indication of the saturation of wetland media due to accumulation of pollutants. Moreover, the NO_3-N concentrations within the effluent were higher than those values linked to the influent. However, wetlands with a high loading rate led to a significant ($p<0.05$) improvement of the reduction of COD, BOD and SS over time if compared to other wetland designs.

Findings related to treatment comparisons between wetlands and ponds showed that COD and SS removals were significantly ($p<0.05$) higher in mature wetlands compared to ponds. With the exception of aerated ponds, NH_4-N and PO_4-P

removals were better in mature wetlands compared to ponds. These findings revealed a significantly ($p<0.05$) higher ability of the aerated ponds planted with reeds to remove NH_4-N and PO_4-P, if compared with other pond designs and mature wetlands. In the aerated ponds, dissolved oxygen concentration played an important role in the NH_4-N transformation processes to NO_3-N. Wetlands with high and low contact times were higher in BOD removal than ponds. For high loading rates, the BOD reduction was similar ($p>0.05$) in both wetlands and ponds planted with reeds.

6- Findings of recycling performance highlights, for the first time, the optimum environmental conditions for effective growth of the example fruiting vegetable chilli in greenhouses using urban wastewater pre-treated by mature vertical-flow wetlands. An encouraging solution has been successfully proposed to effectively treat and subsequently re-use domestic wastewater in a more sustainable way, particularly for water-constrained systems and climates, even when capital investment is low. Vertical-flow constructed wetlands subject to hydrocarbon contamination are associated with an encouraging treatment performance. However, the corresponding yields are rather low. Filters associated with a high loading rate release more nutrients into their effluents, which results in a greater marketable profit. This applies to both uncontaminated vertical-flow constructed wetlands and those with hydrocarbon contamination. Marketable yields were substantially higher for filters lacking hydrocarbon pollution. A subset of these wetlands, containing small aggregates and where the contact time and loading rate were low, provided good yields. In comparison, for wetlands subject to diesel spills, high yields of chillies in terms of economic return were linked to small aggregate size, high contact time, high loading rate and irrigation water based on

concentrated wastewater. Some findings presented in this research show a good agreement with what has recently been published in the literature. Regarding food contamination by poisonous elements, only slight zinc contamination was detected in harvested chillies for filter F8 (characterized predominantly by a low wetland resting time) based on European standards for vegetables. Furthermore, considering that the economic return for chillies irrigated with diesel-contaminated irrigation water is usually rather low, the author recommends not releasing the corresponding harvest to the market. The productivity of chillies was independent of the water consumption. In general, the first 8 months of the experiment showed the best growth of fruits for all plants. After that, the growth of fruits for plants receiving rain water and artificial grey water decreased gradually, possibly because of a lack of nutrients in these two types of water.

5.2 Recommendations

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

1- Long-term process assessments of water quality parameters in vertical-flow constructed wetlands treating wastewater subjected to different petroleum hydrocarbon one-off and regular dosages of diesel spills is recommended for further investigation. This could form a data base for an improved Wang-Scholz model and could help to assess the long lasting impacts of hydrocarbons on microbiological communities. Future research should also assess the relationship between clogging and substrate porosity variation, which is a function of parameters such as clogging by solids, and growth of roots and rhizomes.

However, such experimental investigations are destructive in nature and would require access to a much larger wetland system, so further research undertaken in field-scale conditions is recommended.

2- A further study is proposed to compare the capabilities of both treatment systems (VF CWs and artificial ponds) to meet the demand for a greater removal of pathogenic organisms compared to conventional treatment units. Other types of wastewater could also be applied to compare the efficiencies of both systems in removing pollutants.

3- Regarding recycling treated wastewater for crops irrigation, more long-term research is needed to understand the cumulative effects of pollutants on the chemical and biological properties of the soil and crop production. Further research to optimize nutrient and trace mineral provision using precision agriculture, which is, however, too inexpensive for most developing countries, is recommended. The role of top soil and bark in reducing pollutants could also be investigated. Finally, research on chilli fruit contamination by recycled treated domestic wastewater from constructed wetland systems should be performed at a field-scale to assess the impact of accumulated contaminants on the growth of chilli fruits and their productivity in terms of yield and economic return, and evaluate the ability of these accumulated pollutants to reach groundwater.

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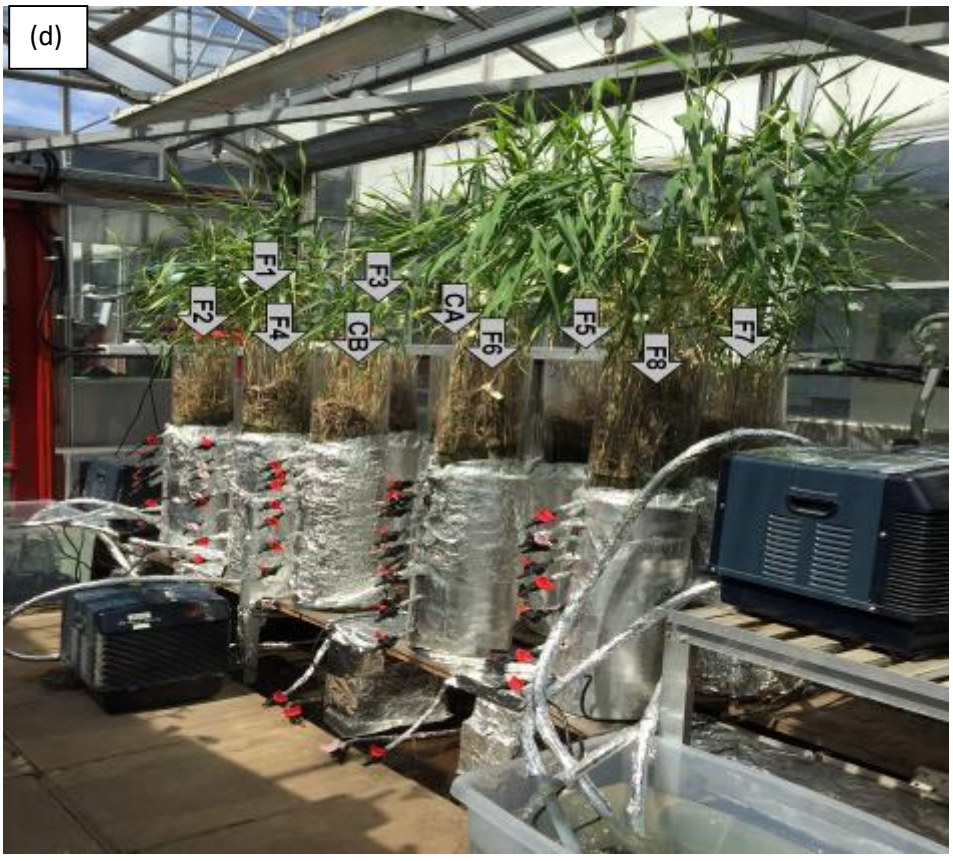
APPENDIX A

Experimental constructed wetlands pictures in different periods of operation

(a)

(b)


(c)
F1
F2
F3
F4
CA
CB
F5
F6
F7
F8


(d)
F1
F2
F3
F4
CA
CB
F5
F6
F7
F8

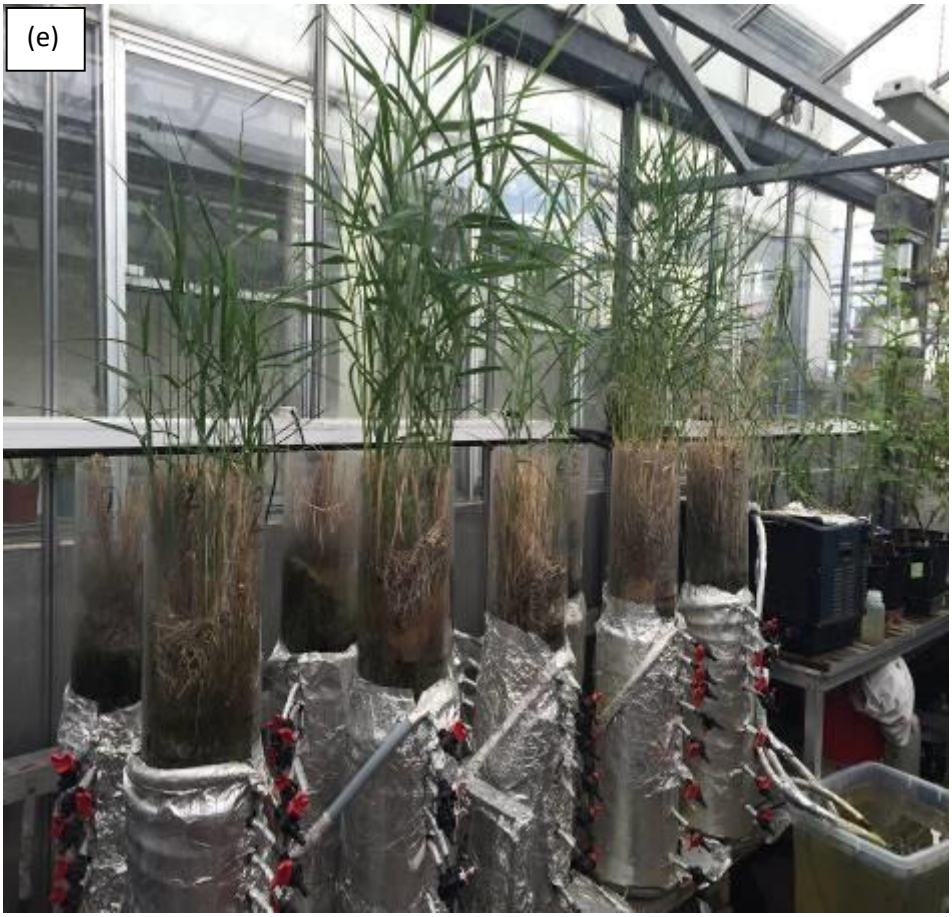


Figure A.1 Laboratory vertical-flow constructed wetlands in various operation periods (a) set-up period, (b) October 2011, (c) July 2013, (d) July 2015, and (e) May 2016.

APPENDIX B

Description of urban wastewater collection site

Urban wastewater is collected periodically from Davyhulme wastewater treatment and sewerage plant. Davyhulme site is the largest wastewater treatment works in North West England and one of the biggest in the UK, treating a flow rate up to 785 Ml/d and serving a population equivalent of 1.5 million. It is located in the Urmston area of Manchester, adjacent to the historic Manchester Ship Canal and within a stone's throw of the Trafford Centre (see satellite photo Figure B.1a). The wastewater is collected from a place that is located directly after preliminary treatment unit (Figure B.1b, photo from Google Earth shows the location point of wastewater collection). Wastewater is collected every week to ensure having fresh wastewater, the same as the wastewater in the treatment plant. During the period of wastewater storage, in order to achieve natural wastewater (i.e. the quality of wastewater being the same as the water flowing in the channels of treatment plant), aeration is provided by installing air pumps in a cool building (Peel Building, Salford University) to provide the wastewater with oxygen to keep the micro-organisms alive and ensure good activity in the wastewater. The Davyhulme wastewater is the catchment of various waters (of different types and volumes) which differ depending on human activities, urban runoff and weather conditions (seasons), therefore the pollutants concentration varies from time to time.

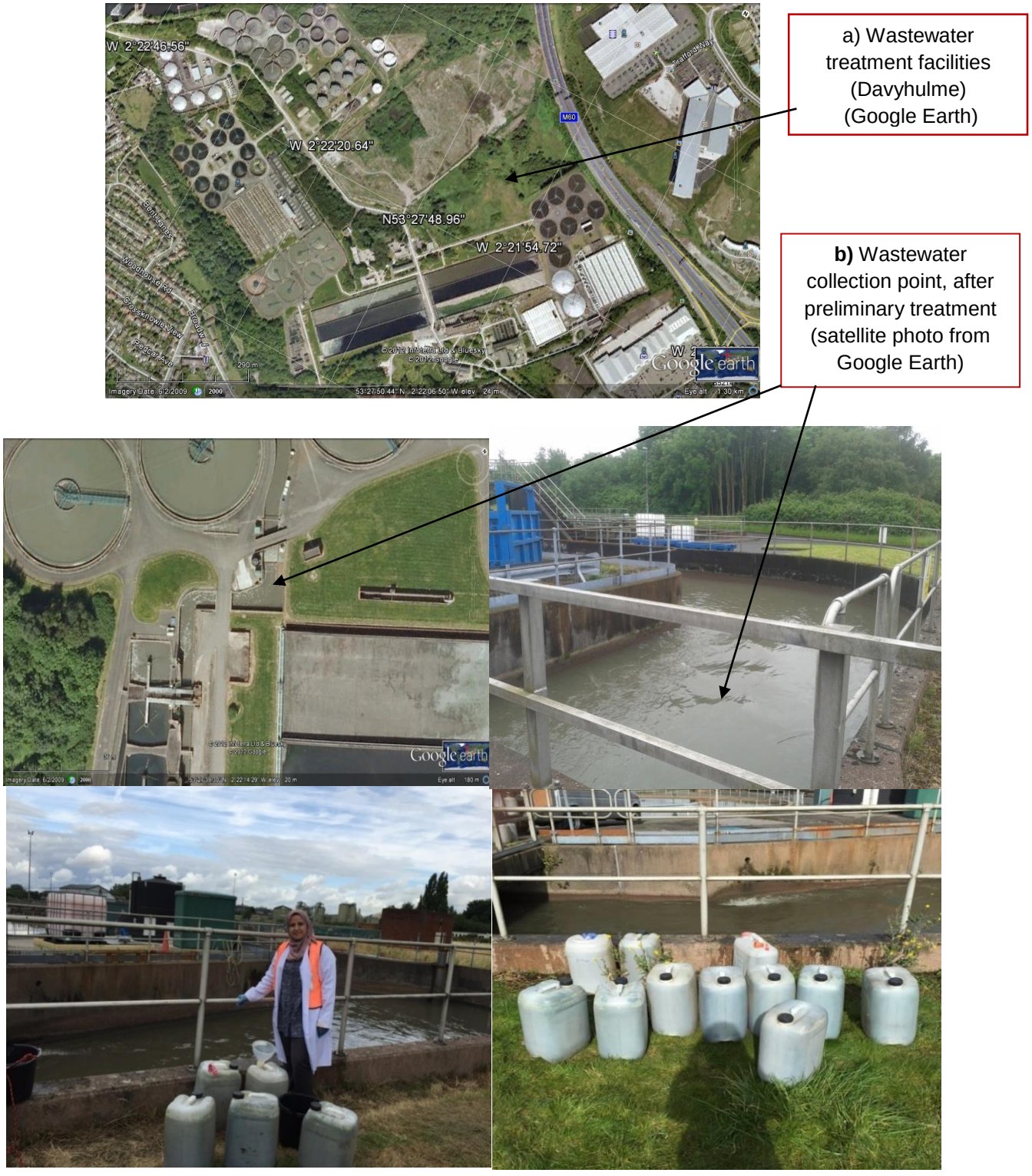


Figure B.1 Davyhulme wastewater and sewerage treatment plant, (a) Site facilities (Google Earth photo), (b) Location of wastewater collection point.

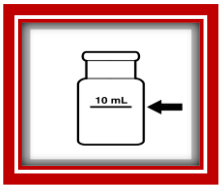
APPENDIX C

Water quality parameter measurements

Suspended solids (SS)

The spectrophotometer DR 2800 Hach Lange (www.hach.com) is used for measuring SS in the water sample. The measurement steps are explained as follows:

Firstly, select the test from main menu and stored programs, by clicking on suspended solids test. Then, take 500 ml of water sample and pour it in appropriate bottle and shake it for exactly two minutes. After that, the mixed sample is poured into a 600-ml beaker. To prepare the sample, pour 10 ml of the mixed sample into a sample cell.



For spectrophotometer calibration: zero the spectrophotometer by inserting the samples cell filled with drinking water and the press the READ button and the display will show: 0 mg/l TSS. Then, swirl the prepared sample to remove any gas bubbles and uniformly suspend any residue. Wipe and insert the sample which was prepared earlier into the cell holder and finally, press the READ button and the results will appear on the screen in mg/l TSS.

Turbidity (TBD)

Switch on the equipment for turbidity measurement and press **Start**. After that, shake 250 ml of water sample for 2 minutes. Finally, pour an amount from the shaken sample into a sample cell to the black mark and press **Read**, the display will show the turbidity value in NTU.

Chemical oxygen demand (COD):

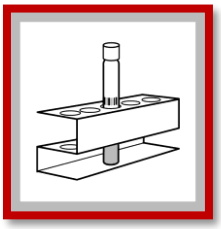
(LCK314 / COD cuvette test / measuring range 15-150 mg/l)

LCI400 / COD cuvette test / measuring range 0-1000 mg/l

The spectrophotometer DR 2800 Hach Lange (www.hach.com) is used for analysis of COD, and the DRB200 Reactor for digestion is used to heat the water sample.

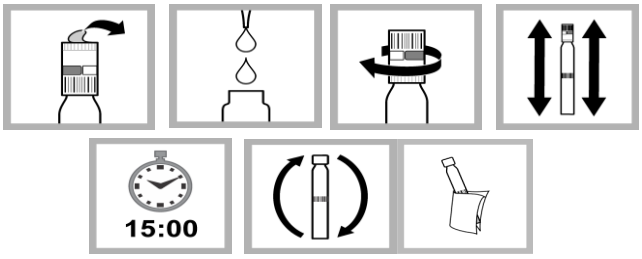
The first step for using the TNTplus, reactor digestion method, is to turn on the DRB200 Reactor to preheat it to 150 °C. To make sure that a representative portion of the sample is analysed, pour 250-ml of sample into an appropriate bottle and shake thoroughly. Secondly, use the pipette to fill 2.0 ml of the sample carefully into the reagent. After that, hold the vial from the head and shake it to ensure the chemicals are mixed and then place it in the reactor and close the protective lid.

Leave the vial in the reactor for two hours for heating. After that, turn off the reactor and shake the vial a few times while it is still hot, wait 20 minutes to cool the vials to 120 °C or less. Finally, clean the vial thoroughly and put it in the spectrophotometer instrument to read the barcode. Results will appear on the screen in mg/l COD.



Ammonia-Nitrogen (NH_4-N): by dimethylphenol method

(LCK303 / Ammonium cuvette test / measuring range 2.0-47 mg/l NH4-N)



Firstly, remove the lid carefully, from the DosiCap™ Zip cap and remove the cap from the test vial. Then use a pipette to add 0.2 ml of sample to the test vial. Immediately continue to the next step. Turn the DosiCap Zip over the test vial so that the reagent side goes on the vial. After that, tighten the cap on the vial. Secondly, shake the vial 2-3 times to dissolve the reagent in the cap. Then, look through the open end of the DosiCap to make sure that the reagent has dissolved. After that, start the reaction time of 15 minutes. When 15 minutes have passed, invert the vial 2-3 times. Finally, clean the vial and insert it into the cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/l NH_4. No instrument zero is required.

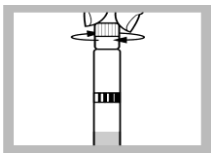
Nitrate-Nitrogen (NO_3-N): by dimethylphenol method

The spectrophotometer DR 2800 Hach Lange (www.hach.com) is used for analysis of NO_3-N. The first step is to pipette 1.0 ml of sample into the reagent vial. Then pipette 0.2 ml of Solution A into the vial. After that, the vial must be capped and shaken 2-3 times until no more streaks can be seen in the reaction tube solution and then wait for 15 minutes. After the 15 minutes, wipe the vial and place it into the cell holder. The instrument reads the barcode in mg/L NO_3–N. No zero of the instrument is required.

Orthophosphate-Phosphorous (PO_4-P)

(LCK049 / Phosphate (ortho) cuvette test / measuring range 1.6-30 mg/l PO4-P)

For the first step, pipette 5.0 ml of sample into the cuvette then close it and invert a few times. After that, wait for 10 minutes. Install the Light Shield if applicable and clean the outside of the vial and insert it into the cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/l PO_4. No instrument zero is required.



Biochemical oxygen demand (BOD):

Measurements are done by using the OxiTop® system

1. Estimate the measuring range of the sample to be analysed.
2. Before filling the overflow measuring flask, add all the additional solutions.
3. If required, add the nitrification inhibitor.
4. If necessary, seed the sample (caution: blank test determination!).
5. If necessary, add nutrient solutions, mineral solutions and buffer solutions (caution: Blank test determination!).
6. Take the selected volume of homogenized sample with the aid of the overflow measuring flask.
7. By means of a funnel, transfer the measured solution into the graduated measuring flask.
8. Insert a magnetic stirrer bar into the bottle.
9. Place 2 sodium hydroxide pellets in the rubber sleeve.
10. Insert the rubber sleeve onto the bottle. (Samples that come into contact with

sodium hydroxide can no longer be used for measurement.)

11. Screw on the OxiTop® measuring head tightly. The rubber sleeve ensures the necessary sealing of the system. (Do not use any sealing lubricant!)

12. Start the measurement on the OxiTop® head, or on the controller, if the OxiTop® C is used.

13. Place the graduated measuring flask in an incubator for five days at 20 °C.

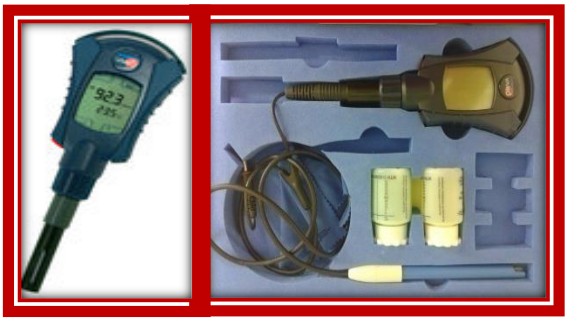
14. Read the results after five days.

pH (Hach sensION™+ MM374)

Basically, measuring pH consists of calibrating the instrument, placing the electrodes in a well-mixed sample, and then reading the pH directly from the pH meter.

The calibration of pH meter was carried out every 7 days and three buffers were used to calibrate the meter (buffer solution with a pH of 7.0, a buffer solution with a pH of 4.0, and a buffer solution with a pH of 10.0). During calibration, we placed the electrode in a series of buffer solutions and set the meter to those values. The next step after the meter calibration is preparing the water sample which includes filling the sample water in an appropriate cup then putting the magnetic stirrer in the sample water and turning on the stirrer to ensure that the sample is well mixed. The final step is placing the electrode in the sample with continued stirring of the sample as the pH is measured by the meter.

Redox potential (mV)



The redox potential measurement is made by inserting the probe of the meter into the sample to be measured. The resulting potential is read directly in millivolts from the meter screen. The probes with measurement beaker (and all glassware used in this procedure) were cleaned before the first run, and after each sampling run, with deionized water.

Electrical conductivity (EC)

This is a measurement of the conductive material in the water sample.

It is measured with a probe and a meter. A voltage is applied between the two electrodes in the probe immersed in the sample water. By inserting the probe in a sufficient water sample, the meter will read the conductivity in micro- Siemens per centimetre.

Dissolved oxygen (DO)

DO is measured by the rate of consumption of oxygen at the tip of the probe of a DO meter.

The first step is preparing the meter by pressing the ON/OFF button to turn on the meter then the

instrument will be activated after a few seconds. The second step is preparing the sample which includes inserting the black probe of the instrument in the water sample container, using the tip of the probe to make continual movement of water in the sample container while ensuring the probe tip is submerged. Finally, after waiting a few seconds for the water sample to be stable, the DO concentration can be read in mg/l.

APPENDIX D

Petroleum hydrocarbon measurements

The first step for hydrocarbon measurements is preparing the water samples. Glass bottles (one-litre capacity), were filled with the effluents from wetland filters by opening the main valve in the bottom of each filter and releasing outflow water. Also one sample was taken from the inflow to analyse the hydrocarbon removal efficiency for the wetland system and another bottle was filled with raw diesel for comparison with all filters (Figure D.1). Moreover, glass vials were also, filled with water samples and used to measure volatile hydrocarbon components. The bottles of samples with their vials were kept in a cool box provided with ice before transfer to the Exova Lab for analysis.

Figure D.1 Wastewater collection for hydrocarbon analysis

The following procedure for hydrocarbon measurements which be used by Exova Hillington Lab (Mc Eleny et al., 2013):

1.0 SCOPE AND FIELD OF APPLICATION

1.1 This method is applicable to the determination of Total Extractable Petroleum Hydrocarbons in waters in the carbon range C8-C44. Results can be reported in various styles including TPH Speciation (GRO C8-C10, DRO C10-C28, and MRO C28-C44) or TPH Banded (bands as requested by client analysed from a total extraction).

1.2 The method also permits the determination of the aliphatic and aromatic hydrocarbon fractions in the samples in the ranges C8-C10, >C10-C12, >C12-C16, >C16-C21, >C21-C35 and >C35-C44 inclusive, based on carbon number. This method when used in conjunction with a method giving values for aliphatics and aromatics in the C5-C8 band may be used to report Total Petroleum Hydrocarbon (CWG) values.

1.3 TPHs are extracted from liquids into Pentane with vigorous shaking. Water is removed from the system by the inclusion of anhydrous sodium sulphate during the shaking step.

1.4 Using a GC-FID the hydrocarbons in the sample can be separated according to size as related to carbon number. By looking at the peak areas the quantities of extractable petroleum hydrocarbon material in each of these bands can be determined.

1.5 The samples should be tested within 7 days of sampling date. If not the sample will be classed as ‘deviating’.

1.6 Accreditation to ISO17025 is pending for the for extractable petroleum hydrocabons only.

1.7 The aliphatic/aromatic process is not accredited.

2.0 NORMATIVE REFERENCES

2.2 SOP007 – Technical reporting

2.3 SP003 – Sample Handling

3.0 TERMS AND DEFINITIONS

3.1 Petroleum hydrocarbons may be defined as hydrocarbons derived from the processing of crude oil (petroleum). The refining process produces mixtures of hydrocarbons with a variety of boiling ranges with the different fractions having

different uses, e.g. petrol, aviation fuel, motor oil, etc.

3.2 The term 'mineral oil' is sometimes encountered and may be used (or misused) to indicate a product of a particular type, generally in the diesel oil/lubricating oil carbon range. Generically, however, any hydrocarbon mixture from a 'mineral' source (i.e. taken from the ground) can be regarded as a mineral oil and this being the case the term can be confusing.

3.3 To avoid this confusion the target hydrocarbons may be banded according to carbon number with C6 to C10 being classed as gasoline range hydrocarbons (GROs), >C10 to C28 being the Diesel Range Organics (DROs) and the group >C28 to C44 being classed as Mineral Range Organics (MROs).

3.4 The current method bands the hydrocarbon fractions as follows:

Aliphatics - C8-C10, >C10- C12, >C12-C16, >C16-C21, >C21-C35 and >C35-C44 inclusive

Aromatics - C8-C10, >C10-C12, >C12-C16, >C16-C21, >C21-C35 and >C35-C44 inclusive

4.0 HEALTH AND SAFETY

4.1 It is the policy of Exova to provide and maintain a safe and healthy working environment. All laboratory practices will be carried out in accordance with guidelines laid down in the Exova Health and Safety Manual.

4.2 The 'Hazardous Substance' and 'Risk Assessment' information is available in the Managers office.

5.0 PROCEDURE

5.1 Equipment

5.1.1 Gas chromatograph with flame ionisation detection with suitable data collection and handling software. An Thermo Finnigan Trace GC operating with Chromecard software has been shown to be suitable. Other systems may be used if similar performance can be demonstrated.

5.1.2 Zebron inferno 15 m x 0.32 mm GC column with 0.1 µm film thickness. Other columns may be used if a similar performance can be demonstrated.

5.1.3 Turbovap sample concentrator at 45 ºC.

5.1.4 Turbovap tubes.

5.1.5 Glass SPE tubes, 8ml.

5.1.6 Frits – Chromabond Filters for glass columns – 730192.

5.1.7 2 ml sample vials with crimp caps.

5.1.8 Bottle top dispenser suitable for use with organic solvents and capable of accurately dispensing 20 ml.

5.1.9 Glass filter funnels.

5.1.10 Filter Papers, Munktell Grade 12/N or equivalent.

5.1.11 Glass syringes capable of accurately dispensing from 2 µl to 1000µl.

5.1.12 Balance capable of accurately weighing to 1 decimal place.

5.1.13 Volumetric flasks, various sizes, Grade B or better.

5.2 Reagents

5.2.1 All reagents should be labelled with preparation date, expiry date and initials of the person who prepared it.

5.2.2 n-pentane, Hipersolv grade or better.

5.2.3 Acetone, GPR grade or better.

5.2.4 Dichloromethane (DCM), GPR grade or better.

5.2.5 Granular anhydrous sodium sulphate powder, GPR grade or better.

5.2.6 Silica gel 60 (0.063-0.200 mm).

5.2.7 Activated Alumina, Brockman 1, Standard Grade, approx 150 mesh, 58 Å.

5.2.8 Compressed Nitrogen.

5.3 Standard Preparation

5.3.1 *Internal Standard/Surrogate*

5.3.1.1 n-Heneicosane standard to be purchased from VWR (Cat No. A18198) and logged in to the appropriate standard receipt log.

5.3.1.2 From this stock a 2500 mg/l working solution is required.

5.3.1.3 Weigh 0.25 g of the heneicosane and make up to 100 ml with pentane in a volumetric flask.

5.3.1.4 Preparation of this standard should be recorded and an expiry date of 1 year and ID noted on the flask. This standard should be stored in the fridge when not in use.

5.3.2 *TPH Calibration Standard*

5.3.2.1 This is a combined standard made up of diesel fuel and motor oil.

5.3.2.2 Diesel Fuel #2 Composite Standard – 50000 mg/l standard should be purchased from Thames Restek (Cat No. 31259). This comes as a 5ml ampule

5.3.2.3 Motor Oil Composite Standard – 50000 mg/l standard should be purchased from Thames Restek (Cat No. 31464) this comes as a 1ml ampule, 5ml is required for making the working standard.

5.3.2.4 A 10000 mg/l TPH Cal Mix is required (i.e. 5000 mg/l diesel and 5000 mg/l motor oil).

5.3.2.5 Take 5 ml of 50000 mg/l diesel fuel standard and 5ml of 50000 mg/l motor oil standard and make up to 50 ml in a volumetric flask with pentane.

5.3.2.6 This combined working mix has a 12-month expiry and should be labelled accordingly, standard prep sheets filled out and stored in the fridge when not in use.

5.3.2.7 A carbon marker solution ranging from C8 – C44 should be run with each calibration.

Working TPH Calibration (Freshly Prepared at Time of Use)

5.3.2.7 A 6-point calibration range should be running at least once a week.

5.3.2.8 Using the 10000 mg/l combined calibration stock, prepare the calibration concentrations below:

Calibration Level (mg/l)	Volume TPH Cal Mix (µl)	Volume Pentane (µl)
1000	100	900
500	50	950
250	25	975
100	10	990
25	2.5	997.5
0	0	1000

5.3.2.9 To each of these calibration vials add 10 µl of 2500 mg/l internal standard (section 5.3.1).

5.3.2.10 Once ran these vials can be discarded.

5.3.3 *Calibration Check Standard (CCS)*

5.3.3.1 This standard is required to be analysed at the start and end of every run to check the system is still performing suitably. The result must be

recorded on the QC spreadsheet and be within +/-15% of the prepared concentration.

5.3.3.2 For this prepare a 250 mg/l CCS using the 10000 mg/l TPH Cal Mix as below:

CCS (mg/l)	Volume TPH Cal Mix (µl)	Volume Pentane(µl)
250	25	975

5.3.3.3 To this add 10 µl of 2500 mg/l internal standard.

5.3.4 *AQC Standard*

5.3.4.1 A 45,000 mg/l standard should be produced from commercial diesel and gear oil EP90 or equivalent mineral oil.

5.3.4.2 Weigh 1.5 g (+/-0.001) of commercial diesel and 3g (+/-0.001) of gear oil and making up to 100 ml with pentane in a volumetric flask.

5.3.4.3 This solution should be labelled and kept in the fridge at <8 °C when not in use. Standard production sheets should be prepared at time of preparation.

5.3.4.4 This solution is stable for 1 year.

5.3.5 Florida TPH standard (500 ppm) - Alkane standard mix.

A reference mixture of straight chain aliphatic hydrocarbons in solution is used to determine the limits of the bands. The final concentration of the mixture injected into the GC should be around 20 ppm with any necessary dilutions being made in n-hexane. Store at <8 ºC for up to 1 year.

5.4 Sample Preparation

5.4.1 *Extraction procedure*

The extraction of water samples is dependent both upon the nature of the samples and on whether or not a dedicated sample has been supplied by the client.

(a) Waters with little or no sediment

The entire contents of the bottle are used where possible.

(b) Waters with some sediment

Allow the sample to settle, then transfer an appropriate volume to the separating funnel for extraction.

If the entire contents of the sample bottle are to be extracted, weigh the sample bottle and contents to the nearest 1g before extraction. Record all weights on the appropriate laboratory worksheets. If the entire contents are not to be used, weigh back the bottle and subtract from the initial weight.

If high analyte concentrations are anticipated, a smaller sample volume may be taken and diluted to 1 liter with DI water, or samples may be collected in smaller sample bottles and the whole sample used.

It should be noted that the preferred method shall be to use the entire sample for extraction.

5.4.1.1 Transfer the sample from the sample bottle to the separating funnel, reweigh the bottle and record the weight on the appropriate laboratory worksheet. The sample volume can then be determined by difference (assume density of water 1.00 g/ml).

5.4.1.2 Add 25 ml of pentane then seal and shake the separating funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.

5.4.1.3 Allow the organic layer to separate from the water phase for between 2 and 10 minutes depending on the nature of the sample. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample and may include stirring, filtration of the emulsion through glass wool, centrifugation, ultrasonic bath or other physical methods. Collect the solvent extract in a 100 ml glass measuring cylinder.

5.4.1.4 Repeat the extraction using a fresh portion of solvent. Combine the two solvent extracts in the measuring cylinder.

5.4.1.5 The extract is now ready for concentration, and analysis. Excess water present in the extract may be removed by filtering the extract through

a bed of anhydrous sodium sulphate. If drying is carried out, the whole sample must be dried.

5.4.1.6 Transfer the extract to a Zymark (turbovap) concentration tube. Rinse the measuring cylinder, which contained the solvent extract, with a suitable volume of extract solvent and add it to the concentration tube to complete the quantitative transfer.

5.4.1.7 Perform the concentration using the Turbovap II until the volume of the extract is less than 2 ml.

5.4.1.8 Transfer the extract to a 5 ml measuring cylinder. Wash the side of the concentrator tube with pentane and gently pipette the washings into the measuring cylinder up to 4ml sample. Transfer to a labelled 5 ml snap cap vial.

5.4.1.9 <u>Total TPH</u>

Transfer 1 ml aliquot into a 2 ml GC vial. Add 10 ul of internal standard. Crimp the vial.

<u>Aromatic / aliphatic banding (unaccredited)</u>

Clean aliphatic portion by putting extract through an activated florisil column (minimum 5 mm). Transfer 1ml aliquot into a 2 ml GC vial. Add 10 ul of internal standard. Crimp the vial.

5.4.1.10 the extract may now be analysed for TPH.

5.4.2 *Extraction Blank*

5.4.2.1 For every 20 samples at least 1 blank should be extracted.

5.4.2.2 Measure approx. 950 ml of tap water and extract as steps 5.4.1.3 to 5.4.1.9 above.

5.4.2.3 The concentration in the blank should be <1 mg/l.

5.4.3 *AQC – Matrix Spike (Total TPH, TPH Speciated and TPH Banded)*

5.4.3.1 For every 20 samples at least 1 AQC matrix spike should be extracted.

5.4.3.2 Measure approx. 950 ml of tap water.

5.4.3.3 Using a calibrated syringe add 80 ul of 45,000 mg/l diesel/gear oil standard (section 5.3.4.) to the water.

5.4.3.4 Follow sections 5.4.1.3 to 5.4.1.9 above.

5.4.4 *AQC – Matrix Spike (Aliphatic/Aromatic Splits)*

5.4.4.1 In each batch a split check sample (5.2.14) is run. This sample is not extracted but is treated as an extract during splitting. No more than 10% of the components of the FTPH mix should be seen in the DCM extracted fraction of the split and no more than 10% of PAHs should be apparent in the hexane extracted fraction. If there is evidence that these limits have been exceeded then the section supervisor must be informed and the splits for all samples in this batch must be repeated.

5.5 Aliphatic Aromatic Split

5.5.1 Columns for splitting the aliphatic fraction from the aromatic fraction of the sample are prepared in 8ml glass SPE tubes mounted on SPE tank.

5.5.2 To the SPE tube add a frit, pushing it to the bottom of the tube and tamping down gently.

5.5.3 Add 1cm depth of activated silica to the column, followed by 0.25 cm of activated alumina. NB. Silica must be freshly activated on the day of use.

5.5.4 Wash the columns with 4 ml of DCM followed by 4 ml of hexane, adding the solvents in 1ml aliquots and allowing each volume to run into the column before adding the next.

5.5.5 Place clean 30 ml glass vials into tank underneath each SPE tube.

5.5.6 Using glass Pasteur pipettes transfer the contents of the Turbovap tubes to the tops of the splitting columns, allowing the samples to run into the columns and leaving for between 30 s and 1min before proceeding.

5.5.7 Add, in 1 ml aliquots, 3 mls of hexane to the top of the column, allowing each to run fully into the 30 ml vial before adding the next. This step washes the aliphatic portion of the sample into the Turbovap tube and care must be taken at this stage as too much hexane will carry some of the aromatic fraction into the vial whilst too little will result in some of the aliphatic portion remaining on the column.

5.5.8 When all the hexane has run through the SPE tubes, transfer the extract in the vials to clean Turbovap tubes rinsing the 30 ml vials with a small amount of

hexane.

5.5.9 Place fresh 30 ml vials below the SPE tubes. Add, in 1ml aliquots, 4 mls of DCM to the top of each SPE tube, allowing each aliquot to run fully into the column before adding the next. This step washes the aromatic fraction from the column into the collecting vials.

5.5.10 When all the DCM has run through the SPE tubes, set them aside & store for refilling & re-use.

5.5.11 Transfer DCM eluted aromatic fraction to Turbovap tubes, rinsing the 30ml vials with a small amount of DCM.

5.5.12 Concentrate the samples down to approx 0.5 ml under flowing nitrogen on a Turbovap at 45 ºC, washing down the sides of the tubes once with a small volume of DCM as the volume decreases towards 1ml.

5.5.13 Using a calibrated 1ml syringe, wash the sample down the sides of the Turbovap tube with a small amount of DCM and make up to the 1ml mark on syringe. Transfer to a 2 ml autosampler vial and cap immediately.

5.5.14 Samples should be stored at <8 ºC.

5.5.15 Repeat 5.6.12 to 5.6.13 for the aliphatic fractions substituting DCM with hexane.

5.6 Instrument Conditions

5.6.1 See appendix 1 for current GC running conditions

5.6.2 These conditions are subject to change and the appendix should be updated accordingly when required.

5.6.3 The GC is a duel column instrument and when required both columns can be installed and ran using the conditions outlined in appendix one. Both columns should be independently calibrated when being used.

5.7 Calibration

5.7.1 A full calibration should be ran weekly as outlined in section 5.3.2.

5.7.2 The total TPH area and internal standard area should be integrated and the responses entered into the current controlled calculations spreadsheet.

5.7.3 The R^2 value of this calibration should be >0.995 in order for the calibration to be acceptable.

5.7.4 The calculation for the calibration is as follows:

Total TPH area of standard x Mean of Internal Standard of Calibration

Internal Standard Area of Standard

5.7.5 This calculation spreadsheet should be saved with the current date for use throughout the week.

5.8 CCS Monitoring and System Suitability

5.8.1 A calibration check standard should be running at the start and end of each run to obtain that the system is working under suitable conditions and the calibration has not drifted.

5.8.2 CCS samples should be prepared as outlined in section 5.3.3.

5.8.3 System suitability should be carried out on both CCS samples for every run and must meet the following parameters:

- Internal Standard Symmetry <2
- Internal Standard Area as per current control chart limits
- Result in mg/l 212.5-287.5 (15%)

5.8.4 If any of the above parameters fail an investigation into the instrument working conditions must be carried out and maintenance carried out if necessary.

5.8.5 A fresh full calibration should be running and samples re-ran before reporting.

5.8.6 Any areas for concern should be raised to the section head as soon as possible.

5.9 AQC Matrix Spike Monitoring

5.9.1 1 AQC should be extracted for every 20 samples as outlined in section 5.3.4.

5.9.2 AQC spike recovery values should be recorded on the appropriate control chart and be within the set acceptable limits before results of sample can be accepted.

5.9.3 If any failures occur analysis should be stopped and the situation investigated by looking at the following areas:

- Spike stock used was in date.
- Syringes used where within calibration
- The corrected spike volume was added to sample
- Instrument condition is suitable

5.9.4 Once all this has been checked the whole run should be repeated.

5.9.5 If the problem persists the section head should be informed and a full investigation carried out before any more analysis is carried out.

5.10 Results

5.10.1 The chromatograms are for each sample are integrated based on client requirements and reported as such (section 1.1).

5.10.2 The area for each chromatogram along with the area of internal standard is entered into the current calibration calculation spreadsheet and the concentration in mg/l automatically calculated taking into account any dilutions that have been done on the sample.

5.10.3 The CCS and AQC results should also be entered into this spreadsheet and the results checked by another analyst before being entered into LIMs.

5.11 Uncertainty, Precision, Bias and LOD

5.11.1 The uncertainty, precision, bias was calculated using data from QC chart from 21/05/13 to 14/08/13. Limit of detection is currently being determined.

Uncertainty % (k=2)	% RSD	%Bias	LOD (mg/l)	Reporting Limit (mg/l)
26	12.4	-20.6		0.01

6.0 REVISION HISTORY

Rev	Reason for revision	Reviewed by	Date
0	First issue	A Hardie G Greene	02 August 2004
1	Validation data removed to validation folders.	G Greene	02 August 2006
2	Review date extended by 12 months to enable transition from weekly QC to per batch QC. Per batch AQC added	AJ /DT	11 September 2006
3	Comprehensive review by DT/AJ. Sample extraction added to the method	AJ	08/03/11
4	Full review of method. Updated to reflect format of soil method AN51as calibration ranges etc are now the same		01/08/13

GC Conditions

The following chromatographic conditions have been found to be suitable. Other conditions may be substituted if it is found that performance is equivalent or better:

Carrier pressure (helium): 75 ml/min

Make up flow (nitrogen): 30 ml/min

Hydrogen flow: 40 ml/min

Air flow: 400 ml/min

Injector temperature: 280 ^{o}C

Detector temperature: 300^{o}C

Programme: 47 ^{o}C hold for 1 min

Ramp 1 - 27.5 ^{o}C/min to 100 ^{o}C/min, hold for 0 mins

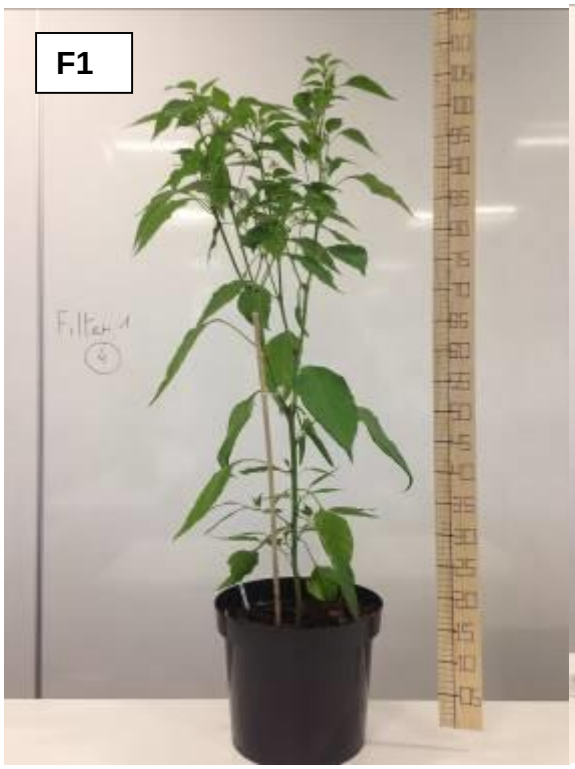
Ramp 2 – 37 ^{o}C/min to 350 ^{o}C/min, hold for 2 mins

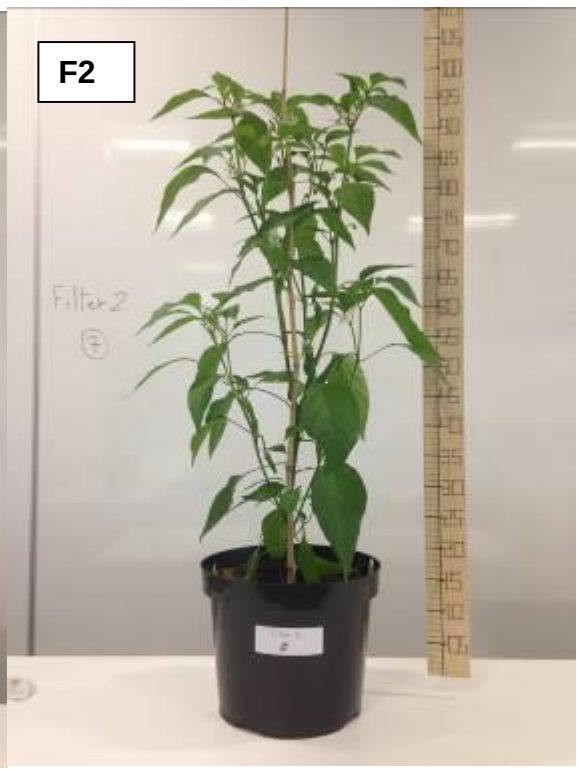
Split ratio: 2

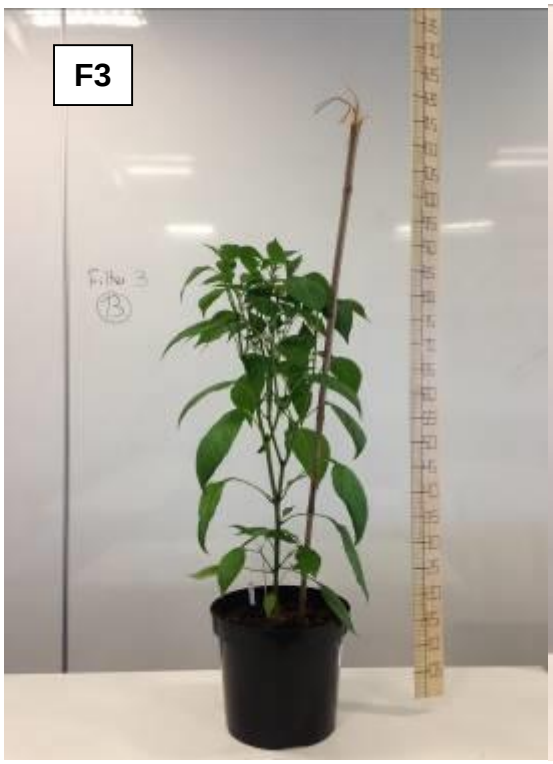
Injection volume: 1 μl

APPENDIX E

Experimental chilli plant photographs in different periods of plant growth



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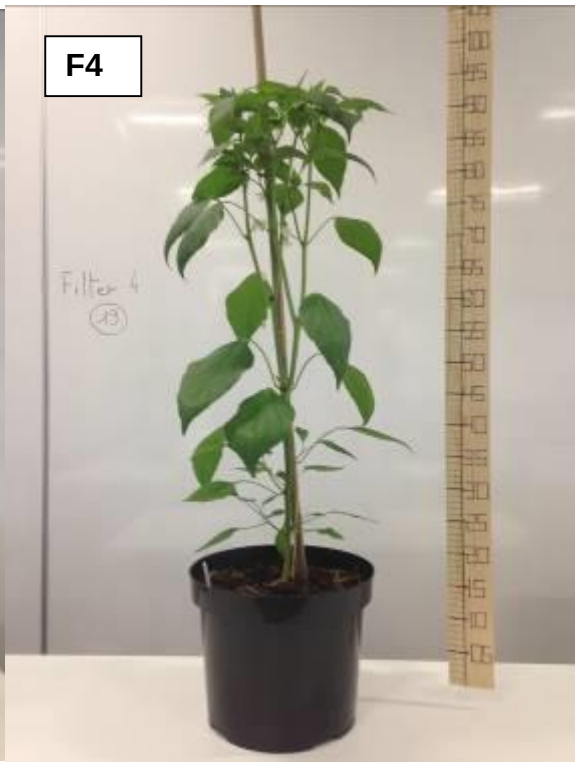
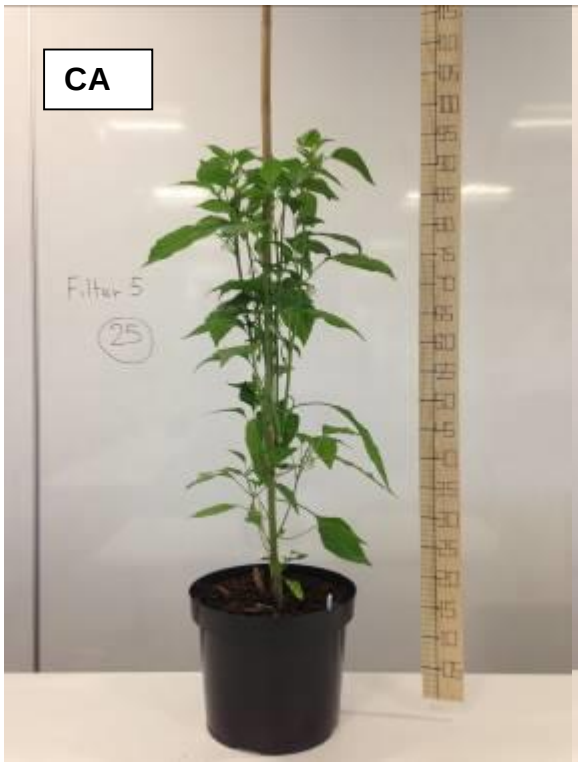
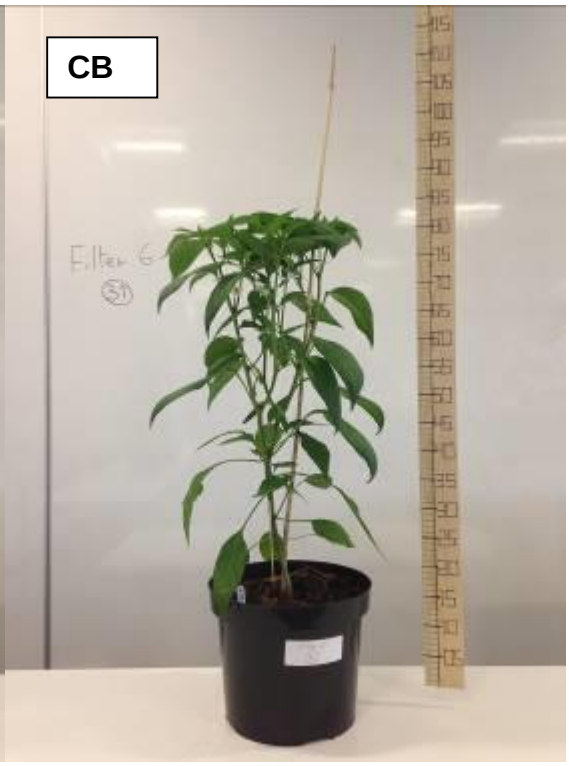
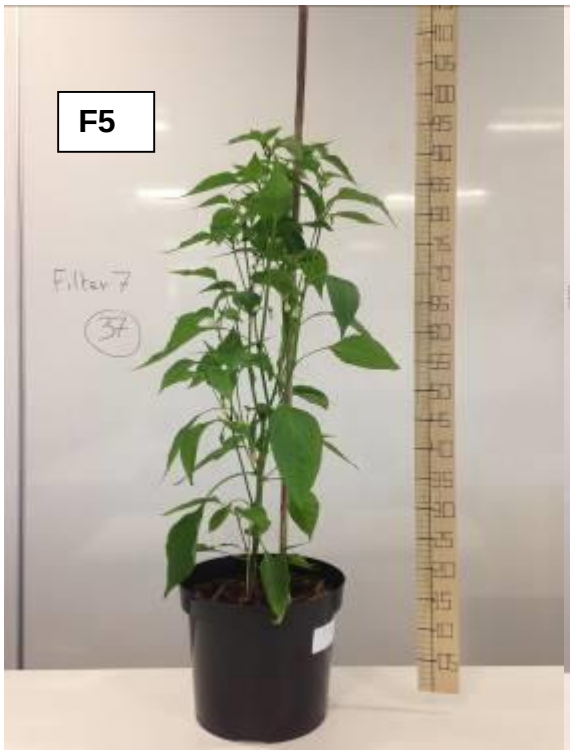
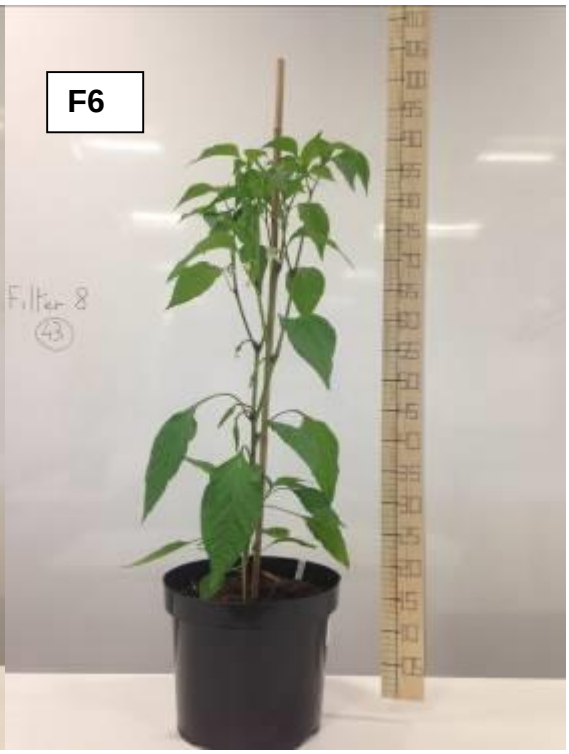
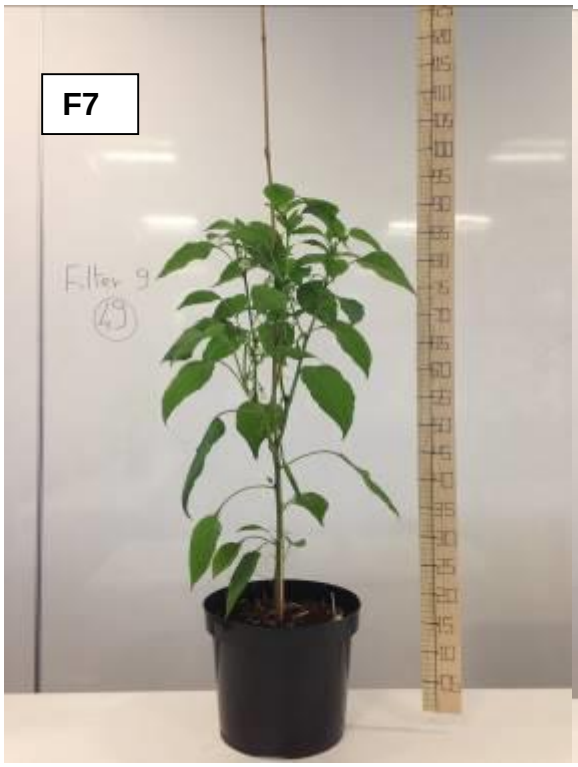

F4

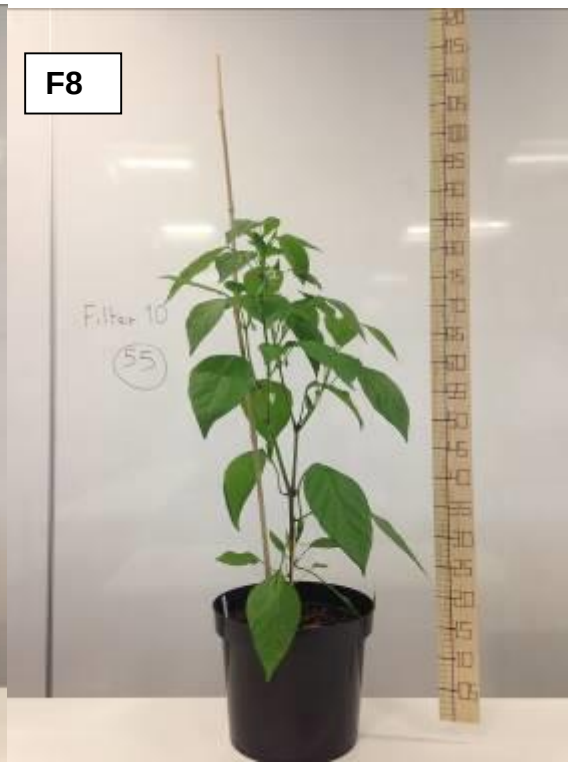

CA

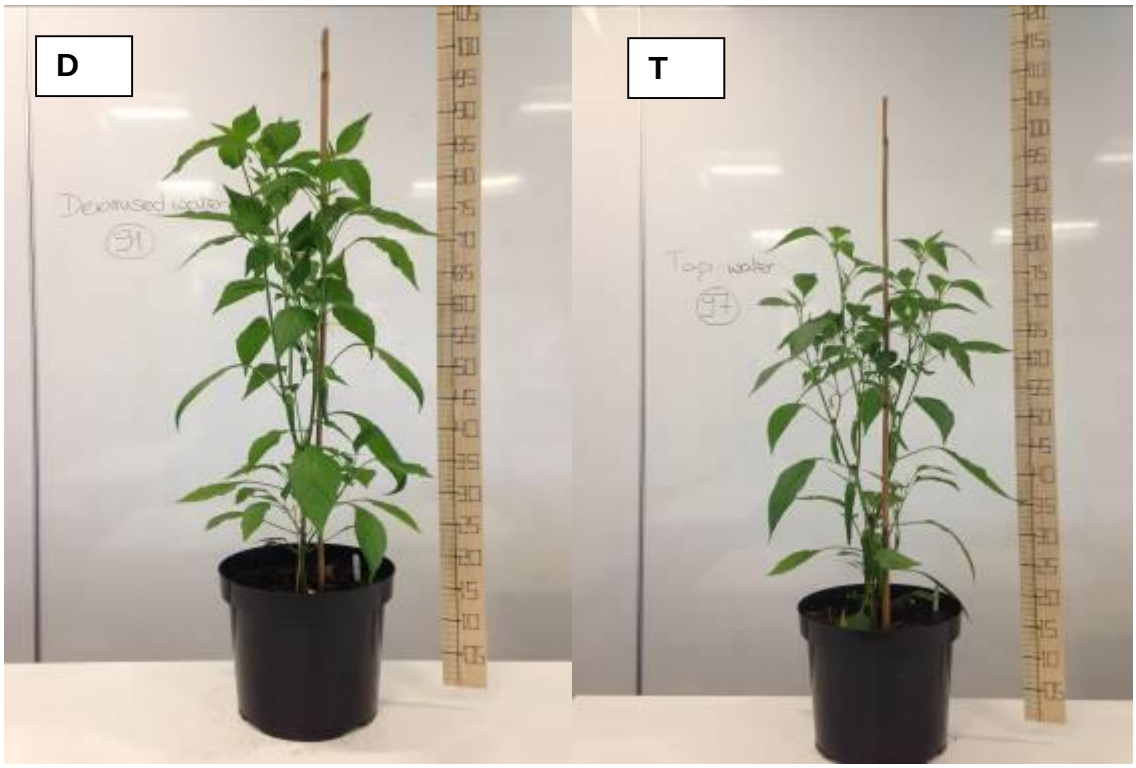

CB

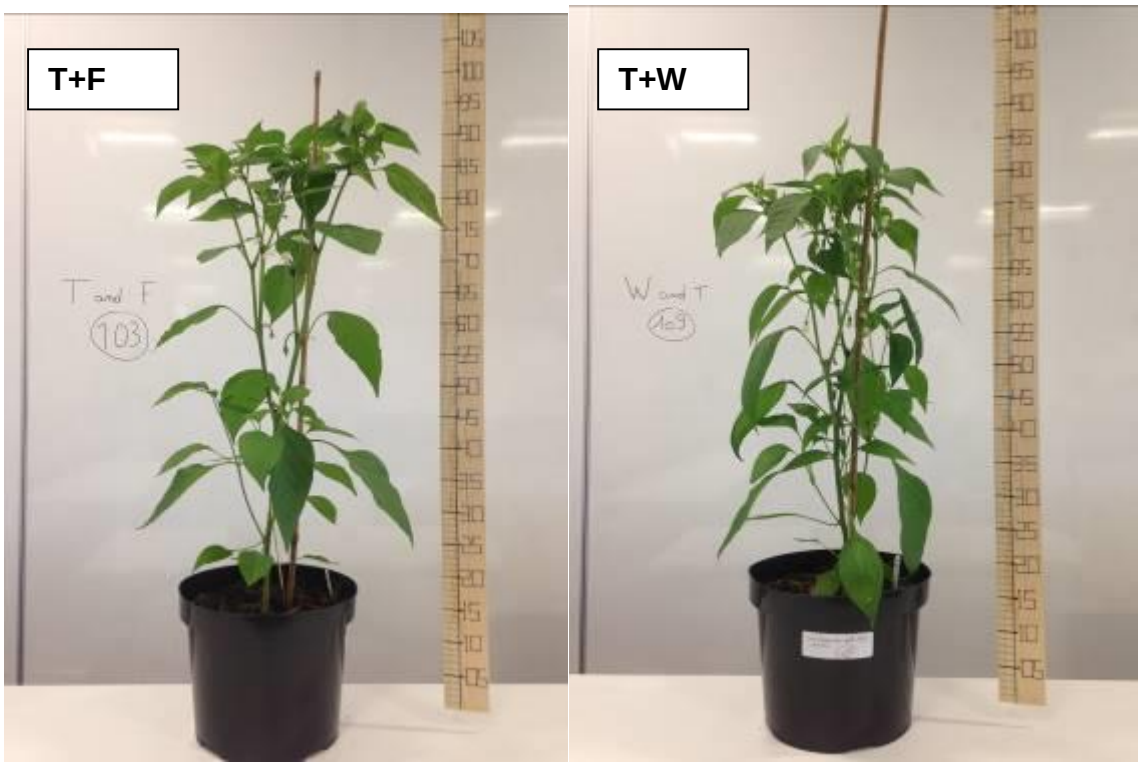

F5
Filter 7
37

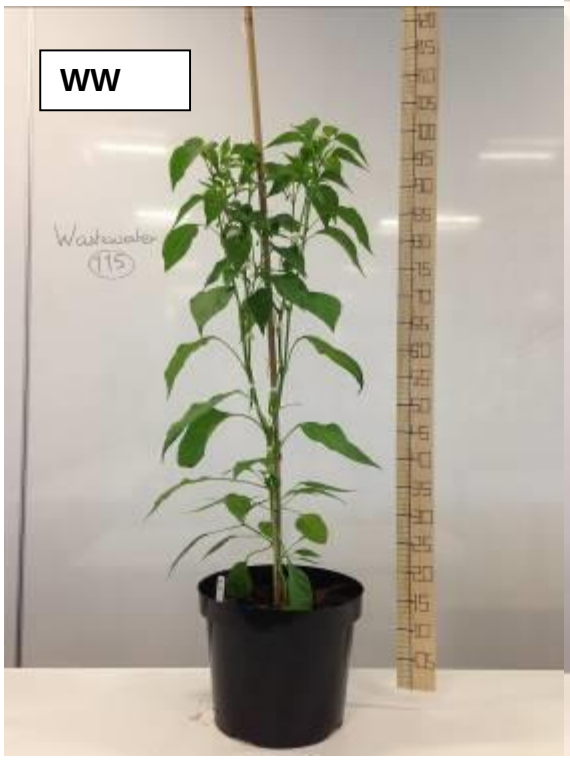

F6
Filter 8
43

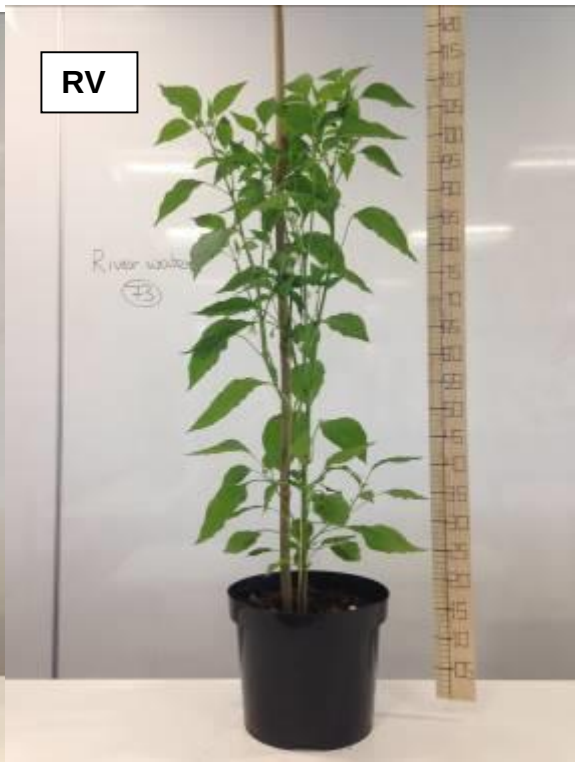

F7
Filter 9
49

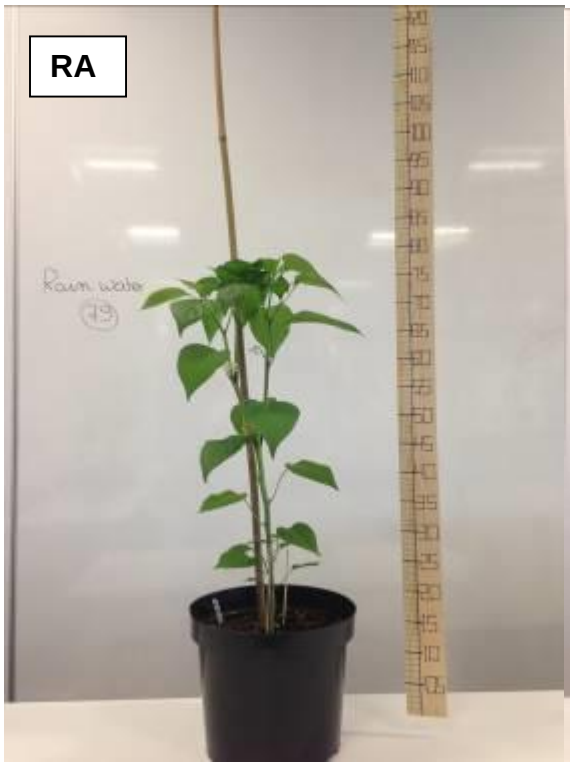

F8
Filter 10
55

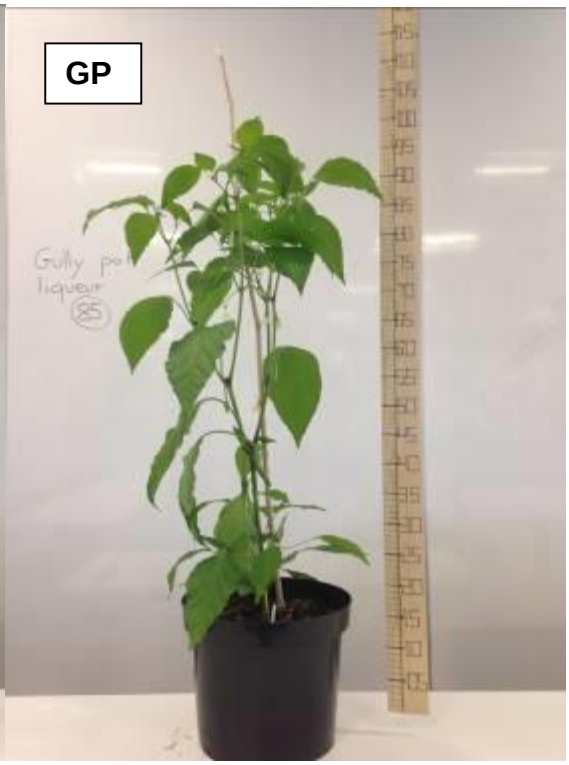

D
T

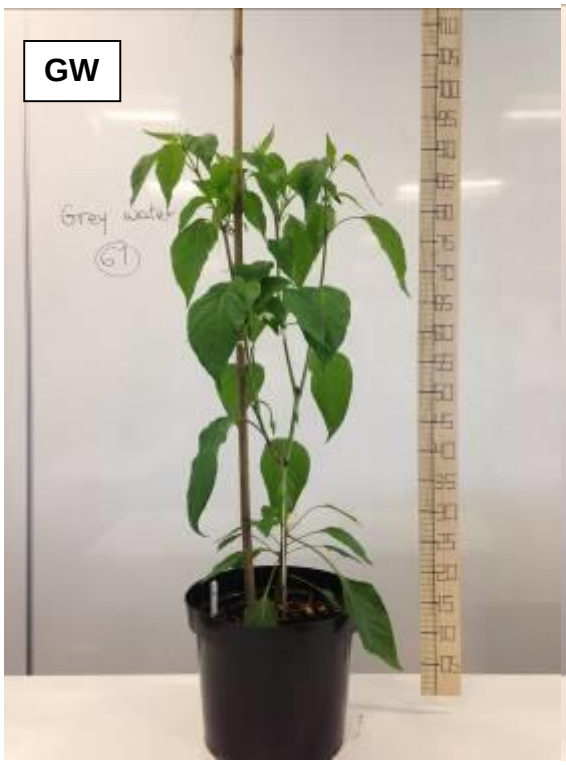

T+F
T+W

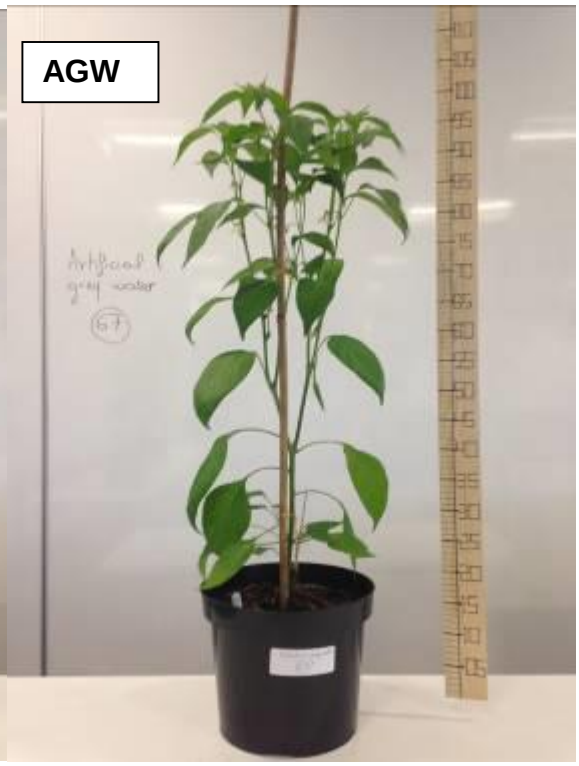

WW
Wastewater
175


RV
River water
73

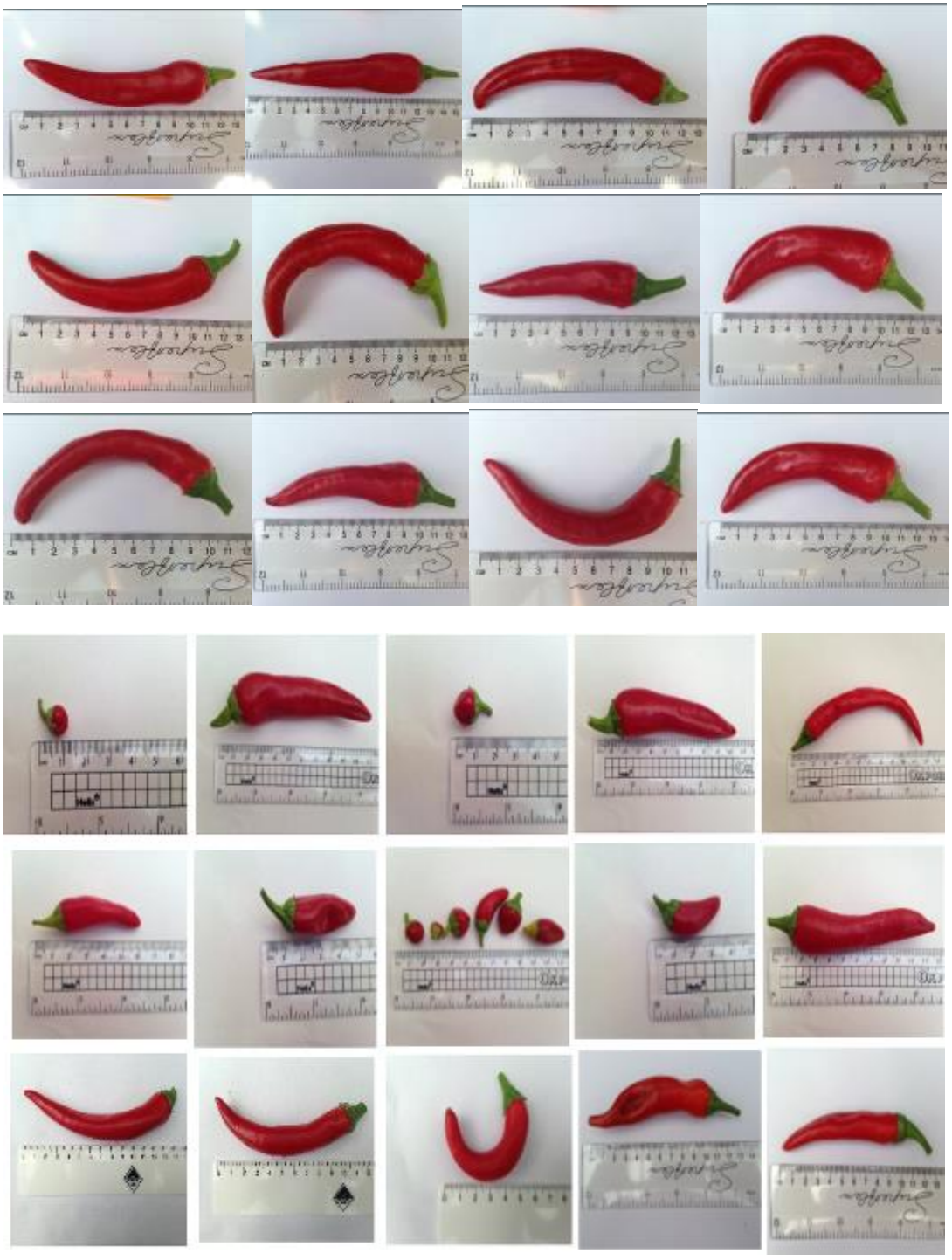

RA
Rain wate
19


GP
Gully po
liqueur
85


GW


AGW



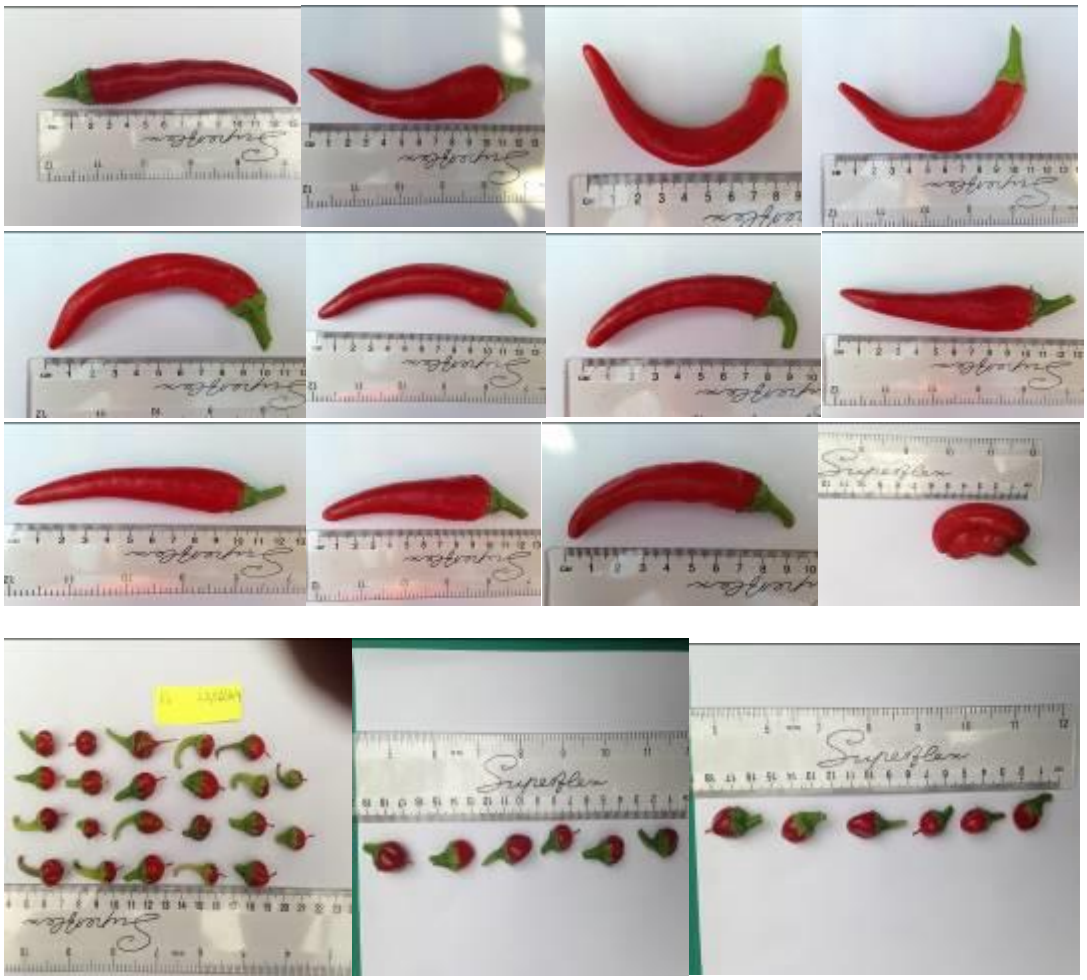


Figure E.1 Photographs of example chilli plants linked to Filter 2, 4, 6, 7, 8 and Control B (without diesel contamination) and Filters 1, 3 and 5 and Control A (diesel contaminated). Note: F1, wetland filter 1; F2, wetland filter 2; F3, wetland filter 3; F4, wetland filter 4; F5, wetland filter 5; F6, wetland filter 6; F7, wetland filter 7; F8, wetland filter 8; CA and CB, Controls A and B (wetland filters receiving tap water); D, deionized water; T, tap water; T+F, tap water mixed with fertilizer (0.7 ml/l); WW+T, one part wastewater mixed with four parts tap water; and WW, preliminarily treated wastewater.