Hydrocarbon removal with constructed wetlands

Design and operation of experimental hybrid constructed wetlands applied for hydrocarbon treatment, and application of an artificial neural network to support constructed wetlands optimization and management

Paul Emeka Eke (B.Eng., MSc.)

A thesis submitted for the degree of doctor of philosophy

The University of Edinburgh

February 2008

Declaration

I hereby declare that the research reported in this thesis is original and have been completed independently by myself (Paul Emeka Eke), under the supervision of Dr Miklas Scholz, Dr Stephen Welch and Mr Scott Wallace. This PhD thesis has not been submitted for the award of any other degree or professional qualification.

Where other sources are quoted full references are given.

Paul Emeka Eke

February 2008

Dedication

To God Almighty – Ancient of days, who makes all things possible

To my Love - Mama, who supports me in all I do

To my mentor – Prof Ozo Nweke Ozo, for his immeasurable love and support

Executive Summary

Wetlands have long played a significant role as natural purification systems, and have been effectively used to treat domestic, agricultural and industrial wastewater. However, very little is known about the biochemical processes involved, and the use of constructed treatment wetlands in the removal of petroleum aromatic hydrocarbons from produced and/or processed water. Wastewaters from the oil industry contain aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (ortho, meta and para isomers), which are highly soluble, neurotoxic and cause cancer. The components of the hydrocarbon and the processes of its transformation, metabolism and degradation are complex, the mechanisms of treatment within constructed wetlands are not yet entirely known. This has limited the effective application of this sustainable technology in the oil and gas industries. Sound knowledge of hydrocarbon treatment processes in the various constructed wetlands is needed to make guided judgments about the probable effects of a given suite of impacts. Moreover, most of the traditional treatment technologies used by the oil industry such as hydrocyclones, coalescence, flotation, centrifuges and various separators are not efficient concerning the removal of dissolved organic components including aromatics in the dissolved water phase.

Twelve experimental wetlands have been designed and constructed at The King's Buildings campus (The University of Edinburgh, Scotland) using different compositions. Selected wetlands were planted with *Phragmites australis* (Cav.) Trin. ex Steud (common reeds). The wetlands were operated in batch-flow mode to avoid pumping

costs. Six wetlands were located indoors, and six corresponding wetlands were placed outdoors to allow for a direct comparison of controlled and uncontrolled environmental conditions. The experimental wetlands were designed to optimize the chemical, physical and microbiological processes naturally occurring within wetlands. The outdoor rig simulates natural weather conditions while the indoor rig operates under controlled environmental conditions such as regulated temperature, humidity and light. Benzene was used as an example of a low molecular weight petroleum hydrocarbon within the inflow of selected wetlands. This chemical is part of the aromatic hydrocarbon group known as BTEX (acronym for benzene, toluene, ethylbenzene and xylene), and was used as a pollutant together with tap water spiked also with essential nutrients.

The study period was from spring 2005 to autumn 2007. The research focused on the advancing of the understanding of biochemical processes and the application of constructed wetlands for hydrocarbon removal. The study investigated the seasonal internal interactions of benzene with other individual water quality variables in the constructed wetlands. Variables and boundary conditions (e.g. temperature, macrophytes and aggregates) impacting on the design, operation and treatment performance; and the efficiency of different wetland set-ups in removing benzene, chemical oxygen demand (COD), five-day @ 20°C N-Allylthiourea biochemical oxygen demand (BOD_5) and major nutrients were monitored.

Findings indicate that the constructed wetlands successfully remove benzene (inflow concentration of 1 g/l) and other water quality variables from simulated hydrocarbon contaminated wastewater streams with better indoor (controlled environment) than outdoor treatment performances. The benzene removal efficiency was high (97-100%) during the first year of operation and without visible seasonal variations. Seasonal variability in benzene removal was apparent after spring 2006, the highest and lowest benzene removal efficiencies occurred in spring and winter, respectively. In 2006, for example, benzene removal in spring was 44.4% higher than in winter. However, no seasonal variability was detected in the effluent ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N) and ortho-phosphorus-phosphate (PO_4^{3-}-P) concentrations. Their outflow concentrations increased or decreased with corresponding changes of the influent nutrient supply. In addition, benzene treatment led to trends of decreasing effluent pH and redox potential (redox) values but increasing effluent dissolved oxygen (DO) concentrations. Approximately 8 g (added to the influent every second week) of the well balanced slow-releasing N-P-K Miracle-Gro fertilizer was sufficient to treat 1000 mg/l benzene.

Results based on linear regression indicated that the seasonal benzene removal efficiency was negatively correlated and closely linked to the seasonal effluent DO and NO_3-N concentrations, while positively correlated and closely linked to the seasonal effluent pH and redox values. Temperature, effluent NH_4-N and PO_4^{3-}-P concentrations were weakly linked to seasonal benzene removal efficiencies. During the entire running period, the seasonal benzene removal efficiency reached up to 90%, while the effluent DO, NO_3-N, pH and redox values ranged between 0.8 and 2.3 mg/l, 0.56 and 3.68 mg/l, 7.03 and 7.17, and 178.2 and 268.93 mV, respectively.

Novel techniques and tools such as Artificial Neural Network (self-organizing map (SOM)), Multivariable regression and hierarchical cluster analysis were applied to predict benzene, COD and BOD, and to demonstrate an alternative method of analyzing

water quality performance indicators. The results suggest that cost-effective and easily to measure online variables such as DO, EC, redox, T and pH efficiently predicted effluent benzene concentrations by applying artificial neural network and multivariable regression model. The performances of these models are encouraging and support their potential for future use as promising tools for real time optimization, monitoring and prediction of benzene removal in constructed wetlands. These also improved understanding of the physical and biochemical processes within vertical-flow constructed wetlands, particularly of the role of the different constituents of the constructed wetlands in removal of hydrocarbon. These techniques also helped to provide answers to original research questions such as: What does the job? Physical design, filter media, macrophytes or micro-organisms?

The overall outcome of this research is a significant contribution to the development of constructed wetland technology for petroleum industry and other related industrial application.

Awards and Publications

Awards:

Won the first place in the Society of Petroleum Engineers (SPE) European Student Paper Contest (PhD Division), for the paper "Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry" **Eke, P. E**., **Scholz, M.** and **Wallace, S. D.** The award was presented at the Offshore Europe Oil & Gas Conference & Exhibition held on 4-7 September 2007 in Aberdeen, Scotland, United Kingdom. Consequently, was invited and presented the paper again at the SPE Annual Technical Conference and Exhibition (ATCE) in California, 11-14 November 2007. Available online in Society of Petroleum Engineers elibrary (http://www.spe.org/elibrary), SPE 113644.

Won the Student Paper Award from the American Academy of Sciences paper competition for the paper "Constructed Treatment Wetlands: Sustainable Technology for the Petroleum Industry" **Eke, P. E**., **Scholz, M.** and **Wallace, S. D.** The award was presented at the Third International Conference on Environmental Science and Technology held on 6-9 August 2007. American Academy of Sciences, Houston, Texas, USA, Volume 2, pp. 174-179, ISBN: 978-0976885399. American Science Press, Houston, USA.

Publications:

The following publications have been produced as a result of this research.

Eke, P. E. and **Scholz, M.** (2008). Benzene removal with vertical-flow constructed treatment wetlands. *Journal of Chemical Technology & Biotechnology* 83(1), 55-63 DOI: 10.1002/jctb.1778.

Tang, X., Eke, P. E., Scholz, M. and **Huang, S.** (2008). Processes Impacting Benzene Removal in Vertical-Flow Constructed Wetlands. *Bioresource Technology*, in press.

Eke, P. E., **Scholz, M.** and **Wallace, S. D.** (2007). Constructed Treatment Wetlands: Sustainable Technology for the Petroleum Industry. Starrett S. K., Hong J., Wilcock R. J., Li Q., Carson J. H., and Arnold S. (Eds.). *Proceedings of the Third International Conference on Environmental Science and Technology* (6-9/08/2007), American Academy of Sciences, Houston, Texas, USA, Volume 2, pp. 174-179, ISBN: 978-0976885399. American Science Press, Houston, USA.

Tang X., **Scholz M.**, **Eke P. E.** and **Huang S.** (2008). Nutrient Removal in Vertical-flow Constructed Wetlands Treating Benzene, *Environment Technology*, submitted.

Tang, T., Eke, P. E., Scholz, M. and **Huang, S.** (2008). Sustainable management of the seasonal variability in benzene removal by planted vertical-flow constructed wetlands to prevent pollution. *Journal of Environmental Management*, submitted.

Eke, P. E. and **Scholz, M.** (2006). Hydrocarbon Removal with Constructed Treatment Wetlands for the Benefit of the Petroleum Industry. *Proceedings of the 10th International Conference on Wetland Systems for Water Pollution Control* (23-29/09/2006), ed by Dias V and Vymazal J. International Water Association, Lisbon, Portugal, Volume **3**:1707-1714, ISBN: 989-20-0361-6.

Eke, P. E., Scholz, M., and **Wallace, S. D.** (2007). Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry. Paper presented at the 2007 Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition, and SPE International Student Paper Contest being held on 11-14 November in Anaheim, California, USA. Available online in Society of Petroleum Engineers elibrary (http://www.spe.org/elibrary), SPE 113644, DOI: 10.2118/113644-STU.

Eke, P. E. and **Scholz, M.** (2007). Hydrocarbon Removal with Constructed treatment Wetlands. Kungolos A., Aravossis K., Karagiannidis A. and Samaras P. (Eds.). *Proceedings of the Society for Ecotoxicology Conference and the International Conference on Environmental Management, Engineering, Planning and Economics* (24-28/06/2007), Volume 2, pp. 1095-1100, ISBN: 978-960-89818-0-5, Skiathos Island, Greece. Grafima Publishing, Thessaloniki, Greece.

Sawalha, O., Scholz, M. and **Eke, P. E.** (2007). Improving the Accuracy of Capillary Suction Time Testing. Kungolos A., Aravossis K., Karagiannidis A. and Samaras P. (Eds.). *Proceedings of the Society for Ecotoxicology Conference and the International Conference on Environmental Management, Engineering, Planning and Economics* (24-28/06/2007), Volume 2, pp. 1431-1436, ISBN: 978-960-89818-0-5, Skiathos Island, Greece. Grafima Publishing, Thessaloniki, Greece.

Eke, P. E. and **Scholz, M.** (2006). Hydrocarbon Removal with Constructed Treatment Wetlands. *4th National Conference* held by The Chartered Institution of Water and Environmental Management (CIWEM) and Aqua Enviro Technology Transfer in Newcastle (12-14/09/04), Aqua Enviro Technology Transfer, Leeds, UK, 8 pages on CD, ISBN: 1-903958-18-0.

Eke, E. P. and **Scholz, M.** (2007). Hydrocarbon Removal with Constructed Treatment Wetlands. Poster presentation; extended abstract. In: Mander Ü., Kõiv M. and Vohla C, eds. 2nd *International Symposium on Wetland Pollutant Dynamics and Control – WETPOL 2007* (16-20/09/2007), Tartu, Estonia, pp. 412-414. ISBN 978-9949-11-689-8.

Eke, P. E., **Scholz, M.** and **Wallace, S. D.** (2007). Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry. Paper presented at the Petroleum Engineers (SPE) European Student Paper Contest (PhD Division) during the Offshore Europe Oil & Gas Conference & Exhibition held on 4-7 September 2007 in Aberdeen, Scotland, United Kingdom.

Eke, P. E. and **Scholz, M.** (2005). Constructed Wetlands Used for the Treatment of Produced Water Contaminated with Hydrocarbons. In *Abstract Booklet of the Institute of Environmental Management and Assessments Seminar on 'Wetlands: Environmental Management and Assessment'* (15/0905), IEMA, Edinburgh, Scotland, UK.

Acknowledgements

The author would like to express his heartfelt thanks to his supervisor, Dr Miklas Scholz, for his excellent academic guidance, advice, encouragement and continuous support throughout the PhD. His competent supervision, warm personality, and friendship to all students have encouraged him and furthered his development as a scientific researcher. He wishes to thank the other members of supervisory panel; Dr Stephen Welch (University of Edinburgh) and Scott D. Wallace (North American Wetland Engineering, Forest Lake, Minnesota, USA) for their assistance, review and comments on the study.

He is grateful to all his colleagues in School of Engineering and Electronics, Institute for Infrastructure and Environment, especially Environmental Engineering and Urban Water Research group (Byoung-Hwa Lee, Sara Kazemi-Yazdi, Piotr Grabowiecki, Ola Sawalha and Atif Mustafa) for their support. He would like to thank numerous final year project and visiting students (visiting PhD students from China; Liang Zhang and Xianqiang Tang, and several other visiting researchers worldwide) that were part of the research group and have assisted him in the laboratory work. He would like to thank Dr. Peter Anderson for his assistance in benzene sample analysis and Derek Jardine for his technical support in both rig construction and laboratory materials set up. He would also like to thank the computing support team especially David Stewart for the special attention and support.

Sincere thanks also go to all his friends outside the university for all their support, encouragement and love prior and during his research study. Special thanks to his true friend, Mr/Mrs Emeka & Nkiruka Okeke and family of Boston Massachusetts USA, for their unconditional love and selfless support. He appreciates the supports and friendship of the following great families; Kelechi Idimogu of PTDF Abuja, Prof Babs Oyeneyin, Dr Afam Ituma, Dr Peter Okey, Dr Ike-Elechi Ogba, Dr Clement Otto and Dr Elias Igwe. He is grateful for the supports of several PTDF new breed scholars especially Benjamin Ogbeche and Comrade Evong ESB and family. He appreciates the support and prayers of his pastor (Peter Anderson and family of Destiny Church Edinburgh). To all his friends and families too numerous to mention, he appreciates all your support.

His heart felt gratitude goes to all his family and in-laws, especially his elder brother (His Royal Highness Chief Dr John Eke (*Onwa Ngbo*)) and family, for his moldings and restoring in him the nuggets of life. He would like to thank his dear wife (Hope Nkiruka Eke) and sons (Donald Emeka Eke (DEE) and Joel Ebuka Eke (JEE)) for their love, considerations, encouragement, tolerance and constant sources of inspiration. They were his anchor, he could not have the opportunity to finish this study without their supports.

Finally, special thanks go to the first and second Executive Governor of Ebonyi State (Dr Sam Egwu) and the Petroleum Technology Development Fund (Nigeria), for funding this research. A big thanks goes to the Secretary to Ebonyi State Government (Prof Ozo Nweke Ozo - his mentor), for his love and unflinching support.

Sound of mystery

High pitched croaks of the frog at dusk

Resonating with the voices and sounds from nature

Clapping sounds of thunder

Vibrating and rumbling as lightening flashes

Roar of stormy rain

Rushing to streams and unleashing the natural energy

Noisy pounding pace of the human race

Invading the tranquility of the night

Quick quacking sound of a duck

Complex multi-note melody whistles of birds

Greeting each day with song and revealing the morning

Whispers of the ocean. Pinching and rolling with a rhythm

I could not interpret the sounds. But they were sounds of life

Nature's secrets, sound of mystery that is inexplicable

Life like the sound is a mirage

Interwoven jumble of contradiction

The strands that give breathe

Awesome master piece. An epitome of riddle

Hidden by architect of the web of life

beyond great intellectual yearning and comprehension

Unwrapped in Divine Revelation

Contents

Abbreviations

ANN	artificial neural network
ANOVA	analysis of variance
BMU	best-matching unit
BOD_5	five-day @ 20 °C N-Allylthiourea biochemical oxygen demand (mg/l)
BTEX	benzene, toluene, ethlbenzene and xylene
CFU	colony forming units
CWs	constructed wetlands
COD	chemical oxygen demand (mg/l)
COSHH	control of substances hazardous to health regulations
DO	dissolved oxygen (mg/l)
FID	flame ionization detector
FWS	free water surface
GC	gas chromatography
HF	horizontal flow
HPC	heterotrophic plate count
HRT	hydraulic retention time
HS	hazardous substances
LSD	least significant difference
MSDS	Material Safety Data Sheets
NTU	nephelometric turbidity units

OEL	occupational exposure limit
PPE	personal protective equipment
Redox	redox potential (mV)
SOM	self-organizing map
SF	surface flow
SSF	sub-surface flow
SSW	safe system of work
TE	topographic error
TOC	total organic carbon
QE	quantization error
U-matrix	unified distance matrix
VF	vertical flow

List of figures

List of tables

1

Introduction

1.1. Background of the Research

The era of escalating environmental crisis such as pollution, water shortages, climatic changes (Hartemink, 2006), rapid population growth and several compelling reasons justify the need for sustainable wastewater treatment technology that could be environmental friendly, easy to operate, less energy-intensive and cost-effective. Natural systems such as constructed wetlands (CWs) have been used to attain wastewater treatment goals by using natural components and processes which significantly reduce the use of energy intensive mechanical devices and technical complexity. Furthermore, CWs involves natural processes resulting in the efficient conversion of hazardous compounds (Ye et al., 2006).

Natural wetlands are complex and integrated ecosystems in which water, plants, micro-organisms and the environment interact to improve the water quality (Guirguis, 2004). Constructed treatment wetlands are manmade wetlands developed and managed to treat contaminants in wastewater that flows through them. Constructed wetlands are designed to imitate physical, chemical and biological processes found in natural wetland ecosystems to remove contaminants from the wastewater. Constructed wetlands rely on natural ecological processes as a preferred alternative to more energy and chemical intensive "mechanical" systems. Successful applications CWs for the treatment of municipal wastewater have led to the exploration of the technology for the treatment of wastewater from several sources, including industrial, agricultural, storm water, acid mine drainage, landfill leachate, urban, airport runoff, gully pot liquor, etc (Moshiri, 1993; Kadlec and Brix, 1995; Kadlec and Knight, 1996; Vymazal et al., 1998; Haberl, 1999; Rew and Mulamoottil, 1999; Moshiri, 2000; Scholz, 2002; Zhao et al., 2004; Scholz and Lee, 2005). The use of constructed wetlands to treat various wastewaters is rapidly emerging as a viable alternative everywhere in the world.

A recent literature survey reported by Wallace and Knight (2006) stated that more than 1,640 CWs have been constructed in the past 15 years in 19 countries.

This study is part of a comprehensive effort of Environmental Engineering Research Group (Urban Water Research group specifically) at the University of Edinburgh (Scotland, United Kingdom) in assessing and advancing understanding of the application of constructed treatment wetlands technology by improving the design, operation and management of CWs applied to wastewaters from various sources (urban, storm water, rural, industrial, agricultural, etc) (Scholz and Lee, 2005; Scholz, 2006; Scholz et al, 2007). The results of the design, operation and performance of the hybrid (combined vertical subsurface-flow and pond) CWs applied for hydrocarbon removal is documented here in this thesis.

The specific design concepts for hydrocarbon treatment with constructed wetland systems have not been examined as precisely as for wastewaters from other sources and the literature on this specific application has been sparse. Zhao et al., (2004) reported that performance of wetland could be greatly improved by modifying the operation conditions. One of such innovative modifications is to introduce more oxygen into system (we introduced aeration pipe, intermittent flooding and draining), which has shown a great potential for the treatment. This research adopted the innovative modification in design and operation of the novel hybrid vertical-flow CWs used for the study. Furthermore, in an attempt to assess and optimize hydrocarbon removal with constructed wetlands, the control of several environmental parameters such as temperature, humidity and nutrients is required. The environmental variability, internal processes and removal mechanism of the CWs were studied in the state-of-the-art advanced ecological engineering indoor test room equipped with a high specification unit for climatic research. The equipment was used in evaluating and optimizing the environmental variables in the constructed wetlands was explained in detail in chapter 3.

Exploration, production, refining, storage, transportation, distribution and utilization of petroleum hydrocarbons have brought about frequent occurrences of water and soil contamination with hydrocarbon (Atlas and Cerniglia, 1995). The pollution of the environment increases as petroleum hydrocarbon continues to be used as the principle source of energy. These problems often result in huge disturbances and disastrous consequences for the biotic and abiotic components of the ecosystem (Mueller et al., 1992). Even small releases of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in excess of regulatory limits (Spence et al., 2005). Produced and wastewaters represent the largest volume waste stream in the exploration and production of oil. As the producing field gets to maturity stage, the volume of produced water exceeds up to ten times the total volume of hydrocarbon produced (Stephenson, 1992). Treatment and disposal of such large volume is of great concern to the operator and the environment. Wastewaters from the

Petroleum industry contain aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (ortho, meta and para isomers), which are highly soluble, neurotoxic and cause cancer (Hiegel, 2004). Petroleum hydrocarbon wastewaters contain also pollutants such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), nitrogen and phosphorus (Knight et al, 1999). However, the major focus of the petroleum industry is on assessing the removal efficiency of hydrocarbons. Due to hydrocarbons’ toxic properties and persistence in nature, biodegradation processes and wetland remediation methods have attracted great attention (Ilker et al., 2000, Wallace and Knight, 2006). Most of the traditional treatment technologies used by the oil industry such as hydrocyclones, coalescence, flotation, centrifuges and various separators are not efficient concerning the removal of dissolved organic components including aromatics in the dissolved water phase (Descousse et al., 2004; International Association of Oil and Gas Producers, 2002). CWs have the potential of removing organics such as aromatic components in the dissolved water phase (Eke et al, 2007b) and inorganic compounds in wastewater (Wallace and Knight, 2006). Historically, the removal of organic compounds from water by most of the traditional treatment technologies are expensive and has relied upon exploiting density differences between water and the oils and/or organic compounds to be removed (Alper, 2003). The 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 water pollution especially in petroleum industry.

The CWs technology has diverse applications and has established roots as cost effective and natural wastewater technology around the world. Cole (1998) reported that although there is a rapid spread and diversification of constructed wetland technology, they are, however, running ahead of the mechanistic understanding of how they work, which is needed to develop detailed design criteria.

Despite the recorded great breakthrough in other sectors, CWs technology has not yet gained full acceptance in the petroleum industry Cole (1998). In part, this reluctance in acceptance exists because the technology is not yet completely understood or trusted by those who must approve its use in this field, and its success is a hotly debated issue. Very little is known about the internal processes involved and the use of constructed treatment wetlands in the removal of petroleum hydrocarbons from processed water. This has limited the effective application of this technology in the oil and gas industries, as a firm scientific basis for design and operation currently does not yet exist. These complex natural systems are still somewhat of a "black box," in some specific field application (Cole, 1998) such as petroleum industry. One of the major arguments in especially upstream sector of petroleum industry is that the Knowledge of how the wetland works is not far enough advanced to provide engineers with detailed processes and predictive models. And, being natural systems, their performance is variable, subject to the changing seasons and environmental factors (this need to be investigated). Another primary reason for the lack of understanding and mistrust of CWs is that the technology requires knowledge not only of such fields as environmental engineering and hydrology, which are important in conventional cleanup methods, but also of the complex workings of microorganisms. There is no detailed study on the treatment of hydrocarbon contaminated wastewater using constructed wetlands known in the literature. However, the working mechanism of this treatment technology consists of interconnected interactions of chemical, physical and biological processes and a concerted action between soil, plant rhizomes and the bacteria acclimatized to the toxic effluents, which is insufficiently known or tested, especially as applied to hydrocarbon removal. Considering also that the components of the hydrocarbon and the processes of its transformation, metabolism and degradation are complicated, the mechanisms of treatment within constructed wetlands are not yet entirely known. Moreover, there are no practicable or academically established criteria to assess the mechanisms and performances of hydrocarbon removal within constructed wetlands.

It is also clear that there is still a long way to go before such systems will be considered for routine use in petroleum industry specifically the upstream sector.

Although this CWs technology has been applied now for several decades, few quantitative researches have been done on the complex processes that occur inside this man-made ecosystem (Cole, 1998). Indeed, most studies adopted a black box approach where low-frequent or seasonally-averaged data were applied to feed the empirical models, thereby largely ignoring the intrinsic variability of such treatment systems. Prominent researchers in this field showed concern on the black box nature of the technology by making the following comments:

R. Kadlec (in Cole, 1998): *"We've got a huge, functioning mess called wetlands out there with all sorts of interesting things going on inside it. But we do not have enough information about what goes on inside the system. We have a solid foundation of empirical understanding, but to advance our knowledge, we need to understand the internal processes that lead to the observed performance."*

R. Gearheart (in Cole, 1998): "*Basically, all we know is that they work … But if you want to be able to say, for example, what happens if you double the loading* rate, *we're not there yet. We can not model it.*"

Constructed wetlands are complex systems in terms of biology, hydraulics and water chemistry. Furthermore, there is little or a lack of quality data of sufficient detail, on full-scale constructed wetlands applied specifically for petroleum industry. In most reported data concerning constructed wetland performance, there was a consistency in removing the total nitrogen, total phosphorus and biochemical oxygen demand. However, this was not the case regarding hydrocarbon removal. This may be due to the involvement of sequence of processes needed to be accomplished before achieving the hydrocarbon removal and the huge variation in hydrocarbon contaminated wastewater characteristics, operating regime and wetland configuration.

Considering also that currently, there is very little practical information on how the characteristics of the influent wastewater and the physical state of the hydrocarbon affect the efficiency and hydraulic properties of wetlands. A clear understanding of the biological, physical and chemical processes involved is essential. Moreover, successful implementation of the constructed treatment wetland technology in both upstream and downstream sectors of petroleum industry will save cost for producers and have great commercial spin-off for the environmental scientists, engineers and policymakers. Sound knowledge of hydrocarbon treatment processes in the various constructed wetlands is needed to make guided judgments about the probable effects of a given suite of impacts. This research focused specifically on a more thorough understanding of the science, underlying environmental variables and mechanisms of hydrocarbon removal with constructed wetlands.

1.2. Problem statement

The above introduction identified gaps in knowledge. The highlights suggests that constructed wetlands are a versatile, natural and cost effective technology (Rew and Mulamoottil, 1999) that is suitable for removing several pollutants from different types of wastewater (Moshiri, 2000; Scholz, 2002; Zhao et al., 2004), at varying loading rates and under a range of climatological conditions. The study seeks to provide a better understanding and application of the technology as it expands to this new area by assessing the processes and verifying how effective novel hybrid (combined vertical-flow and stabilization ponds) constructed wetlands could be at removing pollutants from simulated hydrocarbon contaminated wastewater. In designing, constructing and operating the experimental wetlands to solve the identified problems (gaps in knowledge), the research sought to answer specific questions which surfaced such as:

(1) To what extent would hydrocarbons and other water quality parameters such as COD, five-day @ 20°C N-Allylthiourea biochemical oxygen demand (BOD_5), etc be removed in different constructed wetland set-ups?

(2) How is the efficiency of experimental wetland system variables affected by the hydrocarbon in the wastewater influent?

(3) How effective would major experimental constructed wetland components and control parameter such as filter media, plants, nutrients and oxygen be in treating hydrocarbon contaminated wastewater?

(4) What is the mechanism of removal of hydrocarbons in constructed wetlands?

(5) How do extreme hydrocarbon loading rates (high strength aromatic hydrocarbon) influence treatment efficiency?

(6) Can addition of nutrients in the hydrocarbon contaminated wastewater improve the efficiency of CWs?

(7) How and to what extent does environmental and seasonal variability (summer, winter, etc) affect treatment efficiency?

(8) How do the quantity and quality of hydrocarbons accumulated in wetland

substrate affect hydraulic conductivity, treatment efficacy and long-term system sustainability?

The operational experience including intensive monitoring data and research results this study sought to provide will be useful in providing solution to the above mentioned problems and in managing constructed wetlands systems applied for hydrocarbon contaminated wastewaters treatment opportunities for the future.

In an attempt to meet the required knowledge and understanding in this research, the study did evaluate in-depth basic internal workings of the constructed wetland components and the interrelationships that compose the system processes as detailed in this thesis. The observations and results obtained were thus reported in this thesis. The findings have been very encouraging and consequently, expected to go a long way in reducing the existing skepticism by providing the technological insight required by oil producers, regulators and engineers in the petroleum industry.

1.3. Rationale, Aims and objectives of the project

Constructed wetlands are already widely used to treat wastewaters polluted with various compounds (Scholz, 2006). This research interest has been driven by growing recognition of the natural treatment functions performed by natural and constructed wetlands. However, the working mechanisms of wetland treatment technology consist of interconnected interactions of chemical, physical and biological processes and interactions between soil, plant rhizomes and the bacteria acclimatized to the toxic effluents. The use of constructed wetlands to treat specific industrial wastewater such as hydrocarbons is a relatively new ecological engineering technique compared with conventional treatment systems; therefore, a proper understanding of their operations and functions is required.

In an attempt to improve the scientific knowledge and optimize hydrocarbon removal with constructed wetlands, the control of several environmental parameters such as temperature, humidity and nutrients is required. Pioneering work on the use of subsurface-flow wetlands to treat industrial organic compounds was undertaken by Seidel (1973) in Germany. The Seidel’s approach was modified and used in this research to verify the use of vertical-flow subsurface constructed wetlands to treat benzene, a representative hydrocarbon species. The research uses data gathered from experimental-scale wetlands to assess the efficiency of hydrocarbon removal in each wetland and to compare different operational conditions. The research covers the assessment of environmental, physical, chemical and microbial processes. This enhances operational knowledge and understanding of treatment wetlands and provides data that could be used to design full-scale wetland systems for efficient hydrocarbon treatment, and to model biodegradation and operational processes. Improved system control should include knowledge and understanding concerning environmental requirements such as oxygen availability, water inundation duration and temperature variability, fertilizer requirements for wetland microbes, and characterization of microbes capable of degrading petroleum hydrocarbons.

The overall aim is therefore to advance understanding of the application of constructed treatment wetlands for hydrocarbon removal.

The specific objectives are to assess:

(1) The current literature on hydrocarbon removal with constructed wetland systems;

(2) The efficiency of different wetland set-ups in removing hydrocarbon, COD, BOD_5 and nutrients;

(3) Variables and boundary conditions impacting on the operation and treatment

performance (e.g. temperature, macrophytes and aggregates);

(4) The effect of nutrient concentration increases on hydrocarbon removal within wetlands;

(5) The role of environmental factors and seasonal variability on hydrocarbon removal with vertical-flow constructed wetlands operated in both controlled and uncontrolled environments. Literally assessing the specific impact of seasonal changes and environmental control on the treatment efficiency of hydrocarbon and other water quality variables such as COD, BOD_5, DO, redox potential, turbidity and nutrients;

(6) The sustainability and cumulative impact of the constructed wetlands by assessing the long-term performance in treating petroleum hydrocarbons such as benzene, which are associated with considerable human health and environmental concerns; and

(7) The main hydrocarbon removal pathways and biochemical mechanisms

1.4. Thesis outline

This thesis started the investigation by reviewing of the existing information on wetlands and constructed wetlands applied for hydrocarbon removal. The study then investigated the performances of the laboratory-scale experimental constructed wetlands applied for hydrocarbon removal.

Chapter 1 described the background, statement of the problem, outline, rationale, aim and objectives of the study.

Chapter 2 presented the review of literature on hydrocarbon removal with wetland systems. An overview of the constructed wetlands enumerating the role of main wetland components (nutrients, temperature, etc) and types of wetland flow systems

(Surface Flow, Subsurface Flow [Vertical and Horizontal] and hybrid types). A significant proportion of the chapter is devoted to the published literature on the removal mechanisms of pollutants.

Chapter 3 described the materials, the experimental set-up and operation methods applied for the study. This chapter explained the experimental filter design, construction, parking order, compositions as well as operational and/or controlled environmental condition. The chapter also documented the design and operational limitations as well as the risk assessment done prior to the study.

Chapter 4 presented the overall treatment results and discussions. The chapter shows the water quality performance of each wetland filter for the entire study period and also analyzed statistically to establish the relationship between constructed wetland components. The result of the variables essential for control, efficiency monitoring and optimization of the wetland were presented.

Chapter 5 discusses hydrocarbon removal performance of the system. Hydrocarbon removal mechanism in vertical-flow experimental constructed wetlands was examined with additional column experiment. The role of macrophytes, filter media, nutrients and cumulative impact of long-term performance of the system in hydrocarbon treatment discussed in detail.

Chapter 6 examined the seasonal variability and monthly performances of hydrocarbon and the corresponding impacts of other water quality variables.

Chapter 7 described application of artificial neural networks to support constructed wetlands operation, optimization and management. Artificial neural networks are introduced as a tool for the prediction of experimental constructed wetland performance. The self-organizing map (SOM) and Multivariable testing are applied to predict water quality indicators such as COD, BOD_5, nutrient and hydrocarbon, which is expensive and labour intensive to estimate. Variables such as dissolved oxygen, pH and conductivity, which can be monitored in real time is used as input of models.

Chapter 8 finally brought together the outcomes of this thesis, compared and discussed the results from the different chapters and provides some overall conclusions. The chapter gave some suggestions for further research as well. References and appendix were documented after chapter 8.

2

Constructed treatment wetlands for the petroleum industry application

2.1. Overview

This chapter presented the in-depth historical and technical review of existing information about natural and constructed wetlands, showing the hydrology, components, types and the removal mechanisms of contaminants in wetlands.

The chapter is grouped into sections for specific descriptions as follows. Sections 2.1 introduced the chapter, 2.2 described historical development of constructed treatment wetlands and 2.3 described components of wetland. 2.4 described wetland hydrology and 2.5 described constructed wetlands types with the subsections describing major wetland types, while section 2.6 described the applications of temporarily flooded vertical-flow wetlands. Section 2.7 presented the removal mechanisms of contaminants with the subsections dealing with various removal processes with special emphasis on application of constructed wetlands for hydrocarbon treatment. 2.8 presented wetland treatment model, 2.9 described the role of temperature in constructed wetland, while section, 2.10 role of nutrients and 2.11. summarized the chapter.

2.2. Historical development of constructed treatment wetlands

The use of wetlands to remove pollutants from wastewater is not a new idea. Despite poor documentation of this technology at early stage of development, there were several indications that wetlands were used for decades in many different forms and applications (Moshiri, 1993; Kadlec and Knight, 1996). Kadlec and Knight (1996) documented a good historical account of the use of natural and constructed wetlands for wastewater treatment and disposal. As they pointed out, natural wetlands have probably been used for wastewater disposal for as long as wastewater has been collected, with documented discharges dating back to 1912. Some early constructed wetlands researchers probably began their efforts based on observations of the apparent treatment capacity of natural wetlands. Research studies on the use of constructed wetlands for wastewater treatment began in Europe in the 1950's, and in the United States of America (USA) in the late 1960's. Research efforts in the USA increased throughout the 1970's and 1980's, with significant federal involvement by the Tennessee Valley Authority (TVA) and the US Department of Agriculture in the late 1980's and early 1990's. USEPA has had a limited role in constructed wetlands research which might explain the dearth of useful, quality-assured data (USEPA, 2000).

Another school of thought believe that since the practice of building sewers in urban areas dates back to about 7,000 BC, it is likely indication that natural wetlands have been used as receiving waters ever since. The sewage farming experiences of the 1870s in the United Kingdom led to an appreciation of the link between wastewater application rates, wetland hydrology, plant adaptation, and wastewater purification. It was noted in 1877 that wastewater loadings at about 0.25 m^2 per person per day were sufficient to maintain wastewater application areas as “grass plots” (Wallace, 2004). Thousands of years ago, natural wetlands were also used by the Egyptians and the Chinese to clarify liquid effluent. However, the first botanical treatment of wastewater was reported in Europe in the 1950s (Fujita, 98). Gessner et al (2005) reported that America’s research into the field was reported in the 1970s, in 1973–1976, the first intentionally engineered, constructed wetland treatment pilot systems in North America were constructed at Brookhaven National Laboratory near Brookhaven, New York. However, the use of a wetland within a deliberately engineered treatment vessel was also documented in a US Patent dating back to 1901 (Monjeau, 1901; Wallace, 2004). Furthermore, early concept about “constructed” wetland was reported 1904 (in Australia). This apparently indicated that by 1900, the idea of creating wetlands specifically for wastewater treatment had been developed. Wallace (2004) and Brix (1994a) reported that this early wetland concept was clearly documented in an essay to the Hornsby Literary Institute, NSW, Australia in 1904 which states in part as follows:

“If every householder disposed of his own drainage on his own premises as he might very well do, the health of all of us would be much improved. Anyone who has a little ground about his house can dispose of his dirty water as follows: Dig up a plot of ground thoroughly to a depth of fifteen to eighteen inches. Cut a channel leading from the kitchen and washhouse into the highest side of the plot and let all the dirty water drain into it. Plant the plot with plants that grow rapidly and require a great deal of water such as Arum Lilies, for instance. The dirty water will be all absorbed by the roots of the plants and a most luxuriant garden will be produced which will defy the hottest weather and will always be green and beautiful. By this means a curse will be transformed into a blessing.”

From historical perspective a large spectrum of habitats are regarded naturally as wetlands. There are many different terms for description of wetland 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, spong, stream, saltflat, creek, reservoir and beds of marine algae or seagrasses (Kadlec and Knight 1996; http://www.eco-pros.com/types_of_wetlands.htm). The term "wetland" appears to have been adopted as a euphemistic substitute for the term "swamp" (Wright, 1907). Nineteenth-century scientists used terms such as mire, bog, and fen to describe the lands that are now called wetlands, and these terms are still used by scientists to describe specific kinds of wetland (Mitsch and Gosselink, 1986; Dennison and Berry, 1993). The term wetland has come gradually into common scientific usage only in the second half of the twentieth century. However, what brings all these diverse kinds of habitats together is that the land is wet for a part or whole of the year that the vegetation is quite distinct from that of the adjacent upland areas (Gopal, 1999). Wetlands act as a transition between the terrestrial and aquatic ecosystems and exhibit some characteristics of each (Smith 1980). Essentially a natural wetland occurs where the level of water is near the surface for enough time to keep the soil below saturated. Wetlands systems use various physical, biological and chemical processes to treat its pollutants.

Providing a precise definition of wetlands has been very controversial because of the enormous variety of wetland types and the problems of defining their boundaries. Guirguis (2004) defined wetlands as complex and integrated systems in which water, animals, plants, micro-organisms and the environment interact to improve the water quality. Cowardin et al, (1979) documented a comprehensive definition of wetland by US Fish and Wildlife Service thus: “*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. For the purposes of this classification wetlands must have one or more of the following three attributes: (1) at least periodically, the land supports predominantly hydrophytes; (2) the substrate is predominantly undrained hydric soil; and (3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year*”. Fortunately, despite various definitions and terms for description of the wetland systems, the most widely accepted definition was developed in 1980 by the International Union for the Conservation of Nature and Natural Resources (IUCN) in the Ramsar Convention. This convention defined wetlands as “any areas of swamp, pond, peat, or water, natural or artificial, permanent or temporary, stagnant or flowing water, including estuaries and marine water, the depth of which at low tide does not exceed six meters” (Mitsch and Gosselink, 1993). This definition brings many different bodies of water under the meaning of wetland. The international community has been made aware of the value of wetlands since The Ramsar Convention (Scholz 2006). Over 100 countries have adopted a definition by signing the Ramsar Convention on Wetlands. In wetland systems, animals, plants and micro-organisms thrive and interact with sunlight, soil and air to improve water quality (Guirguis, 2004). Wetlands can be natural or man-made; the latter where humans attempt to mimic the water treatment processes exhibited in natural wetlands, in an attempt to solve various water quality problems. 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).

Patten (1990) reported that wetlands comprise 7.7% of the Earth's landscape, or in other words a total surface area of 11.65 million km^2. How much of the earth's surface is presently composed of wetlands is not known exactly. The UNEP-World Conservation Monitoring Centre has suggested an estimate of about 570 million hectares (5.7 million km^2) – roughly 6% of the Earth's land surface – of which 2% are lakes, 30% bogs, 26% fens, 20% swamps, and 15% floodplains (Ramsar, 1971). Mitsch and Gosselink, in their standard textbook *Wetlands*, 3rd ed. (2000), suggest 4 to 6% of the Earth's land surface. Mangroves cover some 240,000 km^2 of coastal area, and an estimated 600,000 km^2 of coral reefs remain worldwide. Nevertheless, a global review of wetland resources prepared for Ramsar COP7 in 1999, while affirming that "it is not possible to provide an acceptable figure of the areal extent of wetlands at a global scale", indicated a 'best' minimum global estimate at between 748 and 778 million hectares. The same report indicated that this "minimum" could be increased to a total of between 999 and 4,462 million hectares when other sources of information were taken into account (Ramsar, 1971).

Wetlands mean different things to different people with different backgrounds. To some, wetlands are important habitats for numerous kinds of waterfowl and fish whereas to others they are the "kidneys of the earth" and some a leading "green" infrastructure of the 21st century. Sometimes they have been called "biological supermarkets" for the extensive food chain and rich biodiversity they support (Mitsch and Gosselink, 1993). Wetlands vary widely because of regional and local differences in soils, topography, climate, hydrology, water chemistry, vegetation, and other factors, including human interaction. Both freshwater and saltwater wetlands, due to their transitional location and reducing conditions were found to have very significant roles in the natural cycling of organic and inorganic materials (Bastian and Hammer, 1993).

Wetlands are comparable to rain forests and coral reefs as being one of the most productive ecosystems on the planet in performing several ecological functions. The hydrological, biological and biogeochemical functions impart wetlands various values (Sather and Smith, 1984). Vymazal et al (1998) and Denny (1997) summarized some of the values of the wetlands as follows:

1 Hydrological and hydraulic functions (erosion and flood control; recharge of groundwater aquifers; floodplain hydrodynamics),
2 Climatic effects (buffering global warming; carbon fixation and CO_2 balance; micro-climatic influences),
3 Biodiversity functions (wildlife enhancement; breeding grounds for waterfowl, fish and invertebrates like shrimps, crabs, oysters, clams, mussels; preservation of gene pools; conservation of flora and fauna),
4 Mining activities (getting peat, sand, gravel),
5 Usage of plants (staple food plants; grazing land; timber; paper production; roofing; agriculture, horticulture, fertilizers, fodder),
6 Development of aquaculture and integrated systems (fishing, hunting, fish cultivation combined with rice production),
7 Energy production (hydroelectric; solar energy; heat pumps; fuel as gas, solid and liquid),
8 Educational uses (training; nature studies; research activities)
9 Recreational and reclamation uses (sightseeing/ aesthetic benefits; sailing; swimming; canoeing and other water sports).

10 Relatively low capital and operating costs, simplicity of operation (low requirement for operator supervision) and seen as a natural and therefore “green” process.

Unfortunately, most of the above mentioned values were recognized recently by the developed world, which considered the natural wetlands for a long time as “wastelands” (Gopal, 1999) and used them as “convenient wastewater discharge sites” for as long as sewage has been collected (Kadlec and Knight, 1996). Furthermore, they have been drained, ditched, covered, overfilled with toxins and nutrients for long periods (Mitsch and Gosselink, 1993).

Man has started to mimic nature by building wetlands to treat a variety of waters, wastewaters, storm waters, gully pot liquor, acid-mine drainage waters, landfill leachate, irrigation waters, agricultural wastewater, runoff waters, industrial wastewater and produced waters (Moshiri, 1993; Kadlec and Brix, 1995; Kadlec and Knight, 1996; Cooper et al., 1996; Vrhovsek et al., 1996; Vymazal et al., 1998; Haberl, 1999; Rew and Mulamoottil, 1999; Moshiri, 2000; Kivaisi, 2001; Yang et al., 2001; Scholz, 2004; Zhao et al., 2004). Constructed wetlands are man-made systems designed to imitate the optimal treatment conditions found in natural wetlands, which filter out pollutants and act as sinks for nutrients by purifying the water through physical (sedimentation and filtration), physical-chemical (adsorption on plants, soil and organic substrates) and biochemical (biochemical degradation, nitrification, denitrification, decomposition and plant uptake) processes (Novontny and Olem, 1994). Constructed wetlands technology has been efficient in the removal of pollutants in the wastewater and was simple to construct, operate and maintain with low cost, low energy demand, effectiveness and potential for creating biodiversity (Haberl, 1999). Constructed treatment wetlands being an environmentally friendly and low-cost technology system now serves as the potential alternative systems for the treatment of wastewater from various sources throughout the world (Scholz, 2006).

There were an expansion in variety of applications for constructed treatment wetland technology for water quality improvement as a result of the transfer of the knowledge, technical collaboration and co-operation by the scientists in developed countries recently (Haberl, 1999; Kivaisi, 2001; Njau et al., 2003). However, the initial research deliberately investigating wastewater treatment by wetland plants started in 1952 at the Max Planck Institute in Plon, Germany, when a German scientist, Dr. Kathe Seidel, began investigating the water purification capabilities of bulrush (Schoenoplectus lacustris) grown in artificial rooting environments (Bastian and Hammer 1993). She explored the removal of phenols from wastewater by *Scirpus lacustris* and in 1956 began testing dairy wastewater treatment with *S. lacustris* (Bastian and Hammer, 1993). From 1955 through the late 1970s, Seidel published numerous studies on water and wastewater treatment with wetland plants (Seidel, 1955, 1961, 1976). Seidel could be called “Mother of constructed wetlands” because her discoveries gave birth to modern constructed wetlands and marked the earliest documented engineered treatment wetlands research of the western world. However, most of her early publications were in German thus making it difficult for non German speaking scientists to understand, and thus hindering dissemination of the acquired knowledge. Her research also seemed heavily criticised since the investigations and calculations were mainly aimed at nutrient removal through plant uptake which would require a regular harvesting regime and very large surface areas (Vymazal, 1998a). In the early 1960s, in collaboration with Dr. Seidel, Dr. Reinhold Kickuth (one of her students) at the University of Göttingen, Germany, developed a wetland treatment process known as the Root Zone Method, which was first used for a full-scale wetland system at Othfresen, Germany in 1972. Kickuth continued with the experimental work and popularized this concept with his co-workers in Europe, resulting in nearly 200 municipal and industrial waste treatment systems. Interest in these subsurface (the Root Zone Method) flow wetlands spread throughout Europe by the mid 1980s (Bastian and Hammer, 1993). Throughout the 1970s, in the U.S., land treatment alternatives were developed with the support of a significant research and development effort funded by the U.S. Environmental Protection Agency, the U.S. Army Corps of Engineers and other agencies (Bastian and Hammer, 1993). Use of these wetlands expanded dramatically in the United States after the Tennessee Valley Authority published a design manual in 1993 targeted primarily for single-family homes (Wallace, 2004). Constructed treatment wetland systems as engineered and managed “natural systems” are receiving increased worldwide attention for wastewater treatment and recycling (Bastian and Benforado, 1983; Reddy and Smith, 1987; Reed et al., 1988, Hammer, 1989; Cooper and Findlater, 1990; Etnier and Guterstam, 1991; Tchobanoglous and Burton, 1991; Kadlec and Knight 1996; Vymazal et al., 1998; IWA, 2000; Njau et al., 2003; Scholz, 2006). The increased popularity this technology is receiving appears to be due to the growing interest on technologies that supports environmental protection, resource conservation and more reliance on natural ecological processes in comparison to the more energy and chemical intensive “mechanical” (conventional) systems. While experience in research and practical application has been built up over the years, a number of fundamental knowledge of the internal processes that lead to the observed performance of wetland is not yet entirely known. This could be attributed to the technology being natural system with variable performance that depends on the interaction of many different components and subject to changing seasons.

2.3. Components of a wetland

Wetlands consist of basic components such as underlying strata, water, hydric soil, detritus and macrophytes (vegetation). However, other important components of wetlands such as the communities of microorganisms and aquatic invertebrates develop naturally. The water, soil and vegetation are basic components for the characterization of a wetland. The understanding of the components is useful for the manipulation of constructed wetland. Constructed wetlands are wastewater treatment methods that mimic processes that occur in natural wetlands by utilizing the components to cleanse water. However, wetland processes are among the most complicated sets of soil and water chemistry, plant and hydrology interactions occurring within any ecosystem on earth (Campbell and Ogden, 1999). The underlying strata are unaltered organic, mineral or lithic strata which are usually saturated with or impervious to water and are below the active rooting zone of the wetland vegetation (Campbell and Ogden, 1999).

Water. Wetlands are areas where water is the primary factor controlling the environment and the associated plant and animal life. They occur where the water table is at or near the surface of the land, or where the land is covered by shallow water (Ramsar, 1971). A wetland can be built almost anywhere in the landscape by shaping the land surface to collect surface water and by sealing the basin to retain the water. All wetland soils must be *hydric* - saturated with water for at least part of the growing season. Hydrology (explained in detail in section 2.4) is the most important design factor in constructed wetlands because it links all of the functions in a wetland

and because it is often the primary factor in the success or failure of a constructed wetland. While the hydrology of constructed wetlands is not greatly different than that of other surface and near-surface waters, it does differ in several important respects.

Small changes in hydrology can have fairly significant effects on a wetland and its treatment effectiveness because of the large surface area of the water and its shallow depth, a wetland system interacts strongly with the atmosphere through rainfall and evapotranspiration. The density of vegetation of a wetland strongly affects its hydrology, first, by obstructing flow paths as the water finds its sinuous way through the network of stems, leaves, roots, and rhizomes and, second, by blocking exposure to wind and sun (US EPA, 2000). It is important to note that an area is strictly classified as wetland based on three defining characteristics – hydrology, soils, and vegetation. However, just because water exists in an area doesn't mean an area is a wetland, or vice versa; just because there is no obvious water doesn't mean that an area is not a wetland. Wetlands do not always occur at the assumed "bottom of a hill" where water collects. One may come across a wetland at the top of a hill from a perched water table.

Substrate. Substrates (also called aggregates or wetland media) used to construct wetlands include soil, sand, gravel, rock, and organic materials such as compost. Wetland researchers have started to use industrial by-products like alum sludge (waterworks sludge), light weight aggregates and waste materials from industries, as well as natural materials with higher adsorption capacities (Babatunde and Zhao, 2007, Johanson, 1996; Brooks *et al.*, 2000; Zhu *et al.*, 2002). However, soil is the main supporting material for plant growth and microbial films in constructed wetlands. The soil matrix has a decisive influence on the hydraulic processes

(Stottmeister *et al*., 2003). Soils consist of unconsolidated, natural material that supports or is capable of supporting plant life. The upper limit contains air, and the lower limit is either bedrock or limit of biological activity (ITRC, 2003). Soils are generally divided into two different types - mineral and organic. Soils can be further categorized based on the amount of moisture present. Under wetland conditions, soils are considered to be hydric, i.e., saturated, flooded, or ponded long enough during the growing season to develop anaerobic conditions in the upper portion of the soil.

Hydric soils are developed under conditions sufficiently wet to support vegetation typical to wet areas (hydrophytic vegetation) (ITRC, 2003). The physical and chemical characteristics of soils and other substrates are altered when they are flooded. In a saturated substrate, water replaces the atmospheric gases in the pore spaces and microbial metabolism consumes the available oxygen. Since oxygen is consumed more rapidly than it can be replaced by diffusion from the atmosphere, substrates become anoxic (without oxygen) (US EPA 2000). A mixture of sand and gravel is recommended to improve hydraulic condition and the removal of contaminants (IWA specialist group, 2000; Stottmeister *et al*., 2003). For vertical-flow constructed wetland, a relatively small range of effective grain size of 0.06 to 0.1 mm was evaluated, while that for horizontal-flow system was found to be higher at 0.1 mm (Stottmeister *et al.*, 2003). A number of specialty media have been studied to access the possibility of increasing the adsorption capacity of filter media with different substrates. However, there has been contradictory view about the function of expensive filter media in the treatment process of constructed wetlands. Study carried out by Scholz and Xu (2002) demonstrated that there was no additional benefit in using expensive adsorption media like granular activated carbon to enhance filtration performance of constructed wetlands.

Macrophytes. Plant is an important component of a wetland system. Both vascular plants (the higher plants) and non-vascular plants (algae) are important in constructed wetlands. Macrophytes can assimilate pollutants in their tissue, and also provide a surface and an environment for microorganisms to grow (Vymazal, 2002).

The growth of roots within filter medium helps to decompose organic matter and prevents clogging by creating channels for the water to pass through in the intermittent loading vertical-flow system. Photosynthesis by algae increases the dissolved oxygen content of the water. Some wetland plants release sufficient oxygen into the root zone to support aerobic microbial activity (Bodelier et al., 1996; Armstrong et al., 1990), and this may sometimes represent as much as 90% of the total oxygen entering a wetland substrate (Reddy et al., 1989). Nevertheless, the relative contribution of plant oxygen transport to wastewater treatment remains controversial. Some wetland designers assume that plant oxygen transport is significant (DeBusk and DeBusk, 2001), while others dismiss it as negligible (US EPA, 2000). Wetland ecosystems support plant communities dominated by species that are able to tolerate either permanent or periodic saturation. Quantification of oxygen flux from entire root systems has been complicated by species and seasonal differences, spatial heterogeneity and measurement accuracies for variables including the oxygen demand of the root zone solution and the root to solution volume (Sorrell and Armstrong, 1994). The plants' capacity to supply oxygen to the root zone varies among species due to differences in vascular tissues, metabolism, and root distribution (Steinberg and Coonrod, 1994). The potential for plants to release oxygen into the root zone may increase during cold periods, because root and rhizome respiration consumes relatively large proportions of oxygen, which diffuses through plant shoots, and the oxygen demand for root and rhizome respiration declines with temperature (Callaway and King, 1996). Metabolism by the indigenous microflora is a function of the availability of light, oxygen, temperature, nitrogen and phosphorus (Atlas, 1981).

Macrophytes are widely used within treatment wetlands (Cooper et al., 1996; Scholz, 2006). However, the role of macrophytes in treatment wetlands has been controversial. Some researchers have documented that macrophytes can improve pollutant removal (Cooper et al., 1996; Brix, 1997; Vymazal, 1999; Kadlec et al., 2000; Neralla et al., 2000; Kadlec, 2002; Karathanasis et al., 2003). However, despite such an ability of macrophytes, when compared to microorganisms, they only play a secondary role in the degradation of organic matters in wetland systems. (Stottmeister *et al*., 2003). Alternatively, others did not detect any significant difference between planted and unplanted systems (Baldizon et al., 2002; Scholz et al., 2002). Despite the contradiction in the scientific findings, plants play an indirect role in treatment of contaminants in constructed wetland. For example, the growth of roots within filter media helps to decompose organic matter and prevents clogging by creating channels for the water to pass through. The macrophytes transport oxygen into the rhizosphere, which stimulates both aerobic decomposition of organic matter and the growth of nitrifying bacteria (Brix, 1997). The most common plants in wetlands are reed (*Phragmites* sp), cattail (*Typha* sp.), rush (*Juncus* sp.) and bulrush (*Scirpus* sp.).

However, the most frequently used plant species worldwide is *P. australis* (IWA specialist, 2000). Constructed wetlands vegetations attract waterfowl and wading birds, including mallards, green-winged teal, wood ducks, moorhens, green and great blue herons, and bitterns. Snipe, red-winged blackbirds, marsh wrens, bank swallows, redtailed hawks, and Northern harriers feed and/or nest in wetlands (US EPA, 2000).

Microorganisms. Microbes which live virtually ubiquitously in soils are the key player in wetlands. In any wetland, the ecological food web requires microbes, to function in all of its complex transformations of energy. In a constructed wetland, the food web is fueled by influent wastewater, which provides energy stored in organic molecules. Microbial activity is particularly important in the transformations of nutrients into varying biologically useful forms (USEPA, 2000).

Microorganisms that naturally live in water, soil, and on the roots of wetland plants feed on organic materials and/or nutrients thus reducing, breaking down or completely removing a wide variety of contaminants from the wastewater. Functions of wetlands are largely regulated by microorganisms and their metabolism (Wetzel, 1993). Microorganisms are ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily (Francis, 1996). Many of the widely distributed microorganisms in nature possess the ability to utilize hydrocarbons as the sole source of carbon (energy) in their metabolism. The microbial utilization of hydrocarbons was highly dependent on the chemical nature of the components within the petroleum mixture, and environmental determinants (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 microorganisms in presence of sufficient oxygen and nutrients to break down hydrocarbons and other organic contaminants.

Microbial populations adjust to changes in the water delivered to them. Populations of microbes can expand quickly when presented with suitable environment and energy-containing materials. When environmental conditions are no longer suitable, many microorganisms become dormant and can remain dormant for years (Hilton 1993). Microbial performs very important activities in wetlands such as; transforming a great number of organic and inorganic substances into innocuous or insoluble substances, alters the reduction/oxidation (redox) conditions of the substrate and thus affects the processing capacity of the wetland, and is involved in the recycling of nutrients (US EPA, 2000). Many microbes are capable of functioning under both aerobic and anaerobic conditions (facultative anaerobes) in response to changing environmental conditions. In aerobic respiration, microbes use O_2 to oxidize part of the carbon in the contaminant to carbon dioxide (CO_2), with the rest of the carbon used to produce new cell mass. Thus the major byproducts of aerobic respiration are carbon dioxide, water, and an increased population of microorganisms (Francis, 1996, Christensen, et al., 1996, Riser-Roberts, 1992,). 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 (Christensen, et al., 1996).

Microorganisms gain energy for growth and reproduction by catalyzing energy-producing chemical reactions that involve breaking chemical bonds and transferring electrons away from the contaminant. The energy gained from these electron transfers is then "invested," along with some electrons and carbon from the contaminant, to product more cells (Christensen, et al., 1996, Riser-Roberts, 1992).

However, microorganisms do not always gain energy from degradation of contaminants; instead, degradation may be an incidental reaction, commonly referred to as "secondary utilization" or "cometabolism", where the presence of primary substrates to support microbial metabolism is required (NRC, 1993).

2.4. Hydrology

Hydrology is probably the single most important determinant for the establishment and maintenance of specific types of wetlands and wetland processes (Mitsch and Gosselink, 1986). It is the permanent or periodic saturation of a wetland area that results in the anaerobic conditions in the soil under which typical wetland biogeochemical processes occur (Sheoran and Sheoran, 2006). These processes cause the development of characteristic wetland soils, which support a dominant plant community adapted to living in saturated soils (Mitsch and Gosselink, 1993; ITRC, 2003). Gosselink and Turner, (1978) described the hydrology of a wetland to be dependent on two parameters (the hydroperiod and depth of flooding). The hydroperiod represents the integration of all inflow and outflow components of the water budget and is affected by numerous natural factors such as topography, geology, groundwater, subsurface soil characteristics, and weather conditions. The hydroperiod is the seasonal pattern of the water level within a wetland and hydrologically characterizes each type of wetland (Mitsch and Gosselink, 1986). The hydroperiod is the time during which the soil is flooded or saturated, expressed in percentage, while the depth of flooding in a natural wetland varies between +2 m and –1 m relatively to the ground surface, with an average of approximately +1 m. These two parameters highly affect the characteristics (oxygen concentration, pH, nutrients, plants, etc) and stability of the wetlands (Scholz, 2006). However, this research showcases the case scenario where the hydrological cycle are designed in a such a way that the wetlands are temporarily flooded and drained on a regular basis, allowing oxygen to be drawn in and regenerated in the lower levels of the wetlands.

When the wetland is drained, the retreating water acts as a passive pump to draw air from the atmosphere into the matrix (Green *et al.*, 1998; Sun *et al.*, 2003, Zhao, 2004). The hydrological cycle can therefore be determined with precision. Hydraulic Retention Time (HRT) is the average time that water remains in the wetland and is an important variable in designing and evaluating treatment efficiency of wetland treatment systems (Hammer and Kadlec, 1983). HRT is one of the most crucial factors in designing and operating a constructed wetland and variable in determining the efficiency of settling solids, biochemical processes, and plant uptake (Kedlec and Knight, 1996). Nominal HRT is, in some instances, not necessarily indicative of the actual HRT because it is based on the assumption that the entire volume of water in the wetland is involved in the flow (Kadlec and Knight, 1996).

This can generate considerable errors in wetland HRT estimations when a relatively large volume of water remains stagnant without taking part in the flow movement. Under these circumstances, the actual HRT will tend to be shorter than the nominal HRT. One of the design consideration options could be the estimations of HRT with the assumption that the hydrodynamic processes occur under steady-state flow conditions. Existing wetlands are designed with a wide range of HRT, generally ranging from 2 to 20 days. However, wetlands with longer HRT will result in an increase of dissolved organic carbon leached from plant derived material (Pinney *et al.*, 2000). It is suggested that wetlands should have a minimum retention time of at least 10-15 hours to achieve a high level of removal efficiency (Shutes *et al.*, 1999; Ellis *et al.*, 2003). However, hydrodynamics (fluid dynamics) controls the retention time of a wetland and thus the time available for water quality enhancement to take place. Precipitation, surface water inflow and outflow, groundwater exchange, and evapotranspiration are the major factors influencing the hydrology of most wetlands.

A wetland's hydroperiod integrates all aspects of its water budget (rainfall, evapotranspiration, runoff from adjacent areas, flooding, net seepage of ground water). In a hydrologic balance, these components are represented by the following equation (Reinelt et al., 1993):

$$P + I +/- G +/- S = ET + O \quad 2\text{-}1$$

Where P = precipitation; I = surface inflow; G = groundwater exchange, S = change in wetland storage, ET = evapotranspiration; and O = surface outflow.

The balance of inflows and outflows of water through a wetland defines the water budget and determines the amount of water stored within the wetland. Furthermore, the simplified general equation describing the hydrologic balance of a wetland was presented by Mariano (1999) as follows:

$$I - O = \Delta V \quad 2\text{-}2$$

Where I are the different inflows into the wetland, O the different outflows out of the wetland and ΔV the change in volume of water storage within the wetland.

In addition to the water pumped into the wetland, other positive inflow sources are precipitation, groundwater inflow, and seepage. Together with the water pumped from the wetland, there are other negative outflow sources (loss): evapotranspiration, seepage, and aquifer recharge (Kadlec, 1983). The term ΔV represents the net change in storage in the wetland, and is an important component associated with any water budget. With knowledge of the wetland area, the term ΔV describes the wetland water regime, or seasonal pattern of the water stages within the wetland. The terms in Eq. (2-2) are not equally susceptible to measurement. Some terms are difficult to measure and, therefore, are determined as a whole component by solving the budget equation. That combined component also includes the residual error associated with all terms (Kadlec, 1983). The duration and frequency of saturation or inundation of a site vary according to the site's hydrogeologic setting, and they depend on regional differences in physiography and climate and on antecedent moisture conditions (Skaggs et al., 1991; Winter, 1992; Brinson, 1993a; Mausbach and Richardson, 1994). It is pertinent to note that the constructed wetland area must have sufficient detention volume to store the design inflow volume which contains the pollutants for required retention time.

2.5. Types of constructed wetlands

There are several design approaches available for constructed wetlands (Kadlec and Knight, 1996). The basic classification of wetlands is based on the type of macrophytic growth (emergent, submerged, free floating and rooted with floating leaves) (figure 2-1), further classification is usually based on the water flow regime (surface flow, sub-surface vertical or horizontal flow (figures 2-1) (IWA, 2000).

Recently, the combinations of various types of constructed wetland systems (so-called hybrid systems) have been used to enhance the treatment efficiency

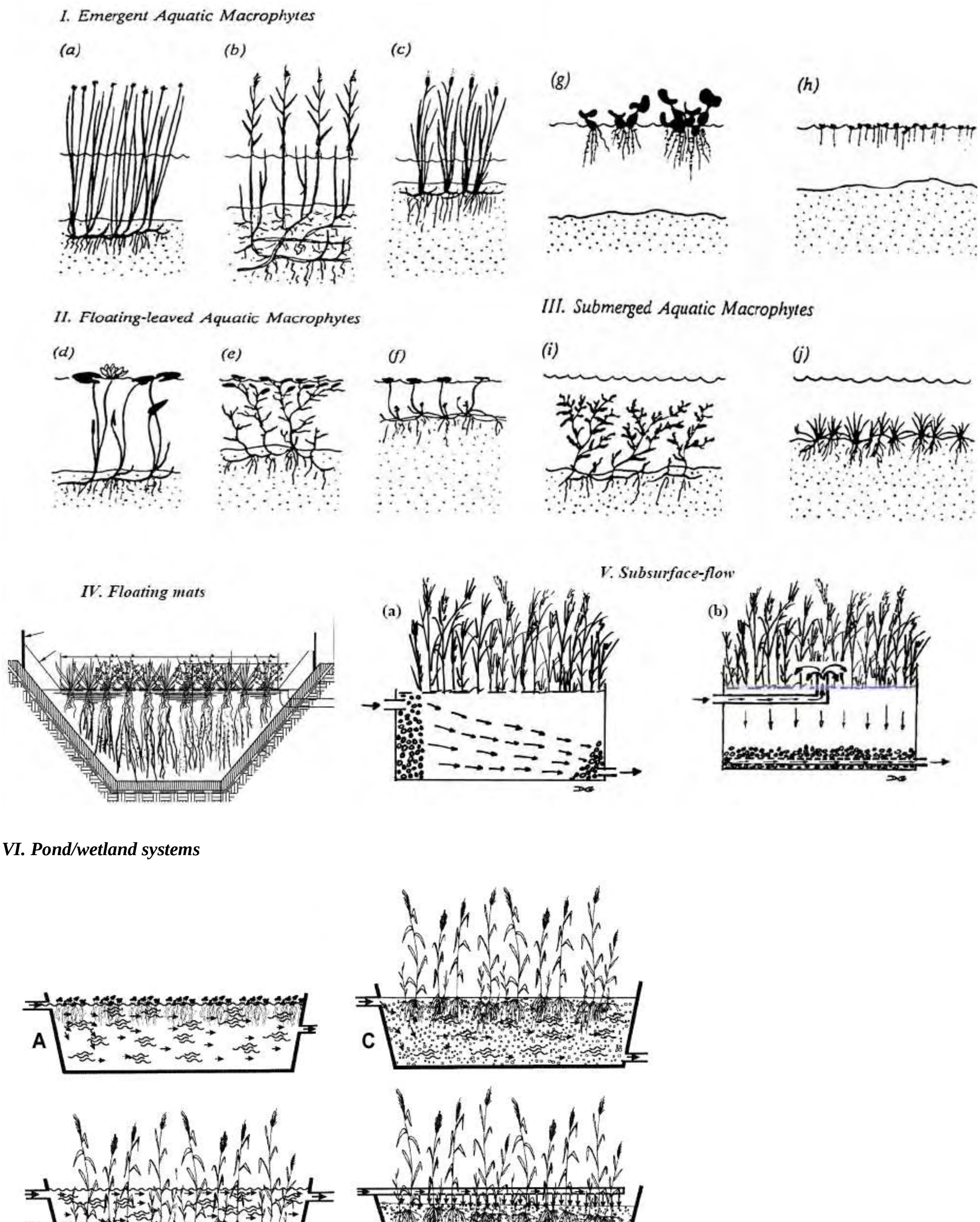


Figure 2-1. Schematic representation of different types of constructed wetlands (I, II, III after Vymazal *et al*., 1998b; IV after Van Acker *et al*., 2005; V after De Wilde and Geenens, 2003; VI after Stottmeister et al, 2003 (A, pond with free-floating plants; B, horizontal surface flow wetland or pond with emergent water plants; C, horizontal subsurface flow wetland; D, vertical flow wetland)).(Vymazal, 2005).

However, for the purpose of practicability, description of constructed wetlands types in this content focuses on water flow regime types such as; surface flow, subsurface flow and hybrid system. Subsurface flow constructed wetlands are further subdivided into horizontal and vertical flow.

2.5.1. Surface-flow system

The Surface Flow (SF) also known as Free Water Surface (FWS) wetland (Figures 2-1b and 2-2) typically consists of a shallow basin or channels with some type of barrier to prevent seepage, soil or any other media to support the roots of the emergent vegetation, and water at a relatively shallow depth flowing through the substrate at low velocities in a slow moving manner. Surface flow treatment wetlands mimic the hydrologic regime of natural wetlands, where water flows over the soil surface from an inlet point to an outlet point or, in few cases, is totally lost to evapotranspiration and infiltration within the wetland (Knight et al., 1999; Scholz et al., 2007). The wetland is flooded from the top and water flows horizontally on top of the wetland soil, infiltrates the soil or is evaporated as the water surface is exposed to the atmosphere (US EPA, 1993). The water is distributed on the ground surface and allowed to flow on top of the ground surface until collected at the outlet. The first full-scale surface flow constructed wetland (CW) was built in The Netherlands to treat wastewaters from a camping site during the period 1967–1969 (Vymazal, 2005). Reed and Brown (1992) characterize this type of wetland as most closely mimicking natural marshes. These wetlands involve dense vegetation and the water is treated as it flows along the surface. Surface wetlands may also have a pond with standing water several feet deep for either aesthetic or wildlife value. Surface flow wetlands have some characteristics in common with facultative lagoons (ITRC, 2003). Tchobanoglous and

Burton (1991) observed that facultative ponds are useful for pretreatment of primary effluent or certain industrial wastes. Wetland processes occurring in deeper zones are nearly identical to processes in the deeper zones of ponds with a surface autotrophic zone dominated by planktonic or filamentous algae, or by floating or submerged aquatic macrophytes (Kadlec, 2001). Anaerobic microbes dominate these deeper zones in the treatment wetland due to the absence of oxygen and light. However, the wetland does not resemble a facultative lagoon at zones closer to the surface. This could be attributable to wetland plants that cool and shade the surface therefore lowering algal growth and limiting water column processes that produce dissolved oxygen (Kadlec, 2001). Another important difference is that surface flow wetlands tend to have higher net carbon production than facultative ponds. This is because of the high gross primary production in the form of structural carbon accompanied by the resistance to degradation and low organic carbon decomposition rates in the oxygen deficient zones (Kadlec, 2001). These differences between wetlands and ponds/lagoons results in differences in biogeochemical cycling and therefore wetlands can not be treated as ponds or lagoons. Surface wetlands are usually not the preferred type in cold climates.

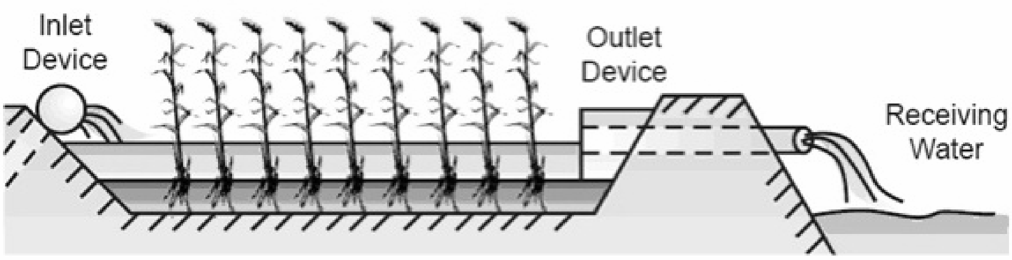


Figure 2-2. Typical configuration of a surface flow wetland system (after Kadlec and Knight, 1996)

This is because they tend to freeze over in the wintertime, which results in significantly lower contaminant removal rates. Further reductions in removal efficiencies arise from the loss of volatilization and oxygen transfer (ITRC, 2003).

2.5.2. Sub-surface-flow

Subsurface Flow (SSF) Wetlands are generally constructed with a porous material such as soil, sand, or gravel for a substrate. SSF 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. Subsurface flow constructed wetlands first emerged as a wastewater treatment technology in Western Europe based on research by Seidel (1966) commencing in the 1960s, and by Kickuth (1977) in the late 1970s and early 1980s. They are designed so that water flows below ground surface through the substrate (ITRC, 2003). In subsurface flow wetlands, the wastewater flows through a constructed media bed planted with wetland plants (US EPA, 1993). In these wetlands, wastewaters are treated as they enters through an inlet distributor and flows slowly below the ground surface, passing through the shoots and/or root-zone of wetland plants until it reaches the outlet collection system. The depth of the flow-through for a constructed wetland is generally between 0.6 to 0.3 meters (Cooper, 1993). Bed depth is normally shallow because at greater depths the roots and rhizomes get smaller and weaker. Any depth less than 0.3 meters decreases the effectiveness of the treatment zone. The beds are normally sealed on all sides with either clay or a plastic liner/membrane to prevent leakage. Gravel beds that use uniform gravel in the range of 3 to 10 millimeters have been shown to work best (Cooper, 1993). The rhizomes of reeds and other species grow horizontally and vertically, which provides openings in the bed to provide a hydraulic pathway (Cooper, 1993).

The wastewater flowing through the bed comes into contact with various aerobic, anoxic, and anaerobic zones. A SSF wetland combines aerobic, anoxic and anaerobic zones. Water purification, achieved through microbiological, physical a chemical processes, mainly takes place in the aerobic zone, which is situated in the rhizosphere. SSF wetlands have the primary benefit that water is not exposed during the treatment process, minimizing energy losses through evaporation and convection. This makes SSF system more suitable for winter application (Wallace *et al*., 2000). In the rhizosphere, large populations of common anaerobic and aerobic bacteria grow. These bacteria can breakdown the contaminants. It has been shown that bacterial population levels in the rhizosphere are enhanced by oxygen transfer from plants (Kadlec, 2001). Aerobic zones are located around the roots and rhizomes of the plants because of their ability to transport oxygen down from the leaves and stem into the rhizomes and out through the roots (Hiegel, 2004). Wetland plant species do this because of a unique characteristic that allow them to adapt to anaerobic soil conditions. The plants develop internal air spaces called aerenchyma that transport the oxygen into the root zone. These air spaces can occupy up to 60% of the total tissue depending on plant species (Reddy and D'Angelo, 1997). The oxygen is transported through molecular diffusion as a result of partial pressure gradients and mass flow as a result of temperature and humidity induced pressurization (Reddy and D'Angelo, 1997). This then stimulates the growth of aerobic bacteria and helps remove BOD and nitrogen by promoting oxidation-reduction reactions in the rhizosphere.

There are two basic types of SSF wetlands: horizontal flow (HF) and vertical flow (VF). Both allow water to flow through permeable, root-laced media, but some vertical flow systems combine an organic substrate with the permeable media. Large populations of bacteria and beneficial fungi live in the beds as biofilm attached to the media surfaces. VF systems have removal mechanisms similar to those of HF systems but completely different hydraulics.

The advantages of SSF systems include increased treatment efficiencies, fewer pest problems, reduced risk of exposing humans or wildlife to toxics, decreased waterfowl use (advantageous near certain facilities such as airports), and increased accessibility for upkeep (no standing water). Subsurface-flow systems have the advantage of requiring less land area for water treatment, but are not generally as suitable for wildlife habitat as are surface-flow constructed wetlands. The substrate provides more surface area for bacterial biofilm growth over an SF wetland, so increased treatment effectiveness may require smaller land areas. 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 since they are more insulated by the earth. 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).

2.5.3. Horizontal-flow system

Horizontal flow (HF) systems (figures 2-1c and 2-3) are designed in such a way that water flows in a horizontal direction with the inlet at one end and the outlet at the opposite end. Surface Flow constructed wetland systems did not spread

throughout the Europe but constructed wetlands with horizontal sub-surface flow horizontal flow systems became the dominant type of CWs in Europe. However, the first full-scale horizontal flow system was built in 1974 in Othfresen in Germany

(Vymazal, 2005). Vymazal (2005) also stated that the early horizontal flow systems in Germany and Denmark used predominantly heavy soils, often with high content of clay. These systems had a very high treatment effect but because of low hydraulic permeability, clogging occurred shortly and the systems resembled more or less surface flow systems. Kickuth (1977) proposed the use of cohesive soils instead of sand or gravel; the vegetation of preference was *Phragmites* and the design flow path was horizontal through the soil media.

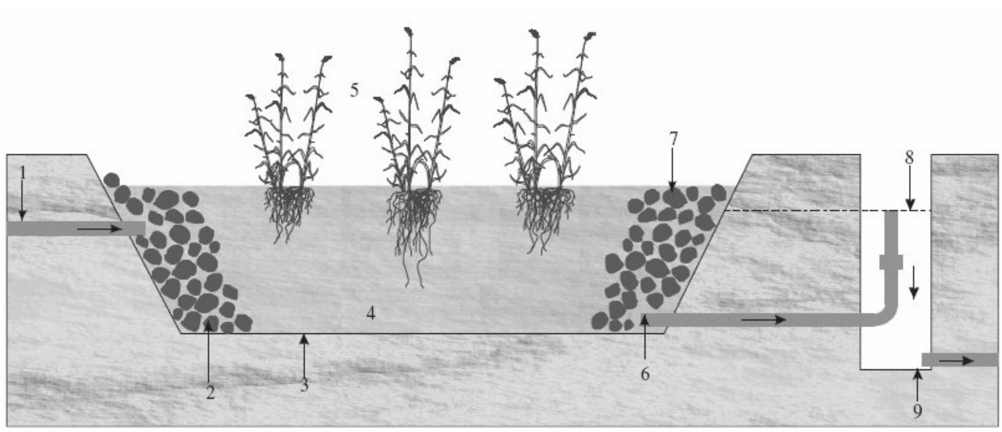


Figure 2-3. Schematic representation of a standard planted constructed wetland with horizontal sub-surface flow (after Vymazal, 2001). 1: inflow; 2: distribution zone filled with large stones; 3: impermeable layer; 4: aggregates (e.g. gravel, sand and crushed stones); 5: macrophytes; 6: outlet collector; 7: collection zone filled with large stones; 8: water level; 9: outflow.

The original concept as developed by Seidel included a series of beds composed of sand or gravel supporting emergent aquatic vegetation such as cattails *(Typha)*,bulrush *(Scirpus)*, and reeds *(Phragmites)*, with *Phragmites* being the most commonly used. Excellent performance for removal of BOD_5, TSS, nitrogen, phosphorus, and more complex organics was claimed (US EPA, 1993).

2.5.4. Vertical-flow system

Vertical flow wetlands (figures 2-4) originally developed by Seidel (1967) are above ground constructions either built of impermeable materials or lined with synthetic or clay materials to prevent seepage to the groundwater. In vertical flow SSF system, the surface of the wetland floods to a depth of several centimeters then slowly percolates downwards through the granular media undergoing filtration and coming into contact with the dense microbial populations on the surface of the media particles and macrophyte roots. Vertical flow wetlands can be saturated with water or dried, thus enabling oxygen to be regenerated in all areas of the wetland which are usually flooded and anaerobic.

Vertical flow systems are becoming more popular than the horizontal flow systems. The reason for growing interest in using vertical flow systems are (i) they have much greater oxygen transfer capacity resulting in good nitrification, (ii) they are considerably smaller (1-2 m^2/pe) (pe means person equivalent (equal to one person living continuously in catchment area for wetland treatment)) than the HF system which need 5-10 m^2/pe for secondary treatment, (iii) they can efficiently remove BOD_5, COD and bacteria (Cooper, 1999, USEPA, 2000). In comparison, horizontal flow systems tend to be oxygen limited because wetland vegetation cannot supply the oxygen at a fast enough rates compared to the wastewater requirement and therefore tend to be unable to nitrify to high levels (Kadlec, 2001).

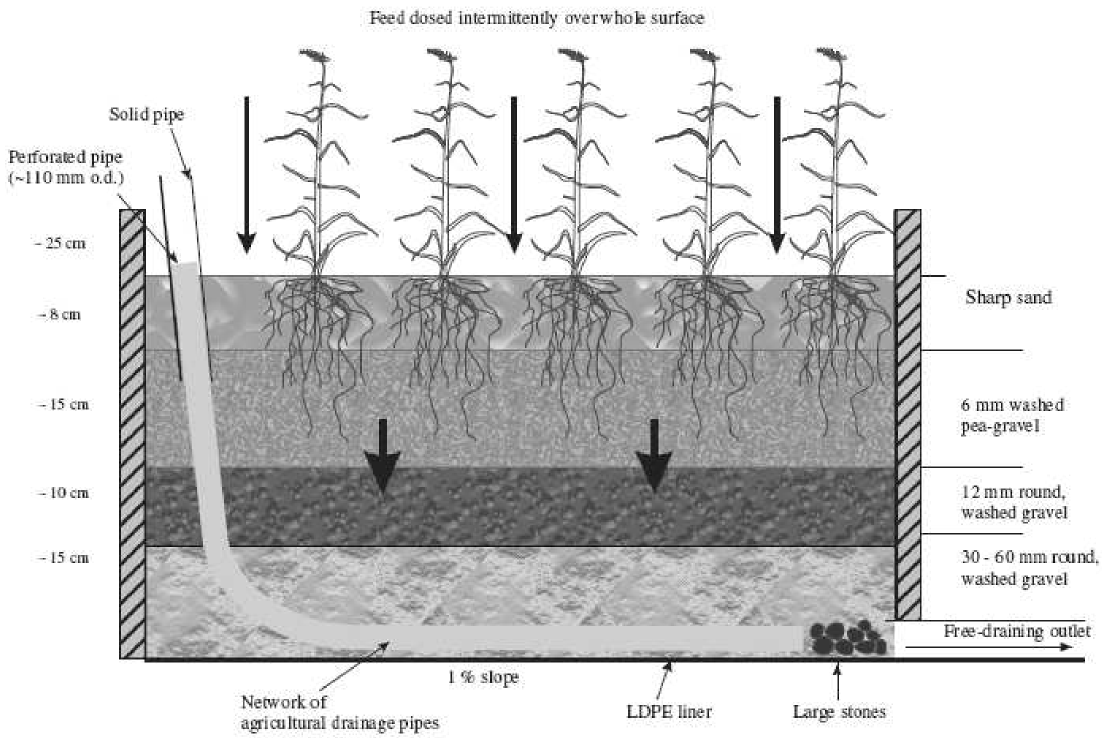


Figure 2-4. Schematic representation of a standard planted constructed wetland with a vertical flow (after Cooper *et al.*, 1996)

Upward vertical flow systems are a very new concept and have only been tested at smaller scale sites and there have not been many long-term studies conducted (Kadlec, 2001). Another variation of the vertical flow system involves the addition of passive aeration (Kadlec, 2001). In these systems the bed contains small diameter gravel over a rock layer. Within the rock layer there is a network of perforated aeration pipes. The network is vented vertically through a riser pipe. Wastewater is applied to the top of the bed and flows vertically downward through the bed until it reaches the drainage system located in the rock layer. When the bed is being drained the water quickly drains out of the bed and air is then drawn into the bottom of the bed. This causes bubbles to migrate to replace the draining water. As wastewater enters from the top and flows to the bottom of the bed, it traps the air and forces it upward through the bed. Draining of bed allows more efficient BOD and ammonia-N removal compared to the continuously saturated and generally anaerobic horizontal-flow system (Cooper, 1996; Magmedov *et al.,* 1996). Vertical flow constructed wetlands have more equal root distribution and water-root contact and fewer problems of bad odor and proliferation of insects since they do not have a free water surface (Haberl *et al.*, 1995; Cooper, 1999).

2.5.5. Hybrid system

A hybrid system is a combination of two or more different systems. Hybrid systems are comprised most frequently of vertical flow (VF), horizontal flow (HF) and stabilization pond systems arranged in a staged manner. Different layouts include single-cell, dual-cell in series, or multiple-cell (parallel or in series). While some constructed wetlands are solely water based systems, Integrated Water Strategies combines soil wetlands with the moisture regimes of periodically flooded wetland environments. These types of Constructed Wetlands, also called hybrid systems, allow for periods of dry, aerobic conditions that sustain more complete pollutant removal. Hybrid system is used in the present research (vertical-flow with stabilization pond). As the wetlands are filled up to the top, the filter media is covered with water. The top layer of the wetlands can then be compared to a stabilization pond and the bottom part of the filter acts as vertical flow wetland (Kedlec and Knight, 1996). The particular system designed for this study can be classified as a combination of a vertical-flow wetland system and a facultative pond. A facultative pond is made of three different strata: the surface zone, which is aerated naturally; an intermediate zone which is both anaerobic and aerobic; and a bottom layer which is anaerobic (more detail presented in chapter 3). The pond must not be too deep, otherwise light penetration is impeded and the anaerobic zone increases.

In late 1990s, the inability to produce simultaneously nitrification and denitrification in a single horizontal flow or vertical flow and thus remove total nitrogen lead to the use of hybrid systems which combine various types of constructed wetlands. The concept of combination of various types of filtration beds was actually suggested by Seidel in Germany in the 1960s but only few fullscale systems were built (e.g. Saint Bohaire in France or Oaklands Park in UK) in 1980s and early 1990s (Vymazal, 2005).

2.6. Application of temporarily flooded wetlands

The temporarily flooded wetlands as applied in this research is a hybrid (a combination of vertical-flow and stabilization pond) design system which is rhythmically (temporarily flooded) filled with wastewater then drained, and similar system (tidal vertical-flow constructed wetlands) (Zhao *et al*., 2004) was used elsewhere. This kept attracting significant attention due to its highly efficient treatment potential and relatively low operation cost. In recent years, several studies

have shown much progress in the design, operation and performance reliability of treatment wetlands by developing novel treatment wetlands, such as tidal flow system (Green *et al*., 1998; Zhao et al., 2004; Sun *et al*., 2005), aerated systems (Bezbaruah and Zhang, 2003; Wallace et al, 2004; Lee, 2005) and combination of constructed wetland with other treatment systems (Obarska-Pempkowiak and Klimkowska,, 1999). Zhao et al., (2004b) demonstrated that a gravel-based tidal flow reed bed system produced the highest pollutant removal efficiencies with a relatively short saturated period and long unsaturated period, highlighting the importance of oxygen transfer into reed bed matrices during the treatment. Furthermore, tidal vertical-flow constructed wetlands have potential to enhance the removal of BOD through aerobic decomposition and removal of ammonium-N through nitrification, as maximum pollutant-biofilm contact is established and the rate of oxygen transfer increased during the operation (Sun *et al*., 2005). Zhao et al., (2004b) also demonstrated that application of the tidal flow system achieved percentage removals of 86% and 78% for COD and BOD_5 from initial levels of 4,254 mg/l and 3,150 mg/l, respectively, under the hydraulic retention time of five hours per day.

2.7. Removal mechanisms of a constructed wetland

Constructed wetlands are highly complex systems that separate and transform contaminants through several mechanisms as the wastewater flows through the system (Figure 2-5). IWA specialist group (2000) described the mechanisms involved in constructed wetlands as follows: 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 effluent and the substrate and litter, breakdown and transformation and up take of pollutants and nutrients by microorganisms and plants, absorption and ion exchange on the surface of the plants, substrate, sediment and litter, predation and natural die off and settling of suspended particulate matter.

The predominant 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. The external input parameters most often of concern include the wastewater quality and quantity and the system hydrological cycle (USEPA, 2000). The mechanisms used for treatment of a contaminant depend on the specific contaminant, site conditions, remedial objectives, and regulatory issues (ITRC, 2003).

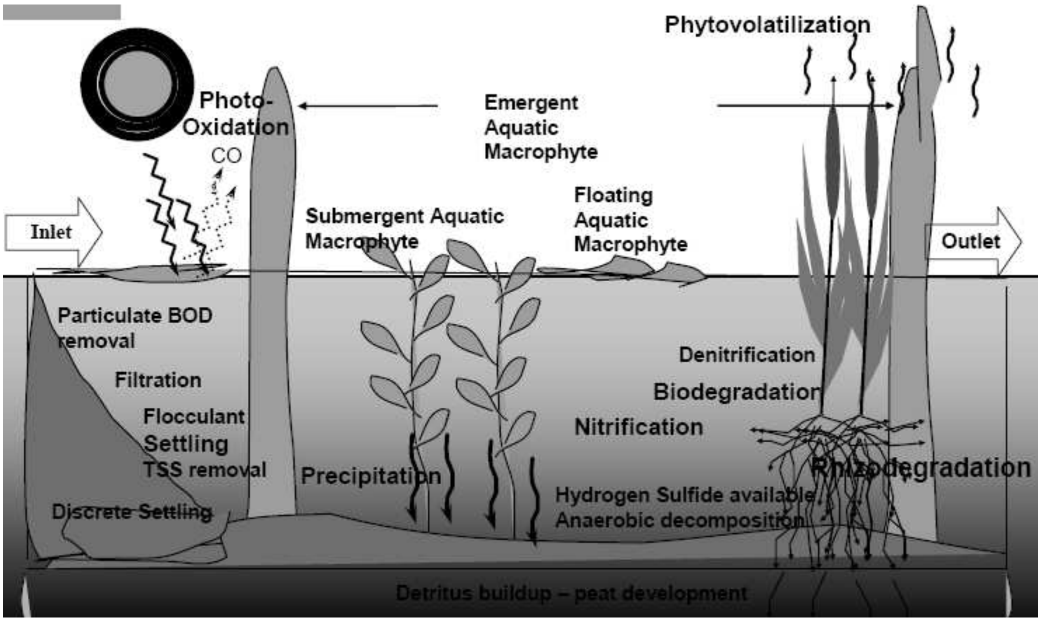


Figure 2-5. Processes Occurring in a Wetland (after ITRC, 2003)

Processes taking place in a constructed wetland may be grouped as abiotic (physical/chemical) or biotic (microbial/ phytological). Wetlands are capable of providing highly efficient physical removal of contaminants associated with particulate matter in the wastewater. The primary physical processes that are responsible for contaminant removal in a constructed wetland include settling and sedimentation. Settling and sedimentation achieve efficient removal of particulate matter and suspended solids (Kadlec and Knight, 1996; ITRC, 2003). Biological removal processes probably is the most important pathway for contaminant removal in wetlands. These transformations are a result of the high microbial activity that occurs in the wetland soils. Biological removal mechanisms include aerobic microbial respiration, anaerobic microbial fermentation and methanogenesis, plant uptake, extracellular and intracellular enzymatic reactions, antibiotic excretion and microbial predation, and die-off (ITRC, 2003). The coupled processes of nitrification and denitrification are universally important in the cycling and bioavailability of nitrogen in wetland and upland soils (Mitsch and Gosselink, 1993; Reddy and D'Angelo, 1994; Kadlec and Knight, 1996; DeBusk, 1999). Most pollutant-transforming chemical reactions occur in wetland water, detritus, and rooted soil zones. In addition to physical and biological processes, a wide range of chemical processes are involved in the removal of contaminants in wetlands. However, the primary contaminant removal mechanisms are summarized in table 2-1 below.

Table 2-1. Summary of Primary Contaminant Removal Mechanisms

Contaminant /	**Mechanism**		
Water quality variable	**Physical**	**Chemical**	**Biological**
Oxygen demand • Biological oxygen demand • Chemical oxygen demand	 Settling Settling	 Oxidation	 Biodegradation
Hydrocarbon: • BTEX, TPHs, Fuels, oil and grease • PAHs, chlorinated and nonchlorinated solvents pesticides, herbicides, insecticides	 Volatilization Diffusion Settling	 Photochemical oxidation	 Biodegradation/ Photodegradation/ Photovolatilization/ Evapotranspiration
Nitrogenous Compounds • Nitrate-nitrogen, Ammonia-nitrogen Organic N, NO_2	 Settling		Biodenitrification Nitrification Plant uptake
Phosphoric Compounds • Ortho-phosphate-phosphorus Organic P	 Settling	 Precipitation Adsorption	 Microbes Plant uptake
Metals • Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Ni, Se, Ag, Zn	 Settling	 Precipitation/ adsorption/ ion exchange	 Biodegradation/ phytodegradation/ phytovolatilization
Pathogens		UV radiation	Die-off Microbes

(Sources: Kadlec and Knight, 1996; Hammer, 1997; Moshiri, 1993; Horner, 1995; ITRC, 2003)

2.7.1. Organic compound removal

Wastewaters contain a wide variety of organic compounds, which are measured as BOD, COD, and total organic carbon (TOC). Hydrocarbons and other priority organic compounds are another group of contaminants that has the potential to affect the habitat value of treatment wetlands. The main routes for organic carbon removal include volatilization, photochemical oxidation, sedimentation, sorption, and biodegradation (ITRC, 2003). Toxic organics can undergo wetland treatment in the same manner as natural organic matter. The organics can be removed by aerobic microbial breakdown, anaerobic microbial breakdown, vegetative uptake, volatilization, photolysis, chemical hydrolysis, sorption and burial in the soil (Reddy and D'Angelo, 1997). The removal method depends on the type of compound and chemical/biological condition of the soil water. Altering the temperature, light intensity, nutrient availability, electron acceptor availability, or organic matter content changes the processes that take place and the degree to which they can occur.

Constructed wetlands usually provide high BOD removal (Vymazal, 1999; Neralla *et al*., 2000; Leuderitz *et al*., 2001). Organic contaminants sorbed onto particles flowing into the wetlands settle out in the quiescent water and are then broken down by the microbiota in the sediment layer. The accumulated organic matter potentially contributes to the clogging of pore spaces in wetlands and may ultimately leads to a decline in wastewater retention time and reduction in the efficiency of nutrient removal (Nguyen, 2000). Volatilization may also be a significant removal mechanism in the microbial breakdown products of organics. Organic matter contains about 45–50% carbon. BOD is a measure of the oxygen required by the microorganisms to oxidize the organic matter. Cooper *et al* (1996) observed that the uptake of organic matter by the constructed treatment wetlands macrophytes is negligible compared to the biological degradation.

2.7.2. Hydrocarbon removal

Hydrocarbons consist of a broad range of compounds, both naturally occurring and anthropogenically developed, whose characteristics are primarily determined 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. Aliphatics can be further divided into three main groupings - the alkanes, the alkenes, and the cycloalkanes. Aromatic compounds have one or more benzene rings as structural components to them. Benzene is a carbon ring that always consists of six carbons atoms and six hydrogen atoms (C_6H_6). The more common simple aromatics encountered as environmental pollutants include benzene, toluene, ethylbenzene and xylene (BTEX) (ITRC, 2003). The classes of compounds are susceptible to the degradation processes typical to constructed wetlands. Benzene contamination is a significant problem. It is used in a wide range of manufacturing processes and is a primary component of petroleum-based fuels. Benzene is a hydrocarbon that is soluble, mobile, toxic and stable, especially in ground and surface waters. It is poorly biodegraded in the absence of oxygen (Coates et al., 1999).

Exploration, production, refining, storage, transportation, distribution and utilization of petroleum hydrocarbons have brought about frequent occurrences of water and soil contamination with hydrocarbon (Atlas and Cerniglia, 1995). The pollution of the environment increases as petroleum hydrocarbon continues to be used as the principle source of energy. These problems often result in huge disturbances and disastrous consequences for the biotic and abiotic components of the ecosystem

(Mueller et al., 1992). Even small releases of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in excess of regulatory limits (Spence et al., 2005). Since 1995, journal articles and symposia proceedings indicate the petroleum industry's interest in using constructed wetlands to manage process wastewater and storm water at a variety of installations including refineries, oil and gas wells, and pumping stations (Knight, 1999). The area of emphasis in this research is the use of constructed wetlands for treatment of dissolved petroleum hydrocarbon compounds. Petroleum hydrocarbon wastewaters contain monoaromatic hydrocarbons (i.e. benzene, toluene, ethylbenzene and xylene) which are commonly found in gasoline, and are highly volatile substances (Coates, 2002). Petroleum hydrocarbon wastewaters contain also pollutants such as COD, BOD, nitrogen and phosphorus (Knight et al, 1999). However, the major focus of the petroleum industry is on assessing the removal efficiency of hydrocarbons.

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). Due to their relatively high solubility and toxicity, they represent a significant health risk in contaminated environments. Of all of the BTEX compounds, benzene is of most concern, because it is the most toxic and a well-known human carcinogen. The benzene ring is a chemical structure that is common in nature. Moreover, the thermodynamic stability of the benzene ring increases its persistence in the environment; therefore, many aromatic compounds are major environmental pollutants (Dagley, 1986; Díaz, 2004). Their major industrial source is petroleum and natural gas, formed geochemically from biomass under high pressure and temperature (Heider et al, 1998). Aromatic hydrocarbons are one of the most abundant class of organic compounds and constituents of petroleum and its refined products. Monocyclic aromatic hydrocarbons are of major concern, because of their toxicity, high solubility and ability to migrate within groundwater. These BTEX compounds are of primary discharge concern for the water quality of receiving waters (Caswell, 1992). The BTEX fraction of total volatile hydrocarbons is primarily responsible for most of the total toxicity in gasoline-contaminated groundwater.

Hence, an attempt to reduce toxicity requires targeting these compounds for destruction. Many components of hydrocarbon mixtures are toxic and relatively soluble in water. In natural gas, benzene concentrations typically range from about 0 to 1,000 mg L^{-1}; in crude oils from virtually zero to10, 000 mg L^{-1} (Janks and Cadena, 1991). Benzene has relatively high water solubility (1,780 mg L^{-1}). Water contamination by oil exploration and production operations, tank farms, underground storage tanks leakage and refineries have become a concern to the oil and gas industry. Kadlec and Knight (1996) indicate that the major mechanisms for the removal of hydrocarbons via constructed wetlands are volatilization and biological or microbial degradation, others were photochemical oxidation, sedimentation, sorption, chemical precipitation and filtration (figure 2-6).

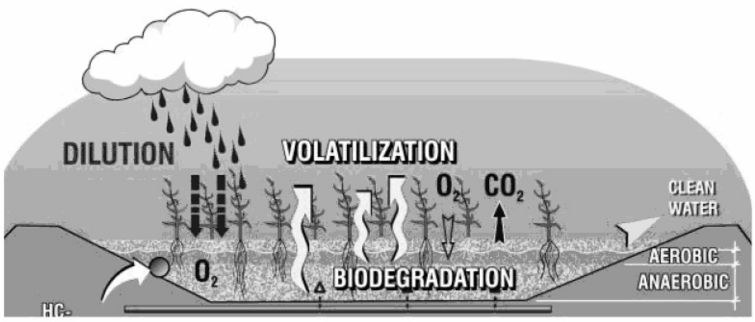


Figure 2-6. Hydrocarbon removal processes in a Wetland (after Komex International Ltd 2001)

Volatilization is the principal degradation pathway for the alkanes, while the aromatic compounds - likely to be more water soluble - tend to be acted upon by other processes upon dissolution in water (Wallace, 2001). In general, high-molecular-weight compounds degrade more slowly than lower-molecular-weight compounds. Significant proportions of hydrocarbon removal in constructed wetlands occur through volatilization and biodegradation.

Degradation occurs both aerobically and anaerobically, depending on the oxygen supply and the molecular structure of the compound. As oxygen is the most thermodynamically favoured electron acceptor used by microbes in the degradation of organic carbon, rates of biodegradation of hydrocarbons in aerobic environments is more rapid than in anaerobic environments. Plants provide oxygen to the rhizosphere thus creating an aerobic environment. This in turn supports microbial communities that can either directly biodegrade or catalyze chemical reactions and maintain the biotransformation process. Bacteria that are capable of degrading volatile organics such as BTEX have been found in the rhizosphere (Sugai et al., 1997). Furthermore, numerous benzene-degrading aerobic microorganisms have been identified; the most notable are the *Pseudomonas* species, which may account for up to 87% of the gasoline-degrading microorganisms in contaminated aquifers (Ridgeway, 1990). Petroleum wastes are documented to degrade in natural wetland environments (Wallace and Knight, 2006; Wemple and Hendricks, 2000). Benzene is biodegradable, particularly in the presence of oxygen (Alexander, 1999). Benzene degradation has also been demonstrated in the presence of nitrate-nitrogen (Burland and Edwards, 1999). Many studies have shown that microbial degradation of petroleum hydrocarbons in the environment is strongly influenced by physical and chemical factors such as temperature, oxygen, nutrients, salinity, pressure, water activity, pH, and the chemical composition, physical state, and concentration of the contaminant; and by biological factors such as the composition and adaptability of the microbial population (Zhou and Crawford, 1995). Because biodegradation and evaporation processes compete in removing petroleum hydrocarbons, biodegradative losses can not be differentiated clearly from volatility losses (Zhou and Crawford, 1995). Wetland plant selection is important but not as significant as having a good microbial community (Baris et al., 2001). However, the presence of other factors common to wetlands (such as nitrate) that will serve as electron receptors during anaerobic biodegradation of hydrocarbon compounds is important (ITRC, 2003).

Hydrocarbon degradation is less dependent on the actual reactions taking place than it is on the processes occurring in the surrounding ecosystem (Sugai et al, 1997). Aerobic biodegradation and volatilization constitute a coupled pathway that contributes significantly to the natural attenuation of hydrocarbon (Lahvis et al, 1999). Achieving high treatment performances within a short time is critical, so the design can be amended to allow manipulations of environmental conditions to enhance dissolved hydrocarbon treatment. Environmental conditions to be taken into consideration include dissolved oxygen (DO), pH, temperature and nutrient requirements (i.e. nitrogen and phosphorous) of the wetland plants and microbes. Associated contamination is therefore a major environmental problem due to the manufacture, transportation and distribution of petroleum (Atlas and Cerniglia, 1995).

Produced and wastewaters represent the largest volume waste stream in the exploration and production of oil. As the producing field gets to maturity stage, the volume of produced water exceeds up to ten times the total volume of hydrocarbon produced (Stephenson, 1992). Treatment and disposal of such large volume is of great concern to the operator and the environment. Wastewaters from the oil industry contain aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (ortho, meta and para isomers), which are highly soluble, neurotoxic and cause cancer (Hiegel, 2004). Due to their toxic properties and persistence in nature, biodegradation processes and wetland remediation methods have attracted great attention (Ilker et al., 2000). Most of the traditional treatment technologies used by the oil industry such as hydrocyclones, coalescence, flotation, centrifuges and various separators are not efficient concerning the removal of dissolved organic components including aromatics in the dissolved water phase (Descousse et al., 2004; International Association of Oil and Gas Producers, 2002). Historically, the removal of organic compounds from water by most of the traditional treatment technologies has relied upon exploiting density differences between water and the oils and/or organic compounds to be removed.

Removal of BTEX compounds in constructed wetlands occurs through volatilization and aerobic biodegradation (Stephenson, 1992). Biodegradation of hydrocarbons is the result of the metabolic activity of microorganisms. Metabolism is a term that embraces the diverse reactions by which a microorganism processes food materials to obtain energy and the compounds from which cell components are made. Biodegradation typically relies on heterotrophic microorganisms; that is, microorganisms that require carbon in the form of relatively complex, reduced organic compounds (e.g., petroleum hydrocarbons). These microbes rely on the oxidation of these reduced organics in exothermic degradation reaction sequences that yield energy and the “building blocks” of biosynthesis (Admire et al, 1995). To biodegrade a given quantity of organic contaminant, a corresponding quantity of oxygen is required. The equation that describes the overall stoichiometry of the oxidation of aromatic hydrocarbon compounds to carbon dioxide and water under aerobic conditions is given below, using benzene as an example:

C_6H_6 (benzene) + 7.5 O_2 (oxygen) ---> $6CO_2$ (carbon dioxide) + $3H_2O$ (water) 2-3

The balanced reaction indicates that 7.5 moles of oxygen are required to metabolize one mole of benzene. Similar calculations can be made for toluene, ethylbenzene and xylenes. Thus in the absence of microbial cell production, each 1.0 mg/L of DO consumed by microbes will mineralize (convert completely to carbon dioxide and water) approximately 0.32 mg/L of benzene or BTEX compounds (Admire et al, 1995). Constructed wetland technology is environmentally friendly and less expensive than other physical–chemical methods, because it involves natural processes resulting in the efficient conversion of hazardous compounds (Ye et al., 2006). Wetland systems are also innovative and inexpensive treatment approaches (Rew and Mulamoottil, 1999), which have the potential of removing organics such as aromatic components in the dissolved water phase and inorganic compounds in wastewater (Wallace and Knight, 2006). Constructed wetlands offer the benefits of natural wetlands, but can be "custom made" to meet the treatment and construction needs of each individual site. Despite the increasing popularity, the effectiveness, environmental friendliness and positive economics of constructed treatment wetlands, the application of this novel wastewater treatment technology is still rare in the petroleum industry. However, latest type of application of treatment wetlands in the petroleum industry is just getting started with numerous pilot studies and a few full-scale systems in operation.

2.8. Treatment wetland models

The use of treatment wetlands continues to increase and so is our understanding of their many varied, yet interconnected processes. Understanding of the physical, chemical and biological processes which interacts to remove pollutants in a wetland is necessary for comprehensive modeling. Contributing to wetland processes are soils, microorganisms, plant litter and macrophytes. A principal controlling factor is water movement patterns in the wetland as it determines the extent of reaction for the pollutants of concern. The investigation of flow patterns and mixing in a fluid system is a well established field of chemical engineering (Werner and Kadlec, 2000). The standard procedure is to develop a model that produces residence time distribution and use the model to provide a simplified view of the very complex system (Werner and Kadlec, 2000). First-order degradation model has been widely used to predict removal performance for all pollutants such as organic matter, suspended solids and nutrients in constructed wetlands. Although there is no convincing evidence that the rate of organic matter removal is first-order, it is still seen as most appropriate equation in light of present knowledge (IWA specialist group, 2000; Sun *et al*., 2005). However, wetlands are a natural system and precise models are not available to size them for specific applications. Wetlands treating municipal wastewater can be sized using a number of different models that each use different design parameters. Models based on detention time, hydraulic loading rate, pollutant loading rate, pollutant uptake rate, percentage of the contributing watershed, design storm detention, and mass balance design have been used depending on the wetland's intended use and waters received (Kadlec and Knight, 1996). In most of the wetlands receiving mainly municipal wastewater, the model parameters are based on the removal of total suspended solids and biochemical oxygen demand. The design and description of treatment wetlands are based on two important parameters: hydraulics and pollutant removal. Some of the design parameters that may be used to model a wetland for design purposes include: evapotranspiration, flow averaging, linear head loss, complicated geometries, mixing, solids trapping efficiency, accretion, bed clogging, and thermal considerations in the summer and winter (Hiegel, 2004).

The first evaluations done on wetlands models involved Darcian flow in subsurface flow systems and vegetated open channel flow in free water surface systems (Kadlec, 1997). These first evaluations suggested using first order irreversible pollutant reduction removal models for treatment wetlands. The first order models can be of two types; the first type is area specific and requires the acreage of the wetland to be determined, while the second is volume specific and requires the wetland water volume to be determined (Kadlec, 1997). These methods represent outlet concentrations based on the inlet concentrations, flow rate, and area or volume. This is not a very accurate representation of what is actually occurring in a treatment wetland. There are a number of other variables that can cause changes in the outlet concentration not represented in the model. Unpredictable fluctuations in input flows and concentrations, changes in internal storages, weather, animal activity, and other ecosystem factors can case the outlet concentration to rise and fall (Hiegel, 2004). Larger more complex models have been used to predict wetland performance based on dynamic behavior of the various ecosystem compartments and processes but these have drawbacks too. The complex models require large amounts of data for proper calibration and use. Flow rates and concentrations for the inlet and outlet are generally insufficient for calibration. Also little is known about the numerous model parameters (Kadlec, 1997). Calibrated compartmental models can provide more details on internal allocations of chemicals, but detailed deterministic models may not provide more accurate descriptions of overall wetland performance (Kadlec, 1997). Because of these problems in modeling wetland treatment systems, only the simple models are used for actual systems. Simple first order area based models have been used by Knight in the petroleum industry which provided a highly simplified description of the complex wetland carbon interactions (Knight et al., 1999). This can represent the system fairly well with about 90% of the intrasystem variability. Simple models assume steady state conditions. However, atmospheric contributions to the water budget can cause temporary deviations from this assumed steady state condition. Evapotranspiration is one of the main deviations. It occurs during all daylight hours but it may be suppressed during cloudy or rainy periods. Evapotranspiration has two effects: first, it lengthens the detention time and second, it concentrates the pollutants. Rainfall also changes steady state conditions. Changes made by rainfall are dependent on frequency distributions of intensity, duration, and inter-event spacing. Rainfall has the opposite effects as evapotranspiration. Rainfall shortens the detention time and dilutes the pollutants. Another potential change to the system is inlet wastewater flows. Inlet flow can be subject to daily, weekly, or seasonal variations, and random upsets. Any of these changes are important to the system because they can change the detention time and dilute or concentrate the pollutants in the wastewater stream. These changes can also cause other problems by altering the hydraulics of the system caused by flooding or drying conditions.

Another important variable that must be considered in modeling wetlands is temperature. Since many biological process rates are temperature sensitive, the rate constants for wetland processes are also temperature sensitive. However, the overall pollutant removal involves many processes occurring simultaneously. These involve physical processes such as sedimentation and sorption, microbially mediated storages and conversions, uptake and storage in biota of varying sizes and life histories, and transfers of other reactants, such as oxygen and carbon dioxide (Kadlec, 1997). Because of all these processes and the seasonal variation, temperature cannot always be assumed to be the cause for changes in the performance of the system.

The simple steady state model used by Kadlec and Knight (1999) in the petroleum industry is

$$J = k_A (C - C^*) \qquad 2.4$$

Where J is the rate of contaminant removal (g m^{-2} yr^{-1}), k_A is the areal removal rate constant (m yr^{-1}), C is the concentration (mg/L), and C^* is the background concentration (mg/L). Assuming the volumetric flow rate (Q) is constant along the length of the wetland (by ignoring infiltration and precipitation), and assuming the background concentration is zero for petroleum hydrocarbons, yields the following first order plug flow equation.

$$(C_o/C_i) = e^{(-k_A A/Q)} = e^{(-k_v \tau)} \qquad 2.5$$

Where C_o is the effluent concentration, C_i is the influent concentration, A is the subsurface area of the wetland, k_v is the volumetric rate constant (day^{-1}), and τ is the hydraulic detention time (days) (Kadlec and Knight, 1996). This model assumes plug flow, which means that the flow is occurring in only one direction and no mixing is occurring, which seldom exists in wetland systems. This areal rate constant is only applicable to one water depth and care must be taken when applying it to another water depth. An alternative method is to use first-order kinetics and a plug-flow reactor with axial dispersion by simulating the actual flow by using a number of complete-mix reactors in series. Studies have shown that a cascade of four to six complete-mix reactors in series can be used to model the performance of constructed wetlands designed as plug-flow reactors (Crites and Tchobanoglous, 1998). The equation for complete-mix reactors is shown below.

$$(C_N/C_o) = 1/(1+kV/NQ)^N \qquad 2.6$$

Where C_N is the effluent concentration from the Nth reactor in series (mg/L), C_o is the influent concentration (mg/L), k is the overall removal rate constant (day^{-1}), V is the total volume of the wetland (ft^3), N is the number of reactors, and Q is the flow rate (ft^3/d).

These mathematical models are derived based on steady-state, plug-flow assumptions which combine removal processes and variables affecting those processes into single, first-order removal rate functions. Werner and Kadlec (2000) suggested that a constructed wetland has an infinite number of ‘micro’ zones of diminished mixing (ZDMs) all along a set of main channels. These zones are not excluded ‘dead zones’, but they only exchange water with the main flows on a limited basis. Comparing wetland system to reactors; the main flow paths, from the inlet to the outlet, are represented by a plug flow stage, and the ZDMs are represented by continuous stirred tank reactor (CSTRs). Thus, there is a plug flow section which has CSTRs attached to it along its length. A parcel of water traveling along the plug flow reactor (PFR) has a small probability of exiting the PFR to enter one of the infinite number of ZDMs.

2.9. Role of temperature

Temperature is a major factor controlling the fate of petroleum hydrocarbons within the aquatic environment, and the hydrocarbon-degrading microbial population within an aquatic ecosystem is not necessarily adapted optimally to the seasonal water temperature (Cooney, 1984). The United States Environmental Protection Agency (US EPA) conducted a subsurface flow wetland technology assessment, and identified high priority research topics including the temperature and seasonal effects on wastewater treatment (US EPA, 1993). It follows that temperature effects on the performance of constructed wetlands are a key factor in the design and optimization of constructed treatment wetlands for hydrocarbon removal. Temperature influences petroleum biodegradation by its effect on the physico-chemical properties of the oil, rate of hydrocarbon metabolism by microorganisms and composition of the microbial community (Atlas, 1981).

Studies on temperature effect on wetland performance have been reported by a number of researchers including Kadlec et al. (2000) and Scholz et al. (2007). However, these studies focused on constructed wetlands for wastewater treatment targeting the removal of biological oxygen demand, nitrogen, and phosphorous. Kadlec and Reddy (2000) studied the temperature dependence of many individual wetland processes and wetland removal of contaminants in surface flow wetland. They concluded that microbial mediated reactions are affected by temperature; the treatment response was much greater to changes at the lower end of the temperature scale (<15ºC) than at the optimal range (20 to 35ºC). Furthermore, they observed that the processes regulating organic matter decomposition were affected by temperature. In colder climates, the overall treatment efficiency is usually relatively low (Kadlec and Reddy, 2000).

There are conflicting opinions concerning temperature dependence within constructed wetlands. Seasonal variations have been reported by several investigators, with the worst performance occurring during the winter (Kuehn et al., 1995; Leonard, 2000; Karathanasis et al., 2003). It is uncertain whether the poor winter performances are due to low temperatures alone or the combined effect with increased hydraulic loadings. Several studies have suggested negligible temperature dependence in wetlands (Harbel et al., 1995; Knight et al., 1999; Vymazal et al., 1999; Neralla et al., 2000). Furthermore, this suggests that soil microbes in winter still have the capacity to decompose organic matter and that low temperatures can enhance aerobic metabolism through the increase of dissolved oxygen saturation. Various studies have also considered the evaluation of the treatment efficiency of constructed wetlands as a function of temperature depending on components such as substrate composition, degree of plant growth, seasonal changes in evapotranspiration rates, and microbial activities (Chunming et al., 1999; Allen et al., 2002). For example, Rosso et al. (1995) demonstrated the effects of temperature and pH on microbial growth.

In a recent report for temperatures and energy flows based on a study of water temperatures in surface flow wetlands in hot arid climate, Kadlec (2006) pointed out three reasons for the importance of water temperature in treatment wetlands:

1 Temperature modifies the rates of several key biological processes;
2 Temperature is sometimes a regulated water quality parameter; and
3 Water temperature is a prime determinant of evaporative water loss processes.

Several biogeochemical processes that regulate the removal of nutrients in wetlands are affected by temperature, thus influencing the overall treatment efficiency

(Kadlec and Reddy, 2000). The temperature conditions in a wetland affect both the physical and the biological activities in the system. The biological reactions responsible for biochemical oxygen demand removal, nitrification and denitrification are known to be temperature dependent (Reed and Brown, 1995).

It follows that temperature is likely to be a significant control parameter for wetlands treating hydrocarbons. At low temperatures, the viscosity of oil increases, while the volatility of toxic low-molecular weight hydrocarbons reduces. This delays the onset of biodegradation (Atlas, 1981). Temperature also variously affects the solubility of hydrocarbons (Foght et al., 1996).

Considering that the above documented research indicates conflicting opinions on the role of temperature, further studies identifying the relationships between microbes, variable climatic conditions and hydrocarbon removal within constructed wetlands are required.

Seasonal variations of BOD removal efficiency in the constructed wetlands have been also reported by several researchers, with the worst performance occurring during the winter (Leonard, 2000; Karathanasis *et al*., 2003). The poor winter performances as pointed out previously is uncertain whether they are due to low temperatures alone or the combined effect with increased hydraulic loadings.

2.10. Role of nutrients

Nutrients (particularly nitrogen and phosphorus) are essential for the successful biodegradation of hydrocarbon pollutants (Cooney, 1984). Mitsch and Gosselink (1993) reveal that freshwater wetlands are typically considered to be nutrient limited due to the heavy demand for nutrients by the plants, and they could also be nutrient traps, as a substantial amount of nutrients may be bound in biomass.

Hence the addition of nutrients is necessary to enhance the biodegradation of oil pollutants (Choi et al, 2000; Kim et al, 2005). However, studies in the past (Chaillan et al, 2006) have shown that excessive nutrient concentrations can inhibit the biodegradation activity, and several authors have reported the negative effect of high nitrogen, phosphorus and potassium levels on the biodegradation of hydrocarbons (Oudot et al 1998; Chaıneau et al, 2005), and particularly on the aromatics (Carmichael and Pfaender, 1997). The use of slow-release fertilizers may provide a continuous supply of nutrients, maintaining a sufficient microbial activity that leads to the reduction of bioremediation costs (Riser-Roberts, 1992; Xu et al., 2003). However, the role of nutrients is presented briefly considering that it is not main forces at work in this study.

2.10.1. Nutrient removal

High contents of ammonium in the wetlands would cause a significant reduction of natural DO content by forming nitrate and nitrite. Nitrate – nitrogen is a nutrient which normally fertilizes the plants in the wetlands. For control of a nitrification/denitrification-process in the constructed wetland ammonia – nitrogen measurement is a must. It is assumed that ammonification process was also facilitated by increased oxygenation in temporarily flooded system used in this study.

Previous studies by Green *et al*., (1998) and Kedlec and Knight, (1996) proves that that microbial nitrification and denitrification are the main nitrogen removal mechanisms in most of the constructed wetlands. Nitrification, the conversion of ammonium-nitrogen to nitrate-nitrogen, is important because *P.australis* used in this study takes up nitrate-nitrogen preferentially to ammonia-nitrogen. Brix (1994) reported that the uptake capacity of macrophytes is roughly in the range 20 to 250 g/m^2/year and this amount can be removed if the biomass is harvested.

Immobilization into microbial cells is also major process of ammonia-nitrogen removal in the constructed wetlands, because a large amount of organic matter is removed by growth of microorganisms in wetlands system. 0.074 g ammonia-nitrogen can be immobilized for 1 g BOD removal by biomass assimilation (Sun *et al*., 2005). Denitrification is the process in which nitrate- nitrogen is reduced to gaseous nitrogen. This transformation is supported by facultative anaerobes. These organisms are capable of breaking down oxygen-containing compounds such as nitrate- nitrogen to obtain oxygen in an anoxic environment that was dominant during the long periods of filter flooding. This anoxic condition was periodically provided by temporarily flooding in both indoor and outdoor rigs.

Intermittently loaded vertical-flow system is known as quite efficient system to provide oxygen. Intermittent loading facilitates oxygen transfer by drawing the water table down periodically to allow oxygen to penetrate into the deeper zones of the wetlands. Furthermore, this study encouraged partial aeration by pipes installed to ventilate the lower media layer. Oxygenation in intermittently loaded vertical-flow system increased several fold compared to the horizontal subsurface flow systems, which may results in efficient nitrification process (Green *et al*., 1998). In addition to that, it is well documented that macrophytes release oxygen from roots into the rhizosphere and this oxygen leakage stimulate growth of nitrifying bacteria (Brix, 1997). In comparison to the present system, despite that the nutrient was increased from 15g/l to 30g/l which was high nutrient loading in an attempt to verify their role in hydrocarbon removal.

Sun *et al*. (2005) also found that less than 10 % of ammonia- nitrogen was removed due to the nitrification in tidal-flow system treating high loads of wastewater. It is believed that high loads of organic matter may have inhibited the nitrification process because oxygen primarily used by heterotrophic microbes to removal organic matter and significant nitrification can not take place until BOD drops to 200mg/l or below (Korkusuz *et al*., 2005; Su and Ouyang, 1996).

2.11. Summary

This chapter presented the historical development of constructed treatment wetlands and documented evidence of early concepts of the technology. The components and types of wetlands, the removal mechanisms of contaminants in the constructed wetlands were covered. The roles of temperature and nutrients in were presented with special interest in their application to hydrocarbon treatment. The chapter discussed hydrocarbon removal as related to this research in detail. The development of models that produces residence time distribution and the use of models to provide a simplified view of the very complex system were discussed.

3

Materials and methods

3.1. Overview

This chapter presents brief description of systems design, construction and analysis used in the study. Section 3.2 describes the experimental set-up, while the sub-section describes wetland design and media compositions. Section 3.3 presented operational conditions and other processes such as the fertilizer addition were also documented. Section 3.4 describes various analytical methods used for the water quality variables determinations. Section 3.5 documented auxiliary experiment to determine major hydrocarbon removal pathway, 3.6 documented risk assessment prepared for the research, 3.7 presented limitations to the experimental design and methods and 3.8 summarized the chapter.

3.2. Experimental set-up

3.2.1. Site *description*

The study was conducted between April 2005 and October 2007. Two experimental constructed wetland rigs treating hydrocarbon contaminated simulated wastewater were designed, constructed and operated at The King's Buildings campus at The University of Edinburgh, Scotland, UK. The experimental rigs were designed to assess the system performance. The constructed wetlands were designed to simulate physical, chemical and microbiological processes occurring in full-scale natural wetlands. Each rig comprised of six constructed wetlands. One rig was operated in a temperature, light and humidity controlled-room to allow control over the major environmental boundary conditions. The use of natural passive treatment systems such as constructed wetlands can be limited by many environmental factors such as temperature and humidity.

The test room was equipped with a high specification unit for climatic research and was used in evaluating and optimizing the environmental factors in the constructed wetland. The indoor control unit is called Denco Local Environmental Control Unit, and was supplied by Denco Limited (East Kilbride, Scotland, UK) (Figure 3-1).

Figure 3-1. Environmental Control Unit

The indoor rig was located below three plant growth lights (Sylvania 15 000 h, 36 W, 1200mm, T8 Grolux Fluorescent Tube; supplied by Lyco Direct Limited (Bletchely, Milton Keynes, England, UK)) to simulate day and night conditions. The temperature and humidity values for the indoor rig fluctuated initially due to technical problems, but constant temperature and humidity specifications of 15^{o}C and 60%, respectively, were reached at a later stage during the experiment. In comparison, the second rig was operated outdoors under natural environmental conditions to assess seasonal changes.

3.2.2. Wetland design and media composition

Round grey polyvinyl chloride drainage pipes which are resistant to hydrocarbons, were used to construct the vertical-flow wetlands. All twelve wetlands were designed with the following dimensions: height = 75 cm, diameter = 10 cm and filled to a depth of 60 cm. Different packing order arrangements of filter media and plants were used to construct the wetlands (Table 3-1, Figure 3-2). The wetlands were packed with various compositions of layers of aggregates (filter media) such as stones, gravel and sand to optimize subsurface hydraulic treatment. The packing order of the experimental constructed wetland set-up for the inside and outside wetland aggregates varied in diameters. The diameters of aggregates were: stones (37.5-75 mm); large gravel (10-20 mm); medium gravel (5-10 mm); small gravel (1.2-5 mm); and sand (0.6-1.2 mm). The outlet valves were located at the centre of the bottom plate of each wetland with 1.2 cm internal diameter vinyl tubing, and were used for the regulation of flow and sampling. Passive aeration was encouraged with a 1.3 cm internal diameter ventilation pipe reaching down to 10 cm above the bottom of each wetland. Selected wetlands were planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Table 3-1, Figure 3-2).

The wetlands had different volumes depending on different layers of aggregates. Different wetlands were similar to various other natural treatment processes. For example, wetlands 5 and 6 (controls) are similar to wastewater stabilization ponds (extended storage) considering that they do not contain any aggregate. Moreover, wetland 6 containing only water and fertilizer can be considered as a ‘blank’. In comparison, wetlands 2 and 4 are similar to gravel and slow sand wetlands. Wetlands 1 and 3 are typical reed beds as they contain gravel, sand substrate and native *P. australis*, all of similar total biomass weight during planting and from the same local source (Alba Trees Public, Lower Winton, Gladsmuir, East Lothian, Scotland). All wetlands were alternatingly inundated and subsequently fully drained two times per week.

Table 3-1. Packing order of the experimental constructed wetland set-up for inside and outside wetlands.

Height (cm)	**Wetland 1**	**Wetland 2**	**Wetland 3**	**Wetland 4**	**Wetland 5**	**Wetland 6**
61-75 (top)	W+B+F	W+F	W+B+F	W+F	W+B+F	W+F
56-60	5+P+W+B+F	5+P+W+F	5+W+B+F	5+W+F	W+B+F	W+F
51-55	5+P+W+B+F	5+P+W+F	5+W+B+F	5+W+F	W+B+F	W+F
36-50	4+P+W+B+F	4+P+W+F	4+W+B+F	4+W+F	W+B+F	W+F
26-35	3+W+B+F	3+W+F	3+W+B+F	3+W+F	W+B+F	W+F
11-25	2+W+B+F	2+W+F	2+W+B+F	2+W+F	W+B+F	W+F
0-10 (bottom)	1+W+B+F	1+W+F	1+W+B+F	1+W+F	W+B+F	W+F

W: water; B: benzene; F: fertilizer (8 g of N-P-K Miracle-Gro fertilizer were added to all wetlands every two weeks until 29 May 2006 when the concentration was increased to 30 g. From 26 June 2006 onwards, the concentration was lowered to 15 g every two weeks.); P: *Phragmites australis* (Cav.) Trin. ex Steud. (nine plants of roughly equal biomass and strength per wetland); 1: stones (37.5-75 mm); 2: large gravel (10-20 mm); 3: medium gravel (5-10 mm); 4: small gravel (1.2-5 mm); 5: sand (0.6-1.2 mm).

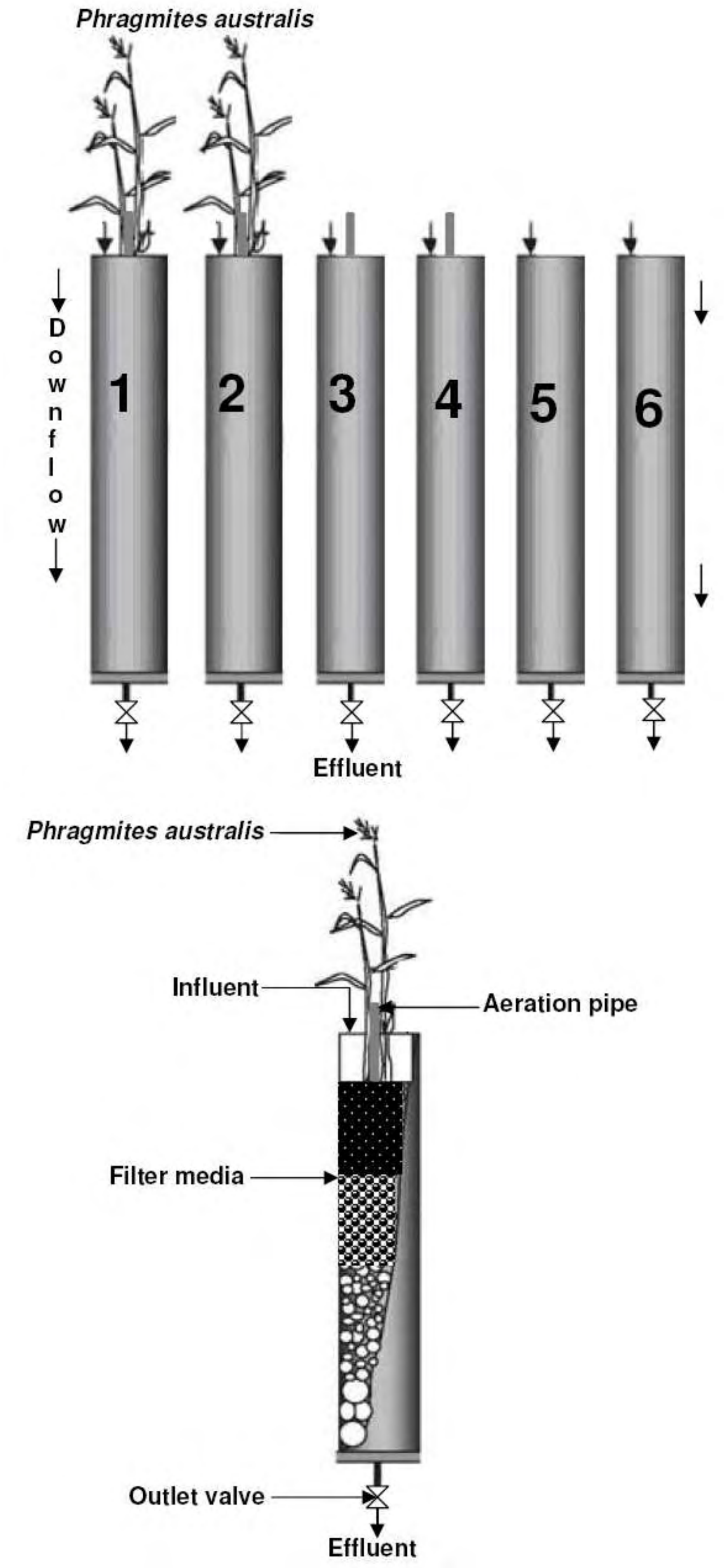


Figure 3-2. Schematic representation showing the wetland set-up and internal structure of the experimental constructed treatment wetland 1

Furthermore, the wetland design of the bottom layer has the highest hydraulic conductivity to ensure fast drainage, while the top layer (made of sand) has the lowest hydraulic conductivity to induce ponding (figure 3-3) of influent and thus uniform distribution of flow across the filter media. The indoor and outdoor wetlands (figures 3-4 and 3-5) designed for this study can be classified as a combination of a vertical-flow wetland system and a facultative (stabilization) pond. A facultative pond is made of three different strata: the surface zone, which is aerated naturally; an intermediate (unsaturated) zone which is both anaerobic and aerobic; and a bottom (saturated zone) layer which is anaerobic (figure 3-3). Effluent flows vertically from the ponding zone through the sand layer to the unsaturated gravel media zone and accumulate at the bottom of the bed (the saturated zone) (figure 3-3).

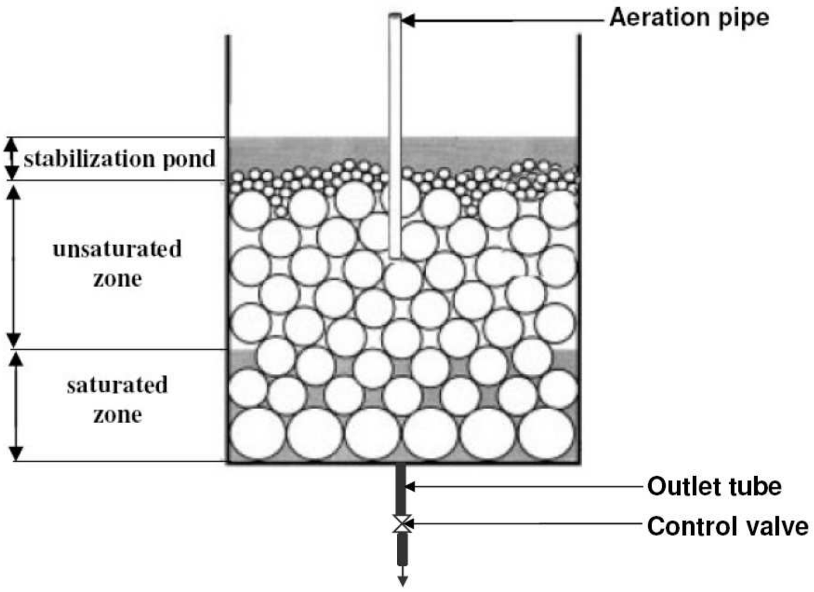


Figure 3-3. Schematic layout of the internal composition of the wetland

Drainage of the effluent from the bottom of the filter causes suction of fresh air through the aeration pipes connecting the outer atmosphere with the confined space of the sub layers. During the full drainage of the wetland, oxygen depleted air is continuously pushed out of the wetland through the aeration pipes due to accumulation of effluent in the saturated zone (detailed description of this operation is documented in subsection 3.3.1 below).

Figure 3-4. Experimental vertical-flow wetland rig located outside The King's Building's campus (June, 2006)

Figure 3-5 (a)

Figure 3-5 (b)

Figure 3-5. Experimental vertical-flow wetland rig located inside (Temperature, humidity and partly controlled room) on The King's Building's campus (a) fully controlled (June, 2006) (b) early control stage (October, 2005).

3.3. Environmental conditions

3.3.1. Operation conditions

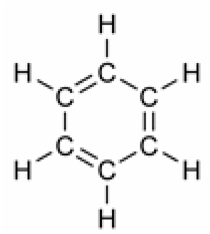
The wetland system was designed to operate in batch flow mode to avoid pumping and computer control costs.

Two types of water were used for the study as the influent: tap water and tap water mixed with benzene. The mean tap water values for BOD_5, COD, PO_4^{3-}, NO_3-N, NH_4-N, temperature, DO, pH, EC, redox and turbidity were <0.1 mg/L, <0.5 mg/L, <0.05 mg/L, <0.1 mg/L, <0.01 mg/L, 11.9ºC, 8.9 mg/L, 7.1, 290 μS, 150 mV and 0.2 NTU, respectively. Wetlands 2, 4 and 6 received tap water, while wetlands 1, 3 and 5 received tap water artificially contaminated with a concentration of 1 g L^{-1} benzene two times per week.

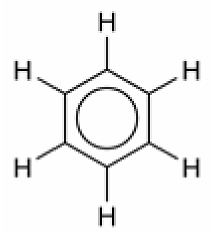
In order to investigate the relationships between nutrient supply and benzene removal, approximately 8 g of N-P-K Miracle-Gro fertilizer (formerly Osmocote, produced by Scot Europe B. V., The Netherlands) was added to all wetlands every two weeks until 29 May 2006 when the amount was increased to 30 g to assess the effect of nutrient concentration increases on benzene removal. Initial findings indicated that excess nutrient supply seems to hamper benzene removal as shown in the result (chapters 5 and 6). Therefore, from 26 June 2006, the nutrient amount was lowered to 15 g, and fertilizer was supplied every two weeks. The purpose was to investigate the effect of the decrease in nutrient supply on potential benzene reduction. Moreover, nutrients were added to enhance plant and microbial growth, and to improve the treatment efficiency of the wetland systems. Furthermore, while hydrocarbons are an excellent source of carbon and thus energy for microbes, they do not contain significant concentrations of other nutrients (such as nitrogen and phosphorus) required for microbial growth and so they are incomplete foods sources (Prince et al., 2002). The input of large quantities of organic carbon sources tends to result in a rapid depletion of available inorganic nutrients (Margesin et. al, 1999), limiting the amount of biodegradation. Thus, bio-stimulation (nutrient addition) can often be used to maximize bioremediation effectiveness (Trinidade et al., 2002).

The rhizomes of the common reeds (*Phragmites australis* (Cav.) Trin. ex Steud) used were washed free of sediments and planted in selected gravel and sand-filled wetlands.

Benzene was used as an example volatile hydrocarbon to assess the removal of low molecular weight petroleum compounds. Benzene (BDH analytical reagent, C_6H_6 (99.7%)) supplied by VWR International Limited (Hunter Boulevard, Lutterworth, England, UK) was used. Benzene represents a special problem in that, to account for all the bonds, there must be alternating double carbon bonds as represented in the structure below:



Benzene is also often depicted with a circle inside a hexagonal arrangement of carbon atoms as represented in the structure below:



As is common in organic chemistry, the carbon atoms in the diagram above have been left unlabeled. The above two benzene structures were created after structures from wikipedia (http://en.wikipedia.org/wiki/Benzene) and (Hemond, and Fechner-Levy, 2000). The major characteristics of benzene are as follows: molar weight, 78.11 g mol^{-1}; density, 0.88 kg L^{-1}; molar volume, 89.11 cm^3; aqueous solubility, 1,780 mg L^{-1}, Henry's law constant 0.55 kPa m^3 mol^{-1}; water partition coefficient, 2.13 log k_{ow}; diffusion coefficient in free solution, 1.16×10^9 m^2 s^{-1});

diffusion coefficient in air, 0.93×10^{5} m^2 s^{-1}; boiling temperature, 80.1°C (Schnoor, 1996; Hemond, and Fechner-Levy, 2000).

Benzene was chosen for various reasons:

- It is a common constituent of liquid fuels;
- Benzene was chosen to represent the aromatic hydrocarbon group, which includes benzene, toluene, ethlbenzene and xylene (BTEX). It is one of the most prevalent organic contaminants in groundwaters (Anderson and Lovley (1997).) and is of major concern owing to its toxicity and relatively high solubility. Benzene has been classified as carcinogenic. Benzene's ability to migrate to and within groundwater is an important water quality concern (Caswell et al., 1992).
- It can be used as a surrogate for a mixture of hydrocarbons to allow for easy interpretation of the data and subsequent modelling.
- The traditional treatment technologies used by the oil industry such as hydrocyclones and separators predominantly remove heavy hydrocarbons but not aromatics components in the dissolved water phase.
- The thermodynamic stability of the benzene ring increases its persistence in the environment; therefore, many aromatic compounds are major environmental pollutants.
- The development of rational strategies for the remediation of petroleum-contaminated waters and aquifers requires an understanding of the ability of microorganisms to degrade the aromatic hydrocarbon contaminants in wetlands. It is well known that aerobic microorganisms can degrade benzene and other aromatic hydrocarbons and limit the spread of benzene plumes in the subsurface (Salanitro, 1993).

All wetlands were fully saturated and flooded to a depth of 10 cm above the level of the packing media. Subsequently the wetlands were fully drained two times per week to encourage air penetration through the aggregates. When the wetlands were flooded, air is removed from the matrix and consequently pond is formed on the top of matrix. When the wetlands are drained, the retreating water acts as a passive pump to draw air from the atmosphere into the matrix (Green et al., 1998; Scholz and Xu,
2002; Sun *et al*., 2005). Theoretically, the oxygen air exchange between the wetlands and the atmosphere is mainly governed by convection and diffusion mechanisms (Green et al., 1998). The air pressure gradient mechanism, caused by gradient of pressure between the space in the wetland and the atmosphere, is the main air exchange mechanism. During the draw phase (when effluent is drained) fresh air flows from the atmosphere (higher pressure zone) into the wetland (lower pressure zone). The major mechanism for oxygen distribution in the wetland is diffusion, where gradient of oxygen partial pressure within the media is the driving force. This gradient is caused by non-uniform initial distribution of fresh air within the media (during the draw phase) and by oxygen consumption by microbial activity (Green et al., 1998). Each filter functioned as an independent batch reactor. Water samples were tested biweekly for chemical oxygen demand, pH, dissolved oxygen, turbidity, redox, conductivity, ammonia-nitrogen, nitrate-nitrogen, ortho-phosphate-phosphorus and temperature. American standard methods (APHA, 1998) were used for all analytical work unless stated otherwise.

Each wetland is fed with hydrocarbon contaminated water or tap water intermittently, as a batch through the surface of the filter, and then gradually percolates downward through it, to the coarser gravel/stone drainage network in the bottom of the wetlands. Vertical-flow wetlands with intermittent loading are the latest generation of constructed wetlands (Haberl et al., 1999). The filter is then completely drained, allowing air to refill it, and the next dose traps this air – leading to much improved oxygen transfer. The treatment technology generally relies on processes similar to those used extensively in gravel "filter beds", enhanced by the extensive rhizomatous root system of the reed plants (*Phragmites australis*) which can transfer limited quantities of oxygen into the surrounding media, stimulating bacterial communities.

3.4. Analytical method

3.4.1. Hydrocarbon determinations

Water samples were collected in a clinically pre-cleaned sample bottle. Samples were analyzed for benzene removal monthly until January 2007. Samples were testing bimonthly afterwards. Water samples were analyzed by Contaminated Land Assessment and Remediation Research Centre (CLARRC), William Rankine Building, The King's Buildings, University of Edinburgh. Benzene was determined with Perkin Elmer gas chromatography/flame ionization detector (GC/FID) (Beaconsfield, England, UK) and headspace sampler (models 9700 and HS-101 respectively) equipment. Details of analysis and operating conditions of the Perkin Elmer GC-FID, model 9700 (Dr Peter Anderson, personal communication) used for the hydrocarbon analysis are as follows:

Oven temperature = 80°C; Detector temperature = 250 °C; Injector temperature = 150 °C. Perkin Elmer Headspace sampler, model HS-101: Oven temperature = 80 °C; Transfer line temperature = 120°C; Needle temperature = 90°C; Operating conditions: Thermostatting time = 5.0 min; Pressurisation time = 0.5; Inject time = 0.08 min;

Withdrawal time = 0.2 min; Sample/standard volume = 2ml; Benzene standards= 0, 1, 1, 50, 100, 500 mg/l.

3.4.2. BOD, nutrient and other water quality determinations

During over 2-year operation, water samples were collected from the constructed wetland system and analyzed for temperature, pH, BOD_5, COD, ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), ortho-phosphate-phosphorus (PO_4^{3-}-P) dissolved oxygen (DO), temperature, turbidity and electrical conductivity at different intervals. Samples were taken and measured biweekly from April 2005 to October 2007. The total number of samples analyzed for each parameter is summarized in chapter 4. pH was measured with a model pHs-25 pHmeter.

COD and BOD_5 were measured by the potassium dichromate-boiling method and incubation method, respectively. All of these parameters were tested using standard laboratory procedures and methods (Standard Method for the Examination of Water and Wastewater Editorial Board) and all analyses were completed within 24 h of sample collection. The BOD_5 in this research was determined in all water samples with the OxiTop IS 12-6 system, a manometric measurement device, supplied by the Wissenschaftlich-Technische Werkstatten (WTW), Weilheim, Germany. The measurement principle is based on measuring pressure differences estimated by piezoresistive electronic pressure sensors. Nitrification was suppressed by adding 0.05 ml of 5 g^{-L} N-Allylthiourea (WTW Chemical Solution No. NTH 600) solution per 50 ml of sample water.

Nutrients were determined by automated precision colorimetry methods using a Palintest Photometer 5000 instrument. Nitrate was reduced to nitrite by cadmium and determined as an azo dye at 540 nm (using a Perstorp Analytical EnviroFlow

3000 flow injection analyzer) following diazotisation with sulfanilamide and subsequent coupling with N-1- naphthylethylendiamine dihydrocloride (Allen, 1974). Ammonia-N and ortho-phosphate-P were determined by automated precision colorimetry in all water samples from reaction with hypochlorite and salicylate ions in solution in the presence of sodium nitrosopentacyanoferrate (nitroprusside), and reaction with acidic molybdate to form a phosphomolybdenum blue complex, respectively (Allen, 1974). The coloured complexes formed were measured spectrometrically at 655 and 882 nm, respectively, using a Bran and Luebbe autoanalyzer (Model AAIII).

A Hanna HI 9142 portable waterproof DO meter, a HACH 2100N turbidity meter and a Mettler Toledo MPC 227 conductivity, TDS and pH meters were used to determine DO, turbidity, and conductivity, TDS and pH, respectively. An ORP HI 98201 redox meter with a platinum tip electrode HI 73201 was used. These handy, easy to use, robust and waterproof instruments perform with low costs the most important parameters for wastewater monitoring. The meter comes complete with sensors, calibration and maintenance solutions for measurement. Composite water samples were analyzed on Mondays and Fridays. All other analytical procedures were performed according to the American standard methods (APHA, 1998).

3.4.3. COD determinations

COD was determined with Palintest Tubetests System. Palintest Tubetests are integrated with the Palintest heater and photometer system so as to provide a complete system for COD measurement. COD analysis was performed with three Palintest Tubetests with product codes; PL450, PL452 and PL454 and the corresponding ranges were 150 mg/l, 400 mg/l and 2000 mg/l respectively. In the Palintest COD method, the water sample is oxidized by digesting in a sealed reaction tube with sulphuric acid and potassium dichromate in the presence of a silver sulphate catalyst. This reaction takes place in Palintest pre-prepared

tubetests that contain the above required reagents. The amount of dichromate reduced is proportional to the COD. The absorbance of the COD samples was read with the Palintest 7000 Interface Photometer model. COD values were recorded as this model is a direct reading user-friendly photometer pre-programmed for Palintest water tests.

The Palintest 7000 Interface Photometer brings a new dimension to the science of water testing as it replaces older models which values were calculated using a calibration curve prepared previously.

3.4.4. Microbiological determinations

The heterotrophic plate count (HPC), formerly known as the standard spread plate method was used for microbiological examinations. This procedure was used to estimate the number of live heterotrophic bacteria and fungi (aromatic hydrocarbon-utilizing bacteria and fungi specifically) in the system and measuring changes during treatment. The method used for this study was spread plate method. The standard plate count procedures were performed according to the American standard methods (APHA, 1998). Before selecting suitable media for aromatic hydrocarbon-degrading bacteria and fungi, different types of agar were tested. Each water sample was diluted. For each dilution, a 100 μl sample was spread on the agar (Atlas, 1995). The tests were replicated three times for verification purposes. All agars used in the examination were bottled prepared media ready for use by manufacturers’. The manufacturers’ instructions were followed as the agar was dissolved in the microwave and then poured onto sterile Petri dishes. Once all (except for controls) Petri dished had been spread with the various sample dilutions, they were placed into the incubator for 48 hours at 35°C. The colonies were counted. In the reporting of data, duplicate plates are averaged to get the observed counts. The results of the plate counts are expressed as colony forming units (CFU). A 'valid' plate count contained between 30 and 300 CFU per plate (Atlas, 1993, 1995; Britton, 1994). During sampling, the air temperature was measured at the study site. Samples were immediately tested after sampling for microbiological indicator organisms.

3.5. Biodegradation and Volatilization removal pathways

Biodegradation and volatilization were also tested in separate experiments. Two extra wetlands (heights: 24 cm; diameters: 5 cm) were set up under controlled environmental conditions; one wetland comprised aggregates and detritus containing mature microbial biomass (284 g detritus was taken from the upper layer of the contaminated parent wetland 3 located indoors) and another wetland was left empty. The small wetlands were constructed in the same way as the large wetlands with the exception of the absence of the ventilation pipes (see above). The purpose of this auxiliary experiment was to assess the main removal pathways of benzene (combined biodegradation and adsorption *versus* volatilization) in constructed treatment wetland. Samples were taken after 1, 2, 3, 6 and 9 d, and benzene was subsequently determined using headspace and gas chromatography.

3.6. Risk assessment

Considering that benzene is highly flammable and carcinogenic, risk assessment was undertaken prior to the commencement of the research. The health hazards of benzene was addressed by undertaking the risk assessment process using the University's latest step by step COSHH HS1 and Safe System of Work (SSW) forms.

This subsection documents the risk assessment and the Safe System of Work (SSW) information regarding the hazardous properties of the substances used in the research. The assessment covered all required for activities involving Hazardous Substances (HS1) and Control of Substances Hazardous to Health Regulations COSHH). The instructions was outlined in order to ensure that the activity is carried out safely and with minimum risk to health, or that of others who may be affected by the acts or omissions involved in the research.

The SSW also gives directions as to the safe manner in which each stage of the activity is carried out and also stated what items of PPE worn at each stage. The lead researcher was given training and advice as to the health risks of working with aromatic hydrocarbon (benzene), what the available exposure routes are: inhalation, absorption through the skin and ingestion. The lead researcher also registered with the University of Edinburgh Occupational Health Unit in accordance to the COSHH Regulations, which state that ‘health surveillance is appropriate for work with carcinogens’.

3.6.1. Risk Assessment for Activities involving Hazardous Substances

This risk assessment was completed and its content conveyed to the users of the hazardous substances and record of their acceptance gained in the appropriate declaration section. The records of the assessment are summarized as follow:

Brief description of work:

1 Secure storage of benzene (small quantity) in a dedicated and suitable

lockable cabin when not in use.

2 Preparation of an aromatic hydrocarbon solution (benzene and water) in a fume cupboard.

3 Transporting the prepared solution from the fume cupboard to the rigs location in an air tight secondary five litre container that is securely sealed.

4 Transferring of the solution from the container into the different experimental rigs.

5 Analysis of treated wastewater.

6 Disposal of treated wastewater via the recognized chemical waste stream.

Hazard Identification: We ensured that Material Safety Data Sheets (MSDS) have been obtained from the supplier for all proprietary (commercial) substances. Where the substance is produced as a result of the activity its hazardous properties and exposure routes were checked with special caution on the following:

(a) The substance or group of substances to be used, or produced, in the above activity were named and listed in the HS1 form. Where the substance presents an inhalation hazard and has been assigned an Occupational Exposure Limit (OEL), caution was taken and the OEL stated.

(b) Each of the substances were classified according to one, or more, of the following categories: - Very toxic; Toxic; Corrosive; Harmful; Dermal Irritant; Respiratory Irritant; Carcinogen; Teratogen; Mutagen. We also, stated if an airborne substance can also be absorbed through the skin (Sk), or is a respiratory sensitiser (Sen).

© Risks phrases denoted in the MSDS were stated.

Hazard Ratings: The ratings were classified as follows:

a. Name of chemical(s) or substances: Benzene

b. Classification: Flammable, carcinogenic and toxic

c. Risk phrases: Known carcinogen; harmful if swallowed, inhaled, or absorbed through skin; restricted use; avoid exposure; R45-11-48/23/24/25 S53A,45

The following exposure routes by which harm may occur were as follow: Skin Contact, Skin Absorption, Eye Contact, Inhalation and Ingestion.

Engineering Control Measures

The work can be carried out on the open bench but Local Exhaust Ventilation (LEV) is required.

Personal Protective Equipment (PPE): PPE must never be used as the first option of control but must only be used where adequate control of exposure to the hazardous substance(s) cannot be achieved by substitution, or engineering controls alone, or where operating practicalities makes their choice unavoidable. (*e.g. transient site working*).

The following types of PPE will be required for part or all of the activity: Eye protection, Face protection and Hand protection and Specialist clothing (Laboratory coat): Safety spectacles, Chemical resistant Goggles and Chemical resistant faceshield. A faceshield will only be used if large quantities are handled, and if splashes are likely to occur. However, the quantities handled are small (<100 ml). Reusable glove, PVA gloves have been provided for direct work involving benzene. Nitrile gloves will only be used for highly diluted solutions or treated effluent.

3.6.2. Safe system of work

The work activity contains procedures requiring a specific scheme of work. Before any activity is undertaken, it is important that engineering controls are in operation, and that protective equipment requirements are met. The safe system of work and personal hygiene measures should be followed as planned. The master risk assessment was located at the William Dudgeon Laboratory (Public Health Lab).

Special Handling and Storage Requirements:

- A small quantity of benzene is stored in tightly closed containers in a cool, dry and fire resistant cupboard with other compatible substances. It will be kept away from oxidizers and all sources of ignition.
- Aromatic hydrocarbons (benzene) are to be prepared for the dosing of the experimental rigs by measuring with a syringe and dissolving known weights of benzene in measured volumes of water (this is to be carried out wholly within the fume cupboard).
- Transporting of the prepared solution from the fume cupboard location to the wetlands must be via the air tight secondary five litre container that is securely sealed.
- Transferring of the solution from the container to the experimental wetlands, which were designed to operate with the influent stream (solution) to be dispersed carefully at the top of each column. The indoor wetlands are provided with an LEV system that must be operational at all times.
- Treated water samples will be collected from the tap at the bottom of the rig using a clean glass sample.
- The analysis of standard water quality variables will be carried out according to best laboratory practice.

Detailed below were procedures to be followed in case of emergency (accident, spillage, accidental release, etc.):

Spill and Accident Procedures

Small Spills (One Liter or less): Ventilate the area and use personal protective equipment as specified in the risk assessment. Absorb the material with an inert absorbent or sand and place in a suitable container for disposal, and arrange disposal through the Chemistry Department's disposal service.

Large Spills (More than a Liter): Note that large spills are unlikely considering that the research scope do not involve large quantities. Turn off the ignition sources first and then notify and evacuate the area as necessary. Call the trained BA team within SME under the direction of Alex Ruthven.

Inhalation: Remove the workers affected to the fresh air. If not breathing, give artificial respiration. Get medical attention immediately.

Ingestion: Induce vomiting. Give large quantities of water or milk. Never give anything by mouth to an unconscious person. Get medical attention immediately.

Skin Contact: Immediately flush skin with copious amounts of water for at least 20 minutes while removing any contaminated clothing. Get medical attention immediately.

Eye Contact: Immediately flush eyes with copious amounts of water for at least 20 minutes. Get medical attention immediately.

Detail waste disposal procedures: Place waste in a dedicated container in the dedicated area within the WD Laboratory. Containers must be closed and labeled with the words 'hazardous waste', and the main constituents (treated water containing traces of benzene). Place waste in the waste collection area, and arrange collection through the School of Chemistry chemical disposal system.

3.6.3. Control of Substances Hazardous to Health Regulations

The COSHH form assigned to all substances used during the research covers: (a) Hazardous substances used, or produced, in this activity. (b) The substances have been assigned the stated hazard classification. An airborne hazard that can also be absorbed through skin is denoted (Sk); a respiratory sensitiser (Sen). (c) The substances have been assigned these standard risk phrases.

Mechanical Controls (Local exhaust ventilation (LEV) and Fume cupboard) must be used during all, or part of the work activity. Detail type (*e.g. cupboard with water wash down*) and when to be used in activity.

- The preparation of the aromatic hydrocarbon (benzene and water) solution is to be done in a fume cupboard.
- The indoor rig was provided with a LEV as specified in the risk assessment.
- For PPE, laboratory coat, gloves (PVA), (disposable) apron and safety glasses are used when preparing the aromatic hydrocarbon solution in the fume cup board, and all time during the experiment, face shield is used in addition when decanting the solution into the rig.

3.7. Limitations to the experimental design and methods.

Highlights and discussions of limitations in this experimental design and methods that may apply to up-scaling were documented in this section.

The experimental wetlands used in this research were very small in comparison to large-scale systems used in industry, but previous findings based on similar column experiments proved that the results obtained were applicable in field scale and thus have been fully accepted by the scientific community. (Omari et al., 2003, Hiegel, 2004, Scholz, 2004, Zhao et al., 2004).

The wetlands studied under controlled conditions may not correspond with other wetlands operated in field scale due to variable environmental factors. However, the results obtained provides insight on the impacts of environmental factors and could serve as a guide in designing and up-scaling field scale wetlands operated in various climates.

Some operational variations could have resulted during nutrient dosage changes and movements of indoor rig to full controlled laboratory on June 2006. However, error associated to these operational hitches was negligible as observed in the results.

The small scale design used in this research could not represent true requirement of large land involved in field scale. Considering that wetland systems use larger land areas and natural energy inputs to establish self-maintaining treatment systems providing environments for many more types of microorganisms because of the diversity of microenvironments in a wetland. Land value is a huge problem in up-scaling due the large surface area required for the construction of field scale wetlands.

Large scale constructed wetlands may be home to a varying number and type of animals and this experimental set up does not take into accounts the effect these will have on wetland processes.

3.8. Summary

This chapter documented the experimental set-up, describing the novel filter design and media composition. The environmental conditions and operation conditions were discussed paying special attention on two basic operational conditions applied such as placing one experimental rig outdoors to assess seasonal changes and the other system was placed indoors to allow a better control over the environmental changes. This chapter also showcases the high specification unit for climatic research and was used in evaluating and optimizing the environmental factors in the constructed wetland.

This unit was of particular interest as it helps not only to monitor the treatment performance but also to expose hidden boundary conditions required to understand internal working of constructed treatment wetlands applied for petroleum hydrocarbon removal.

Summary of risk assessment undertaken were also documented. Limitations of the design and operations were also documented. The chapter finally summarized the overall hydrocarbon and other water quality variables determinations.

4

General Results♣

4.1. Overview

This chapter presented the overall results of most of the variables involved in the study by evaluating the performances of effluent water qualities in the experimental vertical-flow constructed treatment wetlands. The performances of the wetlands assessed were grouped into two categories: variables that show the efficiency of the wetland and variables essential for control and optimization of the wetland. Simple removal models were applied to estimate the removal potentials of the wetlands. The components of each wetland were statistically compared to examine the impact of design components and operation conditions on the removal performance of wetlands. Microbiological examinations of the wetlands were also presented.

This chapter aim at advancing the knowledge of hydrocarbon removal with constructed wetlands and focused specifically on a more thorough understanding of the science, and underlying internal processes by assessing the components of each

♣ Parts of this chapter have been published as:
Eke, P. E. and Scholz, M. (2008). Benzene removal with vertical-flow constructed treatment wetlands. *Journal of Chemical Technology & Biotechnology* 83(1), 55-63 (original copy documented in appendix A).

system. More results were presented in chapters 5, 6 and 7 as evaluation of hydrocarbon performance, seasonal variability and management of the wetlands respectively.

4.2. Variables that show the efficiency of the wetland

The major measurement variables such as COD and BOD_5 were monitored in an attempt to gain proper insight of individual efficiency of the wetlands used in this study and were summarized in Tables 4-1 and 4-2.

Table 4-1. Mean effluent concentrations (mgL^{-1}) for the indoor rig (08/04/05- 18/10/07)

		Wetland 1		Wetland 2		Wetland 3		Wetland 4		Wetland 5		Wetland 6	
Var.	n	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
COD	182	48.1	214.3	37.4	9.2	64.7	168.3	33.4	7.8	51.4	241.6	31.1	8.9
BOD	171	17.6	37.1	11.8	4.7	22.1	42.5	6.1	2.9	11.9	50.9	6.1	2.8
NH_4	167	6.3	40.0	5.7	42.2	5.4	39.1	4.5	38.3	5.0	37.2	4.2	37.1
NO_3	167	1.3	26.5	1.7	47.3	1.8	53.9	1.9	63.5	1.9	25.2	1.9	22.4
PO_4	167	7.8	18.0	9.5	26.0	7.5	27.0	6.6	26.3	8.5	25.1	7.6	27.7
Tem	192	14.3	11.7	14.3	11.7	14.3	11.7	14.3	11.7	14.3	11.7	14.3	11.7
Var.	n	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov
COD	182	704.0	282.5	12.9	18.6	425.3	199.1	15.4	17.4	694.9	293.7	7.3	15.3
BOD	171	41.4	34.0	2.1	5.5	33.3	35.0	1.2	3.1	36.0	37.4	1.5	3.2
NH_4	167	27.1	27.9	35.7	30.7	22.7	26.2	20.3	25.0	14.5	23.3	16.8	23.6
NO_3	167	33.0	21.0	110.0	48.6	33.0	34.9	95.7	53.7	26.5	19.0	61.1	25.4
PO_4	167	7.8	12.9	7.9	17.4	6.5	17.1	6.1	16.4	5.4	16.2	6.8	17.5
Tem	192	13.5	12.9	13.5	12.9	13.5	12.9	13.5	12.9	13.5	12.9	13.5	12.9
Var.	n	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
DO	192	1.1	3.6	1.3	4.0	0.9	3.6	1.5	4.1	3.2	3.5	3.4	5.7
Turb	192	3.1	2.4	2.3	1.7	4.1	4.6	1.7	1.0	0.8	3.3	1.0	0.9
pH	192	7.1	6.7	6.6	6.0	6.9	6.4	6.6	5.5	7.1	6.2	7.1	6.3
Redox	192	206.3	162.3	201.2	164.5	167.0	150.4	213.3	175.4	215.3	159.3	218.5	171.6
Cond	192	280.8	509.0	571.3	670.2	332.0	707.3	295.4	716.0	370.0	580.1	363.8	518.2
Var.	n	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov
DO	192	3.0	2.7	3.5	3.1	3.1	2.7	3.9	3.3	2.6	3.2	5.1	4.9
Turb	192	1.2	2.3	0.9	1.6	3.7	4.2	0.7	1.1	3.6	2.7	0.4	0.8
pH	192	6.2	6.7	5.5	6.0	5.9	6.4	4.8	5.6	5.9	6.4	5.9	6.4
Redox	192	129.7	165.4	134.2	166.3	106.9	143.0	143.2	177.1	122.6	164.6	140.1	175.8
Cond	192	413.4	419.6	589.3	620.6	386.9	515.3	496.0	539.2	229.1	425.2	282.3	410.5

Table 4-2. Mean effluent concentrations (mgL^{-1}) for the outdoor rig (08/04/05- 18/10/07)

		Wetland 1		Wetland 2		Wetland 3		Wetland 4		Wetland 5		Wetland 6	
Var.	n	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
COD	182	58.8	372.3	52.1	12.4	62.2	322.4	40.3	12.6	62.1	339.3	34.3	12.9
BOD	171	38.9	47.4	13.0	3.5	34.6	45.0	9.5	2.9	21.7	42.7	7.3	4.2
NH_4	167	4.9	54.0	4.6	50.0	5.6	46.7	4.8	42.7	4.4	40.5	4.9	36.6
NO_3	167	2.1	42.3	1.8	56.7	1.8	32.8	2.0	38.1	1.6	30.7	1.3	26.7
PO_4	167	9.3	25.6	9.9	40.0	6.9	23.0	6.5	21.9	6.7	17.8	6.1	25.1
Tem	192	21.2	15.7	21.2	15.7	21.2	15.7	21.2	15.7	21.2	15.7	21.2	15.7
Var.	n	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov
COD	182	1100.9	453.7	17.6	25.7	1141.6	441.8	11.3	20.6	955.1	400.3	11.9	19.1
BOD	171	42.1	43.8	3.8	5.8	38.7	40.7	2.7	4.3	37.1	36.2	1.8	4.2
NH_4	167	37.7	36.9	42.8	35.8	37.5	33.4	25.2	28.4	24.0	26.9	19.4	24.1
NO_3	167	61.6	35.5	104.2	52.1	95.6	38.1	100.5	42.0	59.7	29.1	66.7	28.6
PO_4	167	8.5	17.3	13.5	25.8	10.0	15.7	6.6	14.2	6.0	12.1	5.4	15.5
Tem	192	15.0	17.0	15.0	17.0	15.0	17.0	15.0	17.0	15.0	17.0	15.0	17.0
Var.	n	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
DO	192	1.9	5.1	2.4	5.6	2.4	5.2	2.4	7.6	3.7	7.1	3.6	7.6
Turb	192	4.5	2.3	3.3	1.5	2.2	2.0	2.8	1.2	0.8	1.0	0.9	1.0
pH	192	7.1	6.7	6.8	5.5	7.2	6.5	6.9	5.9	7.2	6.5	7.3	6.4
Redox	192	210.1	169.4	228.8	186.9	202.9	161.9	225.3	177.2	210.1	158.7	214.2	162.0
Cond	192	467.9	652.5	549.4	885.5	380.6	551.9	300.2	546.5	284.7	459.5	239.0	464.7
Var.	n	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov	Yr2+	ov
DO	192	4.1	3.9	4.7	4.5	4.5	4.3	5.3	4.7	7.2	6.2	7.4	6.3
Turb	192	2.4	2.9	1.0	1.9	1.7	2.0	0.6	1.5	0.7	0.9	0.5	0.8
pH	192	6.3	6.7	4.6	5.6	6.3	6.7	5.2	6.0	6.5	6.7	6.3	6.6
Redox	192	139.2	172.3	160.2	191.1	145.2	168.6	144.1	181.3	124.8	163.5	118.7	164.5
Cond	192	717.6	374.0	970.2	431.4	632.6	510.1	661.0	526.9	532.6	816.1	362.0	619.5

ov: overall mean for 08/04/05- 18/10/07; Yr1: mean for 08/04/05-27/03/06; Yr2: mean for 28/04/06-30/03/07; Yr2+: mean for 02/04/07-18/10/07; n: sample number; COD: chemical oxygen demand (mg L^{-1}); BOD_5: five-day @ 20^{o}C N-Allythiourea biochemical oxygen demand (mg L^{-1}); NH_4: ammonia-nitrogen (mg L^{-1}); NO_3: nitrate-nitrogen (mg L^{-1}); PO_4: ortho-phosphate-phosphorus (mg L^{-1}); tem: temperature (^{o}C); DO: Dissolved Oxygen (mg L^{-1}); Turb: Turbidity (NTU); pH: Acidity (-); Redox (mV); Cond: Conductivity($_u$S).

These variables are important because knowing the effluent values makes it easy to judge on the efficiency of the wetland. The COD and BOD_5 variables were presented in subsection 4.2.1 and 4.2.2 respectively. In addition to COD and BOD_5, the yearly and overall effluent water quality of nutrient, temperature and other variables were also presented in Tables 4-1 and 4-2.

Furthermore, table 4-3 presented results of the analysis of effluent water quality variables. Means, standard deviations (SD) and standard errors (SE) were calculated. Multiple comparisons using least significant difference (LSD) and homogeneity of variance tests were used to analyze the effluent benzene, BOD_5, COD, NH_4-N, NO_3-N and PO_4^{3-} concentrations and other water quality variables such as DO, EC, redox and turbidity. Tests to determine the significant differences between the above mentioned variables for the indoor and outdoor wetlands were conducted. Multiple comparisons were undertaken with the least significant difference (LSD) and the Duncan's multiple range tests for differences between means (significant level $p \leq 0.05$).

However, the measurement of BOD_5 gave some indication of the impact of benzene on biological processes occurring in wetlands. The ten effluent water quality variables BOD_5, COD, NH_4-N, NO_3-N, PO_4^{3-}, DO, pH, EC, redox and turbidity were summarized in Table 4-3. Ten individual one-way ANOVA were performed to test whether there was any significant difference in these variables among the selected constructed wetlands. Individual examination and analysis of all the variables were also discussed in detail in this chapter. The Duncan's multiple range tests indicated that the mean values of BOD_5, COD NO_3-N, redox and pH for the indoor wetlands were significantly lower than for the outdoor wetlands.

Effluent mean BOD_5 and NO_3-N concentrations for the indoor wetland 1 are significantly ($p<0.05$) lower in comparison to the other wetlands. The indoor wetland 3 had the lowest mean COD, pH and redox values. It is likely that the impact of wind on the outdoor wetlands resulted in higher DO concentrations in comparison to those recorded for the indoor wetlands. The DO concentration ranges were wide. The indoor wetland 3 contained significantly ($p<0.05$) low concentrations of DO, followed by indoor wetlands 1 and 5, where the concentrations were also significantly different from each other. The outdoor wetlands 1 and 3 had relatively high DO concentrations, and were not statistically significantly different from each other (Table 4-3), while the outdoor wetland 5 was the most aerobic with DO concentrations reaching as high as 6.19±2.44 mg/l.

Although mean turbidity values were generally between 1 and 3 NTU (except for the indoor wetland 3 with a mean of 4.21 NTU (Table 4-3)), they were significantly different ($p<0.05$) from each other concerning the indoor and outdoor wetlands. The outdoor wetland 1 has EC significantly ($p<0.05$) higher than those of the other wetlands. The mean EC values for the indoor and outdoor wetlands 3 were significantly ($p<0.05$) higher than those for the other 3 wetlands. Concerning PO_4^{3-} and NH_4-N, a statistical analysis indicated that there were no significant differences ($p>0.05$) between indoor and outdoor wetlands.

Table 4-3. Effluent water quality variables (means ± SD) in contaminated constructed wetlands (mg/l)

Rig. No	N1	BOD mg/l	N2	COD mg/l	N3	PO_4^{3-} mg/l	NO_3-N mg/l	NH_4-N mg/l
1 In	171	33.98±27.25 ^{a}	182	282.48±351.61 ^{a}	149	14.44±27.32	19.07±48.86 ^{a}	27.11±41.14
3 In	171	35.01±27.25 ^{a}	182	199.11±351.61 ^{a}	149	16.43±33.68	34.11±89.76 $^{a, b}$	37.18±83.91
5 In	171	37.39±31.16 ^{a,b}	182	293.71±344.59 ^{a}	149	15.56±32.51	22.38±44.66 $^{a, b}$	26.82±57.05
1Out	171	43.81±30.87 ^{b}	182	453.71±603.49 ^{b}	149	17.93±24.08	34.60±77.47 $^{a, b}$	38.22±60.99
3Out	171	40.69±29.13 ^{a,b}	182	441.83±551.23 ^{b}	149	16.08±26.30	35.37±70.95 $^{a, b}$	35.10±58.34
5Out	171	36.18±27.69 ^{a,b}	182	400.26±517.67 ^{b}	149	14.84±38.32	47.36±137.98 ^{b}	45.08±72.23

Rig. No	N4	DO mg/l	pH	EC µS	Redox mV	Turbidity NTU
1 In	193	2.72±1.63 ^{a}	6.67±0.43 ^{b}	419.64±195.63 ^{a}	165.43±58.45 ^{b}	2.25±1.97 ^{b}
3 In	193	2.69±1.64 ^{a}	6.37±0.49 ^{a}	515.25±306.57 ^{b}	142.96±56.07 ^{a}	4.21±1.71 ^{d}
5 In	193	3.15±1.39 ^{b}	6.38±0.58 ^{a}	425.22±304.58 ^{a}	164.64±53.78 ^{b}	2.69±1.93 ^{c}
1Out	193	3.94±1.99 ^{c}	6.71±0.42 ^{b}	619.52±319.39 ^{c}	172.29±59.45 ^{b}	2.93±2.15 ^{c}
3Out	193	4.25±1.79 ^{c}	6.65±0.43 ^{b}	526.89±351.77 ^{b}	168.60±45.75 ^{b}	1.97±1.27 ^{b}
5Out	193	6.19±2.44 ^{d}	6.70±0.47 ^{b}	431.39±541.06 ^{a}	163.53±51.35 ^{b}	0.86±0.73 ^{a}

In and Out represent indoor and outdoor selected constructed wetlands; N1,N2, N3 and N4, sampling numbers for different water quality variables, data collected between April 2005 and October 2007 ; SD, standard deviation; BOD, biochemical oxygen demand(mg/l); COD, chemical oxygen demand(mg/l); PO_4^{3-}, total phosphorus(mg/l); NO_3-N, nitrite(mg/l); NH_4-N, ammonia(mg/l); DO, dissolved oxygen (mg/l); EC, electronic conductivity (µS); Redox, potential of reduction/oxidation reaction (mV); Turbidity, cloudiness or haziness of effluent (NTU). In any one column, values marked with different letters are significantly different from each other at $p\leq 0.05$ according to the Duncan's multiple range tests.

The results obtained in this study suggest that PO_4^{3-} and NH_4-N play a similar role in benzene removal in constructed wetlands with different operational conditions such as the presence or absence of plants, aggregates and temperature control. No strong relationships between benzene and both $PO_4^{3-}$and NH_4-Nwere detected.

4.2.1. Analysis of variance (ANOVA)

It appears that graphical representations of the result alone could not expose the roles of internal components of these systems, their responses and interactions in the constructed wetlands. Hence, the effluent data of individual variables were further analysed statistically, to compare major components and operational conditions in the wetland. The comparisons would lead to better methods for assessing the water quality effects of impacts to individual wetlands. The statistical procedures were carried out using the MINITAB statistical software package (Minitab Ltd. Brandon Court, Unit E 1, Progress Way, Coventry CV3 2TE, United Kingdom) and SPSS, Analytical Software (Statistical Package for the Social Sciences (SPSS) Headquarters, 233 S. Wacker Drive, Chicago, Illonois, USA). One-way ANOVA methods were used to check the influence of each variable considered (Controlled environment: indoor rig versus outdoor rig; Macrophytes: planted versus unplanted; Hydrocarbon: contaminated versus uncontaminated; Aggregates: filter media versus no filter media; and Annual performances: year 1 versus year 2.) for every water quality parameter of the effluent and to evaluate interactions between variables. Prior to the statistical analysis of data, effluent concentrations were checked to ensure that the variables were normally distributed. Otherwise, the effluent concentrations were log-transformed ($\log_{10}$-transformed), which was the most suitable transformation function to bring the variance closer to the mean. This is in accordance with ANOVA rules and conventional practice.

The level of significance applied for analysing (alpha value) in this study is 0.05 (p-value). The alpha level is a significance level related to the probability of having a type I error (rejecting a true hypothesis). In this case the hypothesis is that one set is significantly difference to another set, for instant planted versus unplanted performance. Typically, in any set of comparison when the p-value is equal or less than 0.05 ($P \leq 0.05$), the result is said to be "statistically significant". It follows that pairs of data associated with $P \geq 0.05$ can be regarded "not statistically significant". Further analysis was carried in chapter 7 to establish the effect of group reactions and relationship with multiple comparison tests.

4.2.2. Chemical Oxygen Demand (COD) removal

The COD measurement is based on a thermal reaction of the sample with chemicals during a heating period of 2 hours at 148°C. This reaction caused a change in colour which was measured with a photometer. This study show that there is consistently more COD in contaminated wetlands than uncontaminated wetlands. This is expected due to the fact that benzene contributes a larger amount (740g per year for 3.5L loads) to inflow COD levels in comparison to fertilizer (140g per year for 3.5L loads). Benzene could be toxic but also provides a carbon source for hydrocarbon degrading bacteria to consume, thus its presence in the contaminated wetlands could result to higher chemical oxygen demand noticed in contaminated wetlands.

Figure 4-1 shows improved COD treatment performances for hydrocarbon contaminated wetlands (wetlands 1, 3 and 5) of the indoor rig in comparison to the outdoor rig. However, the treatment performances of COD of uncontaminated wetlands (wetlands 2, 4 and 6) of both indoor and outdoor rigs were similar.

Furthermore, the overall results show that COD removal efficiencies were considerably high in all wetlands (70 to 98 %) of both indoor and out door rigs (Fig. 4-1).

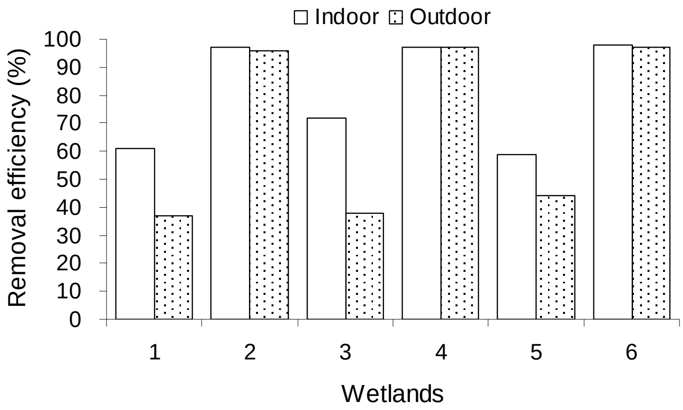


Figure 4-1. Mean COD treatment efficiencies for the indoor and outdoor wetlands

Figure 4-2 shows yearly COD treatment efficiencies (%) for indoor wetlands from 2005-2007. While first year of operation show a better treatment performance for hydrocarbon contaminated wetlands (wetlands 1, 3 and 5), in comparison the uncontaminated filter 2 show similar performance in both first and second year, while uncontaminated wetlands (wetlands 4 and 6) show slightly higher performance in the second year.

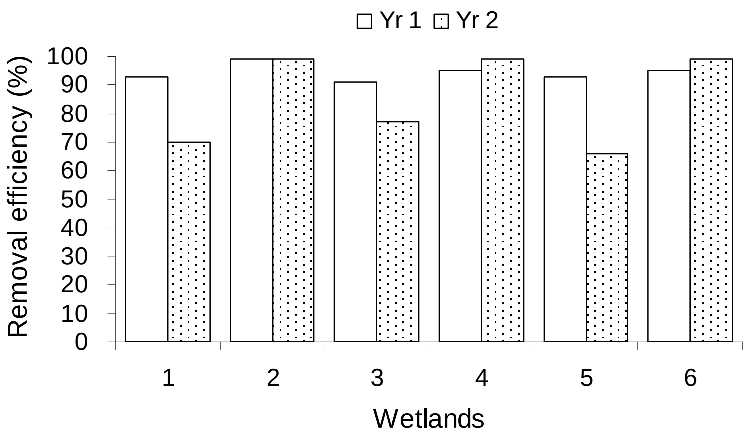


Figure 4-2. Mean COD treatment efficiencies (%) for indoor wetlands

COD performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar ($p \geq 0.05$) from those operated outdoor (Table 4-4). Though COD involves such a powerful oxidizing reaction, weather extremes outside did not result in a significant difference to the inside wetlands.

Table 4-4. Comparison of effluent COD concentrations for constructed wetlands.

Wetland	P values		
	Indoor vs Outdoor		
1 In and 1 Out	0.770		
2 In and 2 Out	0.156		
3 In and 3 Out	0.615		
4 In and 4 Out	0.399		
5 In and 5 Out	0.560		
6 In and 6 Out	0.401		
Wetland	P values		
	Planted vs unplanted		
1 In and 3 In	0.482		
1 Out and 3 Out	0.854		
2 In and 4 In	0.355		
2 Out and 4 Out	0.271		
Wetland	P values		
	Contaminated vs uncontaminated		
In 1and 2	0.000		
In 3 and 4	0.000		
In 5 and 6	0.000		
Out 1and 2	0.000		
Out 3 and 4	0.000		
Out 5 and 6	0.000		
Wetland	P values		
	Filter media vs No filter media		
3 In and 5 In	1.000		
3 Out and 5 Out	0.919		
4 In and 6 In	0.750		
4 Out and 6 Out	0.727		
Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.001	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.003	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were analysed against unplanted wetlands and the COD results indicates that they were similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor (Table 4-4).

COD results of wetlands contaminated with benzene indicate clearly that they were statistical significantly different ($p \leq 0.05$) from uncontaminated wetlands operated both indoor and outdoor (Table 4-4).

However, the analysis of COD effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to wetlands with no filter media operated both indoor and outdoor (Table 4-4).

While, yearly analysis of COD effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation of both indoor and outdoor (Table 4-4).

4.2.3. Biological Oxygen Demand (BOD_5) removal

Knowing the effluent BOD_5 (or simply written as BOD) levels of the wetlands makes calculation of the efficiency easy. The rigs were monitored and the BOD_5 results presented in this subsection.

Figure 4-3 shows improved BOD_5 treatment performances for uncontaminated wetlands (wetlands 2, 4 and 6) of both indoor and outdoor rigs. In comparison, the treatment performances of BOD_5 of hydrocarbon contaminated wetlands (wetlands 1, 3 and 5) of both indoor and outdoor rigs show reduced treatment performances. This suggests that hydrocarbon contamination of the wetlands resulted to apparent influence on the BOD_5 treatment performance. However, overall result shows slightly better treatment performances for wetlands 1, 3, 4 and 6 of the indoor rig. The lower efficiency of the outside wetlands is an indication of a lower average yearly temperature and weather extremes, including freezing in winter, which all put stress on the system that was not endured by the wetlands operated indoors. This result partly supports the theory that the biological reactions responsible for the decomposition of organic matter (BOD_5), nitrification, dentrification and removal of pathogens are generally known to be temperature dependant in all wastewater treatment processes, including Constructed Wetlands (Reed et al., 1995). In comparison, filter 2 show similar performances for both indoor and outdoor rigs, while filter 5 show slightly better performances for outdoor rig.

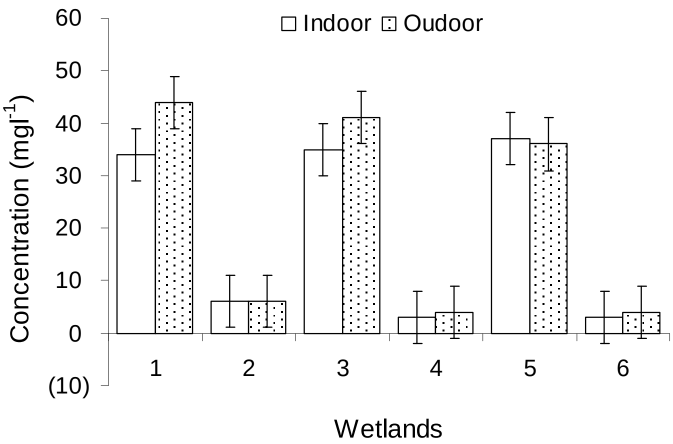


Figure 4-3. Overall BOD_5 effluent mean for the indoor and outdoor wetlands

Figure 4-4 shows mean BOD_5 effluent for indoor wetlands from 2005-2007. First year of operation show a better treatment performance for hydrocarbon contaminated wetlands (wetlands 1, 3 and 5), in comparison the uncontaminated wetlands shows slightly higher performance in the second year. However, the results show better BOD_5 treatment performances for uncontaminated wetlands throughout the duration of the study. It is pertinent to note that reductions in BOD_5 observed in uncontaminated wetlands were satisfactory for most wetlands if compared to minimum American and European standards (<20 mg/l) for the secondary treatment of effluent. In comparison, the BOD_5 treatment performances of hydrocarbon contaminated wetlands show reduced treatment performances in both first and second year of operations (Fig. 4-4). This suggests that addition of toxic benzene could be responsible for the BOD_5 reduction efficiencies in wetlands 1, 3 and 5 compared with improved removal efficiencies in wetlands 2, 4 and 6. The BOD_5 concentrations were similar for the effluents from planted wetlands when compared to unplanted gravel and sand wetlands. Comparing wetlands 1 (planted and contaminated) with Filter 2 (planted but not contaminated), the potential negative effect of benzene contamination on the overall BOD_5 reduction efficiency for wetland 1 were apparent (Figures 4-3 and 4-4, Table 4-5).

This result clearly indicates that high hydrocarbon contamination of the wetlands might harm the microorganism responsible for low BOD_5 treatment during the processes. The effect of high hydrocarbon (benzene) concentration on the BOD_5 treatment as observed in this study could also be attributed to be a major factor causing poor BOD_5 treatment by leading to plant stress and affecting the metabolism function of the organism. Though it can be inferred that toxicity of hydrocarbon did affect BOD_5 removal performance, BOD_5 treatment observed does show that there was some treatment by biological processes.

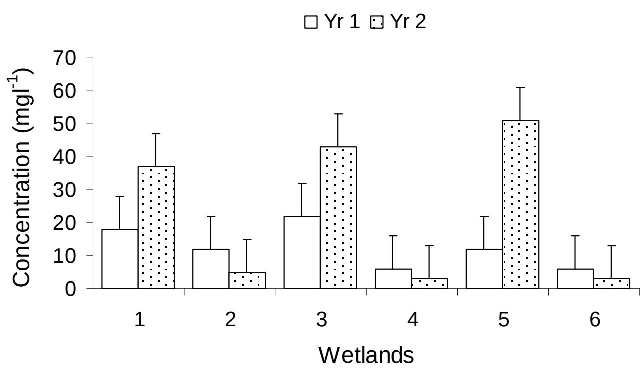


Figure 4-4. Annual BOD_5 effluent mean for the indoor wetlands

Overall BOD_5 performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar ($p \geq 0.05$) from those operated outdoor in all wetlands (Table 4-5).

Table 4-5. Comparison of effluent BOD_5 concentrations for constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.077
2 In and 2 Out	0.964
3 In and 3 Out	0.499
4 In and 4 Out	0.171
5 In and 5 Out	0.505
6 In and 6 Out	0.931
Wetland	P values
	Planted vs unplanted
1 In and 3 In	0.551
1 Out and 3 Out	0.576
2 In and 4 In	0.108
2 Out and 4 Out	0.722
Wetland	P values
	Contaminated vs uncontaminated
In 1and 2	0.000
In 3 and 4	0.000
In 5 and 6	0.000
Out 1and 2	0.000
Out 3 and 4	0.000
Out 5 and 6	0.000
Wetland	P values
	Filter media vs No filter media
3 In and 5 In	0.441
3 Out and 5 Out	0.458
4 In and 6 In	0.452
4 Out and 6 Out	0.450

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
2 In	0.001	2 Out	0.000
3 In	0.005	3 Out	0.000
4 In	0.002	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the BOD_5 results indicates that they were similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor (Table 4-5).

BOD_5 effluent analysis of wetlands contaminated with benzene indicates clearly that they were statistical significantly different ($p \leq 0.05$) from uncontaminated wetlands operated both indoor and outdoor (Table 4-5).

However, the analysis of BOD_5 effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to wetlands with no filter media operated both indoor and outdoor (Table 4-5).

While yearly analysis of BOD_5 effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from uncontaminated wetlands operated both indoor and outdoor in year 2 (Table 4-5).

4.3. Variables essential for control and optimization of the wetland

The water quality variables essential for control and optimization of the wetland used in this study was monitored and present in this section. These variables were DO, pH, Nutrients (Nitrate-nitrogen, Ortho-phosphate-phosphorus and Ammonia-nitrogen), Conductivity, Redox and Turbidity.

The above mentioned variables of the wetlands have been monitored and analysed (Tables 4-2 and 4-3) in an attempt to establish their relationship and to ensure optimal performance in the system. Furthermore, this section presented detailed ANOVA to further support management of constructed treatment wetlands.

4.3.1. Dissolved Oxygen

Sufficient amount of dissolved oxygen for growth and metabolism of microorganism has to be ensured to maintain optimum process in the wetland. A DO concentration of 1-2 mg/l is sufficient in treatment wetland. However, higher DO content did not necessarily increase the treatment efficiency of hydrocarbon in the constructed wetland as seen in the results shown in chapter 6. The aeration of the wetland if provided should be adjusted to that DO concentration of 2 mg/L, more is just wasted energy. The result show that the rig operated outdoor has higher DO than indoor rig (Figure 4-5). More DO observed in the

Wetlands operated in the outdoor environment could be attributed to rain and wind etc, diffusing oxygen into the wetlands. Results also show a higher DO in the outdoor wetlands during the first year of operation (Figure 4-6). In comparison, DO concentrations were similar in subsequent years with higher DO concentrations in uncontaminated wetlands (Figure 4-7).

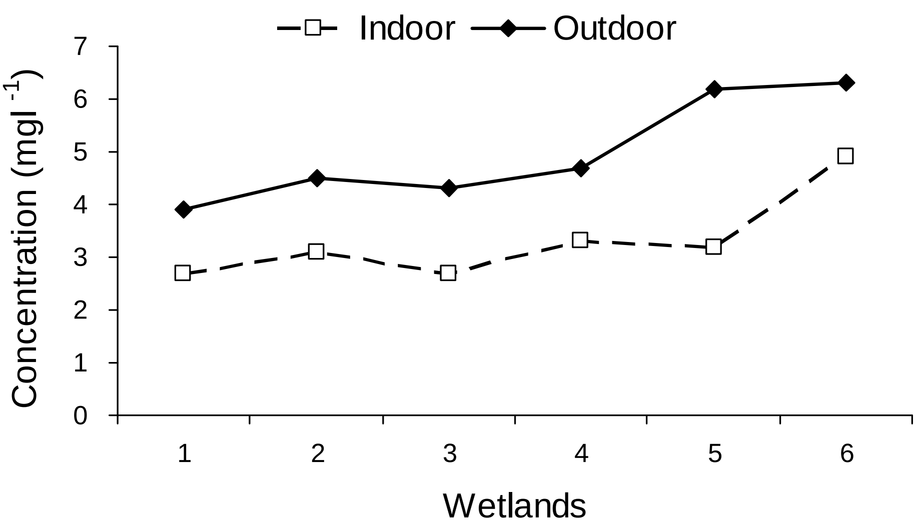


Figure 4-5. Overall DO effluent mean for the indoor and outdoor wetlands (08/04/05- 18/10/07)

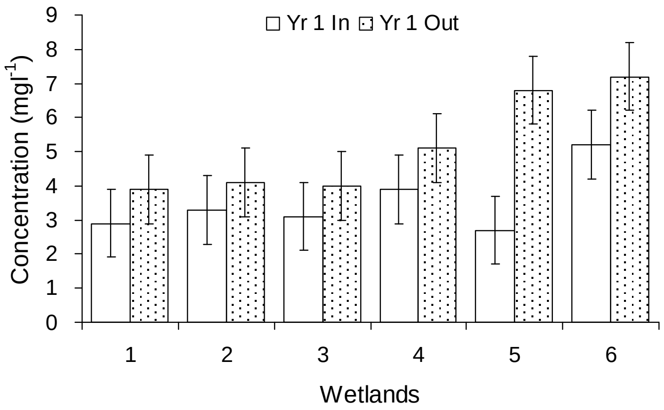


Figure 4-6. First year DO effluent for the indoor and outdoor wetlands

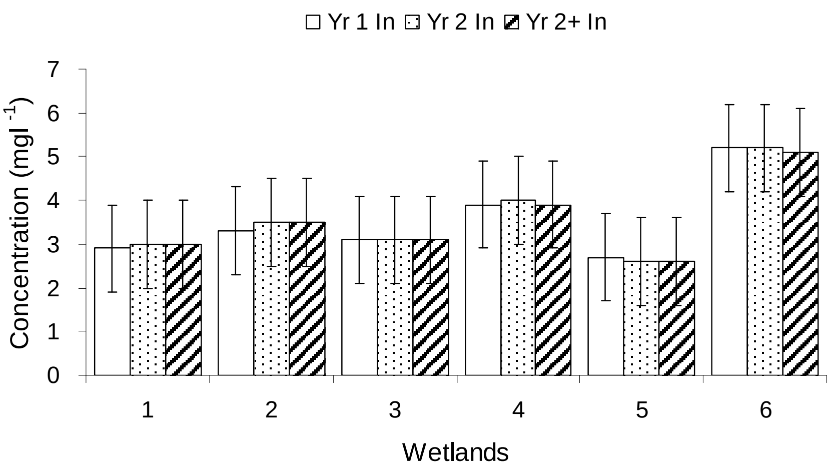


Figure 4-7. Annual DO effluent for the indoor wetlands

Overall DO performances of wetlands operated indoor were also statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistical significantly different ($p \leq 0.05$) from those operated outdoor in all wetlands (Table 4-6).

Table 4-6. Comparison of effluent DO concentrations for constructed wetlands.

Wetland	P values Indoor vs Outdoor
1 In and 1 Out	0.015
2 In and 2 Out	0.006
3 In and 3 Out	0.004
4 In and 4 Out	0.000
5 In and 5 Out	0.000
6 In and 6 Out	0.013

Wetland	P values Planted vs unplanted
1 In and 3 In	0.730
1 Out and 3 Out	0.174
2 In and 4 In	0.408
2 Out and 4 Out	0.589

Wetland	P values Contaminated vs uncontaminated
In 1and 2	0.492
In 3 and 4	0.060
In 5 and 6	0.000
Out 1and 2	0.198
Out 3 and 4	0.562
Out 5 and 6	0.882

Wetland	P values Filter media vs No filter media
3 In and 5 In	0.005
3 Out and 5 Out	0.000
4 In and 6 In	0.000
4 Out and 6 Out	0.002

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.000	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.749	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the DO results indicates that they were similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor (Table 4-6). DO effluent analysis of wetlands contaminated with benzene indicates that wetlands 5(contaminated) and 6 (uncontaminated) operated indoor were statistical significantly different ($p \leq 0.05$). In comparison, all other contaminated and uncontaminated wetlands of the rig operated both indoor and outdoor were statistically similar ($p \geq 0.05$) (Table 4-6).

However, the analysis of DO effluent indicates that wetlands with filter media were statistical significantly different ($p \leq 0.05$) to wetlands with no filter media operated both indoor and outdoor (Table 4-6).

The yearly analysis of DO effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation of both indoor and outdoor, with exception of filter 5 operated indoor which was statistically similar ($p \geq 0.05$) in both years (Table 4-6).

4.3.2. pH

Extreme pH conditions are expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons. System with a pH-value outside of a range from 6.0 to 9.0 will likely cease the activity the microorganism needed for the efficient process. However, pH was monitored to access its role in this study.

The result show that the rig operated outdoor has slightly higher pH than indoor rig, with exception of filter 2 which has higher pH indoor (Figure 4-8). Results also show a higher pH in the outdoor wetlands during the first year of operation (Figure 4-9). In comparison, pH concentrations were higher in the first year and slightly decline in subsequent years (Figure 4-10). Overall pH values were all higher in outside cells except for the planted wetlands which showed higher or similar pH indoor.

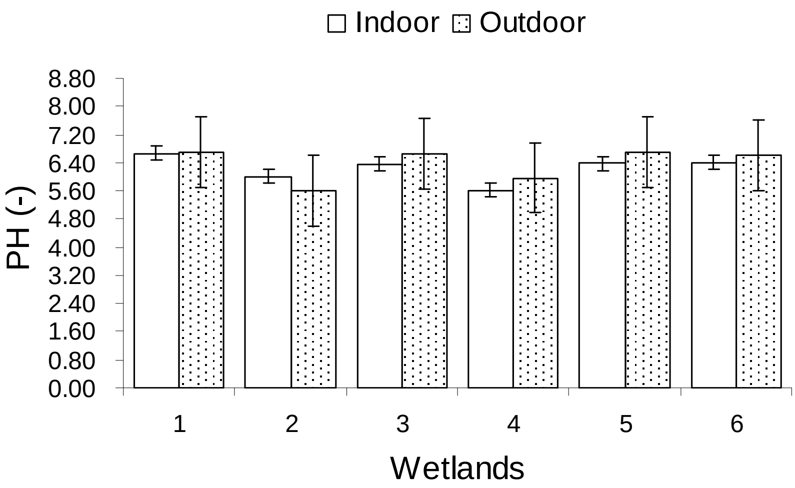


Figure 4-8. Overall mean pH for the indoor and outdoor wetlands

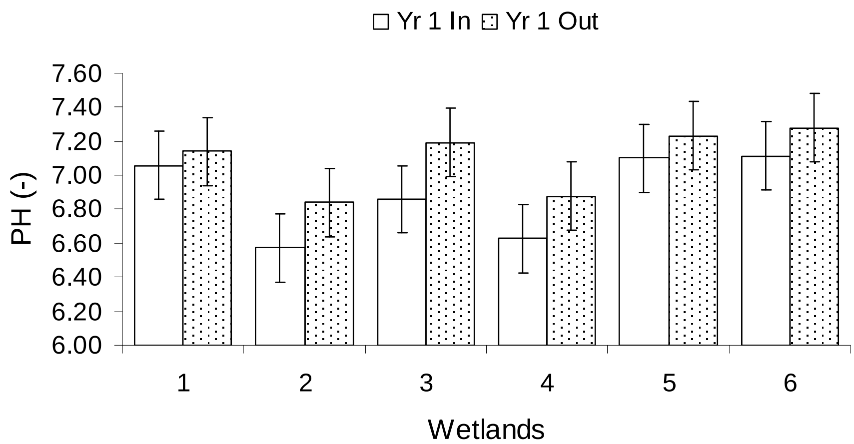


Figure 4-9. First year mean pH for the indoor and outdoor wetlands

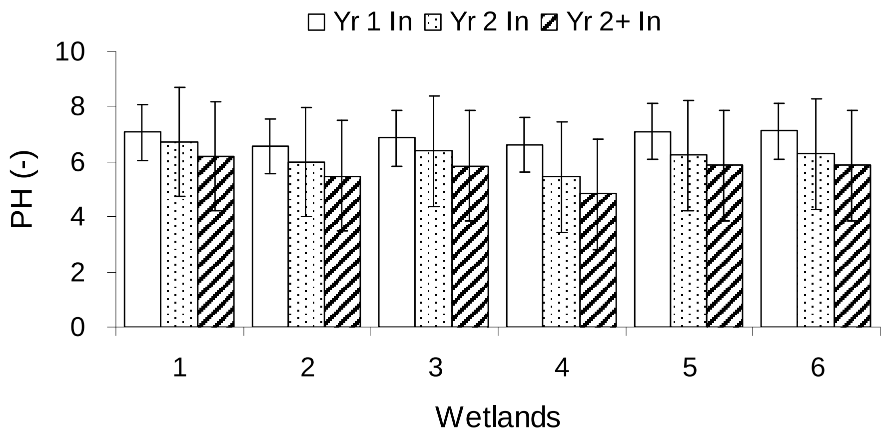


Figure 4-10. Annual pH for the indoor wetlands

Furthermore, pH data were statistically analysed to establish the relationship of the major components. The results indicates that wetlands operated indoor were statistical significantly different ($p \leq 0.05$) from those operated outdoor in wetlands 3 and 5, while other wetlands were statistically similar ($p \geq 0.05$) (Table 4-7).

Table 4-7. Comparison of effluent pH concentrations for constructed wetlands.

Wetland	P values		
	Indoor vs Outdoor		
1 In and 1 Out	0.603		
2 In and 2 Out	0.217		
3 In and 3 Out	0.003		
4 In and 4 Out	0.152		
5 In and 5 Out	0.021		
6 In and 6 Out	0.062		
Wetland	P values		
	Planted vs unplanted		
1 In and 3 In	0.029		
1 Out and 3 Out	0.800		
2 In and 4 In	0.273		
2 Out and 4 Out	0.121		
Wetland	P values		
	Contaminated vs uncontaminated		
In 1and 2	0.000		
In 3 and 4	0.000		
In 5 and 6	0.596		
Out 1and 2	0.000		
Out 3 and 4	0.000		
Out 5 and 6	0.814		
Wetland	P values		
	Filter media vs No filter media		
3 In and 5 In	0.512		
3 Out and 5 Out	0.802		
4 In and 6 In	0.000		
4 Out and 6 Out	0.000		
Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.001	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.001	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were analysed against unplanted wetlands and the pH results indicates that they were statistically similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor, with exception of wetlands 1 and 3 operated indoor rig which was significantly different ($p \leq 0.05$) (Table 4-7).

pH effluent analysis of wetlands contaminated with benzene indicates clearly that they were statistical significantly different ($p \leq 0.05$) from uncontaminated wetlands operated both indoor and outdoor, with exception of wetlands 5 and 6 which was statistically similar ($p \geq 0.05$) in both indoor and outdoor rigs (Table 4-7).

The analysis of pH effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) in wetlands with no filter media of the contaminated wetlands (3 and 5) operated both indoor and outdoor. In comparison, uncontaminated wetlands (4 and 6) were statistical significantly different ($p \leq 0.05$) in both indoor and outdoor (Table 4-7). This analysis suggests that the differences observed could mean that presence of benzene in the contaminated wetlands (3 and 5) could account for pH differences in the corresponding uncontaminated wetlands (4 and 6).

The yearly analysis of pH effluent indicates that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operations of both indoor and outdoor (Table 4-7).

4.3.3. Nutrient removal

The nutrients were important parameters to qualify wastewater in the study, and were assessed in three parameters NH_4-N, NO_3-N and PO_4^{3-}. Nutrient removal performances of the experimental wetland systems were assessed in an attempt to understand their role in hydrocarbon removal and removal mechanisms. The overall removal performance observed in the system operated in both indoors and outdoor could be attributed to oxygen release and nitrogen uptake by macrophytes significantly contributed to removal as presented in subsections 4.3.3.1. to 4.3.3.3. The initial performance of the system was encouraging but decreased as the nutrient loading was increased. The observed performance was in agreement with the report which states that nitrogen removal performance of subsurface flow constructed wetlands treating ammonia-rich wastewater is often relatively poor (IWA specialist group, 2000). Previous findings also indicate that 40% of ammonia- nitrogen was reduced, indicating that nitrification was not active in subsurface flow constructed system (Neralla *et al*., 2000).

The nutrient removal performances were assessed in the present study. The statistical model used to assess the relationship between main wetland components and nutrient removal processes was analysis of variance (ANOVA) and the results thus presented (subsections 4.3.3.1 - 4.3.3.3).

Though ANOVA showed there were significant difference in nutrient values between year 1 and year 2 (Tables 4-8, 4-9 and 4-10) with exception of wetlands 2 and 5 of the ortho-phosphate-phosphorus that were similar for the indoor rig (Table 4-9), removal rates did decrease in year 2. However this was the case with all pollutants. Nutrient removal rates of Ortho-Phosphate-phosphorus, Ammonia-nitrogen and Nitrate-nitrogen all consistently showed the same pattern: there were no significant difference in nutrient for all variables analysed (Tables 4-8 to 4-10).

However, better effluent ammonia-nitrogen concentrations observed as presented in figure 4-13 could be due to increased aeration and better weather conditions in the indoor wetlands, promoting more volatilization and biodegradation (Cooper et al., 1996; Gervin et al., 2001).

4.3.3. 1. Nitrate-nitrogen

Figure 4-11 shows the mean effluent nitrate-nitrogen concentrations in both indoor and outdoor wetlands. The figure shows that nitrate-nitrogen concentrations were slightly higher in wetlands operated outdoors with exception of filter 4 with higher indoor performance. The contaminated wetlands 1, 3 and 5 performed better than the corresponding uncontaminated wetlands 2, 4 and 6. This is an indication that nitrate-nitrogen was directly involved in hydrocarbon removal as an alternative electron acceptor during anaerobic periods of full inundation as reported in previous publication (documented in appendix A). Nitrate-nitrogen was also used partly by hydrocarbon degrading microbes thus enhancing the removal performance in the system. The performance of wetlands without filter media (5 and 6) were similar with filter 5 (contaminated) operated indoor performing slightly better than corresponding filter 6 (uncontaminated) indoor.

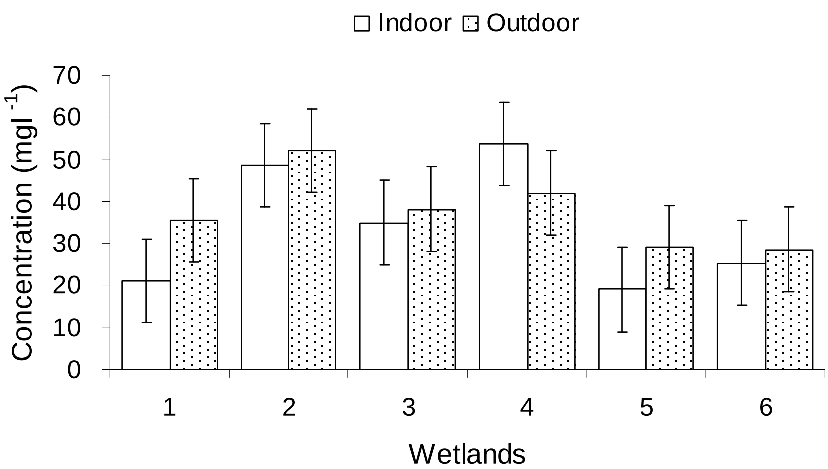


Figure 4-11. Overall nitrate-nitrogen effluent mean for the indoor and outdoor wetlands

However, the performances observed above (figure 4-11) were not enough to establish the significances of the relationship between the variables and processes that contributed to the observed results. Hence, further several statistical analyses were done and the outcome presented in Tables 4-8.

Nitrate-nitrogen performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar (p≥0.05) to those operated outdoor (Table 4-8).

Table 4-8. Comparison of effluent Nitrate-nitrogen concentrations for constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.385
2 In and 2 Out	0.850
3 In and 3 Out	0.664
4 In and 4 Out	0.927
5 In and 5 Out	0.605
6 In and 6 Out	0.925
Wetland	P values
	Planted vs unplanted
1 In and 3 In	0.384
1 Out and 3 Out	0.734
2 In and 4 In	0.704
2 Out and 4 Out	0.904
Wetland	P values
	Contaminated vs uncontaminated
In 1and 2	0.310
In 3 and 4	0.431
In 5 and 6	0.671
Out 1and 2	0.644
Out 3 and 4	0.790
Out 5 and 6	0.862
Wetland	P values
	Filter media vs No filter media
3 In and 5 In	0.556
3 Out and 5 Out	0.637
4 In and 6 In	0.375
4 Out and 6 Out	0.385

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.001	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.001	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were analysed against unplanted wetlands and the nitrate-nitrogen results indicates that they were statistically similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor (Table 4-8).

Nitrate-nitrogen effluent analysis of wetlands contaminated with benzene indicates clearly that they were statistically similar ($p \geq 0.05$) to uncontaminated wetlands operated in both indoor and outdoor rigs (Table 4-8).

The analysis of nitrate-nitrogen effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to the wetlands with no media (Table 4-8). The yearly analysis of nitrate-nitrogen effluent indicates that the operations in indoor rig and outdoor rig were statistical significantly different ($p \leq 0.05$) in both years of operations (Table 4-8).

4.3.3.2. Ortho-phosphate-phosphorus

Figure 4-12 shows the mean effluent ortho-phosphate-phosphorus concentrations in both indoor and outdoor wetlands. The figure shows that ortho-phosphate-phosphorus concentrations were slightly higher in wetlands 1 and 2 operated outdoors, in comparison wetlands 3, 4, 5 and 6 show slightly higher performance in these wetlands operated indoors.

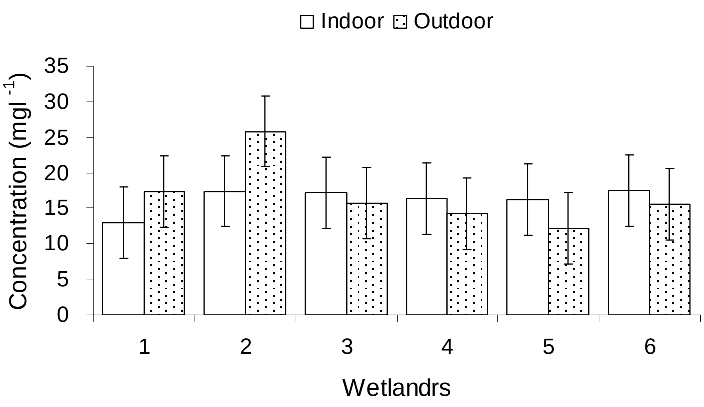


Figure 4-12. Overall ortho-phosphate-phosphorus effluent mean for the indoor and outdoor wetlands

Ortho-phosphate-phosphorus performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar (p≥0.05) to those operated outdoor (Table 4-9).

Table 4-9. Comparison of effluent Ortho-phosphate-phosphorus concentrations for constructed wetlands.

Wetland	P values		
	Indoor vs Outdoor		
1 In and 1 Out	0.296		
2 In and 2 Out	0.353		
3 In and 3 Out	0.786		
4 In and 4 Out	0.970		
5 In and 5 Out	0.496		
6 In and 6 Out	0.650		
Wetland	P values		
	Planted vs unplanted		
1 In and 3 In	0.629		
1 Out and 3 Out	0.287		
2 In and 4 In	0.172		
2 Out and 4 Out	0.019		
Wetland	P values		
	Contaminated vs uncontaminated		
In 1and 2	0.398		
In 3 and 4	0.799		
In 5 and 6	0.570		
Out 1and 2	0.398		
Out 3 and 4	0.544		
Out 5 and 6	0.636		
Wetland	P values		
	Filter media vs No filter media		
3 In and 5 In	0.830		
3 Out and 5 Out	0.433		
4 In and 6 In	0.892		
4 Out and 6 Out	0.543		
Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.001	1 Out	0.000
2 In	0.074	2 Out	0.018
3 In	0.000	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.099	5 Out	0.000
6 In	0.004	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were analysed against unplanted wetlands and the ortho-phosphate-phosphorus results indicates that they were statistically similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor, with exception of wetlands 2 (planted) and 4 (unplanted) operated outdoor which were statistical significantly different ($p \leq 0.05$) (Table 4-9).

Ortho-phosphate-phosphorus effluent analysis of wetlands contaminated with benzene indicates clearly that they were statistically similar ($p \geq 0.05$) to uncontaminated wetlands (Table 4-9). The analysis of ortho-phosphate-phosphorus effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to the wetlands with no media (Table 4-9). The yearly analysis of ortho-phosphate-phosphorus effluent indicates that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operations of both indoor and outdoor rigs, with exception of wetlands 2 and 5 that were similar for the indoor rig (Table 4-9).

4.3.3.3. Ammonia-nitrogen

Figure 4-13 shows the mean effluent ammonia-nitrogen concentrations in both indoor and outdoor wetlands. The figure shows that ammonia-nitrogen concentrations were slightly higher in all wetlands operated outdoors; in comparison all wetlands operated indoors show slightly lower ammonia-nitrogen removal.

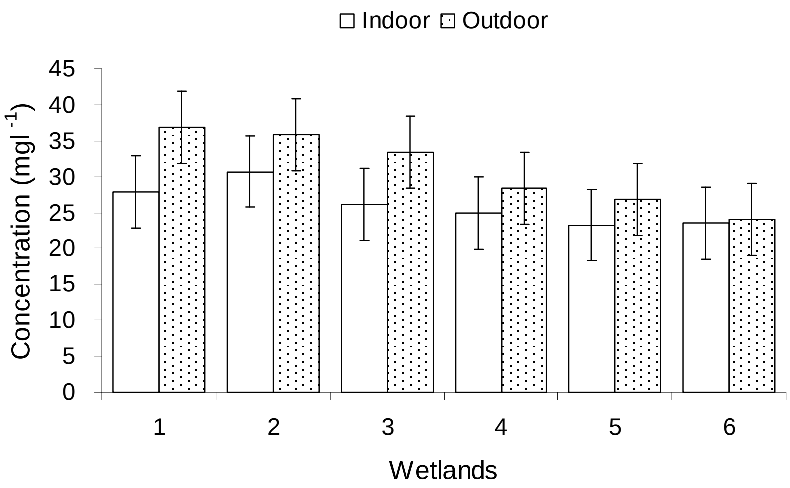


Figure 4-13. Overall ammonia-nitrogen effluent mean for the indoor and outdoor wetlands

Ammonia-nitrogen performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar ($p \geq 0.05$) to those operated outdoor (Table 4-10). Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were analyzed against unplanted wetlands and the ammonia-nitrogen results indicates that they were statistically similar ($p \geq 0.05$) to the corresponding unplanted wetlands operated both indoor and outdoor (Table 4-10).

Ammonia-nitrogen effluent analysis of wetlands contaminated with benzene indicates clearly that they were statistically similar ($p \geq 0.05$) to uncontaminated wetlands operated in both indoor and outdoor rigs (Table 4-10).

The analysis of ammonia-nitrogen effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to the wetlands with no media (Table 4-10). The yearly analysis of ammonia-nitrogen effluent indicates that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operations of both indoor and outdoor rigs (Table 4-10).

Table 4-10. Comparison of effluent Ammonia-nitrogen concentrations for constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.957
2 In and 2 Out	0.646
3 In and 3 Out	0.627
4 In and 4 Out	0.555
5 In and 5 Out	0.643
6 In and 6 Out	0.793
Wetland	**P values**
	Planted vs unplanted
1 In and 3 In	0.424
1 Out and 3 Out	0.861
2 In and 4 In	0.109
2 Out and 4 Out	0.630
Wetland	**P values**
	Contaminated vs uncontaminated
In 1and 2	0.953
In 3 and 4	0.330
In 5 and 6	0.747
Out 1and 2	0.666
Out 3 and 4	0.438
Out 5 and 6	0.623
Wetland	**P values**
	Filter media vs No filter media
3 In and 5 In	0.273
3 Out and 5 Out	0.313
4 In and 6 In	0.701
4 Out and 6 Out	0.480

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.000	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

4.3.4. Electrical Conductivity

Electrical conductivity is a useful indicator essential for control and optimization of the wetland among other variables. It was monitored to access its role in this research. The result show that wetlands 1 and 2 performed slightly better in outdoor rig, wetlands 3 and 4 had a similar performance in both indoor and outdoor rigs while wetlands 5 and 6 show higher performance in indoor rig (Figure 4-14). Comparison of year 1 performances shows a higher conductivity in the indoor wetlands 1 and 3, similar performance in filter 4, while wetlands 2, 5 and 6 show higher performances outdoors (Figure 4-15). However, yearly performance comparison show best performance in year 1 but decreased geometrically as the years of operation increases (Figure 4-16) with exception of filter 6 that show better performance in year1 and decreased in year 2 but the performance increased again after the second year.

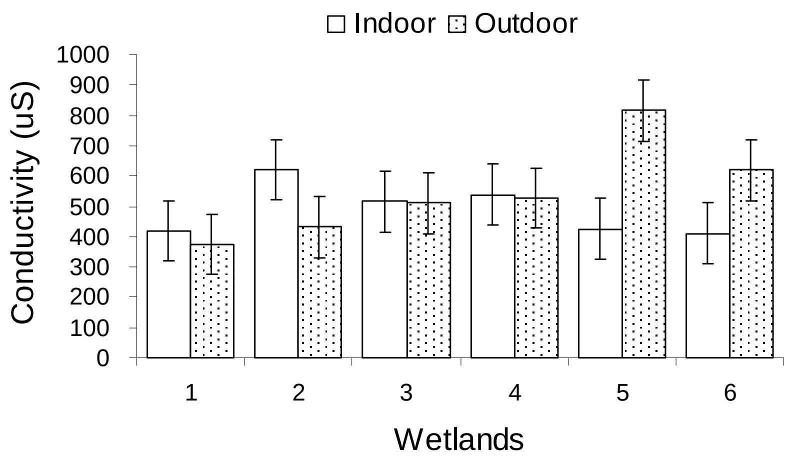


Figure 4-14. Overall conductivity effluent mean for the indoor and outdoor wetlands

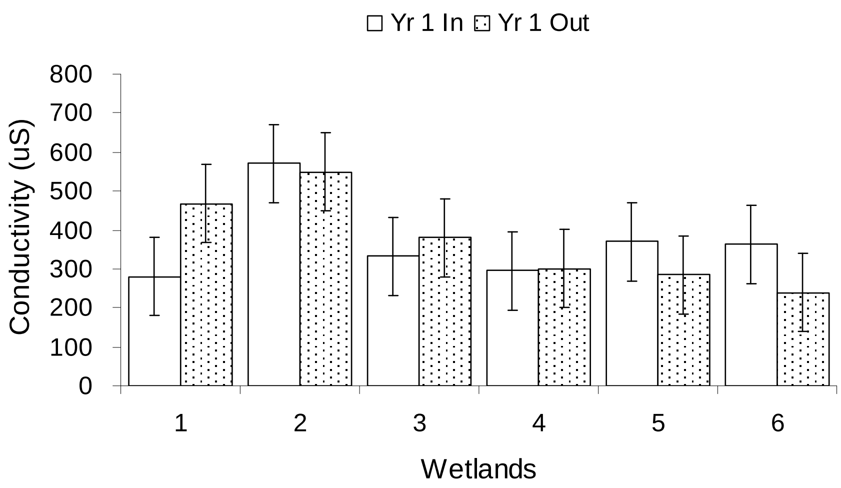


Figure 4-15. First year conductivity effluent for the indoor and outdoor wetlands

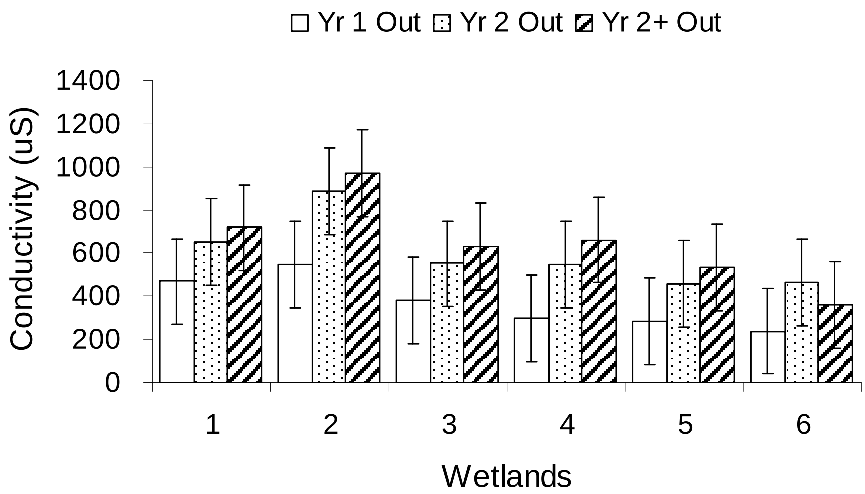


Figure 4-16. Annual conductivity effluent for the outdoor wetlands

Overall conductivity performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were statistically similar ($p \geq 0.05$) to those operated outdoor in all wetlands (Table 4-11).

Table 4-11. Comparison of effluent Conductivity concentrations for constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.121
2 In and 2 Out	0.327
3 In and 3 Out	0.823
4 In and 4 Out	1.000
5 In and 5 Out	0.889
6 In and 6 Out	0.854
Wetland	P values
	Planted vs unplanted
1 In and 3 In	0.117
1 Out and 3 Out	0.871
2 In and 4 In	0.428
2 Out and 4 Out	0.091
Wetland	P values
	Contaminated vs uncontaminated
In 1and 2	0.025
In 3 and 4	1.000
In 5 and 6	0.857
Out 1and 2	0.188
Out 3 and 4	0.863
Out 5 and 6	0.917
Wetland	P values
	Filter media vs No filter media
3 In and 5 In	0.596
3 Out and 5 Out	0.385
4 In and 6 In	0.839
4 Out and 6 Out	0.656

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
2 In	0.001	2 Out	0.000
3 In	0.000	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the conductivity results indicates that they were statistically similar ($p \geq 0.05$) in all wetlands operated both indoor and outdoor (Table 4-11).

Conductivity effluent analysis of wetlands contaminated with benzene indicates that they were statistically similar ($p \geq 0.05$) to uncontaminated wetlands operated in both indoor and outdoor rigs, with exception of wetlands 1(contaminated) and 2 (uncontaminated) that were statistical significantly different ($p \leq 0.05$) indoor (Table 4-11).

The analysis of conductivity effluent indicates that wetlands with filter media were statistically similar ($p \geq 0.05$) to wetlands with no filter media operated both indoor and outdoor (Table 4-11).

The yearly analyses of conductivity effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation of both indoor and outdoor (Table 4-11).

4.3.5. Redox potential

Redox was monitored to access its role in this research. The result show that slightly better performance in rig operated indoor with exception of filter 5 with similar performance and slightly lower performance in filter 6 (Figure 4-17). Maurer and Rittmann (2004a) observed that BTEX are more easily degraded under high redox conditions, with the degradation ability decreased in the order: aerobic oxidation, denitrification, iron reduction, sulphate reduction and methanogenesis. The observed redox performances were constantly high enough to stimulate benzene degradation. However, yearly performance comparison show reduction in the wetland redox as the years increases (Figure 4-18). This could contribute to reduced benzene removal efficiency observed (chapter 5) as the year increases.

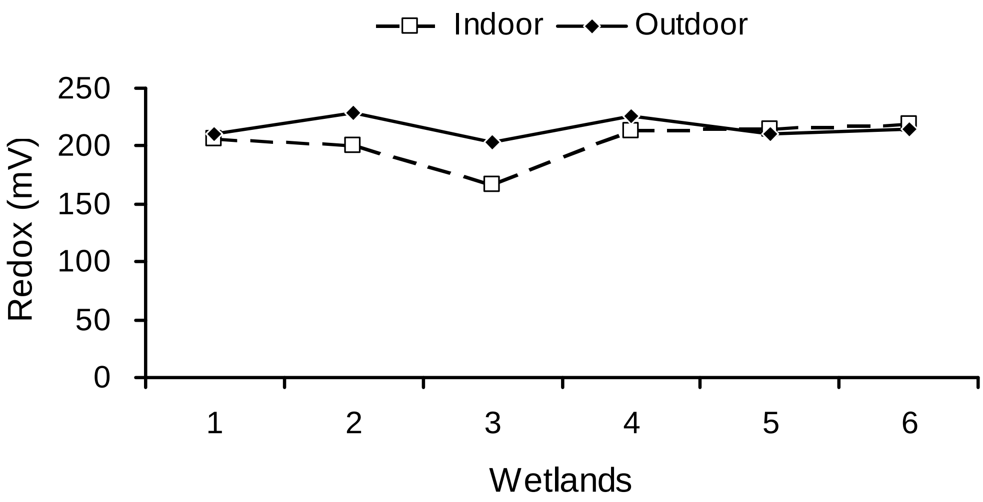


Figure 4-17. Overall Redox effluent mean for the indoor and outdoor wetlands

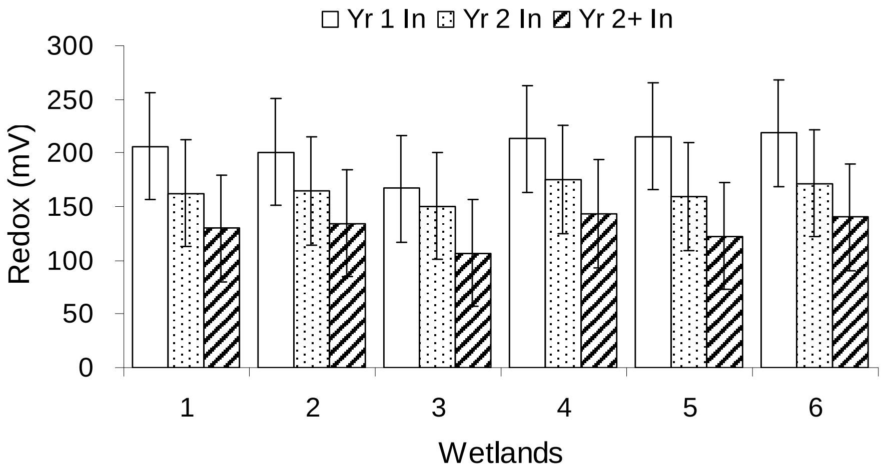


Figure 4-18. Annual Redox effluent for the indoor wetlands

Overall Redox performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were similar ($p \geq 0.05$) to the corresponding wetlands operated outdoor, with exception to filter 3 operated indoor that was statistical significantly different ($p \leq 0.05$) from filter 3 operated outdoor (Table 4-12).

Table 4-12. Comparison of effluent Redox concentrations for constructed wetlands.

Wetland	P values		
	Indoor vs Outdoor		
1 In and 1 Out	0.618		
2 In and 2 Out	0.057		
3 In and 3 Out	0.015		
4 In and 4 Out	0.674		
5 In and 5 Out	0.926		
6 In and 6 Out	0.537		
Wetland	P values		
	Planted vs unplanted		
1 In and 3 In	0.046		
1 Out and 3 Out	0.816		
2 In and 4 In	0.499		
2 Out and 4 Out	0.371		
Wetland	P values		
	Contaminated vs uncontaminated		
In 1and 2	0.706		
In 3 and 4	0.004		
In 5 and 6	0.566		
Out 1and 2	0.053		
Out 3 and 4	0.154		
Out 5 and 6	0.921		
Wetland	P values		
	Filter media vs No filter media		
3 In and 5 In	0.045		
3 Out and 5 Out	0.736		
4 In and 6 In	0.678		
4 Out and 6 Out	0.148		
Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.830	3 Out	0.000
4 In	0.000	4 Out	0.000
5 In	0.000	5 Out	0.000
6 In	0.000	6 Out	0.000

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the Redox results indicates that they were similar ($p \geq 0.05$) to the corresponding unplanted wetlands, with exception of wetlands 1 (planted) and 3 (unplanted) both operated indoor that were statistical significantly different from each other ($p \leq 0.05$ (Table 4-12).

Redox effluent analysis of wetlands contaminated with benzene indicates that they were statistically similar ($p \geq 0.05$) to uncontaminated wetlands operated in both indoor and outdoor rigs. However, wetlands 3 and 4 operated indoor as well as wetlands 1 and 2 operated outdoor were statistical significantly different ($p \leq 0.05$) (Table 4-12).

The analysis of Redox effluent indicates that wetlands with filter media were statistical significantly different ($p \leq 0.05$) to wetlands with no filter media operated in wetlands 3 and 5 indoor. While other wetlands operated both indoor and outdoor were statistically similar ($p \geq 0.05$) (Table 4-12).

The yearly analyses of Redox effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation of both indoor and outdoor, with exception of filter 3 operated indoor that was statistically similar ($p \geq 0.05$) in both years (Table 4-12).

4.3.6. Turbidity

Turbidity was monitored to access its role in this research. The result show that wetlands 1, 2 and 4 performed better in indoor rig, filter 6 performance was similar in both indoor and outdoor rigs while wetlands 3 and 5 show lower turbidity in outdoor rig (Figure 4-19). Comparison of year 1 performances show that wetlands 1, 2 and 4 performed better in indoor rig, filter 5 performance was similar in both indoor and outdoor rigs while wetlands 3 and 6 show lower turbidity in outdoor rig (Figure 4-20).

However, yearly performance comparison show turbidity reduces as year increases in wetlands 1, 2, 4 and 6, while filter 3 shows lower turbidity in year 1 but increased during year 2 and reduced again after year 2. Filter 5 show that turbidity increases with years of operation (Figure 4-21).

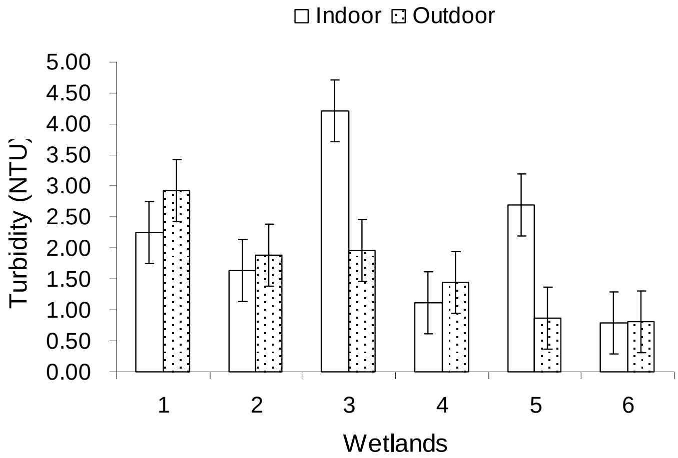


Figure 4-19. Overall Turbidity effluent mean for the indoor and outdoor wetlands

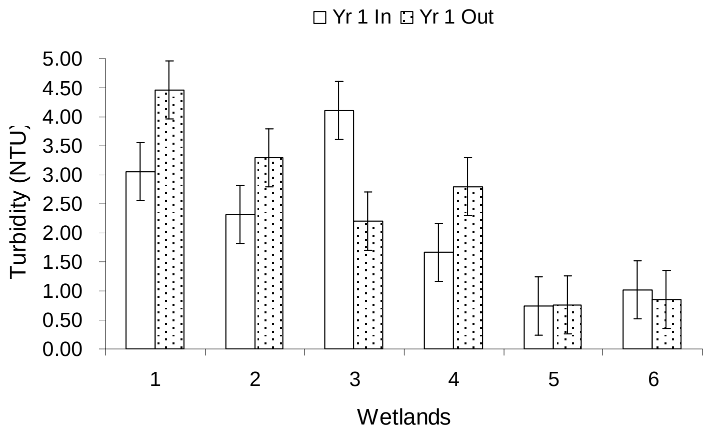


Figure 4-20. First year turbidity effluent for the indoor and outdoor wetlands

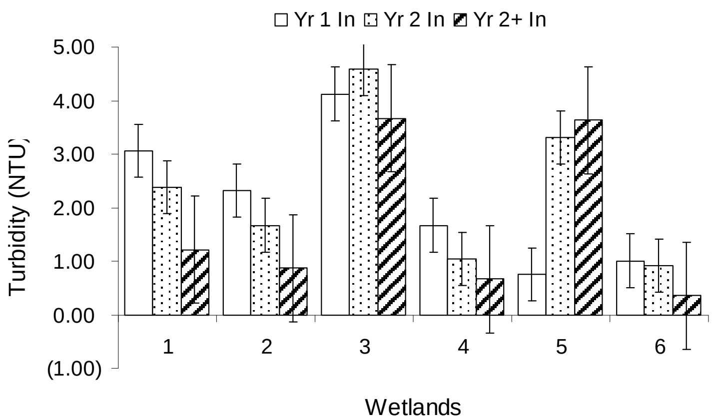


Figure 4-21. Annual Turbidity effluent for the indoor wetlands

Overall Turbidity performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were similar (p≥0.05) to the corresponding wetlands operated outdoor in uncontaminated wetlands (2, 4 and 6), while statistical significantly different (p≤ 0.05) in contaminated wetlands (1, 3 and 5) (Table 4-13). This could be an indication that biodegradation and other reactions taking place in contaminated wetlands were responsible for turbidity differences observed.

Table 4-13. Comparison of effluent Turbidity concentrations for constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.030
2 In and 2 Out	0.461
3 In and 3 Out	0.000
4 In and 4 Out	0.503
5 In and 5 Out	0.000
6 In and 6 Out	0.490
Wetland	**P values**
	Planted vs unplanted
1 In and 3 In	0.000
1 Out and 3 Out	0.003
2 In and 4 In	0.045
2 Out and 4 Out	0.128
Wetland	**P values**
	Contaminated vs uncontaminated
In 1and 2	0.294
In 3 and 4	0.000
In 5 and 6	0.000
Out 1and 2	0.007
Out 3 and 4	0.070
Out 5 and 6	0.840
Wetland	**P values**
	Filter media vs No filter media
3 In and 5 In	0.000
3 Out and 5 Out	0.000
4 In and 6 In	0.008
4 Out and 6 Out	0.010

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.023	1 Out	0.000
2 In	0.000	2 Out	0.000
3 In	0.169	3 Out	0.970
4 In	0.002	4 Out	0.001
5 In	0.000	5 Out	0.031
6 In	0.244	6 Out	0.021

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the Turbidity results indicates that they were statistical significantly different from each other ($p \leq 0.05$), with exception of wetlands 2 (planted) and 4 (unplanted) operated outdoor but were similar ($p \geq 0.05$) (Table 4-13).

Turbidity effluent analysis of wetlands contaminated with benzene versus corresponding uncontaminated wetlands indicates that wetlands (3 and 4 indoor, 5 and 6 indoor, and 1 and 2 outdoor) were statistical significantly different ($p \leq 0.05$), while wetlands (1 and 2 indoor, 3 and 4 outdoor, and 5 and 6 outdoor) were statistically similar ($p \geq 0.05$) (Table 4-13).

The analysis of Turbidity effluent indicates that wetlands with filter media were statistical significantly different ($p \leq 0.05$) to wetlands with no filter media operated both indoor and outdoor (Table 4-13).

The yearly analyses of Turbidity effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation of both indoor and outdoor, with exception of filter (3 and 6 operated indoor, and filter 3 operated outdoor) that were statistically similar ($p \geq 0.05$) (Table 4-13).

4.4. Microbiological examination

Wetlands contain diverse microbial populations which include the flora of bacteria, fungi and algae. These microbes are important for pollutant transformations which help wetland ecosystems to operate consistently to treat wastewater. This subsection documented the findings of the microbial populations' examination. In an attempt to enumerate the aromatic degrading microbes involved in this study, microbiological examination was carried out. Heterotrophic Plate Count (HPC) was used to estimate the number and distribution of live heterotrophic bacteria in the wetland. The microbiological examination results show Pseudomonas species to be the major microorganism in this research among other microbes tested. This observation supported previous findings which states that numerous benzene-degrading aerobic microorganisms have been identified, the most notable of which are the Pseudomonas species, which may account for up to 87% of the petrol-degrading microorganisms in contaminated aquifers (Ridgeway et al., 1990). Biologically-mediated degradation reactions involve electron transfer, and the preferred degradation pathway for a given compound in the subsurface is dependent on the oxidation state of the organic compound and on the local water chemistry and microbial populations. Microorganisms gain energy for growth and reproduction by catalyzing oxidation reduction reactions, which require an electron donor and an electron acceptor. Organic contaminants (aromatic hydrocarbon) can be degraded by serving as either an electron donor that becomes oxidized or as an electron acceptor that becomes reduced.

This suggests that soil microbes have the capacity to decompose organic matter and aerobic metabolism can be enhanced because of DO saturation (Kadlec and Knight 1996). Moreover, previous research showed that microorganisms can be bioindicators to determine the water quality and identify microbiological processes in constructed wetlands (Scholz *et al*., 2002).

Figure 4-22 shows the mean HPC result of the hydrocarbon degrading microbes thriving in contaminated and uncontaminated wetlands operated in both in indoor (environmentally controlled) and outdoors.

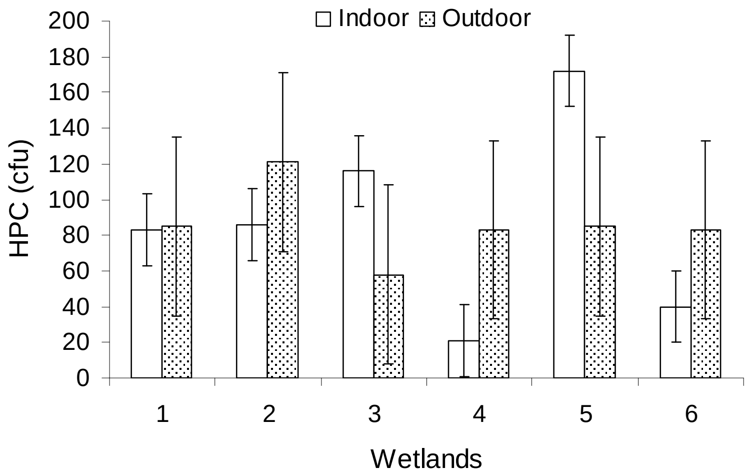


Figure 4-22. Overall HPC for the indoor and outdoor wetlands

The HPC result as presented in the bar chat below show more microbes in contaminated wetlands of the indoor rig (Figure 4-23) with exception of filter 1 that was similar, which is an indication that hydrocarbon degrading microbes thrives best in the indoor rig with better environmental conditions. The result could be one of the evidence for a better hydrocarbon removal performance as seen (chapter 5) in the rig operated indoor. This finding suggests that the extent of hydrocarbon biodegradation in wetlands is critically dependent upon the creation of optimal environmental conditions to stimulate biodegradative activity (Figure 4-24). Furthermore, the result show that the microbial species in wetlands functions in a wide range of physical and chemical conditions. Overall wetland parameters, such as dissolved oxygen, water temperature and influent constituent concentrations, must be controlled through design and system operational control to keep the microbial community in harmony for optimal treatment.

In comparison, uncontaminated wetlands show more microbes in outdoor rig (Figure 31) and fewer microbes in the corresponding contaminated wetlands operated outdoors.

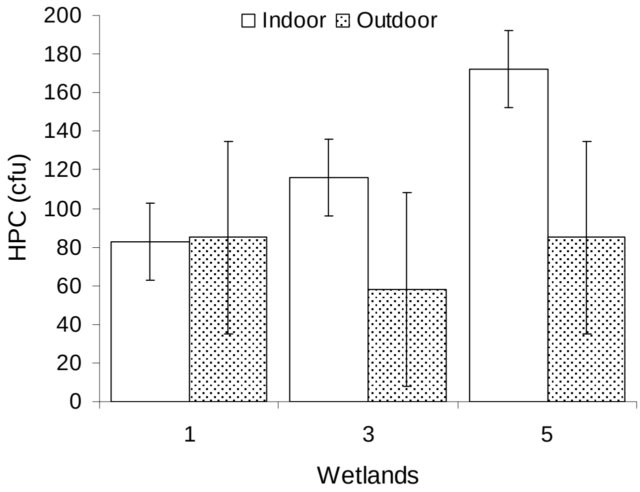


Figure 4-23. Microbial distribution (HPC) in the contaminated wetlands

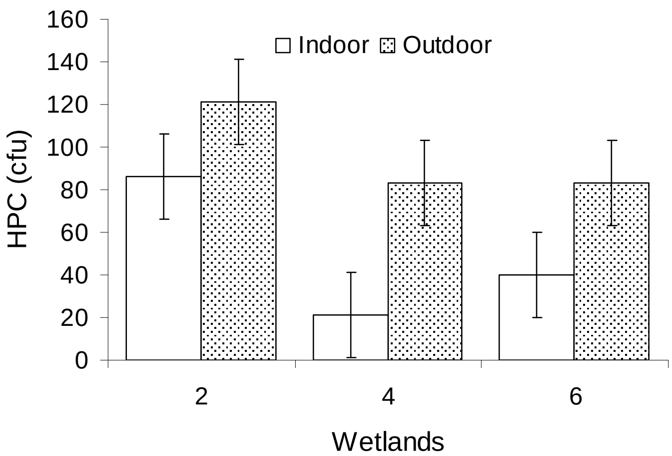


Figure 4-24. Microbial distribution (HPC) in the uncontaminated wetlands

Furthermore, the corresponding HPC Percentage microbial distribution in the indoor wetlands presented in figure 4-25 show more microbes in contaminated wetlands 3 and 5 to be 22 and 33% respectively, with slightly similar distribution in wetlands 1 (16%) and 2 (17%) for the indoor rig, while uncontaminated filter 4 and 6 were 4 and 6% respectively. These were an indication that hydrocarbon degrading microbes thrives best in contaminated wetlands of the indoor rig with better environmental conditions. These results established the link with better indoor hydrocarbon performance observed (chapter 5) in this research.

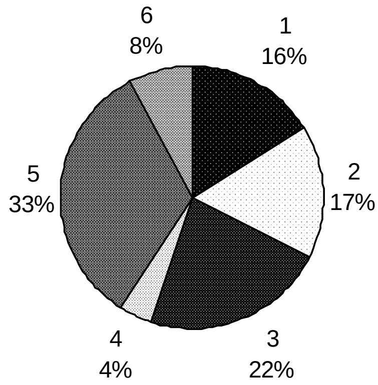


Figure 4-25. Percentage microbial distribution in the indoor wetlands

In comparison, uncontaminated wetlands Percentage microbial distribution in the outdoor wetlands presented in figure 4-26 show more microbes in uncontaminated wetlands 2 and 4 to be 23 and 16% respectively, with slightly similar distribution in wetlands 5 (17%) and 6 (16%) for the outdoor rig, while the chart show fewer microbes in the corresponding contaminated wetlands (1 and 3 were 17 and 11% respectively) operated outdoors.

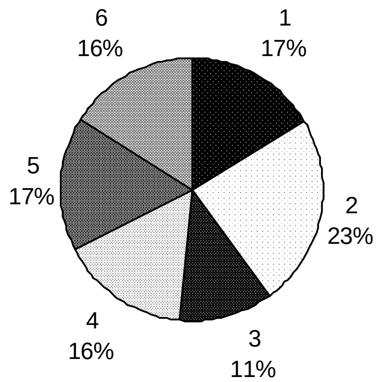


Figure 4-26. Percentage microbial distribution in the outdoor wetlands

Despite the chat presentations above, microbial distributions of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were similar (p≥0.05) to the corresponding wetlands operated outdoor in uncontaminated wetlands (2, 4 and 6) and contaminated filter1, while statistical significantly different (p≤ 0.05) in contaminated wetlands (3 and 5) (Table 4-14). This analysis is in agreement with the chat presented above (figure 4-26) and is an indication that microbes thrive best in appropriate controlled and steadier environment.

Table 4-14. Comparison of microbial distributions in constructed wetlands.

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	0.454
2 In and 2 Out	0.762
3 In and 3 Out	0.025
4 In and 4 Out	0.135
5 In and 5 Out	0.024
6 In and 6 Out	0.594
Wetland	P values
	Planted vs unplanted
1 In and 3 In	0.327
1 Out and 3 Out	0.379
2 In and 4 In	0.005
2 Out and 4 Out	0.253
Wetland	P values
	Contaminated vs uncontaminated
In 1and 2	0.862
In 3 and 4	0.001
In 5 and 6	0.003
Out 1and 2	0.293
Out 3 and 4	0.446
Out 5 and 6	1.000
Wetland	P values
	Filter media vs No filter media
3 In and 5 In	0.374
3 Out and 5 Out	0.498
4 In and 6 In	0.331
4 Out and 6 Out	0.930

Wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also analysed against unplanted wetlands and the Heterotrophic Plate Count results indicates that they were statistically similar from each other($p \geq 0.05$), with exception of wetlands 2 (planted) and 4 (unplanted) operated indoor that were significantly different ($p \leq 0.05$) (Table 4-14).

Heterotrophic Plate Count of microbes in wetlands contaminated with benzene versus corresponding uncontaminated wetlands indicates that wetlands (3 and 4 indoor, and 5 and 6 indoor) were statistical significantly different ($p \leq 0.05$), while other wetlands were statistically similar ($p \geq 0.05$) (Table 4-14).

The analysis of microbes in the effluent indicates that the microbe distributions were statistically similar ($p \geq 0.05$) in all wetlands with filter media and those with no filter media operated indoor and outdoor (Table 4-14). This suggests that filter media might not be a big factor but could contribute indirectly by providing surface for microorganism attachment in the wetland.

Overall microbial examination results show a high development of aerobic heterotrophic bacteria and positive hydrocarbon utilizing microbes' response in the experimental constructed wetlands which is in agreement with previous findings by Salmon et al (1998). Moreover, these microbes probably interacted with the plants and other wetland components for the biodegradation of hydrocarbon. Furthermore the comparative wetlands operated outdoors showed slightly lower performances to the wetlands operated indoors studied in parallel.

4.5. Summary

This chapter has investigated extensively, the role of constructed wetland components and demonstrated that intermittently flooded vertical-flow wetlands were highly efficient for COD, BOD, Nutrient and other water quality variables removal. The better HPC result in the rig operated indoor (environmentally controlled) established the link with better indoor hydrocarbon performance observed (chapter 5) and thus demonstrated that the extent of hydrocarbon biodegradation in wetlands is critically dependent upon the creation of optimal environmental conditions which could favour the microbes and stimulate biodegradative activity.

Furthermore, macrophytes presence in the present study does show similarity on all the variables analysed with exception of Conductivity (Table 4-11), pH with exception of indoor wetlands which show difference between planted and unplanted (Table 4-7) and Turbidity with exception of outdoor wetlands 2 and 4 which show difference between planted and unplanted (Table 4-13). However, macrophytes provided good filtration conditions by preventing the filter from clogging and provide surface for microbes' attachment. However it should be remembered that the effects of macrophytes go beyond aesthetics or support but help to maintain the natural processes that are being mimicked in constructed wetlands (Kadlec, 2001).

5

Hydrocarbon performance evaluations♣

5.1. Overview

This chapter presented very vital results of the study as it examines hydrocarbon removal performance in the constructed wetland systems. The internal workings of various wetland designs and major components were evaluated in section 5.2. Section 5.3 presented the result of the additional experiment to investigate hydrocarbon removal mechanism while subsections 5.4 documented factors affecting hydrocarbon removal, in an attempt to assess the roles played by other water quality variables in benzene removal. 5.4.1 and 5.4.2 evaluates the role of temperature, aggregates (filter media) and macrophytes on benzene removal respectively, and 5.4.3 documented role of nutrients. Section 5.5 presented the traced changes of hydrocarbon removal performance with running period and the observed impact of

♣ Parts of this chapter have been published and won first prize in the 2007 Society of Petroleum Engineers (SPE) European Regional paper contest as:
Eke P. E., Scholz, M., and Wallace S.D., (2007b), Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry. The paper was also invited and presented at the 2007 Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition (ATCE), and SPE International Student Paper Contest held on 11-14 November in Anaheim, California, USA. Available online in Society of Petroleum Engineers International elibrary (http://www.spe.org/elibrary), SPE 113644. DOI: 10.2118/113644-STU (original copy documented in appendix A).

An earlier version of parts of this chapter was also published as:
Eke P. E. and Scholz M. (2006), Hydrocarbon Removal with Constructed Treatment Wetlands for the Benefit of the Petroleum Industry. In: Proceedings of the 10th International Conference on Wetland Systems for Water Pollution Control, 23-29 September 2006, ed by Dias V and Vymazal J. International Water Association, Lisbon, Portugal, Volume 3:1707-1714, ISBN: 989-20-0361-6.

long-term operation of hydrocarbon contaminated wastewater in experimental constructed wetlands. Section 5.6 summarized the chapter.

5.2. Removal performance

How well constructed wetlands perform basic physical, biological and chemical treatment functions to remove hydrocarbon has been studied for over two years (31 months) in Edinburgh. Paper documented in appendix A summarized the removal efficiencies of different wetlands while Figure 5-1 and Table 5-1 presented the hydrocarbon treatment performance for both the indoor and outdoor rigs. The benzene removal efficiency varied with time. No obvious decrease of the benzene removal efficiency was observed between April 2005 and October 2005 for both the indoor and outdoor wetlands (Fig. 5-1).

The benzene removal performances for both the indoor and outdoor wetlands are shown in Fig. 5-1. Benzene removal efficiencies were higher for the indoor wetlands than for those located outdoors. The findings indicate very high overall mean removal efficiencies for Benzene to be 90% for wetlands operated indoors in comparison to slightly lower overall mean treatment performances of approximately 81% for wetlands operated outdoors (Figure 5-1 and Table 5-1). These findings were comparable to data published previously by Myers and Jackson (2001).

Table 5-1. Effluent benzene concentrations in selected constructed wetlands

Rig. No	N	Mean mg/l	SD mg/l	SE mg/l	95% Confidence Interval mg/l	Minimum mg/l	Maximum mg/l	Mean Re %
1 In	41	112.87 [a]	146.43	22.87	66.65~159.09	0.00	467.00	88.71
3 In	41	102.73 [a]	134.58	21.02	60.25~145.21	0.00	653.60	89.73
5 In	41	102.34 [a]	129.67	20.25	61.41~143.27	0.00	452.10	89.77
1Out	41	247.99 [b]	243.43	38.02	171.15~ 324.83	0.00	997.50	75.20
3Out	41	273.42 [b]	319.66	49.92	172.52~374.32	0.00	1241.10	72.66
5Out	41	195.41 [a,b]	280.35	43.78	106.93~283.90	0.00	1016.90	80.46

In and Out represent indoor and outdoor selected constructed wetlands; N, sampling number, data collected between April 2005 and October 2007(31 months (Table 5-2)); SD, standard deviation; SE, standard error; Mean Re, Mean removal efficiencies for benzene during the whole observational period. In any one column, values marked with different letters are significantly different from each other at p≤ 0.05 according to the Duncan's multiple range tests.

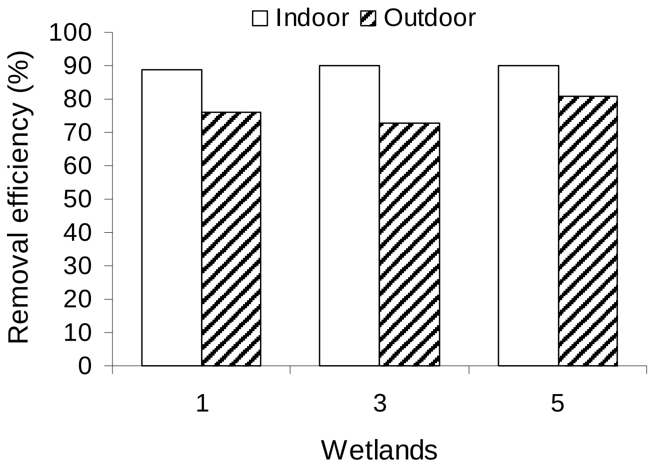


Figure 5-1. Comparison of overall benzene removal efficiencies for the indoor and outdoor wetlands

A common measure of wetland pollutant removal effectiveness is the percentage reduction in pollutant concentration, or the pollutant 'removal efficiency'. The benzene removal efficiency was calculated using Equation 5-1.

$$E\,(\%) = \frac{C_{in} - C_{out}}{C_{in}} * 100 \qquad 5\text{-}1$$

Where; E = removal efficiency, and C_{in} and C_{out} are the mean benzene influent and effluent concentrations, respectively. In the case of unsteady flow and pollutant input conditions, C_{in} and C_{out} are often computed as flow weighted mean concentrations.

The use of E as a measure of wetland effectiveness can often mask the effects of significant influences of the wetland system operating conditions on the wetland system's effectiveness as a water pollution control facility.

These operating conditions include:

- background pollutant concentration levels
- input concentration

- hydraulic loading (ratio of mean discharge to wetland surface area), and
- hydraulic residence time of the pollutant phase

Each of the above factors influences the performance of a wetland, as measured by E, in a non-linear manner. In practice, it will be appropriate when comparing E values derived for different wetlands to incorporate the above four factors to allow a common basis for comparison.

The overall slightly better removal rate thus achieved in rig operated indoor (Table 5-1 and Figure 5-1) could be attributed predominantly to control of environmental variables such as higher and steadier temperature, humidity and light resulted in improved overall treatment performance of the wetlands as indicated by the slightly stable values of the indoor rig (Figure 5-1), see section 5.4 for more detail on factors affecting hydrocarbon removal. It could be partly because of the presence of fertilizer enhancing the biodegradation rate and because some microbial communities are able to utilize the nitrogen component (i.e. nitrate-nitrogen) of the fertilizer, more detail on section 5.4.3. This suggests that during biodegradation, microbes transform available nutrients, including hydrocarbons, into substances useful for energy and cell reproduction. This is in accordance with previous finding by Admire et al (1995) which states that microbes obtain energy by facilitating the transfer of electrons from electron donors to electron acceptors. This results in the oxidation of electron donors and the reduction of electron acceptors. Electron donors include natural organic material and petroleum hydrocarbons. Electron acceptors in this study include dissolved oxygen and nitrate. The use of electron donors by microbes begins with dissolved oxygen (aerobic conditions) which occur more in the upper part of the wetlands in the current study, followed by anaerobic (absent or minimal dissolved oxygen) which occur in the lower part during full inundation of wetlands of the wetlands. This observation supports the findings by Admire et al (1995) which states that when oxygen is not present in sufficient amounts, nitrate, sulfate, ferrous iron, and low carbon dioxide may be used as electron acceptors.

The rate of natural microbial degradation of hydrocarbons is related to the abundance of electron acceptors such as oxygen. During full inundation of wetlands in this study oxygen is depleted (anaerobic conditions), some microbes use electron acceptors such as nitrate. BTEX biodegradation rates under anaerobic (when oxygen is absent) conditions are slower than when oxygen is present (aerobic conditions) as observed in this study. Table 5-2 presented Benzene mean removal efficiency which show more detail as it narrowed the analysis to monthly basis. Both indoor and outdoor wetlands 5 (used as blanks and controls; no aggregates and no planting) exhibited an excellent benzene removal performance in comparison to wetlands filled with aggregates in some instances (Table 5-2). This is attributed to biodegradation and volatilization. Lahvis et al (1999) reported that aerobic biodegradation and volatilization constitute a coupled pathway that contributes significantly to the natural attenuation of hydrocarbon. Findings documented in appendix A deliberated on an experiment to determine the biodegradation and volatilization of benzene, and found that volatilization was the dominant pathway for benzene removal after one day of retention time (more detail in section 5.3.1).

Table 5-2. Mean benzene removal efficiencies (%) for the indoor (i) and outdoor (o) wetlands (F1, F3 and F5)

Month/Wetland	F1i	F1o	F3i	F3o	F5i	F5o
Overall	89	76	90	73	90	81
Apr-05	100	100	98	100	95	99
May-05	98	97	87	95	100	91
Jun-05	97	92	87	94	74	73
Jul-05	100	95	86	99	85	93
Aug-05	100	82	90	83	67	70
Sep-05	100	94	100	100	100	100
Oct-05	100	100	100	100	100	100
Nov-05	100	100	100	100	100	100
Dec-05	100	100	97	100	100	97
Jan-06	100	100	99	96	100	100
Feb-06	100	95	94	100	100	76
Mar-06	100	100	100	100	100	97
Apr-06	100	97	100	100	100	98
May-06	100	93	99	100	100	99
Jun-06	100	90	99	100	100	100
Jul-06	97	82	92	100	100	100
Aug-06	82	64	100	100	100	100
Sep-06	100	46	100	65	100	54
Oct-06	92	97	96	94	92	99
Nov-06	90	78	84	76	89	85
Dec-06	57	35	35	45	68	88
Jan-07	92	75	85	72	91	90
Feb-07	94	64	95	49	89	34
Mar-07	73	38	76	13	66	35
Apr-07	97	95	87	87	99	96
May-07	60	40	82	(7)	89	45
Jun-07	72	64	94	60	86	88
Jul-07	86	51	100	49	93	65
Aug-07	83	78	91	76	99	98
Sep-07	68	62	86	70	75	67
Oct-07	73	55	72	47	68	72

Benzene contained in the wetlands 5, which resemble stabilization ponds (extended storage), could volatilize directly to the atmosphere. Suitable environmental boundary conditions such as a high temperature and turbulent airflow encourage the volatilization process and improve benzene removal (Lee et al., 2004).

Further investigation on the impact of environmental control on operating conditions of the wetland was done on the effluent data of the contaminated wetlands. Overall Benzene performances of wetlands operated indoor were statistically compared to those operated outdoor and the results indicates that wetlands operated indoor were similar ($p \geq 0.05$) to the corresponding wetlands operated outdoor (Table 5-3).

Table 5-3. Comparison of effluent Benzene concentrations for constructed wetlands operated indoor (In) and outdoor (Out).

Wetland	P values
	Indoor vs Outdoor
1 In and 1 Out	1.000
3 In and 3Out	0.369
5 In and 5 Out	0.282

The result of statistical analysis show that wetlands operated indoor were similar with those operated outdoor. This indicates that there was no direct temperature dependence observed in benzene removal efficiency of wetlands operated both indoor and outdoor which is in accordance with findings published elsewhere (Adachi et al., 2001). This relationship differs from the graphical representation as presented in figure 5-1 above. Figure 5-1 is an indication that despite the result of statistical analysis (Table 5-3), suitable environmental boundary conditions such as a high temperature and turbulent airflow encourages the volatilization process and improve benzene removal (Lee et al., 2004). More benzene performances in terms of temperature and seasonal relationship are presented in subsection 5.4.1 and chapter 6.

The yearly analyses of Benzene effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year operation for wetlands 1 (planted with filter media) of both indoor and outdoor. However, other wetlands (3 and 5 operated indoor and outdoor) were statistically similar ($p \geq 0.05$) (Table 5-4).

Table 5-4. Comparison of effluent Benzene concentrations for constructed wetlands operated in year 1 and year 2.

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.000	1 Out	0.000
3 In	0.272	3 Out	0.269
5 In	0.472	5 Out	0.719

5.3. Hydrocarbon removal mechanism

The design and description of treatment wetlands are based on two important parameters: hydraulics and pollutant removal. However, considering that the components of the hydrocarbon and the processes of its transformation, metabolism and degradation are complex, the mechanisms of treatment within constructed wetlands are not yet entirely known. Moreover, there are no known practicable or academically established criteria to assess the mechanisms and performances of hydrocarbon removal within constructed wetlands. The great challenge is the design and operation of wetland systems, which could provide the right environment required for the desired microorganism community to remove high strength and toxic contaminants in wastewater.

A better understanding of the effects of environmental factors such as temperature and humidity, and their possible seasonal interactions with plants, filter media, nutrients and microorganisms is particularly important when optimizing the design, treatment and management of hydrocarbon contaminated wastewater. Taking these factors into considerations, environmental controlled rig was operated to investigate the internal mechanisms and operating conditions. As in other applications of treatment wetlands, there are a variety of mechanisms for the removal of hydrocarbon compounds which include volatilization, biodegradation, adsorption, absorption, biotransformation, chemical precipitation, mineralization, sorption, photolysis, filtration, evapotranspiration, settling, photochemical oxidation and etc. Wetlands as natural bioreactors utilize various species of plants and microbes in the environment to detoxify contaminants (hydrocarbon) present in the water. Scholz (2006) show that naturally developed flora and fauna, including hydrocarbon decomposing bacteria, sulfate reducing bacteria, nitrifying and denitrifying bacteria, algae, etc. biodegrade various contaminants present in wastewater. Biodegradation of hydrocarbons is the result of the metabolic activity of microorganisms, metabolism is a term that embraces the diverse reactions by which a microorganism Processes food materials to obtain energy and the compounds from which cell components are made. The result to establish major hydrocarbon removal mechanism is presented below.

5.3.1. Biodegradation and Volatilization Determination

Literature survey done on hydrocarbon removal mechanisms in constructed wetland show that very few studies have been done on the use of treatment wetlands for the petroleum industry and little or none dedicated to determine the role of volatilization or biodegradation in volatile organic compound removal including benzene. However, studies show that processes such as adsorption, biodegradation and volatilization contributed mostly to benzene removal (Corley et al., 1996; Lee et al., 2004; Li et al., 2006). Extra study investigated the main removal mechanisms in an attempt to understand precisely the internal processes of constructed wetlands. Biodegradation and volatilization were tested in separate experiments. Two extra wetlands (heights: 24 cm; diameters: 5 cm) were set up under controlled environmental conditions; one wetland comprised aggregates and detritus containing mature microbial biomass (284 g detritus was taken from the upper layer of the contaminated parent wetland 3 located indoors) and another wetland was left empty. The small wetlands were constructed in the same way as the large wetlands with the exception of the absence of the ventilation pipes (see chapter 3 for main experimental set up). The purpose of this auxiliary experiment was to assess the main removal pathways of benzene (combined biodegradation and adsorption *versus* volatilization) in constructed treatment wetland. Samples were taken after 1, 2, 3, 6 and 9 d (figure

5-2), and benzene was subsequently determined using headspace and gas chromatography (as described in chapter 3).

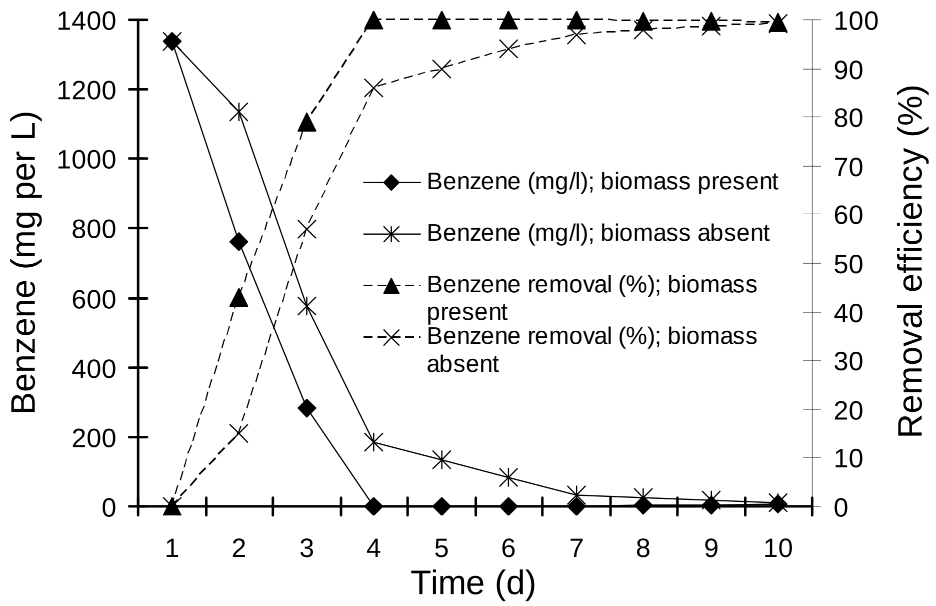


Figure 5-2. Comparison of benzene removal for wetlands with and without biomass

Figure 5-2 above shows a comparison of benzene removal for wetlands with and without biomass. The impacts of volatilization, biodegradation and adsorption on the benzene removal efficiency are often difficult to separate quantitatively from each other. Findings as presented in figure 36 above indicate that biodegradation, volatilization and adsorption support the treatment. This is in agreement with Knight et al (1999) report which observed that aerobic biodegradation contributes significantly to hydrocarbon reduction in constructed wetlands and Salmon et al (1998) who even found that biodegradation was responsible for nearly 80% percent of hydrocarbon reduction with less than 100mg/l influent concentration. Water and oil are likely to separate if the inflow is not in motion. Benzene decanted into tap water was observed to gradually separate into mobile phase and dissolved phase. Volatilization was the dominant mechanism for removal of benzene in mobile phase after one day of retention time and Biodegradation second most removal process for dissolved phase. However, optimizing environmental conditions such as locating wetlands in areas with relatively high temperatures enhances the biodegradation rate. Further research is required in area of removal mechanism to quantify volatilization, aerobic and anaerobic biodegradation, adsorption, absorption, mineralization and other removal mechanisms in large-scale constructed treatment wetlands.

5.4. Factors affecting hydrocarbon removal

This section examines various factors that could affect hydrocarbon removal in constructed wetland such as temperature, nutrients, macrophytes and filter media.

5.4. 1. Role of temperature

Temperature is a major factor controlling the fate of petroleum hydrocarbons within the aquatic environment. Studies on temperature effect on wetland performance have been reported by several researchers including Kadlec et al. (2000) and Scholz et al. (2007). However, these studies focused on constructed wetlands for wastewater treatment targeting the removal of biological oxygen demand, nitrogen, and phosphorous. The current study targets the temperature effect on constructed wetlands applied for hydrocarbon removal. Statistical analysis of benzene effluent versus temperature indicates that wetlands 1 operated in both indoor and outdoor were statistical significantly different ($p \leq 0.05$) while wetlands 3 and 5 operated indoor and outdoor were similar.

Figure 5-3 shows temperature corresponding to benzene removal efficiency trends at the beginning of the operation (April to June 2005), this observed trend at the beginning of the operation is in agreement with Kadlec and Kadlec and Reddy

(2000) which states that several biogeochemical processes in wetlands are affected by temperature, thus influencing the overall treatment efficiency.

In comparison it follows that temperature does not correspond to removal efficiency trends after June 2005, which correspond with previous findings from Cooney (1984) which states that the hydrocarbon-degrading microbial population within an aquatic ecosystem is not necessarily adapted optimally to the seasonal water temperature.

Though the test room was equipped with a high specification unit for climatic research and was used in evaluating and optimizing the environmental factors in the constructed wetland. However, the temperature and humidity values for the indoor rig fluctuated initially due to technical problems, but constant temperature and humidity specifications of 15^{o}C and 60%, respectively, were reached at a later stage during the experiment. In comparison, the second rig was operated outdoors under natural environmental conditions to assess seasonal changes.

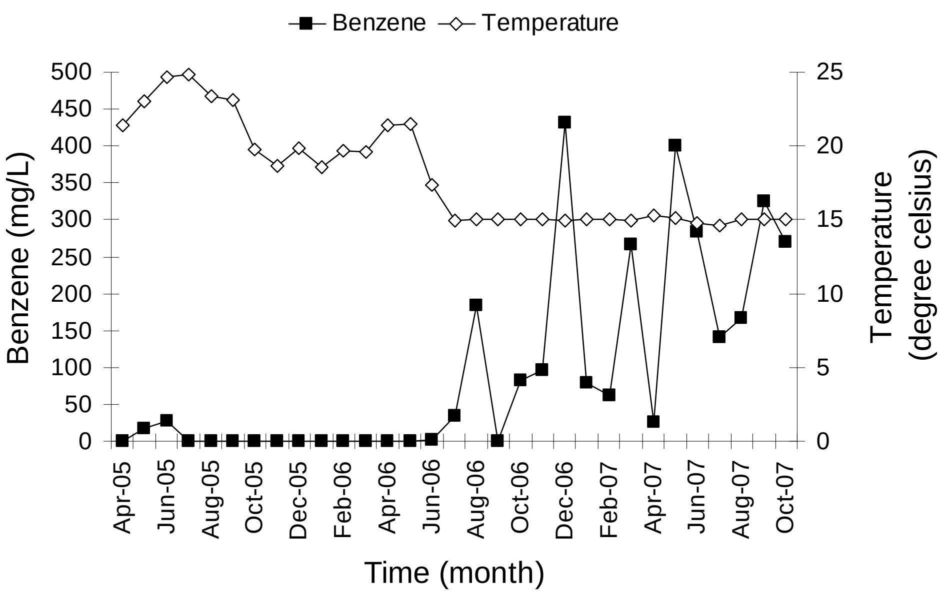


Figure 5-3. Comparison of benzene removal with temperature

It also suggests that the temperature conditions in the wetland affect both the physical and the biological activities in the system. The observed result is an indication that benzene treatment in vertical-flow constructed wetlands did not always respond to temperature changes (Figure 5-3). Despite the deviations from initial trend observed after June 2005, the wetlands still maintain high performances. This also suggests that temperature though do not always correspond to removal efficiency trends but likely a significant control parameter for wetlands treating hydrocarbons. Furthermore, this indicate that there may be distinct environment or seasonal changes required in conjunction with hydraulic retention time, dissolved oxygen, pH and nutrient enrichment to stimulate microorganisms to biodegrade hydrocarbon.

In a recent report for temperatures and energy flows based on a study of water temperatures in surface flow wetlands in hot arid climate, Kadlec (2006) pointed out three reasons for the importance of water temperature in treatment wetlands: temperature modifies the rates of several key biological processes; temperature is sometimes a regulated water quality parameter; and water temperature is a prime determinant of evaporative water loss processes. Atlas (1981) observed that temperature influences petroleum biodegradation by its effect on the physico-chemical properties of the oil, rate of hydrocarbon metabolism by microorganisms and composition of the microbial community. Atlas (1981) also observed that at low temperatures, the viscosity of oil increases, while the volatility of toxic low-molecular weight hydrocarbons reduces. Temperature also variously affects the solubility of hydrocarbons (Foght et al., 1996). Various documented researches indicate conflicting opinions on the role of temperature. Kadlec and Reddy (2000) studied the temperature dependence of many individual wetland processes and wetland removal of contaminants in surface flow wetland. They concluded that microbial mediated reactions are affected by temperature; the treatment response was much greater to changes at the lower end of the temperature scale (<15ºC) than at the optimal range (20 to 35ºC). This observation is partly not in agreement with the observation in this study except during the initial stage of operation (April to June 2005) as pointed above. Furthermore, they observed that the processes regulating organic matter decomposition were affected by temperature. In colder climates, the overall treatment efficiency is usually relatively low (Kadlec and Reddy, 2000).

Considering these conflicting opinions on the role of temperature more analysis on variable climatic conditions and hydrocarbon removal within constructed wetlands were presented in chapter 6. Moreover, further studies identifying the relationships between microbes, temperature and hydrocarbon removal within constructed wetlands are required.

Better control over the indoor environmental conditions such as maintaining a steadier temperature and humidity of 15ºC and 60%, respectively, resulted in an improved overall performance (Figure 5-1). The overall removal efficiencies were slightly lower for the outdoor experimental rig (e.g. benzene, 76-81%) in comparison to the experimental rig placed indoors (e.g. benzene, 89-90%). Furthermore, the mean removal efficiencies for the water quality variables were lowest for the experimental rig placed outdoor (benzene, 76%; COD, 70%; ammonia-nitrogen, 83%; nitrate-nitrogen, 88%; ortho-phosphate-phosphorus, 58%). This is likely due to relatively low and relatively variable (standard deviation: 4.7^{o}C) temperatures in Scotland-Edinburgh (annual mean of approximately 8^{o}C (Met Office (http://www.metoffice.gov.uk/education/secondary/teachers/ukclimate.html#3.2)) (Figure 5-4).

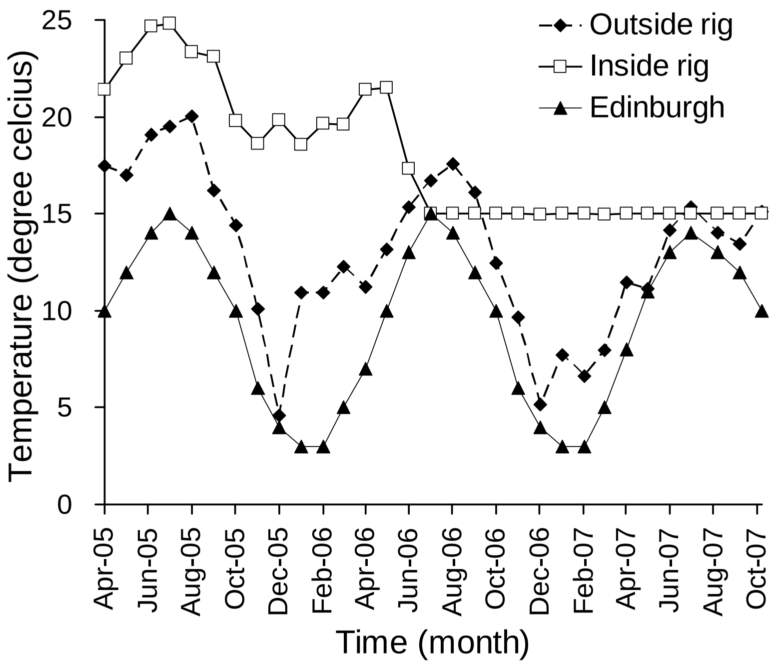


Figure 5-4. Comparison of mean temperature distribution for the inside and outside rigs, and Edinburgh

In comparison, the best overall mean treatment performances were obtained for the experimental rig placed indoors (benzene, 90%; COD, 80%; ammonia-nitrogen, 90%; nitrate-nitrogen, 94%; ortho-phosphate-phosphorus, 66%). The relatively high treatment performance observed indoors is influenced by stable (standard deviation: 3.5^{o}C) and usually relatively high temperatures, particularly after the temperature was fully controlled as described above (Figure 5-4).

The most important constraint in UK applications is that during winter months, water temperatures within the wetland fall, often down to 3 or 4°C or less, and these low temperatures limit rates of treatment, especially rates at which hydrocarbon can be biodegraded. The design (vertical-flow) used in this study was able to provide treatment that coped adequately with seasonal fluctuations in ambient temperature. Nevertheless, significant rates of hydrocarbon treatment have been achieved and observed during colder winter months at temperatures down below 10°C as indicated in figure 5-3 and 5-4. However, the findings of this study provide clue for main concern and the challenge of providing a design that is able to consistently meet specified effluent discharge limits, especially when treating stronger contaminants (hydrocarbon) during winter months in field scale.

5.4. 2. Role of macrophytes and filter media

Macrophytes are widely used within treatment wetlands (Cooper et al., 1996; Sun et al., 2005; Scholz, 2006). However, the role of macrophytes in treatment wetlands has been controversial. Some researchers have documented that macrophytes can improve pollutant removal (Cooper et al., 1996; Brix, 1997; Vymazal, 1999; Kadlec et al., 2000; Neralla et al., 2000; Kadlec, 2002; Karathanasis et al., 2003). Alternatively, others did not detect any significant difference between planted and unplanted systems (Baldizon et al., 2002; Scholz et al., 2002). This subsection documents the result of the findings concerning the role of macrophytes specifically treating hydrocarbon in constructed wetlands.

The trend of the graph showing treatment efficiencies for wetlands planted with *Phragmites australis* (Cav.) Trin. ex Steud. (Filter 1) were similar to the efficiency of the corresponding unplanted wetlands (Filter 3) (Figure 5-5).

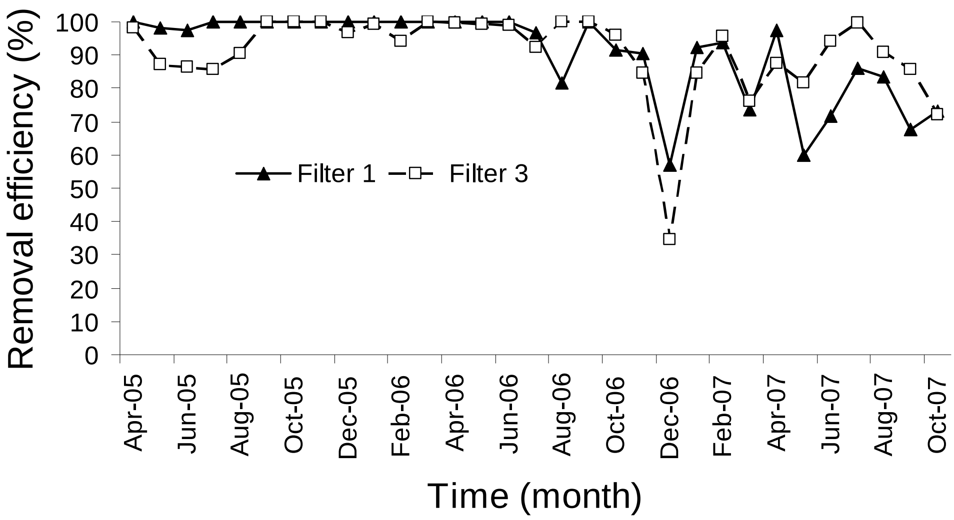


Figure 5-5. Mean benzene treatment efficiencies (%) for the indoor wetlands 1 (planted) and 3 (unplanted)

Furthermore, wetlands (1) planted with *Phragmites australis* (Cav.) Trin. ex Steud. were also statistically analysed against unplanted wetlands and the Benzene results indicates that they were statistical significantly different ($p \leq 0.05$) to wetlands (3) that is not planted (Table 5-5).

Table 5-5. Comparison of effluent Benzene for planted and unplanted constructed wetlands.

Wetland	P values
	Planted vs unplanted
1 In and 3 In	0.000
1 Out and 3 Out	0.000

The presence of wetland plants also resulted in 5-20% additional benzene removal for the outdoor planted wetland 1 when compared with the unplanted wetland 3 (Fig. 5-1). These analyses show an indication that despite the observed similarity in the graph trend lines (Figure 5-5) or the contradiction in the scientific findings, plants play indirect role in treatment of contaminants (especially hydrocarbon) in constructed wetland by preventing clogging and providing oxygen to the rhizosphere thus creating an aerobic environment (Brix, 1997). For example, the growth of roots within filter media helps to decompose organic matter and prevents clogging by creating channels for the water to pass through. The plants in turn provides habitat and supports microbial communities that can either directly biodegrade or catalyze chemical reactions and maintain the hydrocarbon biotransformation process. Considering that bacteria capable of degrading volatile organics such as benzene, toluene, ethylbenzene and o-, m- and p-xylene (BTEX) have been found in the rhizosphere. Baris et al. (2001) also noted that wetland plant selection is important but not as significant as having a good microbial community.

Nevertheless, the relative contribution of plant oxygen transport to wastewater treatment remains controversial as well. Some wetland designers assume strongly that plant oxygen transport is significant (DeBusk and DeBusk, 2001). Same group argues that some wetland plants release sufficient oxygen into the root zone to support aerobic microbial activity (Bodelier et al., 1996; Armstrong et al., 1990), and this may sometimes represent as much as 90% of the total oxygen entering a wetland substrate (Reddy et al., 1989), while others dismiss it as negligible (US EPA, 2000). Sorrell and Armstrong (1994) observed that quantification of oxygen flux from entire root systems has been complicated by species and seasonal differences, spatial heterogeneity and measurement accuracies for variables including the oxygen demand of the root zone solution and the root to solution volume. Steinberg and Coonrod, (1994) states that the plants' capacity to supply oxygen to the root zone varies among species due to differences in vascular tissues, metabolism, and root distribution. The potential for plants to release oxygen into the root zone may increase during cold periods, because root and rhizome respiration consumes relatively large proportions of oxygen, which diffuses through plant shoots, and the oxygen demand for root and rhizome respiration declines with temperature (Callaway and King, 1996). However, despite the controversy on contribution of plant oxygen, the finding in this study suggests that hydrocarbon treatment in vertical-flow constructed wetlands is a function of metabolism by the indigenous microflora which depends directly or indirectly on favourable condition (availability of light, oxygen, temperature, nitrogen and phosphorus) (chapter 4 Figure 30, Figure 37, Table 16c,). This finding is in agreement with findings published elsewhere (Atlas, 1981) and more on role of these conditions is documented in chapter 6.

Furthermore, the graph trend of planted wetlands 1 presented in figure 5-6 show similar performances in wetlands operated both indoor and outdoor. The analysis of variance of the all contaminated wetlands (1, 3 and 5) operated indoor against the corresponding outdoor wetlands show also that they were statistically significantly similar ($p \geq 0.05$).

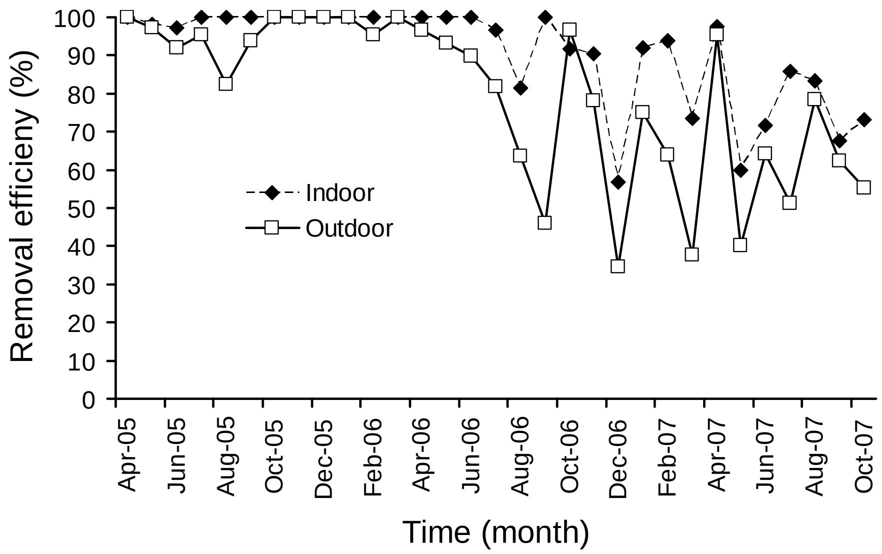


Figure 5-6. Comparison of benzene removal for planted indoor and outdoor wetlands 1

Concerning role of filter media, the analysis of Benzene effluent indicates that wetlands (3) unplanted with filter media were statistically similar ($p \geq 0.05$) to wetlands (5) unplanted with no filter media operated both indoor and outdoor (Table 16d). However, analysis of wetlands that is planted with filter media (1) show that they were statistical significantly different ($p \leq 0.05$) to wetlands (5) unplanted with no filter media operated both indoor and outdoor (Table 5-6). This is an indication that plant with filter media plays indirect role in hydrocarbon treatment by providing surface for attachment of hydrocarbon-degrading microbes.

Table 5-6. Comparison of effluent Benzene concentrations for constructed wetlands plant/unplanted with media and without media.

Wetland	P values
	Filter media (unplanted) vs No filter media
3 In and 5 In	0.177
3 Out and 5 Out	0.456

Wetland	P values
	Filter media (planted) vs No filter media
1 In and 5 In	0.000
1 Out and 5 Out	0.000

5.4.3. Role of nutrients

Hydrocarbon degradation was a function of nutrient availability. Nutrients (particularly nitrogen and phosphorus) are essential for the successful biodegradation of hydrocarbon pollutants (Cooney, 1984). Natural bioattenuation recognizes that petroleum hydrocarbons are readily biodegradable where nutrients and electron acceptors are present in sufficient concentrations.

The use of slow-release fertilizers may provide a continuous supply of nutrients, maintaining a sufficient microbial activity that leads to the reduction of bioremediation costs (Riser-Roberts, 1992; Xu et al., 2003). This study used slow-release fertilizers to provide a continuous supply of nutrients as well. The initial nutrient dosage (8 g of N-P-K Miracle-Gro fertilizer) led to encouraging findings (Figure 5-7).

Figure (5-7a)

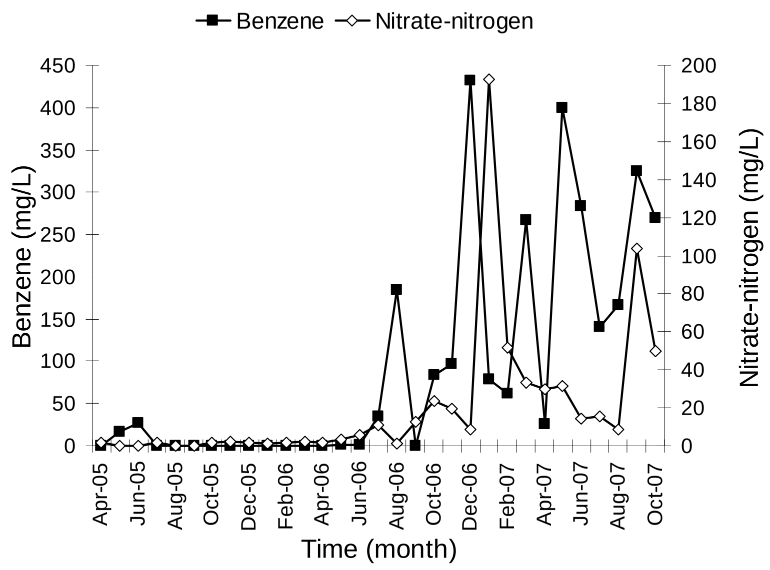


Figure (5-7b)

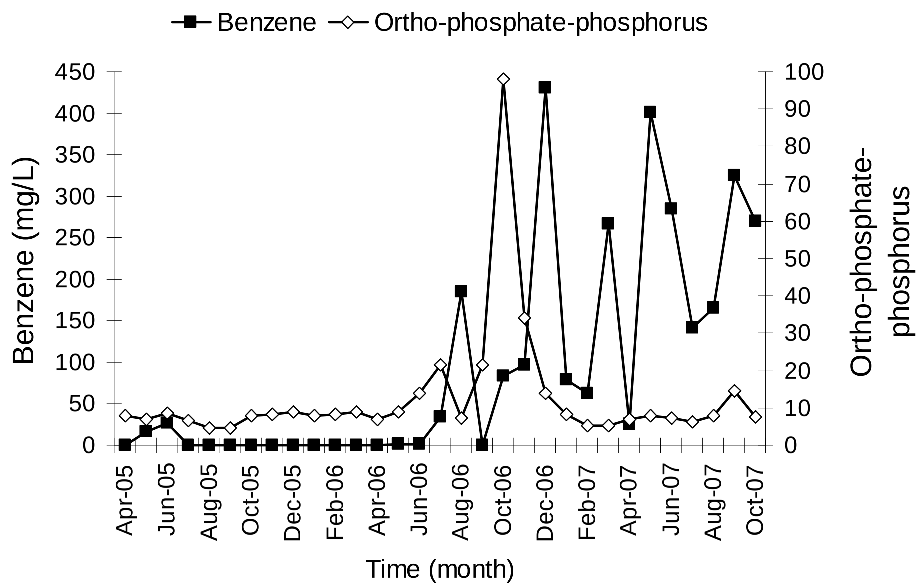


Figure 5-7c

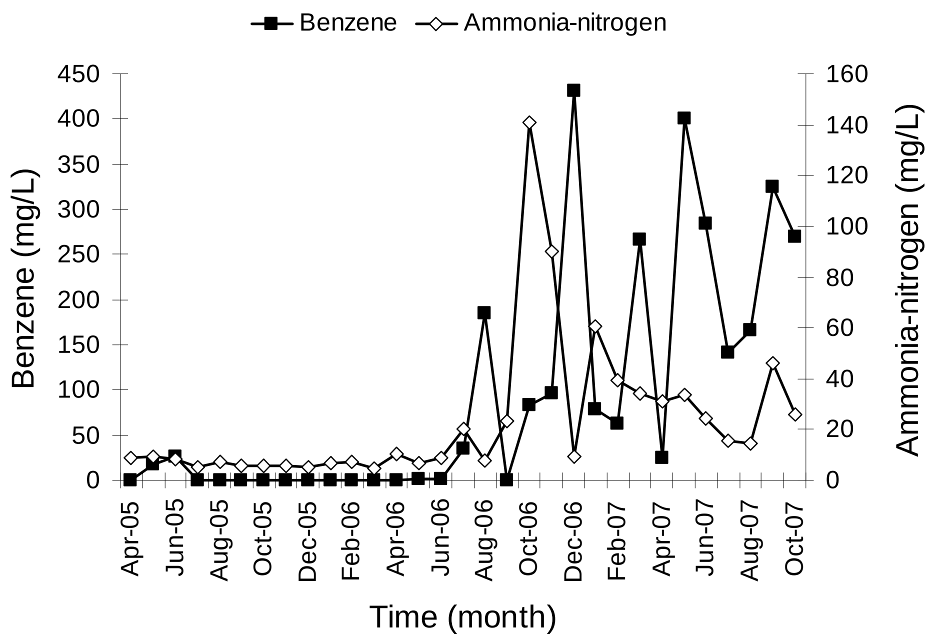


Figure 5-7. Impact of nutrients on benzene removal in vertical-flow constructed wetland filter 1 operated indoors (April 2005 to October 2007): (a) nitrate-nitrogen versus benzene; (b) ortho-phosphate-phosphorus versus benzene; and ammonia-nitrogen versus benzene (c).

During the mid stage of the operation, the nutrients were increased (30 g) in an attempt to determine whether excess fertilization of the constructed wetlands would increase the hydrocarbon treatment efficiency. However, the result (Figure 5-7a, b and c) shows that excess fertilization did not seem to increase the removal of benzene. An excess nutrient virtually seems to be the primary factor hampering the rate of hydrocarbon biodegradation (Figure 5-7a, b and c). This result is in agreement with the published findings which documented that excess nutrient supply would hamper the rate of hydrocarbon removal (Hutchins et al., 1991, Pritchard et al., 1992; Venosa and Zhu, 2003). The concentration was therefore lowered to 15 g, and the results showed subsequently enhanced microbial growth and improved treatment efficiency (Figure 5-7a, b and c).

Of the anaerobic electron-accepting conditions (applicable in this study), denitrifying conditions (i.e., where nitrate is the primary electron acceptor) were clearly the most supportive of anaerobic BTEX degradation and is in agreement with findings from Burland and Edwards (1999). Fertilizer used in this study was observed to have a variable effect on benzene degradation. In some cases, for instance high dosage (30g) fertilizer retarded benzene degradation, but it occasionally enhanced benzene degradation (lower dosage e.g. 8g) depending on the fertilizer dosage used in the constructed wetland (Figure 5-7a, b and c).

The variable effect of fertilizer on benzene degradation as observed in this study proved to be complex but could be attributed to a function of electron-accepting conditions and the microbial community present in the wetlands. This effect was regarded as insignificant on hydrocarbon degradation activity in short term laboratory experiments, but could potentially hinder the anaerobic hydrocarbon degradation in field scale application of the constructed wetlands. There is a need for improved nutrients dosage to the wetland as inappropriate dosage conditions could favour extensive hydrocarbon accumulation in the constructed wetland. In addition, excess nutrient dosage could cause a decrease in pH (thus hindering biodegradation processes). Enhancement of benzene degradation by fertilizer when electron acceptors (such as nitrate from the fertilizer) are supplied in excess may be attributable to the fortuitous growth of benzene-degrading bacteria during benzene degradation. The results from this study suggest that the relationship between microbial community and hydrocarbon degradation activity in constructed wetland can be complex and environment dependant. See chapter 6 for further documentation on relationship of nutrient in hydrocarbon treatment.

Further comparison of Benzene with Nutrient (Nitrate-nitrogen, Ortho-phosphate-phosphorus and Ammonia-nitrogen) by performing the ANOVA show contaminated wetlands (1, 3 indoor and 1, 5 outdoor) to be statistically significant, while wetlands 5 indoor and 3 outdoor were similar statistically for Nitrate-nitrogen. Analyses of Benzene with Ortho-phosphate-phosphorus show similar trend as Nitrate-nitrogen (wetlands 1, 3 indoor and 1, 5 outdoor were statistically significant, while wetlands 5 indoor and 3 outdoor were similar statistically) (Table 5-7).

Table 5-7. Comparison of effluent Benzene versus Nutrient concentrations for contaminated constructed wetlands.

Wetland	P values		
	Benzene vs Nitrate-Nitrogen	Wetland	Benzene vs Nitrate-Nitrogen
1 In	0.000	1 Out	0.000
3 In	0.006	3 Out	0.534
5 In	0.159	5 Out	0.036
Wetland	P values		
	Benzene vs Ortho-phosphate-phosphorus	Wetland	Benzene vs Ortho-phosphate-phosphorus
1 In	0.000	1 Out	0.000
3 In	0.004	3 Out	0.524
5 In	0.525	5 Out	0.028
Wetland	P values		
	Benzene vs Ammonia-Nitrogen	Wetland	Benzene vs Ammonia-Nitrogen
1 In	0.000	1 Out	0.000
3 In	0.040	3 Out	0.949
5 In	0.491	5 Out	0.088

However, analyses of Benzene with Ammonia-nitrogen show wetlands 1, 3 indoor and 1 outdoor were statistically significant, while wetlands 5 indoor and 3, 5 outdoor were similar statistically (Table 5-6).

The above analysis suggests that nutrient (especially Nitrate-nitrogen and Ortho-phosphate-phosphorus) contribute to stimulate hydrocarbon-adapted bacteria which biodegrade benzene in the wetland. Despite the controversy about the role of nutrient in hydrocarbon treatment, this study shows that an adequate level of fertilization increases biodegradation rates, whereas excessive fertilization has a negative effect.

This finding is in good agreement with previous biodegradation research (Chaıˆneau et al., 2005, Hu et al., 2007) showing that nitrate-nitrogen was a favorable electron acceptor for benzene reduction and excessive fertilization has adverse effect. Furthermore, positive influences of nutrients on the biodegradation of saturated and aromatic hydrocarbons extents were also observed by various researchers (Bossert and Bartha 1984, Chaıˆneau et al., 2000, Morgan and Watkinson 1989, Atlas and Bartha 1992). However, further research on inhibitory effect of excess nutrient on hydrocarbon degradation is recommended.

5.5. Long-term hydrocarbon performance

This section documented analysis dedicated to testing the sustainability of the constructed wetlands by assessing the cumulative impact and the long-term performance in treating petroleum hydrocarbons such as benzene, which are associated with considerable human health and environmental concerns. This was approached by evaluating the monthly as well as year-round operations of the high strength benzene contamination in constructed wetlands, and ascertaining the corresponding performance optimization that could be applied to full-scale treatment. The findings indicate very high overall mean removal efficiencies for benzene (89 to 90%), (73 to 81%) for wetlands operated indoors and outdoors respectively as previously reported (Figure 5-1). However, monthly performances as presented in figure 5-8 show that the treatment performances reduced during July of the second year (2006) with increasing hydrocarbon accumulation within the corresponding wetlands. This finding is in contrast with data presented previously (Cooper et al. 1996, Scholz 2006).

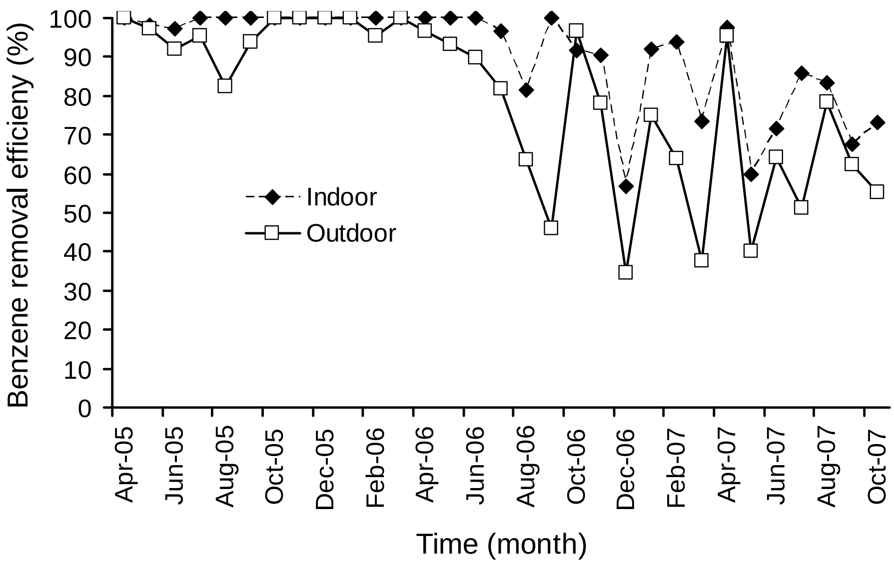


Figure 5-8. Comparison of monthly benzene removal efficiencies for the indoor and outdoor wetlands

Furthermore, yearly performance shows that Benzene was consistently removed with a mean efficiency between 93.41 and 99.63%, and between 90.61 and 97.01 % (Table 5-8) for the indoor and outdoor wetlands, respectively. After the first year of operation, however, a slight deterioration of benzene removal was noted for both the indoor and outdoor wetlands. During the second year of operation (between spring and winter 2006), reductions in terms of removal efficiencies between 2 and 6% and between 11 and 14% (Table 5-8) were noted for the indoor and outdoor wetlands, respectively indicating cumulative impact.

Table 5-8. Year-round benzene removal efficiency (%) of different constructed wetlands

Wetland	Running period		
	Spr-05 ~ Win-05	Spr-06 ~ win-06	Spr-07 ~ Aut-07
1 Indoor	99.63±0.74 [b]	92.15±7.04	76.78±9.44 [a, b]
3 Indoor	94.56±3.91 [a, b]	91.32±9.98	84.99±9.50 [b]
5 Indoor	93.41±7.94 [a, b]	93.22±8.43	81.92±16.06 [a, b]
1 Outdoor	96.13±4.07 [a, b]	76.58±19.59	62.48±9.46 [a]
3 Outdoor	97.03±2.62 [a, b]	82.15±20.83	51.82±10.47 [a]
5 Outdoor	90.61±5.57 [a]	86.62±13.15	68.94±15.64 [a, b]

In any one column, values marked with different letters are significantly different from each other at $p \leq 0.05$ according to the Duncan's multiple range tests.

Benzene removal performances continuously worsen with the extension of the experimental period. Table 5-8 showed that the benzene removal efficiency was between 77 and 85%, and between 51.8 and 68.9% for the indoor and outdoor wetlands between spring 2007 and autumn 2007. After the second year of operation, approximately between 9 and 15%, and between 18 and 25% of the reductions were detected for the indoor and outdoor wetlands. Compared to the indoor wetlands, the decrease of the benzene removal efficiencies for the outdoor wetlands was significantly ($p<0.05$) faster during the whole running period. Benzene showed periodically high removal efficiencies between spring 2005 and autumn 2006. After this period, a decrease of the benzene removal efficiency was observed, especially for outdoor wetland 3 as benzene accumulation was noted. Paper documented in appendix A observed that the hydrocarbon treatment performances reduced during winter. Furthermore, Mann and Bavor (1993) reported that phosphorus removal efficiency for gravel-based systems declined after only one to two years of operation. However, the results suggest that Benzene removal efficiency decreased with increasing hydrocarbon accumulation during summer and autumn 2007.

The result of yearly mean benzene treatment efficiencies for wetlands (wetlands 1, 3 and 5) operated indoor show better removal efficiency for year 1 in filter 1 (planted with filter media) followed by year 2 and 2^+ respectively. Filter 3 (filter media but unplanted) show similar performances in all years while filter 5 (unplanted and no filter media) indicates lower performance in year 1 but similar performances in years 2 and 2^+ (Figure 5-9).

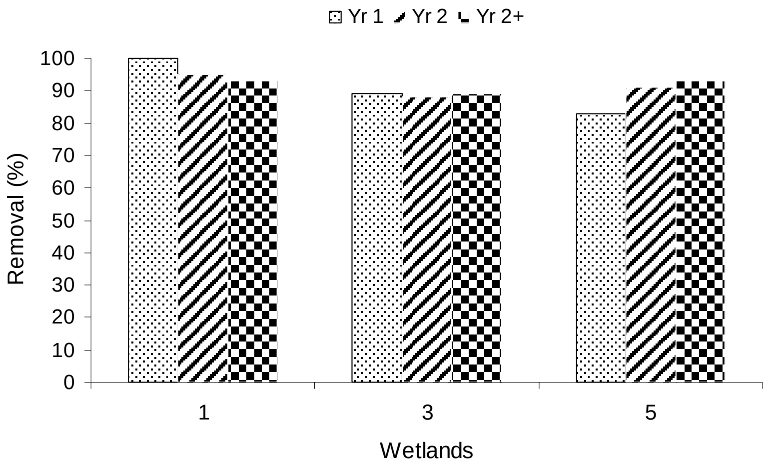


Figure 5-9. Yearly mean benzene treatment efficiencies (%) from 2005-2007

The yearly ANOVA analyses of Benzene effluent indicate that the first year operations were statistical significantly different ($p \leq 0.05$) from second year for wetland filter 1 operated indoor, wetlands 1 and 2 operated outdoor. In comparison, filter 5 operated outdoor, wetlands 3 and 5 operated indoor were statistically similar ($p \geq 0.05$) (Table 5-9).

Table 5-9. Comparison of effluent Benzene concentrations for constructed wetlands operated in year 1 and year 2.

Wetland	P values		
	Year 1 vs Year 2	Wetland	Year 1 vs Year 2
1 In	0.004	1 Out	0.001
3 In	0.077	2 Out	0.003
5 In	0.238	5 Out	0.085

Furthermore, annual variation analysis of the constructed wetland system in removal of Benzene showed that there was a trend of increasing removal efficiency from 2005 to 2006. The overall trend show optimal performance in all the systems and could be theoretically said to attain the ‘steady state’ this period. This may be due to self-adjustment of the constructed wetland as an ecological system during this treatment period (April 2005- June 2006) (figure 5-8). This period of operation could be linked with a well-established microbial population and vegetation, which might improve efficiency. In contrast, the removal efficiency did change as they began to decrease from August 2006 onwards, which could be attributed to cumulative impact of hydrocarbon. Similar analyses for other water quality variables were presented in chapter 6.

The reported decrease of benzene treatment efficiency from the second year operation could be addressed to improve potential engineering application of the results derived from this study. For example, the use of a multi-stage or integrated wetland system for benzene treatment, from engineering point of view could address the decrease in treatment efficiency observed based on the use of a single constructed wetland.

5.5.1. Change of filter volume

The volume of the constructed wetlands was monitored at interval as part of assessment of the rigs’ long-term performance. The inflow water volumes were measured three times by draining the wetlands entirely during the operation period (Table 5-10). The filter volumes of some wetlands decreased during the operation time but wetlands 5 and 6 remain constant since there were blank. The accumulated sediment including macrophytes litter, detritus containing mature microbial biomass and solids reduces the filter volume. However, the decrease in volume of some wetlands was negligible and not associated with observed hydrocarbon treatment efficiency decline.

Table 5-10. Change of filter volume

Date	April-05	June-06		March-08	
Filter Number	Volume (l)	Volume (l)	Reduction (%)	Volume (l)	Reduction (%)
Indoor					
1	4.1	3.5	14.6	3.1	24.4
2	4.1	3.6	12.2	2.7	34.1
3	4.2	3.6	14.3	3.0	28.6
4	4.2	3.5	16.7	3.1	26.2
5	6.3	6.3	0.0	6.3	0.0
6	6.3	6.3	0.0	6.3	0.0
Outdoor					
1	4.1	3.5	14.6	2.7	34.1
2	4.1	3.5	14.6	2.1	48.8
3	4.2	3.6	14.3	3.2	23.8
4	4.2	3.6	14.3	2.2	47.6
5	6.3	6.3	0.0	6.3	0.0
6	6.3	6.3	0.0	6.3	0.0

5.6. Summary

This chapter has demonstrated the findings which suggest that intermittently flooded vertical-flow constructed wetlands treat aromatic hydrocarbon effectively in the presence of sufficient oxygen and fertilizer, which provides nitrate used as an alternative electron acceptor during anaerobic periods of full inundation. The relatively overall high treatment performance observed indoors is influenced by stable and usually relatively high temperatures, particularly after the temperature was fully controlled.

As benzene and its degradation products started to accumulate in the wetlands, removal efficiencies subsequently reduced. Findings show also that benzene removal was highest in wetlands with filter media (aggregates) and biomass providing habitat for hydrocarbon-degrading microbes. However, further studies on estimating the microbial biomass are encouraged.

Metabolic processes of microorganisms are likely to play an important role in removing hydrocarbon compounds in both controlled and semi-natural wetlands. The results show also that *Phragmites australis* (Cav.) Trin. ex Steud. does not play a significant role (despite providing additional oxygen via its rhizomes) in removing benzene, unless sufficient nutrients (including fertilizer) are available.

The study suggests that adequate level of nutrient increases biodegradation rates by contributing to stimulate hydrocarbon-adapted bacteria which biodegrade benzene in the wetland, whereas excessive fertilization has a negative effect. Further research on inhibitory effect of excess nutrient on hydrocarbon degradation is recommended.

Findings indicate also that both biodegradation and volatilization are major removal mechanisms that support the treatment. Volatilization is the dominant mechanism for benzene removal after one day of retention time. These processes suggest that many common wetland interactions probably do entail cumulative impact. However, optimizing environmental conditions such as locating wetlands in areas with relatively high temperatures enhances the biodegradation rate. Further research is required on the specification of biodegradation products and quantification of the proportion of hydrocarbons being lost through volatilization to the atmosphere under varying environmental conditions and the specification of aerobic and anaerobic biodegradation products, adsorption, absorption, mineralization and other removal mechanisms in large-scale constructed treatment wetlands.

The results also suggest that benzene treatment did not always respond to temperature change and nutrient enrichment unless distinct environment or seasonal changes required in conjunction with hydraulic retention time, dissolved oxygen, pH and nutrient enrichment to stimulate microorganisms to biodegrade hydrocarbon.

Though the information already provided by this research has already shed light on long-term impacts. The changes in wetland processes that take place on the scale of years, decades, and longer are not adequately understood. Field studies of hydrocarbon removal over very long periods are needed to examine the long-term effects of wetland impacts. Furthermore, the causes of potential treatment efficiency decline and the effects of cumulative impact of hydrocarbon removal during long-term experimental and field-scale operations needs to be assessed.

6

Seasonal variability and monthly performances of hydrocarbon and water quality variables♣

6.1. Overview

One of the largest uncertainties in constructed treatment wetlands management observed during this research remains seasonal and interannual variations. Characterizing wetlands and their process dynamics is extremely difficult because of constant changes that are directly linked with environments. The basic concern is complexity of both the process dynamics within the wetlands and their corresponding interactions with the surrounding environments. Particularly in a cumulative impact context, it is necessary to understand the interaction of water quality processes that occur in a wetland ecosystem. This chapter presented the results of the detailed wetland studies dedicated to seasonal interactions in an attempt to identify conditions that have relevance for the sustainable functioning of constructed wetlands. This study explored integrated approach by analyzing monthly quality of contaminants treated as well as seasonal variability impact in the constructed wetlands applied for hydrocarbon treatment. The chapter documented the results of investigation into the relationship

♣ An earlier version of this chapter was submitted for publication as:
Xianqiang Tang, Paul Emeka Eke, Miklas Scholz and Suiliang Huang (2008), Sustainable management of the seasonal variability in benzene removal by planted vertical-flow constructed wetlands to prevent pollution. *Journal of Environmental Management* (submitted) (original copy documented in appendix A).

between various variables and hydrocarbon removal in constructed wetlands by assessing the roles played by seasonal changes. This chapter also supports the results that have been documented earlier in chapters 4 and 5. The sustainable management of the seasonal variability in benzene removal by planted vertical-flow constructed wetlands to prevent pollution is therefore the aim of this chapter. In light of the above considerations, a two and half year's investigation was conducted with the following objectives:

- To assess the monthly, annual and seasonal variability in benzene removal by vertical-flow constructed wetlands located indoors and outdoors;
- To qualitatively study the seasonal variability of other effluent variables including ammonia-nitrogen, nitrate-nitrogen, ortho-phosphorus-phosphate, temperature, pH, DO and redox;
- To perform a regression analysis to quantitatively assess the above relationships; and
- To determine the relationships between seasonal benzene removal and the above mentioned effluent variables.

The results of seasonal variability in benzene removal by vertical-flow constructed wetlands and the interactions with other effluent variables including ammonia-nitrogen, nitrate-nitrogen, ortho-phosphorus-phosphate, temperature, pH, dissolved oxygen and redox potential were presented in detailed. The chapter presented the chapter overview in this section (6.1). Section 6.2 documented unit treatment performances such as indoor and outdoor monthly treatment performances, while 6.2.1 compares indoor and outdoor monthly treatment performances. Section 6.3 presents seasonal treatment performances, documented indoor and outdoor seasonal treatment performances and 6.3.1 presents comparison of indoor and outdoor seasonal treatment performances. 6.4 documented seasonal variability in benzene removal and seasonal impacting factors. 6.5 summarized the chapter.

6.2. Monthly treatment performance

Average monthly performance data of over thirty months of wetland operation were analysed in this section. The study monitored changes over time to ensure that optimum treatment performance is maintained. The results show treatment timescales evaluated in an attempt understand major water quality roles and relationship in the system. Figure 6-1 presented mean monthly COD effluent for wetlands operated both indoor (6-1a) and outdoor (6-1b).

Figure 6-1a

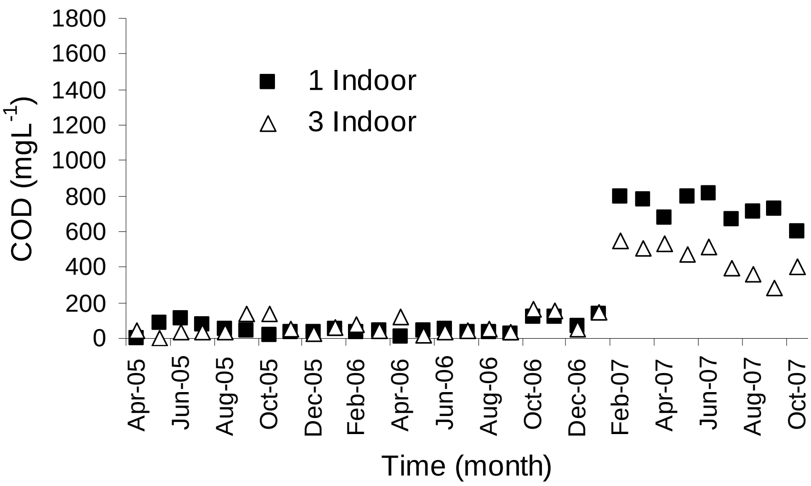


Figure 6-1b

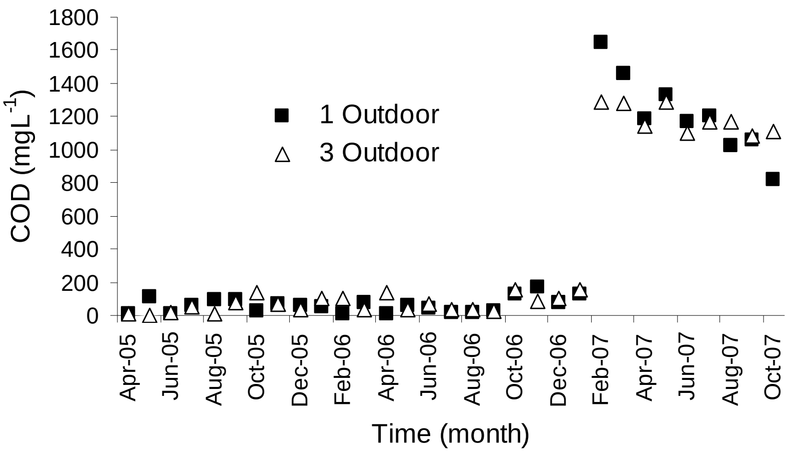


Figure 6-1. Mean monthly COD (a) indoor and (b) outdoor effluents

Figure 6-1 trend showed high COD removal from April 2005 to January 2007 for rigs operated both indoor and outdoor. This trend may be due to a well-established microbial population, vegetation and favourable operating condition that improved the removal efficiency. However, this trend changed as the COD removal efficiency began to decrease from February 2007 onwards in both rigs with slightly better performance in the indoor rig. The observed change could be attributed to accumulation of contaminants in the systems.

Figure 6-2 reported average monthly BOD_5 effluents for wetlands operated both indoor (figure 6-2a) and outdoor (figure 6-2b). The trend in figure 6-2 showed that there BOD removal efficiency was unsteady throughout the period for both indoor and outdoor operated rigs.

Figure 6-2a

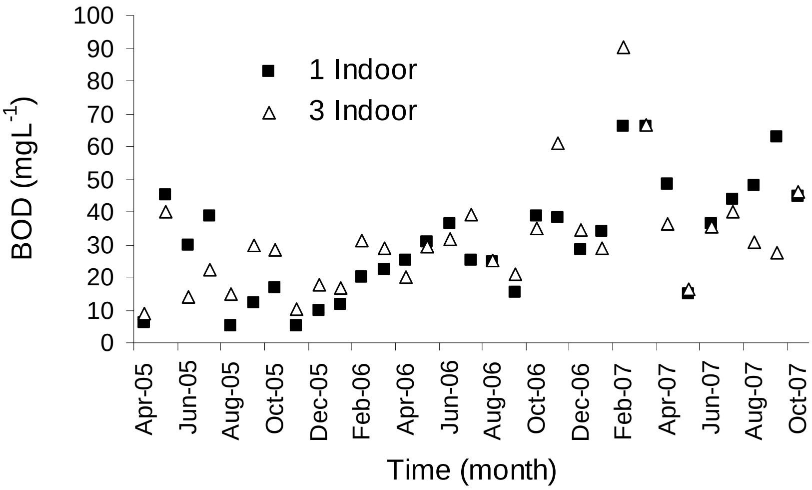


Figure 6-2b

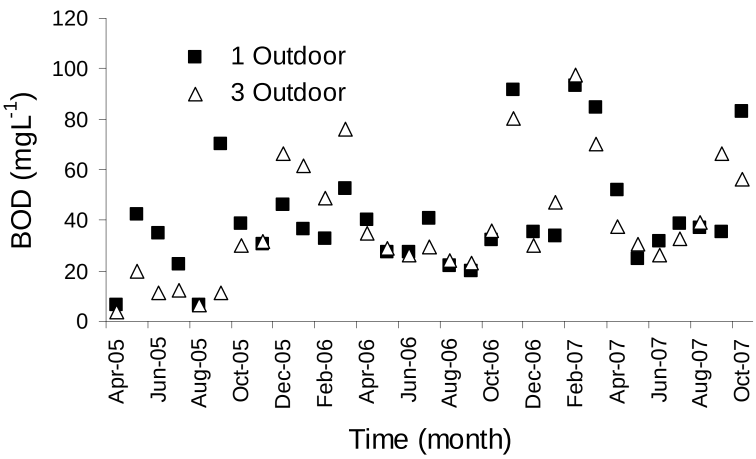


Figure 6-2. Mean monthly BOD_5 (a) indoor and (b) outdoor effluents

Monthly trends of DO as presented in figure 6-3 showed direct relationship with the nutrient. DO increase as nutrient dosage was increased to 30 grams and reduced as the dosage was decreased to 15 grams. This is an indication that when there were excessive nutrient in the wetlands little DO was involved.

The trend also shows DO effluent concentrations decrease as the indoor temperature increases (Figure 6-4). The effluent DO concentrations were slightly higher in outdoor wetlands than in indoor wetlands. DO effluent concentrations for both rigs began to decline after august 2007.

Figure 6-3a

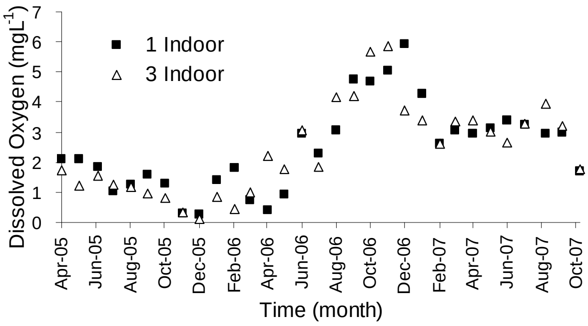


Figure 6-3b

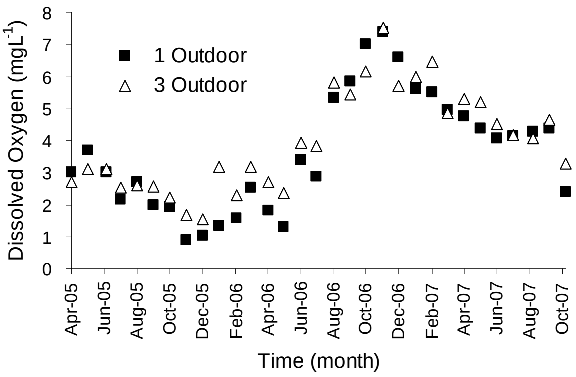


Figure 6-3. Mean monthly (a) indoor and (b) outdoor DO effluents

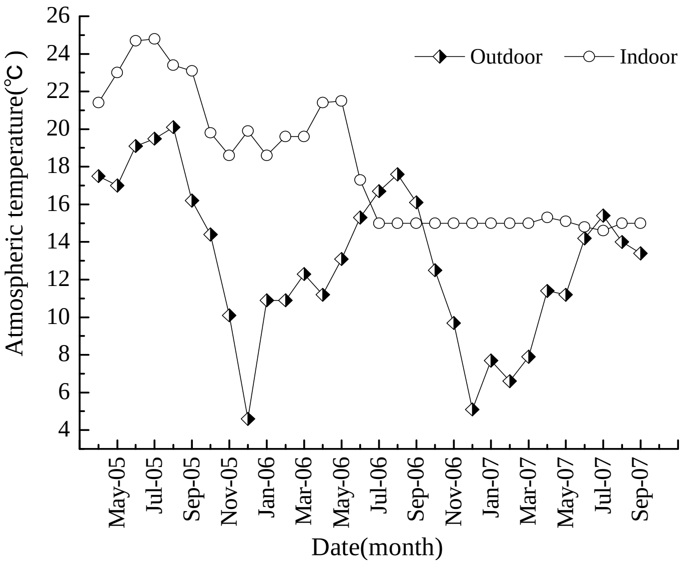


Figure 6-4. Mean monthly temperature

Monthly trends of all water quality variables presented in figure 6-5 except pH (figure 6-5a) showed similar trends. Conductivity, Redox and Turbidity effluent concentrations show unsteady removal trends with better indoor performance for Turbidity (Figure 6-5b-d). Conductivity effluent concentrations were better in the rig operated outdoors during first four months but wetlands operated indoors began to perform better after this period. Redox showed better outdoor performance throughout the period of operation. However, pH shows similar performances in wetlands operated both indoor and outdoor. Monthly pH remains high between 6 and 7.3 mgL^{-1} throughout the operation period (Figure 6-5a).

Figure 6-5a

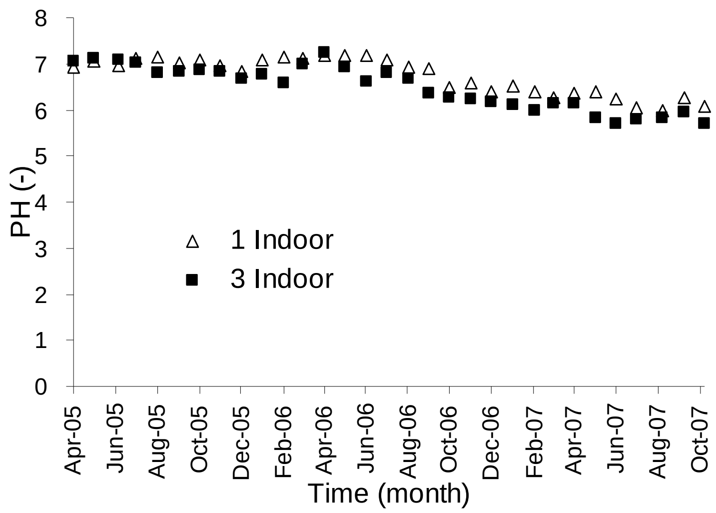


Figure 6-5b

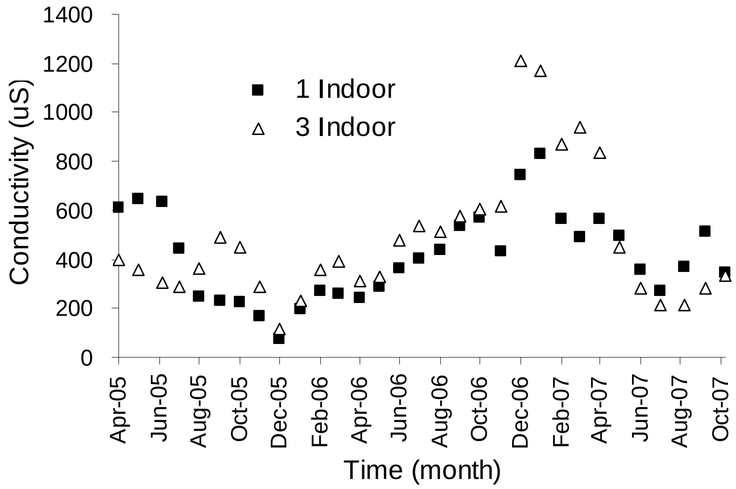


Figure 6-5c

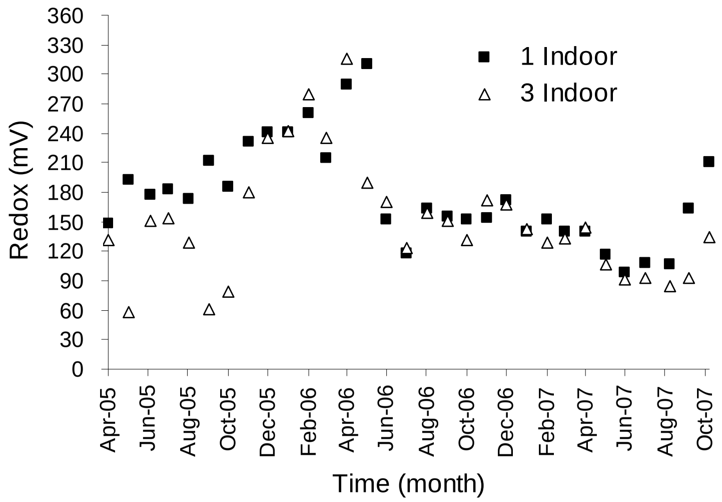


Figure 6-5d

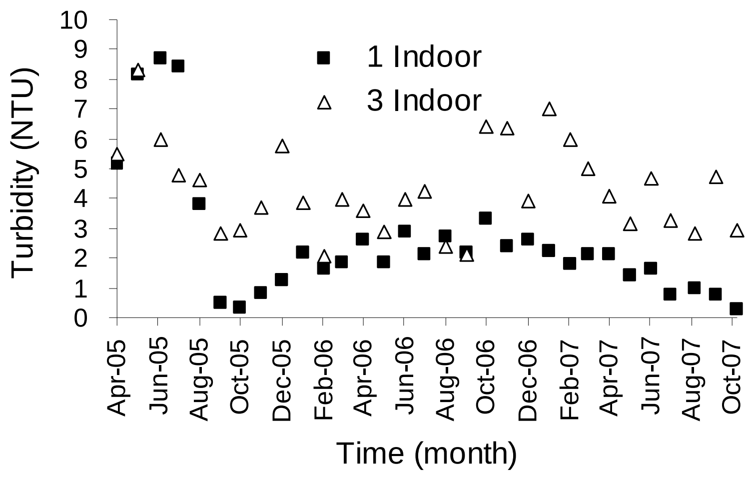


Figure 6-5. Mean monthly (a) pH, (b) Conductivity, (c) Redox and (d) Turbidity effluents

Comparison of monthly temperature with pH effluent concentrations as showed in figure 6-6a indicate stable pH trend. The temperature relationship with pH is directly proportional. pH reduced slightly when temperature was reduced and kept constant at 15^{o}C. Despite this trend, influence of temperature seems very weak because there were no significant variations of the pH effluent concentrations in the wetlands.

However, figure 6-6b show DO effluent concentrations were low and unstable at higher temperature but became DO effluents became high when temperature was reduced to 15^{o}C and kept constant.

Figure 6-6a

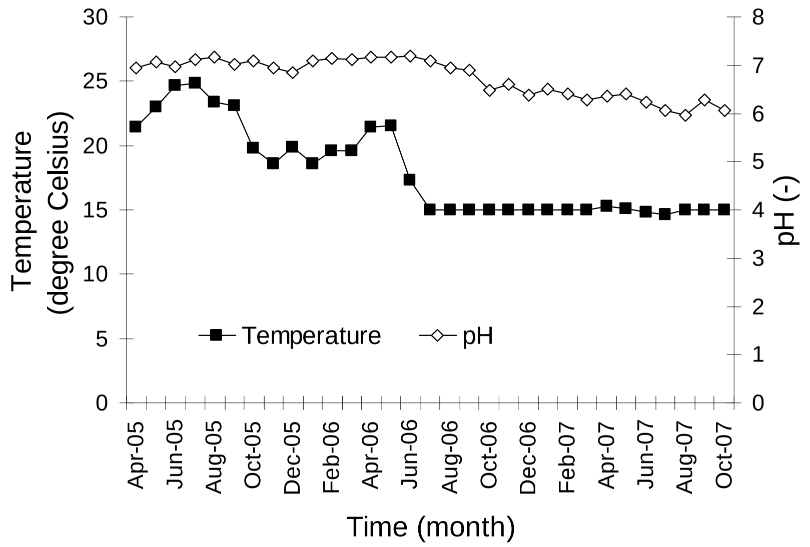


Figure 6-6b

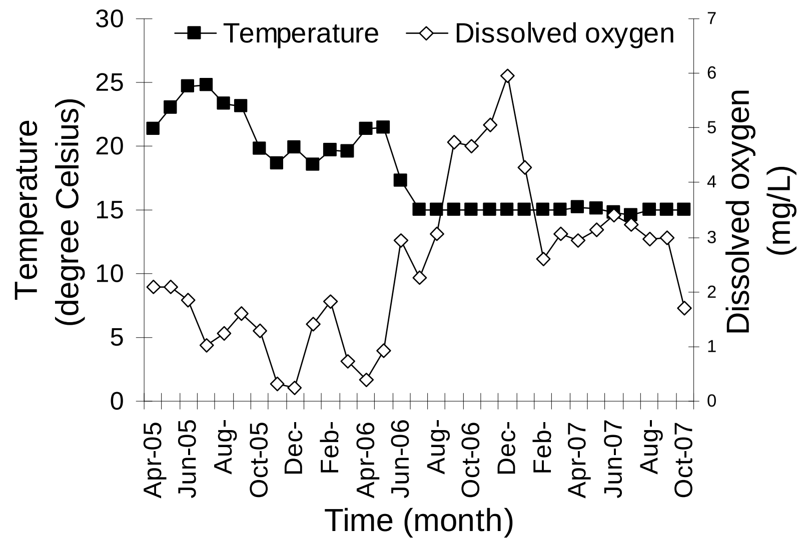


Figure 6-6. Comparison of monthly temperature with (a) pH and (b) dissolved oxygen effluents

6.2.1. Comparison of monthly indoor and outdoor treatment performance

Figure 6-7 presents high removal of COD with no significant difference in the effluent concentration trends until February 2007 when treatment efficiency decreased sharply in both rigs (Figure 6-7a). However, Figure 6-7b showed BOD_5 effluent concentrations to be unsteady with wetlands operated indoor performing better.

Figure 6-7a

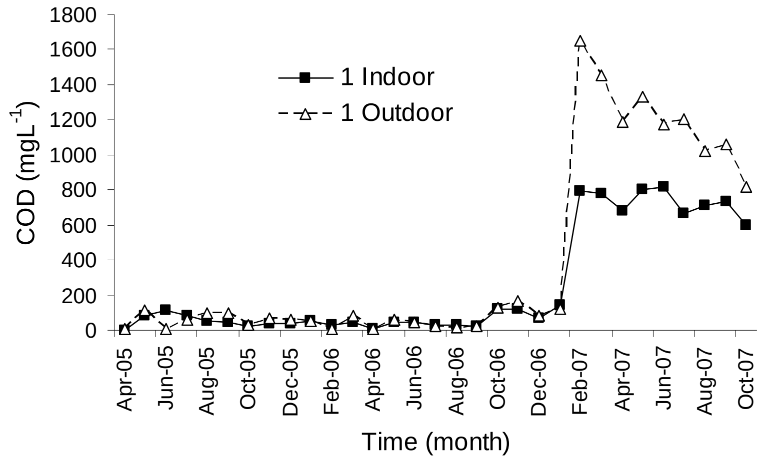


Figure 6-7b

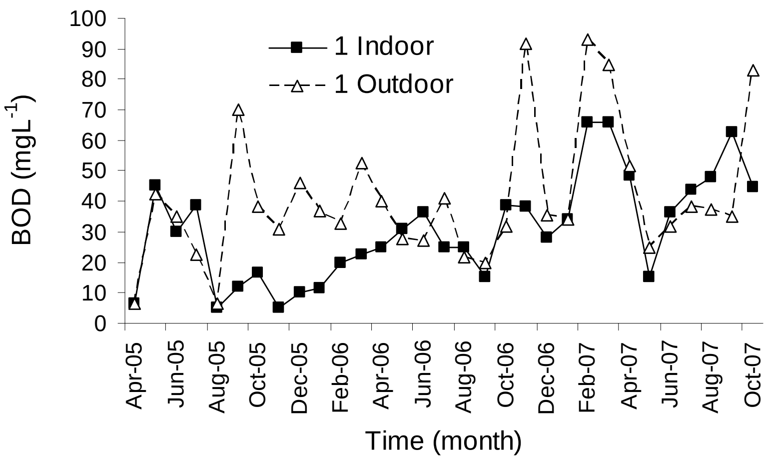


Figure 6-7. Comparison of monthly indoor and outdoor treatment performances for (a) COD and (b) BOD

Figures 6-8 showed similar removal efficiency for the entire nutrient effluents ((a) orth-phosphate-phosphorus, (b) Nitrate-Nitrogen and (c) Ammonia-Nitrogen). The trend show high performance from April 2005 to July 2006 when the nutrient dosage was 8 grams but after a step increase (30 grams) in nutrient loading, treatment efficiency decreased sharply but returned as nutrient was reduced to 15 grams.

These trends were similar for both rigs operated indoor and outdoor.

Figure 6-8a

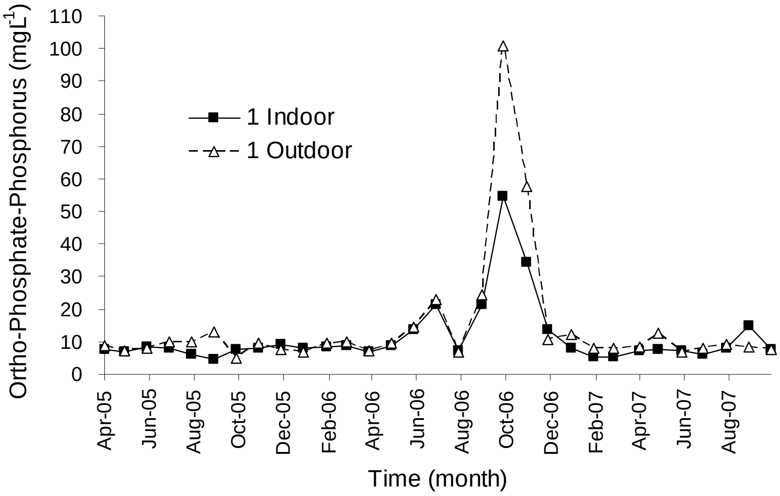


Figure 6-8b

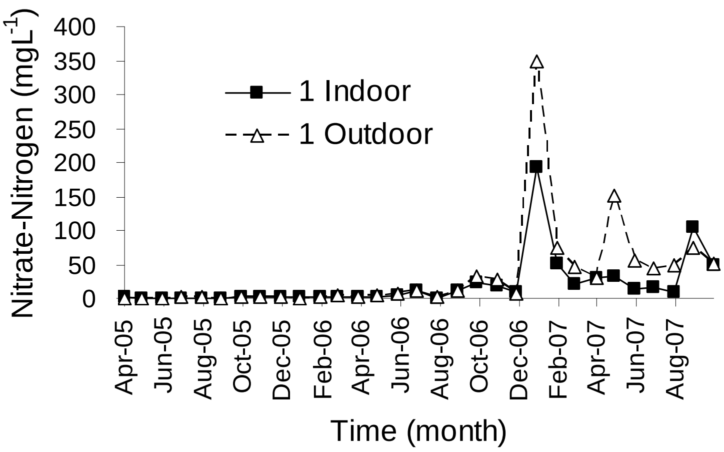


Figure 6-8c

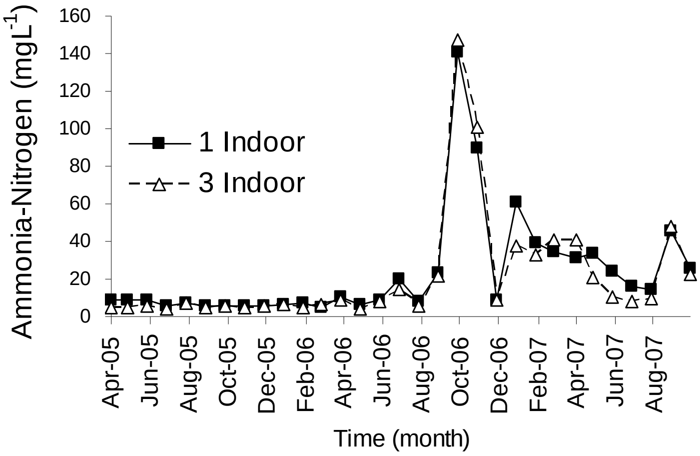


Figure 6-8. Comparison of monthly indoor and outdoor treatment performances for (a) orth-phosphate-phosphorus, (b) Nitrate-Nitrogen and (c) Ammonia-Nitrogen

Comparison of monthly indoor and outdoor treatment performances for (a) DO, (b) pH, (c) Conductivity, (d) Redox and (e) Turbidity effluent concentrations in figure 6-9 showed similar unstable slightly better indoor removal trends except pH. However, pH shows similar performances in wetlands operated both indoor and outdoor. pH monthly effluent concentrations remain high between 6 and 7.3 mgL^{-1} throughout the operation period (Figure 6-9).

Figure 6-9a

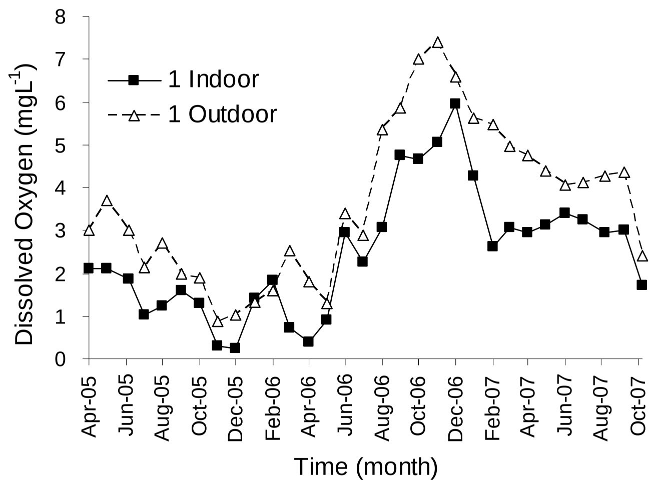


Figure 6-9b

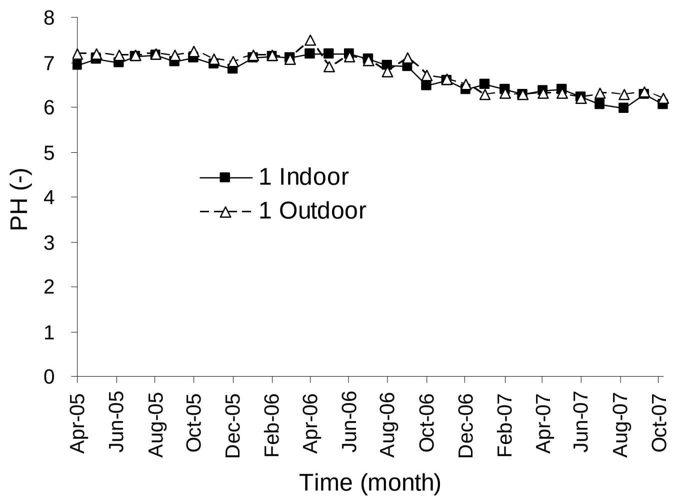


Figure 6-9c

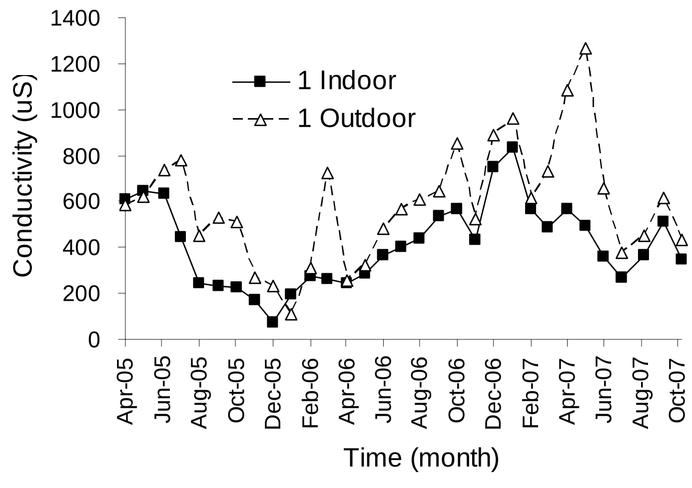


Figure 6-9d

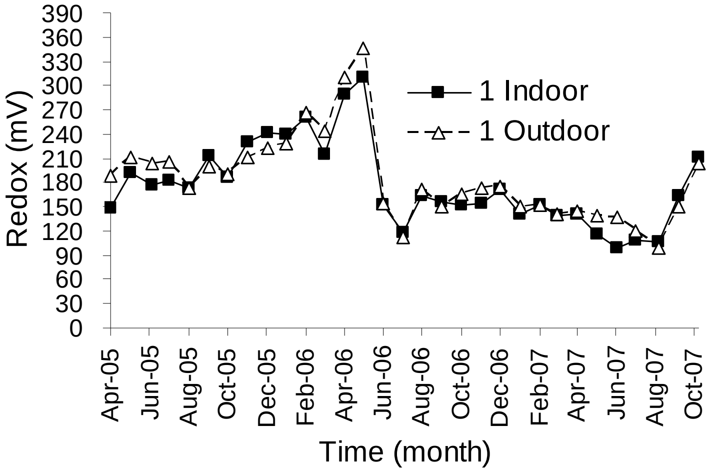


Figure 6-9e

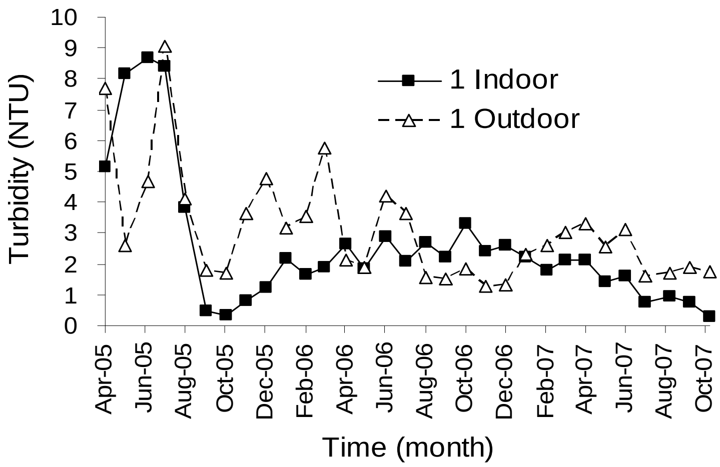


Figure 6-9. Comparison of monthly indoor and outdoor treatment performances for (a) DO, (b) pH, (c) Conductivity, (d) Redox and (e) Turbidity

6.3. Seasonal treatment performance

The impact of seasonal variations was negligible in the wetlands operated indoors and containing hydrocarbon. Similar trend occurred in most of the variables due to environmental control that kept temperature and humidity constant. However, overall seasonal treatment efficiencies and effluent concentrations for the wetland rig operated outdoor and indoor were presented in Tables 6-1, A (Appendix) and figures 6-10.

Table 6-1. Seasonal hydrocarbon removal efficiencies

Season	F1i	F1o	F3i	F3o	F5i	F5o
Overall	89	76	90	73	90	81
Spring 2005	99	96	91	96	90	88
Summer 2005	100	91	92	94	84	88
Autumn 2005	100	100	99	100	100	99
Winter 2005/6	100	98	97	98	100	88
Spring 2006	100	97	100	100	100	98
Summer 2006	94	70	98	91	100	89
Autumn 2006	91	87	90	85	90	92
Winter 2006/7	83	52	78	52	83	68
Spring 2007	79	70	86	40	88	59
Summer 2007	85	66	94	59	94	87
Autumn 2007	66	52	75	56	64	60

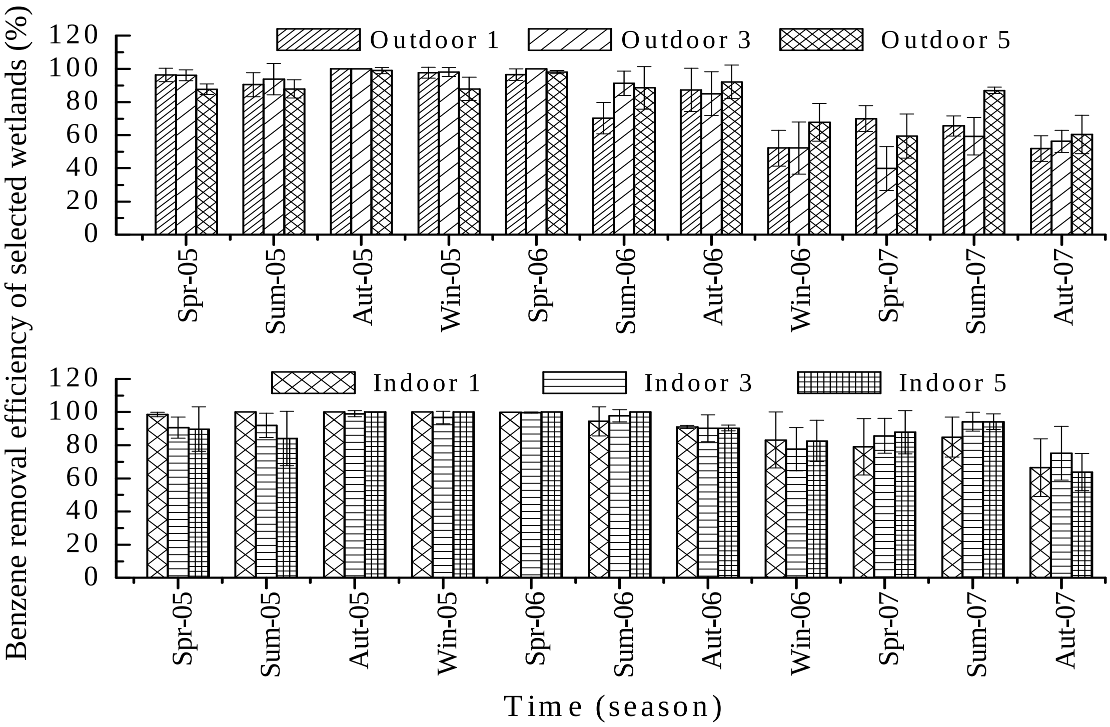


Figure 6-10. Comparison of seasonal benzene removal performance for the indoor and outdoor rigs.

Seasonal variations have been reported by several investigators, with the worst performance occurring during the winter (Kuehn et al., 1995; Leonard, 2000;

Karathanasis et al., 2003).In contrast, the results show less negligible seasonal impact on the treatment performances (Tables 6-1). The benzene treatment efficiencies (Table 6-1) and other water quality effluent concentrations reduced considerably during the winter of the second year (2006/7) in some wetlands operated outdoors which is likely to be due to increasing hydrocarbon accumulation within the corresponding wetlands (Tables 6-1, 6-2 and figures 6-10).

Table 6- 2. presented the seasonal mean effluent water variables (DO, pH, conductivity, Redox, Turbidity, BOD_5, COD, PO_4, NO_3-N and NH_4-N) for the entire study period (2005–2007).

Table 6-2. Mean seasonal water quality variations for the indoor and outdoor wetlands (08/04/05-18/10/07)

Dissolved oxygen (mgL^{-1})

Season	Indoor wetlands						Outdoor wetlands					
	1	2	3	4	5	6	1	2	3	4	5	6
Spring 05	1.7	2.1	1.3	2.5	3.5	4.5	3.1	2.7	3.0	3.4	4.4	4.5
Summer 05	1.2	1.5	1.1	1.5	2.1	2.1	2.3	2.2	2.2	2.8	3.7	3.2
Autumn 05	0.2	0.4	0.0	0.5	2.2	2.3	0.9	1.0	1.4	1.2	3.2	3.4
Winter 05/06	0.0	0.7	0.1	1.5	2.9	3.3	3.5	2.5	3.7	3.2	2.4	3.8
Spring 06	3.4	2.6	2.8	3.0	2.8	2.6	3.8	3.4	3.6	3.8	4.8	5.2
Summer 06	5.2	4.6	4.2	5.1	5.8	6.2	5.8	6.1	5.0	4.4	6.0	5.0
Autumn 06	4.4	4.3	3.9	4.4	4.6	8.7	6.4	7.5	8.0	8.5	9.6	9.0
Winter 06/07	3.2	3.0	3.8	4.2	2.0	4.8	5.2	5.4	4.8	6.0	8.2	7.8
Spring 07	3.3	3.7	2.7	4.2	2.6	5.3	3.4	4.4	3.8	5.2	7.4	8.0
Summer 07	3.3	3.2	2.7	3.4	2.8	4.0	4.9	7.5	5.8	5.5	4.5	5.7
Autumn 07	1.4	1.8	1.9	3.0	0.9	4.4	2.8	5.4	3.6	6.1	9.4	11.4

pH (-)

Season	Indoor wetlands						Outdoor wetlands					
	1	2	3	4	5	6	1	2	3	4	5	6
Spring 05	7.05	6.78	7.10	7.00	7.18	7.24	7.20	6.97	7.12	7.32	7.42	7.80
Summer 05	7.10	6.37	6.76	6.51	7.02	6.95	7.05	6.47	7.20	6.67	7.38	7.26
Autumn 05	6.78	6.42	6.69	6.80	7.42	7.28	7.10	7.00	7.22	6.84	7.20	7.19
Winter 05/06	7.10	6.00	7.20	5.81	6.90	6.49	6.90	6.89	7.32	7.01	6.90	7.32
Spring 06	7.20	6.60	6.24	6.82	6.22	6.24	7.30	6.92	6.86	6.10	6.00	6.18
Summer 06	6.76	6.42	6.19	6.24	6.09	6.20	7.21	6.40	6.72	6.50	6.18	6.10
Autumn 06	6.46	5.32	6.08	4.22	6.21	6.39	7.00	4.07	6.45	5.53	6.47	6.50
Winter 06/07	6.34	5.62	6.24	4.64	6.04	5.76	6.40	5.64	6.04	5.02	6.42	5.96
Spring 07	5.98	5.38	5.44	4.08	5.70	5.78	6.10	4.48	6.06	5.01	6.16	6.24
Summer 07	6.33	5.44	6.05	5.17	5.89	5.55	6.30	4.92	6.39	5.66	6.66	8.20
Autumn 07	5.92	5.31	5.80	4.62	6.08	6.00	6.33	5.37	6.46	5.62	7.30	8.21

Table 6-2 contd.

Conductivity (µS)

Season	Indoor wetlands						Outdoor wetlands					
	1	2	3	4	5	6	1	2	3	4	5	6
Spring 05	642	814	309	357	238	148	780	682	437	286	112	148
Summer 05	226	106	384	438	507	1161	582	775	418	500	166	382
Autumn 05	68	47	105	58	40	55	232	350	316	131	306	158
Winter 05/06	276	517	404	762	726	989	902	777	323	626	140	243
Spring 06	420	480	420	382	684	418	427	482	620	410	725	362
Summer 06	520	424	680	380	682	620	820	624	430	520	446	420
Autumn 06	906	999	1408	1498	1478	773	1849	1936	751	1010	625	536
Winter 06/07	492	802	864	968	420	364	624	830	806	542	420	534
Spring 07	347	482	263	429	161	217	654	906	344	452	261	261
Summer 07	449	423	279	249	168	198	647	371	364	468	235	163
Autumn 07	440	362	338	433	244	236	440	408	256	404	148	89

Redox (mV)

Season	Indoor wetlands						Outdoor wetlands					
	1	2	3	4	5	6	1	2	3	4	5	6
Spring 05	180	161	164	197	219	206	198	226	194	238	219	231
Summer 05	150	109	57	185	186	178	88	121	84	120	103	126
Autumn 05	218	238	226	242	234	254	218	228	218	230	228	218
Winter 05/06	219	231	211	253	244	247	222	206	201	242	201	231
Spring 06	150	170	172	165	178	170	162	170	158	160	184	158
Summer 06	160	184	117	142	130	119	118	145	170	160	152	148
Autumn 06	195	181	186	210	159	157	174	240	179	200	180	179
Winter 06/07	132	168	129	158	132	108	142	168	130	152	124	112
Spring 07	93	99	89	92	96	144	134	150	144	131	96	98
Summer 07	171	147	30	168	161	198	137	206	179	175	155	119
Autumn 07	175	195	188	271	153	220	225	254	177	182	239	217

Turbidity (NTU)

Season	Indoor wetlands						Outdoor wetlands					
	1	2	3	4	5	6	1	2	3	4	5	6
Spring 05	9.06	3.42	5.40	2.80	1.40	0.58	5.32	2.64	2.32	3.02	0.69	1.06
Summer 05	0.60	3.23	3.80	2.64	0.39	0.28	2.81	2.65	1.61	0.39	0.10	0.40
Autumn 05	1.24	1.20	4.80	1.21	0.23	2.10	4.64	5.86	4.10	7.20	1.06	1.79
Winter 05/06	1.78	1.42	4.22	1.44	0.41	1.00	7.10	3.00	3.14	7.50	0.50	0.40
Spring 06	3.00	2.28	3.88	1.32	1.65	1.18	3.49	2.23	2.75	1.42	1.82	1.78
Summer 06	2.02	2.00	1.92	1.60	4.02	1.31	1.08	1.20	1.32	1.40	2.40	1.04
Autumn 06	2.06	1.47	4.23	0.34	1.15	0.49	0.99	0.52	0.75	0.53	0.54	0.88
Winter 06/07	3.04	2.00	5.06	0.72	3.64	0.81	3.50	2.10	1.92	1.64	0.82	0.94
Spring 07	1.19	1.13	5.36	0.16	3.93	0.21	3.18	2.81	1.44	0.34	0.37	0.36
Summer 07	0.75	1.62	7.70	3.73	5.35	0.30	1.37	1.95	2.82	0.78	0.59	0.39
Autumn 07	0.24	0.24	2.17	0.13	1.62	0.11	1.82	0.28	0.33	0.25	0.17	1.38

Table 6-2 contd.

BOD (mgL^{-1})

Season	Indoor wetlands 1	2	3	4	5	6	Outdoor wetlands 1	2	3	4	5	6
Spring 05	28	6	21	5	8	8	25	8	11	8	10	5
Summer 05	20	8	24	9	16	10	37	12	11	11	17	8
Autumn 05	10	13	18	5	8	4	46	15	40	10	16	7
Winter 05/06	17	17	26	6	13	5	35	17	59	9	35	10
Spring 06	34	19	29	4	35	4	36	7	37	7	41	8
Summer 06	23	4	29	4	38	3	28	5	25	3	34	5
Autumn 06	38	2	45	3	46	2	58	2	52	2	42	4
Winter 06/07	54	1	61	1	80	3	72	0	74	1	57	1
Spring 07	37	2	34	1	44	1	41	1	37	3	32	1
Summer 07	44	3	36	2	22	2	35	3	34	3	41	3
Autumn 07	53	1	42	2	61	1	75	14	63	1	49	1

COD (mgL^{-1})

Season	Indoor wetlands 1	2	3	4	5	6	Outdoor wetlands 1	2	3	4	5	6
Spring 05	97	48	18	39	16	22	31	73	11	33	56	30
Summer 05	60	28	57	22	77	24	88	37	39	32	43	22
Autumn 05	30	41	86	33	49	28	45	65	86	37	104	28
Winter 05/06	43	51	66	57	28	52	45	57	89	70	35	67
Spring 06	45	39	38	35	49	39	49	46	57	44	45	62
Summer 06	31	2	38	0	98	1	22	3	34	3	82	1
Autumn 06	98	4	120	4	105	5	131	8	111	3	78	5
Winter 06/07	602	5	412	6	582	8	1035	8	847	14	873	10
Spring 07	706	8	497	18	689	3	1259	15	1217	8	963	8
Summer 07	692	17	376	16	678	11	1102	15	1154	12	979	15
Autumn 07	628	9	375	3	737	5	881	24	1060	14	1000	8

PO_4 (mgL^{-1})

Season	Indoor wetlands 1	2	3	4	5	6	Outdoor wetlands 1	2	3	4	5	6
Spring 05	7.9	11.9	4.1	3.5	12.1	2.9	8.0	11.9	3.7	3.8	11.1	2.9
Summer 05	6.7	11.0	6.6	6.7	7.8	7.8	10.2	10.9	5.9	6.4	5.1	5.9
Autumn 05	8.4	8.0	7.5	6.3	7.6	8.3	8.6	9.4	6.9	6.1	7.3	6.5
Winter 05/06	8.5	7.9	9.8	7.6	9.1	8.1	9.0	8.3	9.7	7.3	6.4	6.4
Spring 06	9.3	8.1	6.3	7.5	8.1	7.6	9.9	8.6	6.8	7.8	8.3	7.5
Summer 06	17.0	15.7	14.6	14.4	14.9	15.0	18.2	16.1	15.0	14.9	14.9	14.9
Autumn 06	36.1	67.8	71.1	68.9	68.4	77.1	60.6	118.5	56.6	51.7	37.6	66.4
Winter 06/07	6.3	8.1	10.9	10.3	4.7	6.3	9.3	9.4	8.5	9.4	7.3	6.9
Spring 07	7.4	6.1	7.3	4.9	5.9	6.7	9.0	14.7	12.1	8.0	8.1	7.2
Summer 07	7.0	7.2	4.3	4.7	3.2	3.6	8.0	12.1	7.0	3.9	3.6	3.6
Autumn 07	9.3	11.5	7.0	9.8	7.3	9.5	7.7	11.0	8.5	6.8	4.2	4.1

Season	Indoor wetlands 1	2	3	4	5	6	Outdoor wetlands 1	2	3	4	5	6
NO_3-N (mgL^{-1})												
Spring 05	0.6	1.4	0.9	1.6	1.8	0.3	0.4	1.3	1.1	1.6	1.5	0.3
Summer 05	0.5	1.5	1.3	1.8	1.6	1.6	1.5	1.9	1.4	1.8	0.9	0.9
Autumn 05	2.1	2.0	2.1	2.2	2.6	2.7	2.3	2.0	2.3	2.4	2.1	1.8
Winter 05/06	1.8	1.7	2.3	1.6	1.6	2.4	3.3	1.3	1.6	1.7	1.8	1.6
Spring 06	3.1	3.7	4.0	3.5	3.4	3.7	3.7	4.1	4.5	3.9	3.7	4.1
Summer 06	8.3	8.6	8.3	8.6	7.7	8.2	8.2	8.6	8.5	8.3	7.9	8.0
Autumn 06	18.0	43.0	34.9	51.2	35.5	46.4	24.2	53.5	25.8	36.3	20.9	29.0
Winter 06/07	75.7	131.5	165.8	189.8	45.4	25.2	133.0	160.8	84.7	102.7	81.8	59.9
Spring 07	25.0	79.8	33.1	76.2	38.9	44.0	66.1	116.4	101.7	87.4	86.2	73.6
Summer 07	24.3	112.9	33.2	70.5	8.5	67.0	42.5	91.2	103.4	88.2	24.0	61.9
Autumn 07	60.5	158.6	41.4	162.7	30.1	86.2	65.3	61.3	58.5	123.4	45.1	47.6
NH_4-N (mgL^{-1})												
Spring 05	8.7	7.4	5.3	1.8	5.5	1.6	7.4	8.5	6.2	2.6	5.8	1.9
Summer 05	6.4	5.1	5.7	4.7	5.1	4.1	5.3	4.6	7.2	5.4	4.3	6.0
Autumn 05	5.4	4.6	4.9	3.5	4.7	4.5	3.9	3.6	3.6	4.2	3.5	3.8
Winter 05/06	5.5	7.1	5.9	6.6	4.5	5.1	3.8	3.9	5.3	5.4	5.1	5.5
Spring 06	6.6	5.6	4.2	3.6	4.9	3.3	6.4	6.0	4.7	3.9	4.1	4.1
Summer 06	16.8	14.6	13.7	12.7	12.9	12.5	17.6	15.8	14.2	12.6	13.1	12.6
Autumn 06	86.3	97.1	92.5	103.2	88.5	96.1	121.8	123.5	114.5	104.3	84.0	89.5
Winter 06/07	40.8	41.6	35.4	25.0	25.5	27.3	57.4	43.7	40.6	39.4	50.4	32.4
Spring 07	32.0	39.9	28.5	22.8	30.1	24.3	51.3	67.5	55.0	36.9	43.4	27.5
Summer 07	19.0	20.6	13.3	8.8	4.3	4.5	27.1	17.7	23.5	15.8	6.5	14.0
Autumn 07	30.1	46.9	27.0	29.5	14.4	18.4	22.9	9.3	16.6	12.4	5.5	7.4

BOD: five-day @ 20°C N-Allythiourea biochemical oxygen demand (mg L^{-1}); COD: chemical oxygen demand (mg L^{-1}); PO_4: ortho-phosphate-phosphorus (mg L^{-1}); NO_3-N: nitrate-nitrogen (mg L^{-1}); NH_4-N: ammonia-nitrogen (mg L^{-1}).

It is uncertain whether the poor winter performances were due to low temperatures alone or the combined effect of operating condition and other variables. Considering that most of the variables did not respond to seasonal variables especially winter (Table 6-2) tend to support several studies that have suggested negligible temperature dependence in wetlands (Harbel et al., 1995; Knight et al., 1999;

Vymazal et al., 1999; Neralla et al., 2000). Furthermore, this suggests that soil microbes in winter still have the capacity to decompose organic matter and that low temperatures can enhance aerobic metabolism through the increase of dissolved oxygen saturation. Various studies have also considered the evaluation of the treatment efficiency of constructed wetlands as a function of temperature depending on components such as substrate composition, degree of plant growth, seasonal changes in evapotranspiration rates, and microbial activities (Chunming et al., 1999; Allen et al., 2002). For example, Rosso et al. (1995) demonstrated the effects of temperature and pH on microbial growth.

The impact of seasonal variations in the wetlands operated outdoors would give a better interpretation of various variables behaviour in the system and were presented in figures 6-11. COD trend lines in figure 6-11a show non impact of season on COD removal until autumn of 2006 when the COD removal efficiency began to decrease. BOD and DO show unsteady trend lines that is slightly responsive to seasonal variations (Figure 6-11b and c). However, pH shows steadier trend lines that do not respond to seasonal variations (Figure 6-11d).

Figure 6-11a

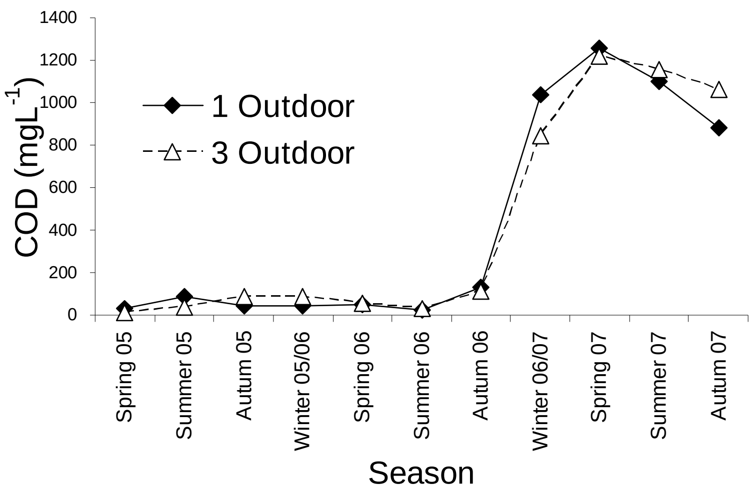


Figure 6-11b

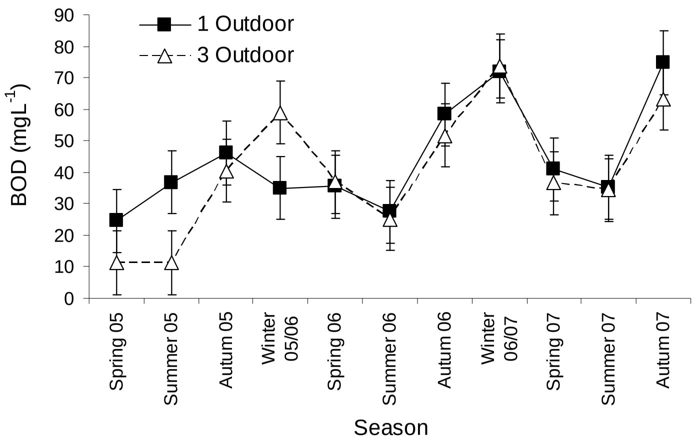


Figure 6-11c

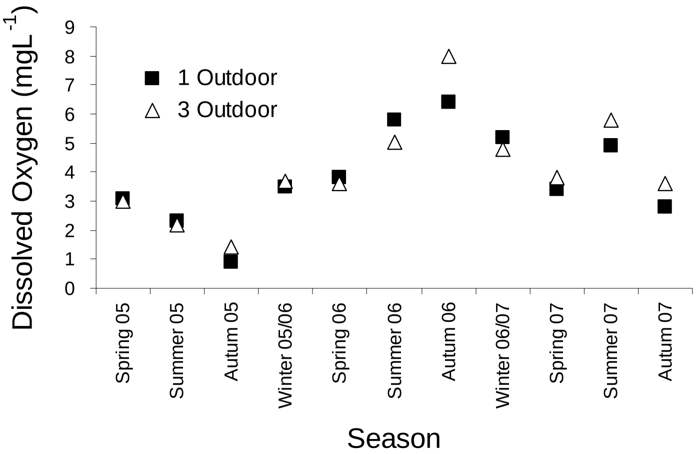


Figure 6-11d

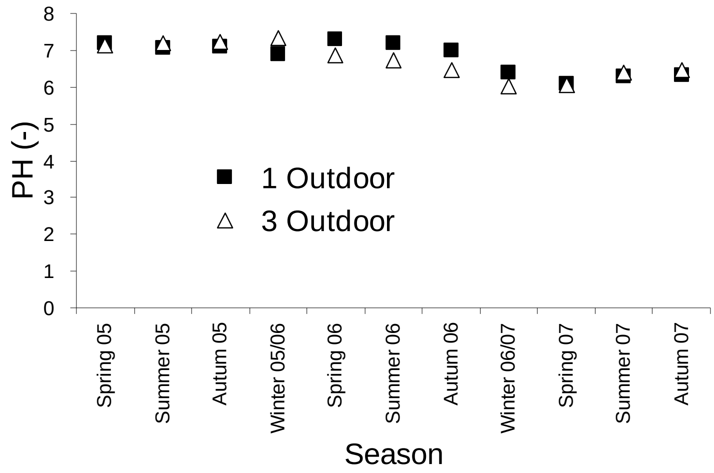


Figure 6-11. Seasonal effluent concentration of outdoor wetlands 1 and 3 for (a) COD, (b) BOD, (c) DO and (d) pH.

6.3.1. Comparison of indoor and outdoor seasonal treatment performance

The result in this subsection often shows similar trend lines in wetlands operated indoor and outdoor irrespective of environmental control of the indoor wetlands. However, the impact of environmental control became more visible from summer of 2006 when control equipment was fully operated. Figure 6-12 show the comparison of seasonal mean benzene removal efficiency for wetlands operated indoor and outdoor. Benzene trends in figure 6-12 show non impact of seasonal changes on benzene removal until autumn of 2006 when the benzene removal efficiency began to decrease. Figure 6-12b show similar trend but unsteady trend line in the filter 1 operated outdoor which is an indication of the impact of seasonal variability in the rig operated outdoors.

Figure 6-12a

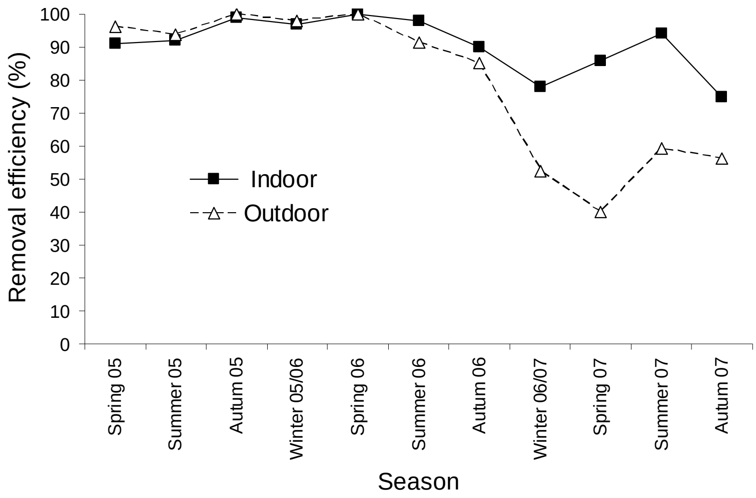


Figure 6-12b

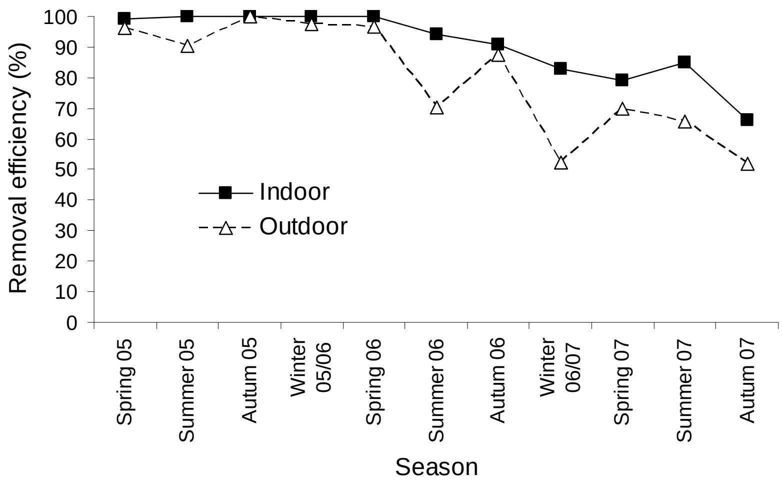


Figure 6-12. Comparison of seasonal mean benzene removal efficiency in the indoor and outdoor (a) wetlands 3 and (b) wetlands 1

pH show steady trend that do not respond to seasonal variations in wetlands operated either indoor or outdoor (Figure 6-13) which is an indication of complete non seasonal dependency of pH in the system.

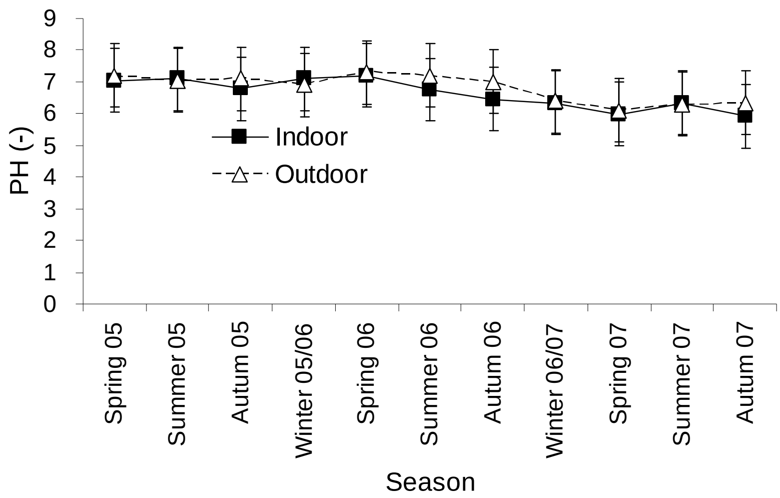


Figure 6-13. comparison of indoor and outdoor seasonal pH treatment performances in wetlands 1

Figure 6-14 show non impact of season changes on benzene removal until winter of 2006/07 in both wetlands. The trend lines also show similarity in planted filter 1 and unplanted filter 3 in addition to similarity in the seasonal variability which is in agreement with the published paper documented in appendix A.

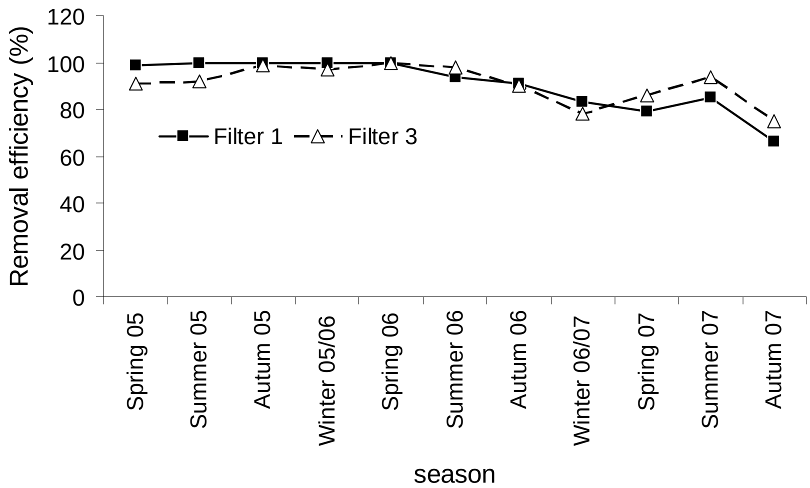


Figure 6-14. Comparison of seasonal mean benzene effluent concentrations for the wetlands 1 and 3

6.4. Seasonal variability in benzene removal and associated impacting factors

The importance of *P. australis* and aggregates concerning benzene removal in vertical-flow constructed wetlands was discussed in chapters 4, 5 and also in the papers documented in appendix A. In this section, the author concentrates on the assessment of the seasonal variability in benzene removal and associated impacting factors. Special emphasis was on seasonal internal interactions of benzene with other individual water quality variables in the constructed wetlands. Therefore, typical reed bed systems were chosen to address this purpose. As shown in Fig. 6-15, from spring 2005 to winter 2005, the seasonal variability in benzene removal was not visually detectable; the benzene removal efficiencies in both indoor and outdoor wetlands were almost constant (97-100%). After the first year of operation, the benzene removal efficiency began to decrease and a visible seasonal fluctuation in benzene removal was noted for the outdoor wetland (Fig. 6-15). For example, the seasonal removal efficiencies were 96.6, 70.3, 87.4 and 52.2% in spring, summer, autumn and winter 2006, respectively, which indicated that benzene removal was higher in spring and autumn than in summer and winter (Fig. 6-15).

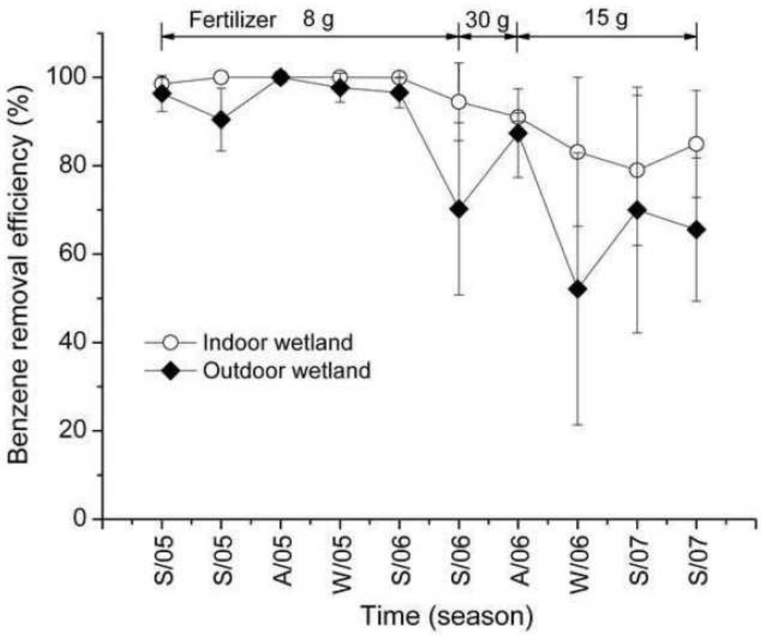


Figure 6-15. Seasonal variability of benzene removal by the indoor and outdoor wetlands for periods of 8, 15 and 30 g fertilizer supply (added to the influent every second week).

This chapter does not discuss the effect of temperature on benzene removal but highlights the associated seasonal variability. Control of temperature caused the absence of seasonal variability in benzene removal for the indoor wetland. However, a gentle decrease was observed with the extension of the running period (Fig. 6-15). Regardless of the indoor and outdoor wetlands, there was no obvious indication of an enhancement of the seasonal benzene removal efficiency with 15 and 30 in comparison to 8 g fertilizer supply (Fig. 6-15). The study provides an indication of the benzene removal at different nutrient concentration levels.

6.4.1. Seasonal nutrient removal and dosage variability

Effluent NH_4-N and NO_3-N concentrations were evaluated to obtain an indication of the seasonal variability in nitrogen removal for vertical-flow constructed wetlands treating benzene. Concerning NH_4-N removal (Fig. 6-16a), no clear seasonal variability was observed during the entire experiments. However, the effluent NH_4-N concentrations were influenced greatly by the amount of nutrients supplied to the treatment wetlands. Concerning the 8 g fertilizer addition, the seasonal effluent NH_4-N concentrations for the indoor wetland was similar to that of the outdoor wetland between spring 2005 and summer 2006. The increase in fertilizer supply led to the significant augmentation ($p<0.05$) of the effluent NH_4-N concentration (Table 6-3). In autumn 2006, the effluent NH_4-N concentrations increased 4.9 and 6.9 times for the indoor and outdoor wetland, respectively, in comparison to summer 2006, after 30 g fertilizer was supplied. A decline of effluent NH_4-N concentrations was observed afterwards, when the fertilizer supply was dropped from 30 to 15 g. Furthermore, relatively low effluent NH_4-N concentrations were observed in the outflows of the indoor wetland during the same running period (Fig. 6-16a).

Figure 6-16a

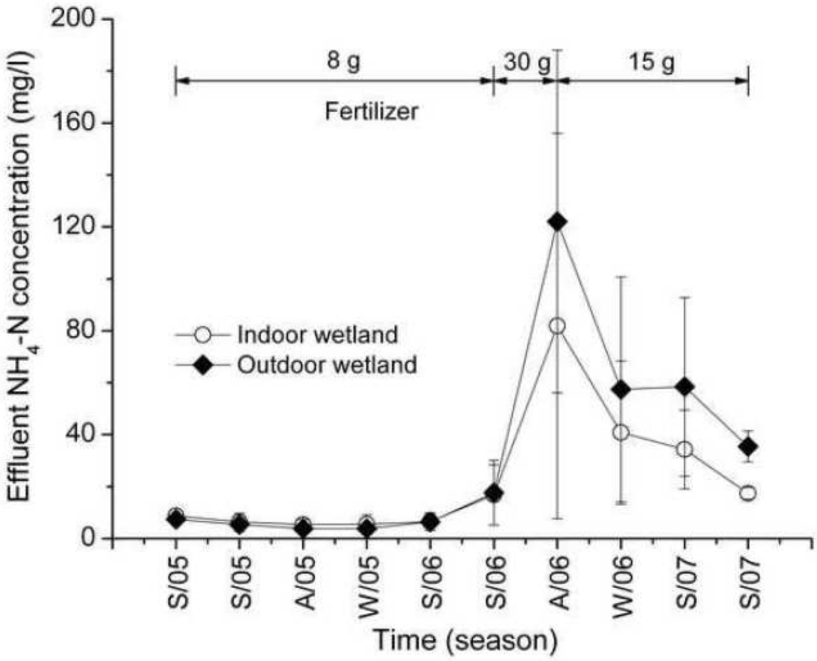


Figure 6-16b

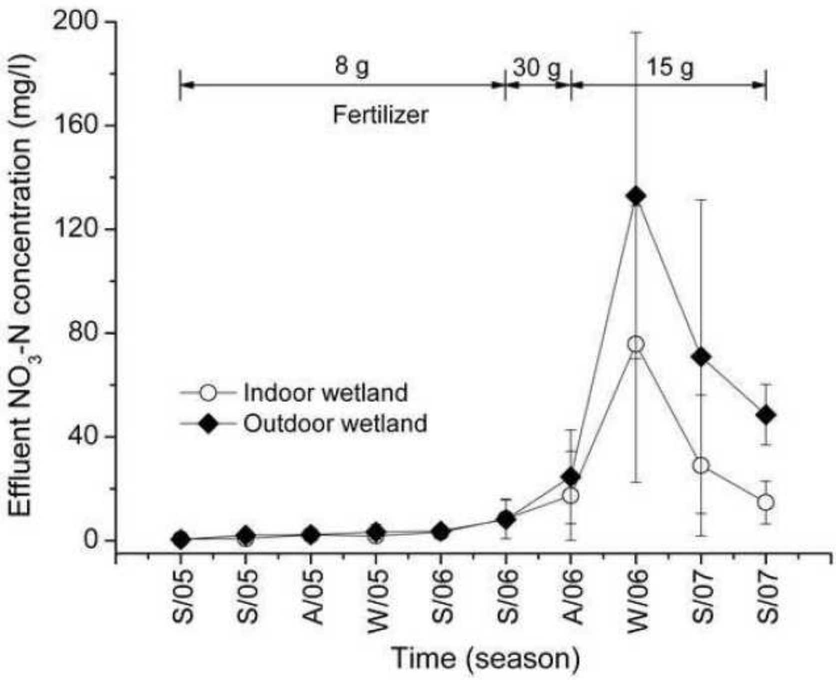


Figure 6-16c

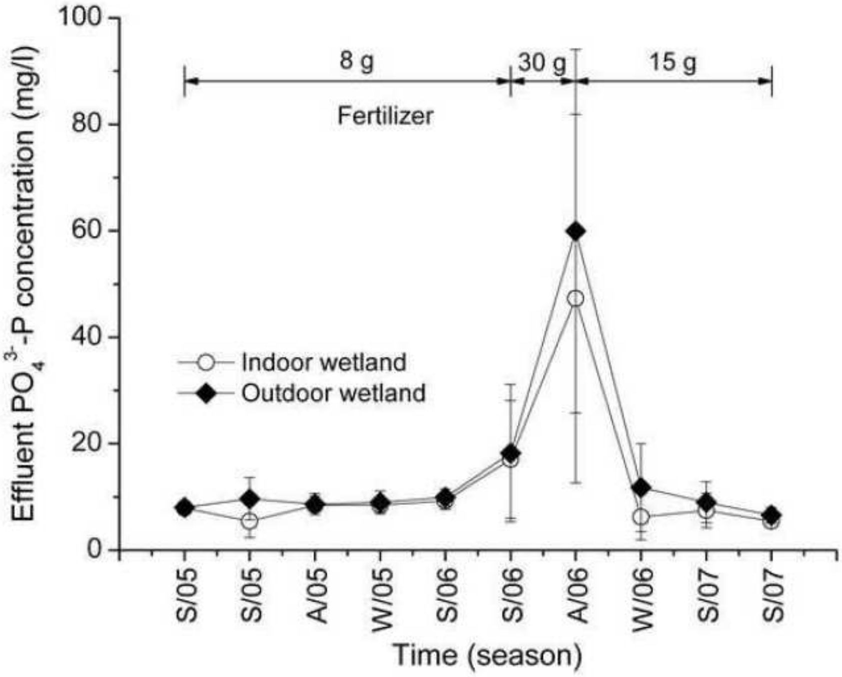


Figure 6-16. Seasonal effluent variability of the (a) ammonia-nitrogen (NH_4-N), (b) nitrate-nitrogen (NO_3-N), and (c) ortho-phosphate-phosphorus (PO_4^{3-}—P) concentrations in the indoor and outdoor wetlands for periods of 8, 15 and 30 g fertilizer supply (added to the influent every second week).

Table 6-3. One-way analysis of variance assessing the effect of fertilizer supply

Parameters		Indoor wetland		Outdoor wetland	
	Unit	F-ratio	*p* value	F-ratio	*p* value
NH_4-N[a]	mg/l	18.48	<0.01	54.88	<0.01
NO_3-N[b]	mg/l	9.19	0.02	23.63	<0.01
PO_4^{3-}P[c]	mg/l	1.61	0.25	0.35	0.57
pH	-	67.09	<0.01	356.02	<0.01
DO[d]	mg/l	9.05	0.02	10.27	0.02
Redox potential	mV	8.28	0.02	7.08	0.03

[a] ammonia-nitrogen; [b] nitrate-nitrogen; [c] ortho-phosphorus-phosphate; [d] dissolved oxygen. Note: Only one seasonal data set was not statistically significance for 30 g (n=1) fertilizer supply. The differences in effluent variables during phases of 8 g (n=6) and 15 g

(n=3) fertilizer supply were evaluated at $p \leq 0.05$. Results of a one-way analysis of variance assessing the effect of fertilizer supply (8 g *versus* 15 g added to the influent every two weeks; see section on experimental operation) on the seasonal effluent nutrient concentrations and other variables for both the indoor and outdoor wetland.

With respect to NO_3-N removal, the absence of seasonal variability was also confirmed with Fig. 6-16b. The relationships between effluent NO_3-N concentrations and nutrient supply were similar to those for NH_4-N. Effluent NO_3-N concentrations significantly increased ($p<0.05$) with increasing nutrient supply and reduced with decreasing nutrient supply (Table 6-3). In contrast to NH_4-N, however, elevated effluent NO_3-N concentrations were not immediately observed after 30 g fertilizer was added, but were noted in the subsequent treatment phase (Fig. 6-16b). Ammonia-nitrogen was the main nitrogen component of the fertilizer. The transformation of NH_4-N to NO_3-N caused inconsistencies in nutrient availability, and led to high effluent NO_3-N concentrations.

Effluent PO_4^{3-}-P concentrations in both indoor and outdoor wetlands were measured to assess the seasonal variability in phosphorus removal by vertical-flow constructed wetlands. As shown in Fig. 6-16c, seasonal variations had a minor impact on phosphorus removal. Nutrient supply changes had a great impact on the distribution of the seasonal effluent phosphorus concentrations during the entire running period. Effluent phosphorus concentrations increased with the augmentation of nutrient supply and decreased with reduced nutrient dosage for both the indoor and outdoor wetlands. In addition, the effluent phosphorus concentrations during the period of 15 g nutrient supply were not significantly lower ($p<0.05$) than those during the phase of 8 g nutrient supply (Fig. 6-16c; Table 6-3). The consumption of phosphorus in benzene treatment increased with greater nutrient availability.

6.4.2. Seasonal variability for other water quality variables

The indoor room temperatures and the outdoor atmospheric temperatures were recorded. As shown in Fig. 6-17, the indoor room temperature was much higher compared to the outdoor atmospheric temperature before full control of the environmental boundary conditions was established in summer 2006. The relatively high indoor temperatures contributed to increased benzene removal efficiencies. Concerning the outdoor wetland, the recorded atmospheric temperatures changed seasonally, and the hottest and coldest season was summer 2005 and winter 2006 with a mean temperature of 19.8 and 7.0°C, respectively.

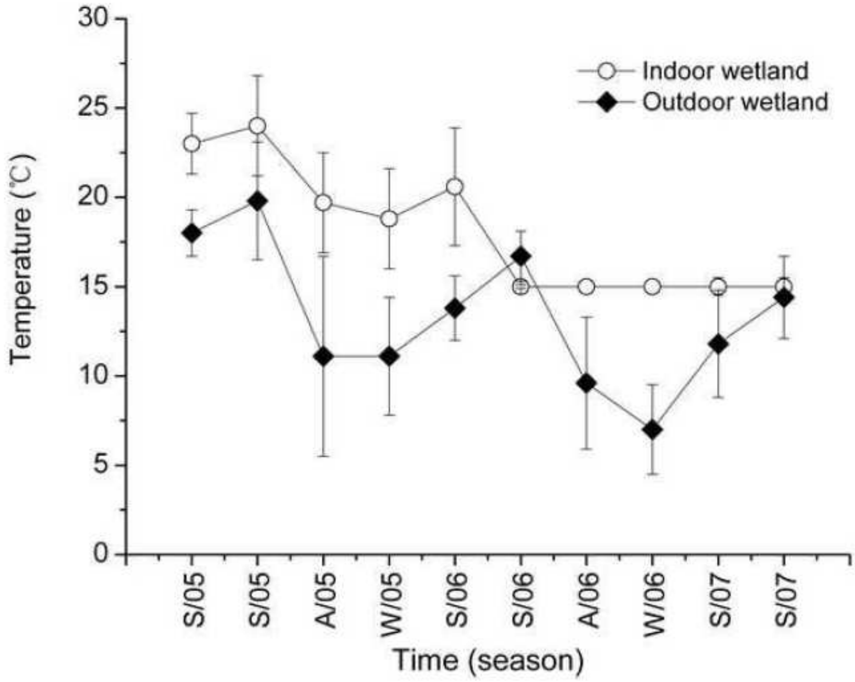


Figure 6-17. Seasonal variability of temperature for the indoor and outdoor wetlands.

The seasonal variability in effluent pH for both the indoor and outdoor wetlands is shown in Fig. 6-18a. No seasonal variations in effluent pH were recorded. Benzene removal resulted in a continuous decline of the effluent pH values for both the indoor and outdoor wetlands, regardless of nutrient concentration changes (Fig. 6-18a). Furthermore, significantly higher ($p<0.05$) effluent pH values were observed with 8 in comparison to 15 g fertilizer supply (Table 6-15).

Figure 6-18a

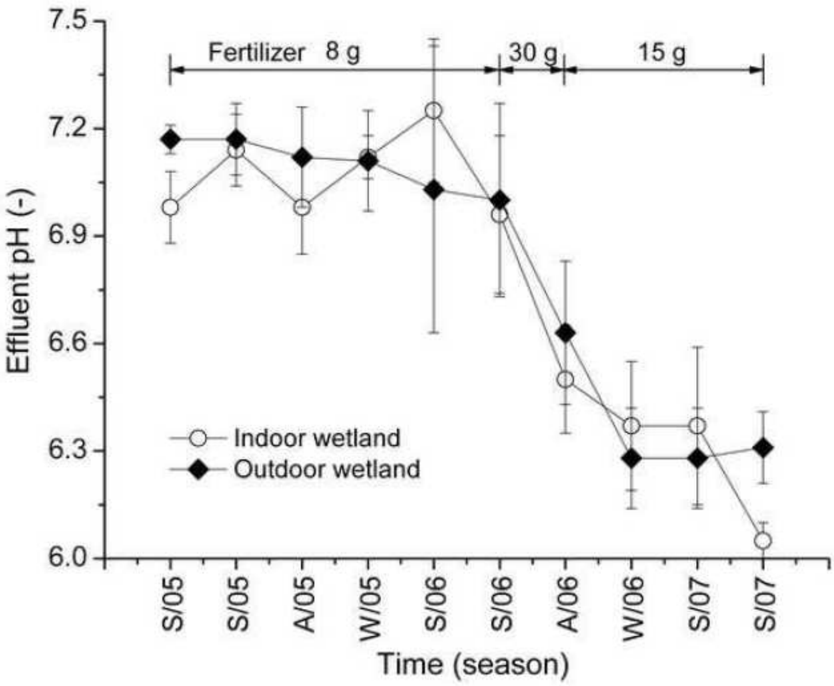


Figure 6-18b

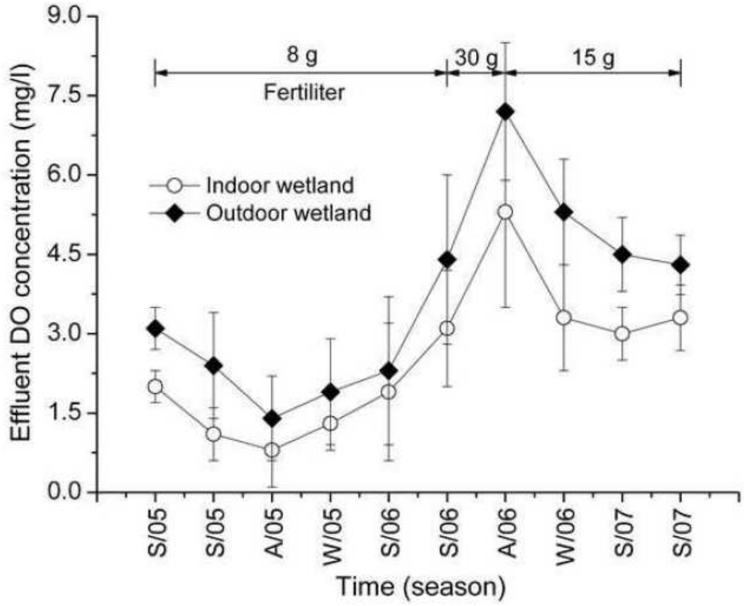


Figure 6-18c

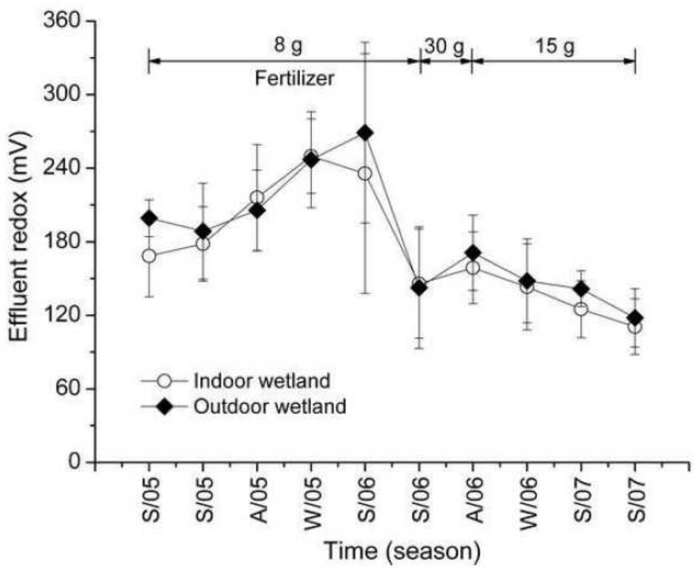


Figure 6-18. Seasonal effluent variability of (a) pH, (b) the dissolved oxygen (DO) concentrations, and (c) the redox potential (redox) in the indoor and outdoor wetlands for periods of 8, 15 and 30 g fertilizer supply (added to the influent every second week).

During the entire experiment, the effluent DO concentrations were lower in the indoor wetland compared to the outdoor wetland (Fig. 6-18b). In the first year of operation, excellent benzene removal for both the indoor and outdoor wetlands was observed. This lowered the corresponding effluent DO concentrations as observed in Fig. 6-18b, especially in autumn 2005. With the decrease of benzene treatment efficiency after spring 2006, the effluent DO concentrations increased virtually independently of seasonal variability. The effluent DO concentrations during periods of 15 and 30 g fertilizer supply were significantly higher ($p<0.05$) than those recorded during the period of 8 g fertilizer supply (Table 6-3). During the entire experiment, the highest effluent DO concentrations in the indoor and outdoor wetlands occurred in autumn 2006 with mean values of 5.3 mg/l and 7.2mg/l, respectively (Fig. 6-18b).

There was no seasonal variability in effluent redox in both indoor and outdoor wetlands (Fig. 6-18c). The effluent redox gradually increased with increased benzene removal observed during the first year of operation but decreased with reduced benzene treatment efficiency. The lowest values of 110.7 and 117.9 mV occurred in summer 2007 for the indoor and outdoor wetlands, respectively. Findings show that an increase of the nutrient supply resulted in a decrease of the effluent redox. Moreover, the effluent redox was significantly higher ($p<0.05$) with 8 in comparison to 15 g fertilizer supply (Table 6-3).

6.4.3. Impact of seasonal temperature on seasonal benzene removal

A number of studies have shown that benzene removal depends on several factors such as temperature, pH, oxygen availability and salinity (Knight *et al.*, 1999; Li, *et al.*, 2006; Lu *et al.*, 2002). The effect of temperature on benzene removal efficiency was not visible during the first year of operation, and the seasonal benzene removal efficiency was almost unchanged for both the indoor and outdoor wetlands (Fig. 6-15). Adsorption by fresh aggregates played an important role in benzene removal as reported by Adachi *et al.* (2003). Accumulation experiments indicated that >50% of the accumulated mass of benzene was located within the intra-particle pores and on the grain surfaces (Corley *et al.*, 1996). Results of linear fits indicated that seasonal benzene removal efficiency was weakly linked to atmospheric temperature in planted vertical-flow constructed wetlands (R^2=0.09; Fig. 6-19). However, seasonal variability in benzene removal efficiency was observed after winter 2005, and much better benzene removal occurred in spring and autumn in contrast to summer and winter (Fig. 6-15). Similar results were also obtained by Salmon *et al.* (1998) showing that the benzene treatment efficiency was significantly lower in winter than in spring and summer. In an early study by Ward and Brock (1976), the highest hydrocarbon removal rate in an oil-contaminated lake occurred during early spring and decreased in the following summer.

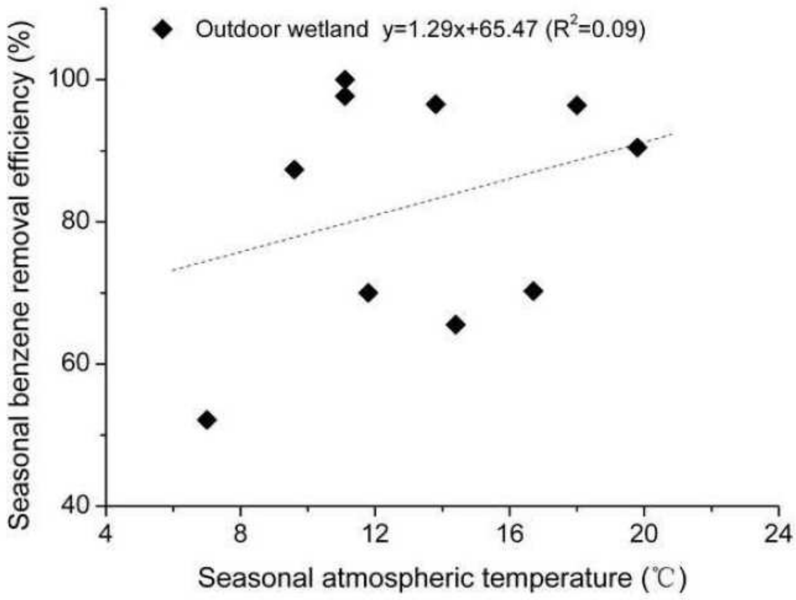


Figure 6-19. Relationship between the seasonal benzene removal efficiency and seasonal atmospheric temperature.

Processes such as adsorption, biodegradation and volatilization contributed to benzene removal (Corley *et al.*, 1996; Lee *et al.*, 2004; Li *et al.*, 2006). Concerning adsorption removal, no obvious temperature dependences were observed and negligible differences in benzene removal efficiency at 4, 10 and 20°C were detected in industrial wastewater adsorption studies (Adachi *et al.*, 2001). In vertical-flow constructed wetlands, benzene removal by volatilization was insignificant, if compared to removal by biodegradation (Salmon *et al*., 1998).

Henry's law constant for benzene can be obtained when temperature increases from 6 to 10°C (Görgényi *et al.*, 2002). However, mesophilic and thermophilic microorganisms are severely limited in degrading benzene at or below 10°C (Kniemeyer *et al.*, 2003). This explains why the lowest benzene removal efficiency was recorded in winter 2006, where the corresponding seasonal temperature was 7°C (Fig. 6-17).

Furthermore, the highest benzene removal efficiency was observed in spring and not in summer as may be expected. It follows that benzene biodegradation does not strictly increase with an increase in temperature. These findings were confirmed by a recent study indicating that biodegradation of benzene was faster at 15 and 25°C than at 30°C. Similar biodegradation rates were noted at 15 and 25°C, while the optimal temperature conditions for biodegradation of benzene were 20°C (Li, *et al.*, 2006). Improved biodegradation rates have also been observed in wetland environments to occur at temperatures between 20 and 30°C (Cooney, 1984).

6.4.4. Impact of nutrient supply on seasonal benzene removal

Salmon *et al.* (1998) introduced a specific cocktail of nutrients (ammonia, 1.5%; nitrate, 2.5%; phosphorus, 1.5%; potassium, 3.8%) to constructed wetlands to encourage benzene biodegradation. In other biodegradation studies, slow-releasing fertilizers have been used to provide a continuous supply of nutrients, particularly nitrogen and phosphorus (e.g. Xu *et al.*, 2003). For groundwater remediation, apatite rock has been used as a phosphorus source for groundwater microbes to improve the biodegradation of monoaromatic hydrocarbon (Granger *et al.*, 1999).

In this study, different N-P-K Miracle-Gro fertilizer amounts were added during different running periods to assess the effect of nutrient supply on benzene removal (see above). The linear trend line fit in Fig. 6-20a indicates that the seasonal benzene removal efficiency was weakly linked to the effluent ammonia-nitrogen (NH_4-N) concentrations

with R^2 values of 0.30 and 0.17 for the indoor and outdoor wetlands, respectively. According to Fig. 6-20b, the seasonal benzene removal efficiency decreased linearly with increasing effluent nitrate-nitrogen (NO_3-N) concentrations with R^2 of 0.55 and 0.73 for indoor and outdoor wetlands, respectively. However, the relationships between seasonal benzene removal efficiencies and effluent ortho-phosphorus-phosphate (PO_4^{3-}-P) concentrations were weak with linear correlation coefficients of R^2=0.001 (zero for all tense and purposes) were obtained for both the indoor and outdoor wetlands (Fig. 6-20c).

Considering the entire experiment, fertilizer supply of up to 30 g did not result in enhanced seasonal benzene removal. The seasonal benzene removal efficiency was 96% with 8 g fertilizer supply and with corresponding effluent NH_4-N concentrations of 3.79 and 8.30 mg/l, effluent NO_3-N concentrations of 0.56 and 3.68 mg/l and effluent PO_4^{3-}-P concentrations of 7.90 and 9.92 mg/l for the indoor and outdoor wetlands, respectively (Fig. 6-20).

Figure 6-20a

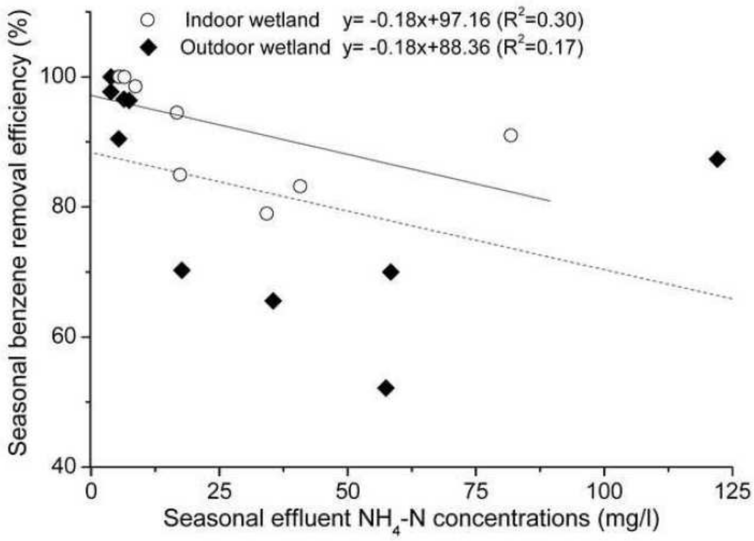


Figure 6-20b

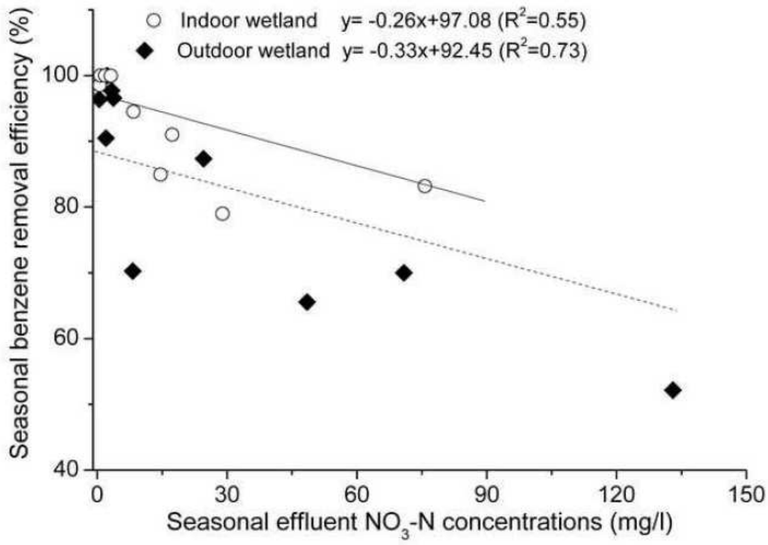


Figure 6-20c

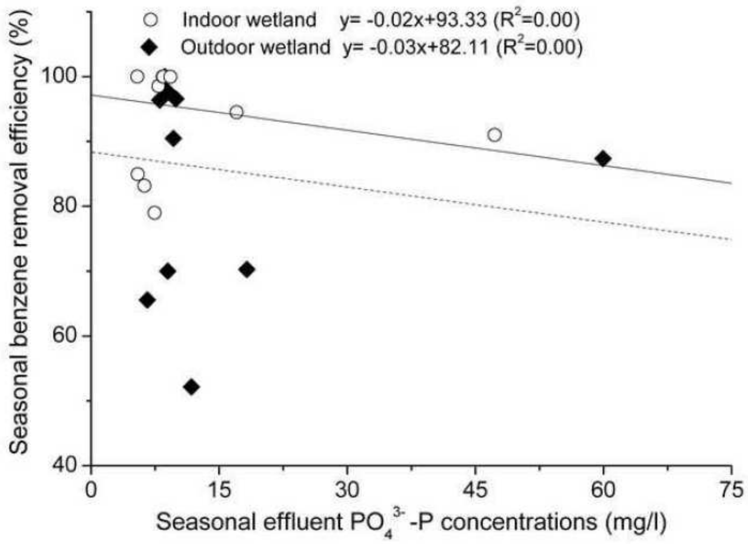


Figure 6-20. Relationships between the seasonal benzene removal efficiency and (a) ammonia-nitrogen (NH_4-N), (b) nitrate-nitrogen (NO_3-N), and (c) ortho-phosphate-phosphorus (PO_4^{3-}–P) effluent concentrations.

Weak linear trendline relationships between seasonal benzene removal efficiency and effluent NH_4-N and PO_4^{3-}-P concentrations were observed. This could be explained with an excess supply of both nutrients, considering that ammonia and phosphate are usually regarded as essential nutrient sources for microorganism growth and propagation in constructed wetlands and microbial biomass (Cannon *et al.*, 2000). As shown in Fig. 6-20b, a good linear fit between seasonal benzene removal and effluent nitrate-nitrogen suggested that nitrate is a more preferred nitrogen source than ammonia in benzene removal processes. Wrenn *et al.* (1994) studied the effects of different forms of nitrogen on biodegradation of light Arabian crude oil in respirometers. Their findings indicated that nitrate is a better nitrogen source than ammonia because acid production associated with ammonia metabolism inhibited oil biodegradation. Furthermore, nitrate was found to be a more favorable electron acceptor in benzene biodegradation (Hu *et al.*, 2007; Johnson *et al.*, 2003; Schreiber and Bahr, 2002; Yang *et al.*, 2008). The most noteworthy paper in this field in recent years described benzene oxidation by two strains of the genus *Dechloromonas* with nitrate as the sole electron acceptor (Coates *et al.*, 2001).

Rosenberg and Ron (1996) calculated that approximately 150 mg of nitrogen and 30 mg of phosphorus are theoretically utilized in the conversion of 1000 mg of hydrocarbon to cell materials. Ahn (1999) further studied the effect of nitrate concentrations under tidal flow conditions on hydrocarbon biodegradation with nitrate concentrations ranging between 6.25 and 400 mg/l. The results from both hydrocarbon analysis (hopane as a biomarker) and microbial growth (phospholipids analysis) showed that the optimal nitrate concentration fed under these conditions was approximately 25 mg/l. Based on the benzene treatment efficiency and corresponding effluent nutrient concentrations during periods of 8, 15 and 30 g fertilizer supply, it can be found that 8 g fertilizer supply satisfies the nutrient requirement for benzene treatment in this study (Figs. 6-15 and 6-16).

6.4.5. Impact of pH on seasonal benzene removal

As shown in Fig. 6-18a, seasonal pH values gradually decreased from 6.98 and 7.17 in spring 2005 to 6.05 and 6.31 in summer 2007 for the indoor and outdoor wetlands, respectively. This implies that the long-term benzene treatment resulted in reduced pH values. Findings showed that the pH values indicated acidic conditions during benzene degradation, which was confirmed by Venosa and Zhu (2003). As remarked previously, adsorption and biodegradation processes were predominantly responsible for benzene removal in this study. Concerning adsorption, the benzene removal efficiency usually increases as pH increases between the range of 1 to 11 (Adachi *et al.*, 2001). Biodegradation studies showed that benzene removal increased with increasing pH. Optimal degradation was observed at neutral or slightly alkaline conditions (Lu *et al.*, 2002; Yang *et al.*, 2008). Jung and Park (2004) reported that the highest benzene biodegradation rate was observed at a pH value of 7. In this study, the seasonal benzene removal efficiency increased with increasing effluent pH values. This has been confirmed by the high linear correlation coefficients R^2 of 0.82 and 0.63 for the indoor and outdoor wetlands, respectively (Fig. 6-21). Furthermore, 95% higher seasonal benzene removal was achieved at effluent pH ranges between 7.03 and 7.17. These findings correspond well with observations by Jung and Park (2004), Lu *et al.* (2002) and Yang *et al.* (2008).

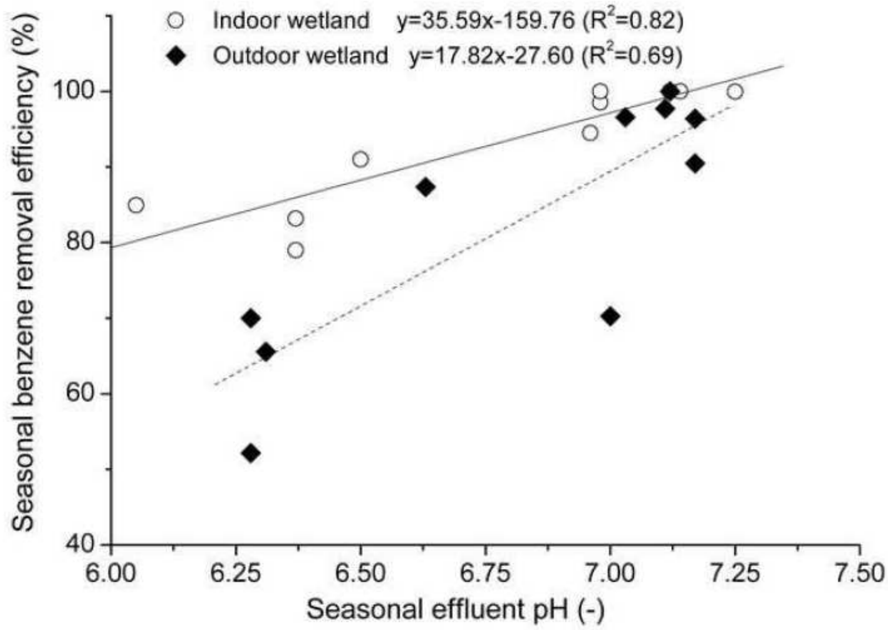


Figure 6-21. Relationship between the seasonal benzene removal efficiency and the pH of the effluent.

6.4.6. Impact of DO and redox on seasonal benzene removal

The release of oxygen by wetland plants was reported in many treatment studies (e.g. Scholz, 2006; Vymazal, 2007). However, freshwater wetlands are typically considered to be nutrient limited due to a heavy demand for nutrients by the aquatic plants (Venosa and Zhu, 2003). They are also viewed as nutrient traps or sinks considering that substantial amount of nutrients can be bound in biomass (Mitsch and Gosselink, 1993). An increase in nutrients led to better plant and root growth in hydrocarbon treatment wetlands, and thus increased the oxygen release from the plant root zone, which subsequently enhanced hydrocarbon degradation as shown by Purandare (1999). This finding helps to explain why the effluent DO concentrations were higher during phases of 15 and 30 g fertilizer supply in comparison to 8 g fertilizer supply (Fig. 6-18b). A further reason for the increased effluent DO concentrations during periods of 30 and 15 g fertilizer supply was the decrease in oxygen consumption due to benzene degradation.

A decline in benzene removal reduces the utilization of available oxygen (Johnson *et al*., 2003). Benzene degradation leads to an increase in acid production (Venosa and Zhu, 2003). This finding was confirmed in this study considering that pH declined continuously (Fig. 6-18a). This increase in acid production resulted in the gradual decrease of effluent redox (Fig. 6-18c).

As shown in Fig. 6-22, higher seasonal benzene removal efficiencies were observed at lower effluent DO concentrations and higher effluent redox values. Seasonal benzene removal efficiency was negatively correlated to effluent DO concentrations with correlation coefficients R^2 of 0.41 and 0.38 (Fig. 6-22a), while positively correlated to effluent redox with R^2 of 0.65 and 0.66 for the indoor and outdoor wetlands, respectively (Fig. 6-22b). In general, the seasonal benzene removal efficiency was 90% higher in both the indoor and outdoor wetlands with corresponding effluent DO concentrations between 0.8 and 2.3 mg/l, and redox values between 178.2 and 268.9 mV, respectively (Fig. 6-22).

Figure 6-22a

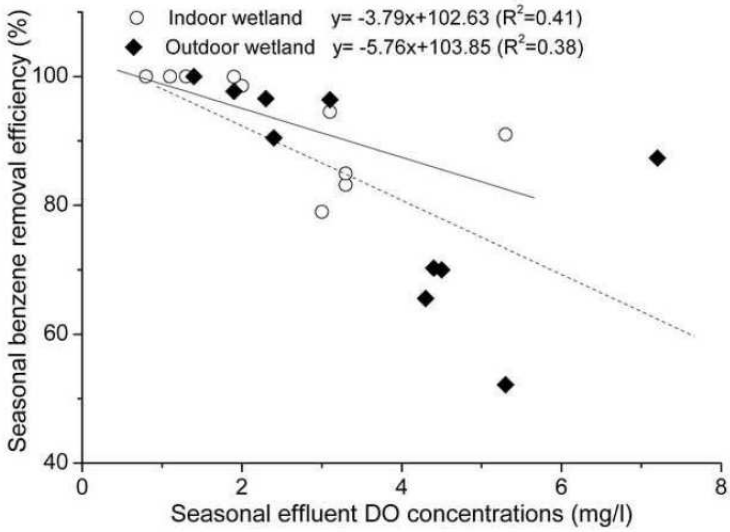


Figure 6-22b

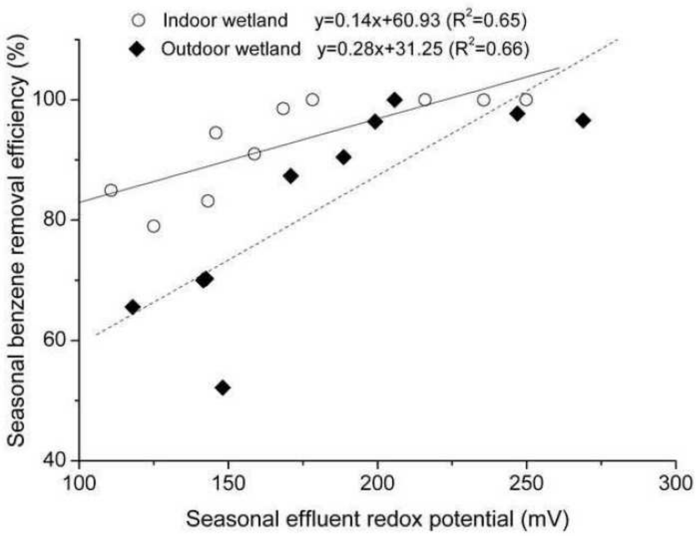


Figure 6-22. Relationships between the seasonal benzene removal efficiency and (a) the dissolved oxygen (DO) effluent concentrations, and (b) the redox potential of the effluent.

Benzene is removed at a faster rate under aerobic compared to anaerobic conditions (Gibson and Subramanian, 1984; Johnson et al, 2003). Similar finding were also reported elsewhere; e.g. the benzene removal efficiency increased with decreasing effluent DO concentrations, and an increasing consumption of oxygen resulted in improved benzene removal (Lu *et al.*, 2002). Yerushalmi and Guiot (2001) defined DO concentrations of <2 mg/l as microaerophilic for benzene biodegradation. The maximum specific rate of benzene biodegradation was approximately 2.6 mg/mg biomass/d at benzene concentrations ranging between 22.0 to 65.9 mg/l.

Considering the importance of oxygen availability for effective benzene removal, a variety of approaches to physically promote oxygen availability have been tested for anoxic soil, sediment and groundwater aquifers. These include biopiles, injection of oxygen, air, aerated water, hydrogen peroxide and chlorite. The latter was degraded by perchlorate-reducing bacteria to yield oxygen *in situ* (Coates *et al.*, 1998; Holder *et al.*, 1999).

Good aeration conditions of vertical-flow constructed wetlands have been demonstrated in previous studies (Scholz *et al.*, 2002; Vymazal, 2007) and these findings are confirmed in this study. As shown in Fig. 6-22, effluent DO concentrations of >2 mg/l were observed during the running period. The observed concentrations of oxygen were sufficient for benzene degradation according to criteria defined by Yerushalmi and Guiot (2001) for comparable experimental settings. Moreover, negative linear correlations between seasonal benzene removal efficiencies and effluent DO concentrations indicated the consumption of oxygen in the benzene biodegradation process (Fig. 6-22a).

The terminal electron acceptor for the biodegradation of benzene is molecular oxygen during aerobic respiration (Johnson *et al.*, 2003). In the absence of oxygen, benzene biodegradation involved common electron acceptors including nitrate, sulphate and carbon dioxide. However, benzene biodegradation in aquifers is predominantly aerobic, with limited amounts being degraded anaerobically (Aronson and Howard, 1997).

Based on these findings, oxygen and nitrate could have been possible electron acceptors for benzene biodegradation in this study. With the supply of these two electron acceptors, positive linear relationships were obtained between the seasonal benzene removal efficiency and effluent redox (Fig. 6-22b). This indicates that benzene removal performed much better under oxidative conditions than under reducing conditions.

6.5. Summary

The research shows the sustainable management of the seasonal variability in benzene removal by planted vertical-flow constructed wetlands to prevent pollution of receiving watercourses. The seasonal variability in benzene removal by planted vertical-flow constructed wetlands was assessed between spring 2005 and autumn 2007.

During the first year of operation, the benzene removal efficiency was virtually constant (97-100%) without any visible signs of seasonal variations in the data distribution. During the following years, benzene removal efficiency varied seasonally in the outdoor wetland. In 2006, the highest and lowest benzene removal efficiencies occurred in spring and winter at mean atmospheric temperatures of 13.8 and 7.0°C, respectively. The highest benzene removal efficiency was noted in spring and not in summer as expected. This indicates that the benzene removal did not solely depend on temperature.

The seasonal variability in effluent ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), ortho-phosphate-phosphorus (PO_4^{3-}-P), pH, dissolved oxygen (DO) and redox potential (redox) was examined. Findings show that the effluent NH_4-N, NO_3-N and PO_4^{3-}-P concentrations did not vary seasonally during the entire period of operation. However, an increase or decrease of fertilizer supply led to the corresponding changes in effluent NH_4-N, NO_3-N and PO_4^{3-}-P concentrations. Except for the above nutrients, no seasonal variability in effluent pH and redox were observed. In both the indoor and outdoor planted wetlands, benzene treatment resulted in a continuous decline in effluent pH and redox. Seasonal variability in effluent DO concentrations were not observed, and the effluent DO concentrations were higher during periods of 15 and 30 g fertilizer supply (added to the influent every second week) in comparison to the period when only 8 g fertilizer was applied for both the indoor and outdoor wetlands.

Results from linear regression analyses indicated that the seasonal benzene removal efficiency was weakly linked to temperature and effluent NH_4-N and PO_4^{3-}-P concentrations. However, the seasonal benzene removal efficiency was negatively correlated and closely related to effluent DO and NO_3-N concentrations, while positively correlated and closely related to effluent pH and redox. Findings show that seasonal benzene removal efficiency increased with an increase in effluent pH and redox potential, but decreased with increasing effluent DO and NO_3-N concentrations.

Concerning nutrient supply, the supply of 8 g fertilizer was sufficient to treat 1000 mg/l benzene influent in vertical-flow constructed wetlands. The seasonal benzene removal efficiency could be as high as 90%, if the effluent pH, redox, DO and NO_3-N values were between 7.03 and 7.17, 178.2 and 268.93 mV, 0.8 and 2.3 mg/l, and 0.56 and 3.68 mg/l, respectively.

This chapter successfully demonstrated advancement of the understanding of wetlands internal processes and optimal operating condition of the water quality variables, as verified by the monthly performances and seasonal variability in benzene removal and associated impacting factors.

7

Application of an artificial neural network and multivariable testing to support constructed wetlands operation, optimization and management[♣]

7.1. Overview

This chapter presents an artificial neural network (Self Organizing Map (SOM)) and multiple variable testing for the prediction of experimental constructed wetland performance. Considering that none of the water quality variables operate in isolation, actual analysis of multiple water quality variables will be required for a thorough understanding of the overall water quality roles of constructed wetlands in treatment of hydrocarbon and the development of assessment techniques. The SOM and multiple variable testing were applied to predict relationship between benzene and other water quality variables. They were also used to assess alternative methods of analyzing water quality performance indicators in constructed wetlands treating hydrocarbon.

[♣] Parts this chapter was submitted for publication as:
Tang X., Eke P. E., Scholz M. and Huang S. (2008), Processes Impacting Benzene Removal in Vertical-Flow Constructed Wetlands. *Bioresource Technology,* in press.

Eke P. E. and Scholz M. (2008), Self Organizing Map applied for management and monitoring of constructed wetlands treating Hydrocarbon. In preparation.

The chapter is structured as follows: Section 7.2 described the aims and objectives of this chapter. Section 7.3 introduced the artificial neural network tool applied for the wastewater treatment system. Section 7.4 gave descriptions of SOM model and its theoretical background. Sections 7.5 presented SOM applied to constructed wetlands treating hydrocarbon, the subsections show methods and software, training, testing of data sets and visualization of results, 7.6 presented benzene removal simulated with selected variables applying multivariate linear regression models, 7.7 presented large Scale Constructed Wetlands applied for Hydrocarbon Treatment: Case Studies, 7.8 presented limitations of the analysis and 7.9 summarized the chapter.

7.2. Aims and objectives

The purpose of this chapter is the development of performance monitoring and data exploration techniques based on self-organizing map. The SOM and multiple variable testing techniques are used to estimate and monitor the diverse states of the water quality variables in the constructed treatment wetlands. Moreover, establishing the correlations among process variables is necessary in order to obtain a knowledge-based system required for effective monitoring of constructed treatment wetlands.

The objectives are to:

1. investigate the processes, interactions and impacts of water quality variables during benzene removal in vertical-flow constructed wetlands;
2. simulate the benzene removal with selected variables applying SOM and multivariate linear regression models;

3. assess novel alternative methods of analyzing water quality performance indicators for constructed treatment wetlands;
4. investigate the potential use of SOM and multiple variable testing techniques as the management and optimization tools to enhance the understanding of 'black box' systems as well as reduce operation costs.

7.3. Artificial neural network applied to wastewater treatment processes

Artificial Neural Network (ANN) technique is part of the research area of artificial intelligence. Artificial neural networks are basically network systems in which various nodes called neurons are interconnected. ANN is artificial and simplified models of the neurons that exist in the human brain. Their ability relies on the quality of the signals used for training and the performance of the training algorithms and their parameters do not contain information that can be directly understood by the human operator or that can easily be related to the physical properties of the system to be modeled (Vieira et al 2004). ANN can be used for finding out the relationship between independent and dependent variables. They can be used as a 'black box' approach to create models of systems profiting of the facility to model non-linear (as well as linear) systems. The advantages of ANN are as follows: ease of use, rapid prototyping, high performance, minor assumptions, reduced expert knowledge required, non-linearity, multi-dimensionality and easy interpretation (Werner and Obach, 2001).

Self-Organizing Map is the most popular artificial neural network algorithm model in the unsupervised learning category. The model was first described as an artificial neural network by the Finnish professor Teuvo Kohonen, and is sometimes called a Kohonen map. About 4000 research articles on it have appeared in the open literature, and many industrial projects use the SOM as a tool for solving hard real-world problems (Kohonen, 2001). Internet search for SOMs turned up 491000 articles in 0.28 seconds. Historically, many fields of science have adopted the SOM as a standard analytical tool: statistics, signal processing, control theory, financial analysis, experimental physics, chemistry and medicine. Considering that SOM solves difficult high-dimensional and nonlinear problems, application of this model to a new area such as constructed treatment wetlands with associated complex wastewater engineering processes control problems is justifiable and makes SOM the ANN of choice in this study.

Constructed treatment wetlands are often seen as complex 'black box' systems, and the processes within an experimental wetland are difficult to model due to the complexity of the relationships between most water quality variables (Gernaey *et al*., 2004). However, it is necessary to monitor, control and predict the treatment processes to meet environmental and sustainability policies, and regulatory requirements such as secondary wastewater treatment standards (Scholz, 2006). Studies have shown that ANN could be applied to establish a mathematical relationship between variables describing a process state and different measured quantities. ANNs such as feed-forward neural networks were developed to predict the effluent concentrations including BOD and COD for wastewater treatment plants (Grieu *et al*., 2005; Hamed *et al*., 2004; Onkal-Engin *et al*., 2005), and to control water treatment processes automatically by modeling the alum dose (Maier *et al*., 2004). The measurement of variables such as COD and BOD which were widely applied for wastewater treatment monitoring to gain proper insight of individual efficiency of the wetlands and general indication of the water quality status. However, taking their measurements can both be expensive (measurements are labour intensive and capital costs of modern on-line equipment are relatively high; approximately £18,000 for COD and £16,000 for BOD) and only of historical value (for BOD_5, results are not available until five days after the sample has been taken). Moreover, it takes at least two and four hours of costly manual labour to obtain Benzene and COD concentrations respectively. Therefore, an indirect method of prediction and monitoring of COD, BOD and Benzene, if it could be made reliable enough, would be advantageous. This could also be used toward real time online monitoring of these key variables in field scale application. Lu and Wang (2005) observed that although ANN methods are cost-effective and highly reliable in analyzing processes, the traditional neural networks have suffered from their inherent drawbacks; i.e. over-training, local minima, poor generalization and difficulties in their practical application.

7.4. Self-organizing map

The Self Organizing Map is an excellent tool in the visualization of high dimensional data. The SOM uses powerful pattern analysis and clustering methods, and at the same time provides excellent visualization capabilities (Garcia and Gonzalez, 2004). The goodness of SOM lies on an unsupervised learning algorithm to establish the relationships among process variables. Mukherjee (1997) stated that the term ‘self organizing’ refers to the ability to learn and organize information without being given the corresponding dependent output values for the input pattern. Lu and Lo (2002) observed that SOM is able to map a structured, highly dimensional data set onto a much lower dimensional network in an ‘orderly’ fashion, and organizes itself by adjusting the weights according to the input patterns. The SOM offers the distinctive ability to gather knowledge by detecting the patterns and relationships from a given data set, learning from relationships and adapting to change. Hong *et al*., (2002) reveals that the SOM potentially outperforms current methods of analysis, because it can successfully deal with the non-linearity of a system, handle ‘noisy’ or irregular data and be easily updated.

Several interesting approaches of SOM have been reported in water quality assessment by various researchers. Verdenius and Broeze (1999) used SOM model as an indexing mechanism in case-based reasoning algorithms to control wastewater treatment processes, and it was employed to diagnose the diverse states of a wastewater treatment plant (Garcia and Gonzalez, 2004; Hong *et al*., 2002). Furthermore, the SOM models were developed to evaluate the state of water quality of a reservoir, and to predict the trophic status of coastal waters, showing a strong ability to identify the diversity between data (Aguilera *et al*., 2001; Gervrey *et al*., 2004). These studies demonstrated that the SOM can assist a process engineer by analyzing multidimensional data and simplifying them into visual information that can be easily applied to control plant performance. However, applications of SOM in water treatment process control are relatively new and were not implemented as much as traditional neural networks such as free forward neural networks (Grieu *et al*., 2005; Hamed *et al*., 2004).

A SOM consists of neurons, which are connected to adjacent neurons by neighborhood relations. In the training step, one vector x from the input set is chosen and all the weight vectors of the SOM are calculated using some distance measure such as the Euclidian distance (Kohonen, 2001). The neuron, whose weight vector is closest to the input x is called the best-matching unit (BMU), subscripted here by c (Equation 7-1):

$$||x - mc|| = \min\{||x - mi||\} \qquad 7\text{-}1$$

Where:

x = input vector;

m = weight vector; and

|| || = the distance measure.

After finding the BMU, the weighting vectors of the SOM are updated, so that the BMU is moved closer to the input vector. This adaptation procedure stretches the BMU and its topological neighbors towards the input vector as shown in Figure 7-1. The SOM update rule for the weight vector of a unit is shown in Equation 7-2.

The detailed algorithm of the SOM can be found in Kohonen (2001) for theoretical considerations:

$$m_i\,(t+1) = m_i\,(t) + \alpha\,(t)\,h_{ci}\,(t)\,[x\,(t) - m_t\,(t)] \qquad 7\text{-}2$$

Where:

m (t) = weight vector indicating the output unit's location in the data space at time t;

$\alpha(t)$ = the learning rate at time t;

h_{ci} (t) = the neighborhood kernel around the 'winner unit' c; and

x (t) = an input vector drawn from the input data set at time t.

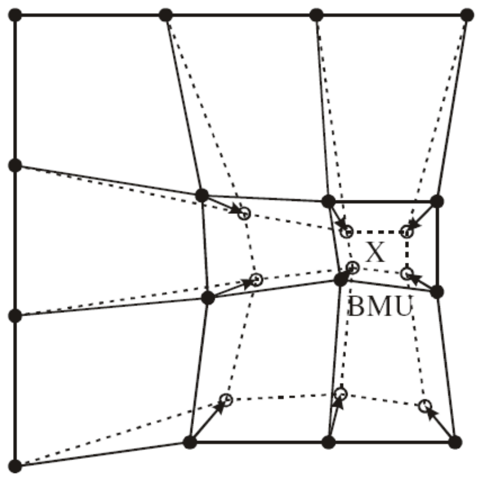


Figure 7-1. Updating the best matching unit (BMU) and its neighbors towards the input vector marked with x. The solids and dashed lines correspond to situations before and after updating respectively (based on Vesanto et al., 1999).

After the SOM has been trained, the map needs to be evaluated to find out if it has been optimally trained or if further training is required. The SOM quality is usually measured with two criteria: quantization error (QE) and topographic error (TE). The QE is the average distance between each data point and its BMU, and TE represents the proportion of all data for which the first and second BMU are not adjacent with respect to the measurement of topology preservation (Kohonen, 2001).

After training the map with different map sizes, the optimum map size was determined on the basis of the minimum QE and minimum TE. The prediction was implemented by finding BMU in the trained map for each test data set.

7.5. SOM applied to constructed wetlands treating hydrocarbon

The SOM model was applied using the data obtained indoor as well as outdoor rigs to identify the relationship between water quality variables and Benzene treatment efficiencies. SOM gives better understanding and had the potential to visualize the relationship between complex biochemical processes (Lee and Scholz 2006). The U-matrix visualizes distances between neighboring map units, and helps to identify the cluster structures of the map. Each component plane shows values for each variable with its corresponding unit. This section considered steps involved such as software requirement, training, testing of data sets and visualization of results.

7.5.1. Methodology and software

The basic software library for implementing the Self-Organizing Map algorithm is known as Somtoolbox. Somtoolbox is a public domain software package, written in C language for UNIX and PC environments. Somtoolbox used was developed by the Laboratory of Information and Computer Science at the Helsinki University of Technology Finland (Vesanto *et al*., 1999). This software package contains all programs necessary for the correct application of the SOM algorithm (Kohonen 2001) in the visualization and analysis of complex experimental data. This toolbox was used to preprocess data, initialize and train SOMs using a range of different kinds of topologies, visualize SOMs in various ways, and analyze the properties of the SOMs and data. The Toolbox can be downloaded for free from http://www.cis.hut.fi/projects/somtoolbox. Some data analysis simulators work in conjunction with other computational environments, such as Microsoft Excel or Matlab. Somtoolbox requires no other toolboxes, just the basic functions of Matlab computing environment. Matlab version R2007a supplied by MathWorks, Inc. (3 Apple Hill Drive Natick, Massachusetts, MA 01760-2098, USA) was used for this analysis.

7.5.2. Training and testing of data sets

Like most artificial neural networks, SOMs operate in two modes: training and mapping. Training builds the map using input examples. It is a competitive process, also called vector quantization. Mapping automatically classifies a new input vector. This makes SOM useful for visualizing low-dimensional views of high-dimensional data, akin to multidimensional scaling.

Experimental data were collected by monitoring the effluent concentrations of the wetlands for over two years (08/04/05 to 18/10/07). The amount of data points used was comparable and even greater than those used in other prediction models (Aguilera *et al*., 2001; Liu *et al*., 2004). In a first stage of the selection of process variables, a selection of the most significant variables has to be carried out. Due to this, the physical process itself was carefully studied and the variables, which have a higher degree of influence on the quality of the treatment process, were chosen. The training of the variables, which are significant to the process condition, is very important. Here, raw data were not taken as input variables to the learning process in a direct way, but their mean value was used. This leads to a much better performance for the SOM. The input variables were; turbidity (NTU), conductivity (μS), redox (mV), atmospheric temperature (°C), DO (mg/l) and pH (-), and output variables; BOD (mg/l), COD (mg/l), nutrients (mg/l) and Benzene (mg/l) were both stored in the data base. The input variables were selected according to their goodness of correlation (Scholz, 2006) with COD, BOD, nutrients and Benzene, because they were more cost-effective and easier to measure in comparison to the output variables.

The SOM could be thought of as a net which is spread to the data cloud. The SOM training algorithm moves the weight vectors so that they span across the data cloud and so that the map is organized: neighboring neurons on the grid get similar weight vectors (Vesanto *et al*., 1999). In the traditional sequential training, samples are presented to the map one at a time, and the algorithm gradually moves the weight vectors towards them. The overall data set was initialized and divided into training and testing data sub-sets. There is a graphical user interface tool for initializing and training SOMs. SOM model was tested for each data sub-set associated with selected wetland filter (Wetlands 1, 3 and 5). The training was performed with the data belonging to the six wetlands contaminated with benzene. The validation process was therefore undertaken with independent data sub-sets that were partly significantly different to the testing data sub-set. For this purpose, a set consisting of input and output vector pairs as mentioned in paragraph one above were required. From this set, some pairs are used for the training, wherein the parameters are adjusted during training in order to minimize the prediction error. After that the network is tested or cross-validated with the remaining pairs.

7.5.3. Visualization of results

Self-organizing map was used to visualize the data structure. Once the SOM has converged, it stores the most relevant information about the process in its codebook vectors. The visualization process allows all this information to be displayed in several ways (U-matrix and the component planes). Interneuron distance matrix (U-matrix) reveals the most important clusters present in the process data. The U-matrix visualizes distances between neighboring map units, and thus shows the cluster structure of the map: high values of the U-matrix indicate a cluster border, uniform areas of low values indicate clusters themselves (Vesanto *et al*., 1999). Each component plane shows the values of one variable in each map unit. On top of these visualizations, additional information can be shown: labels, data histograms and trajectories. The visualization of a data set simply consists of a set of objects, each with a unique position, color and shape.

The result (Figure 7-2) shows overall high performance as there is clear visualization of the relationship between effluent benzene concentrations in wetlands operated indoors and those operated outdoors. This is in agreement with findings of other researchers which states that SOM model showed its high performance in visualization of relationship for non-linear and complex biochemical data sets (Lu and Lo, 2002; Garcia and González, 2004). The U-matrix visualizes distances between neighboring map units, and helps to identify the cluster structures of the map. Each component plane shows values for each variable with its corresponding unit. The SOM map (Figure 7-2) shows that effluent Benzene concentrations for the wetlands operated indoor are directly associated with the corresponding outdoor wetlands. Further observation of the wetlands operated indoor shows relatively lower effluent Benzene concentrations. However, wetland filter 5 operated outdoors, shown in the low and left part in the map, shows impressive better performance in comparison to the corresponding filter 5 indoor.

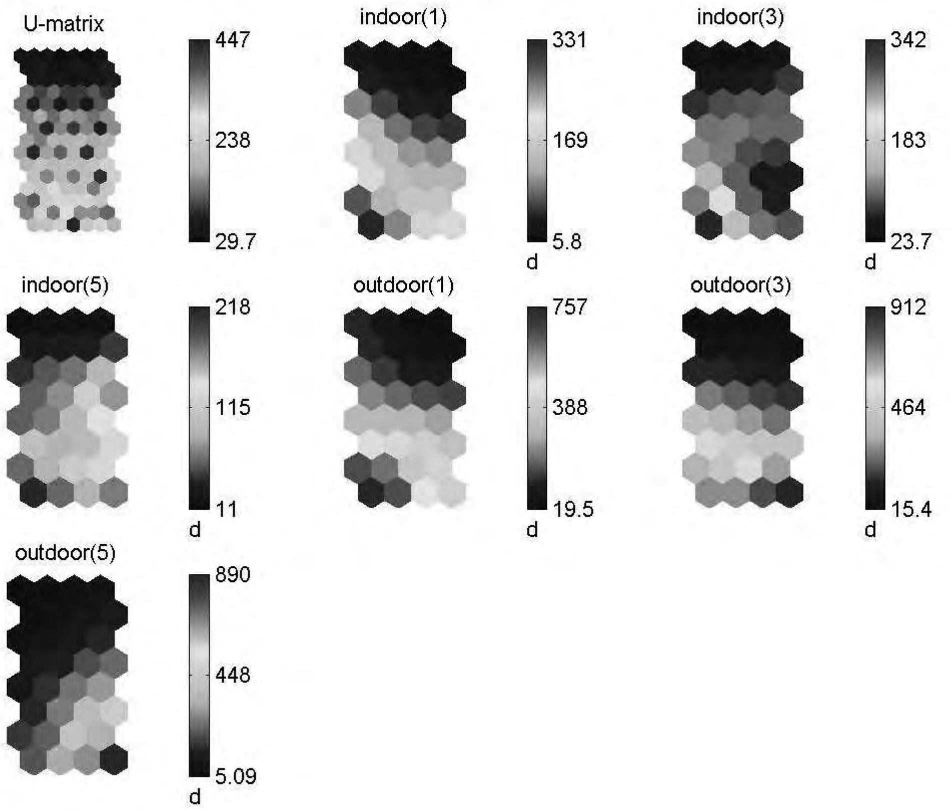


Figure 7-2. Self-Organizing Map visualizing relationship between effluent Benzene concentrations in indoor and outdoor wetlands. U-matrix on top left, then component planes. The seven figures are linked by position: in each figure, the hexagon in a certain position corresponds to the same map unit (Map in original colour documented in appendix B).

Figure 7-3 showed clear visualization of the relationship between benzene and other water quality variables. The SOM map shows that effluent Benzene concentrations as shown in the upper and left part in the map, are directly associated with Nitrate-Nitrogen, COD and associated partially with influent BOD concentrations.

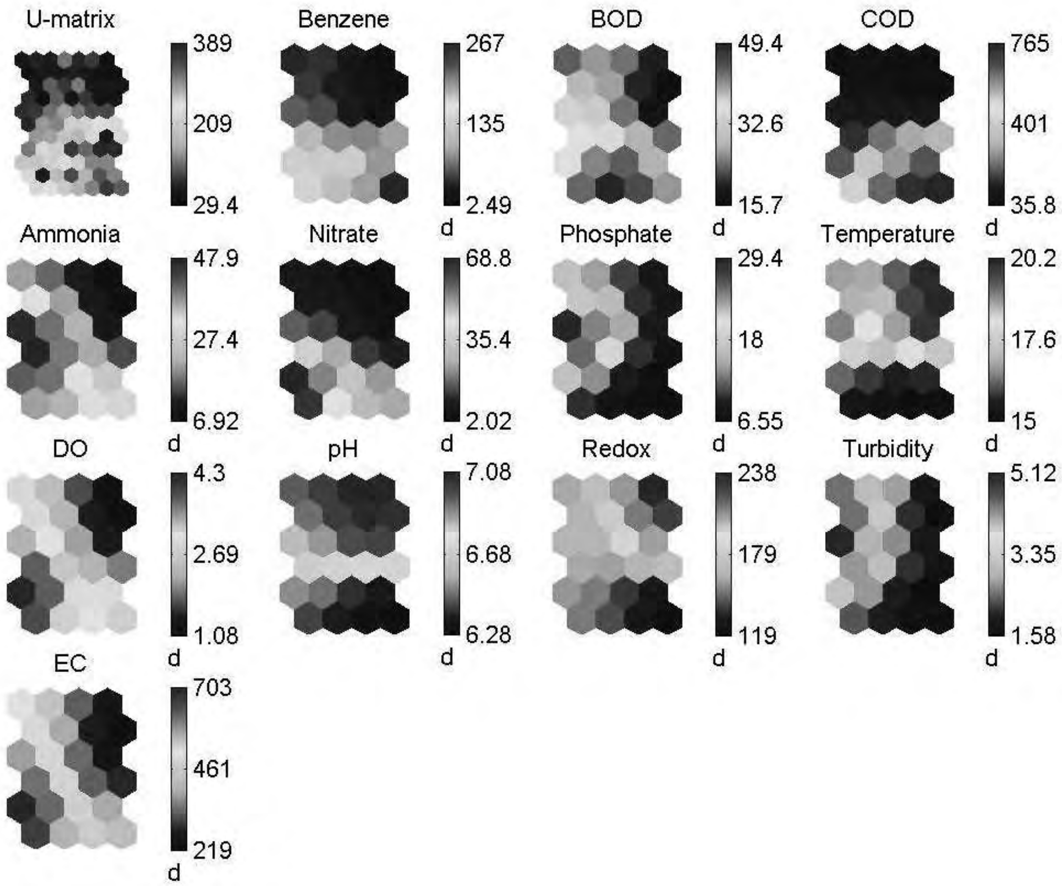


Figure 7-3. Self-Organizing Map visualizing relationship between effluent water quality variables and effluent Benzene concentrations in wetland 1 indoor (Map in original colour documented in appendix B).

Further observation of the indoor variables shows that the relatively high effluent pH did not link to the high effluent Benzene concentrations. Effluent pH (>6) rather linked with relatively low effluent Benzene (<3) concentrations. This clearly indicates that high pH did not harm the microorganism responsible for Benzene degradation during the processes. Effluent Ammonia-Nitrogen, Ortho-phosphate-phosphorus, dissolved oxygen and conductivity, turbidity concentrations show weak link to effluent Benzene concentration. Effluent redox concentration did not link to effluent Benzene concentration. Moderate temperature (>15) did not show any significant relation with the effluent Benzene concentrations in the SOM map (Figure 7-3) but rather indicates that high temperature did not increase Benzene reduction in the wetland. The map also indicates that any temperature ranging from 15 ^{o}C could favour the performance of constructed wetland treating hydrocarbon.

The observed non-effect of temperature in the wetlands is in agreement with previous observation described by Kedlec and Knight (1996), they believed that slowed removal in winter season may not differ considerably from that observed in more biologically active warmer months. This result is in contrast with some researchers that observed negative effect of cold climate on the performance of wetlands (Leonard, 2000; Karathanasis *et al*., 2003), while some have suggested negligible temperature dependence (Kadlec and Knight, 1996). However, Werker *et al* (2002) observed that soil microbes still have the capacity to decompose organic matter in low temperature conditions, even though dormant vegetation and a slow reaction for microbes may reduce biological removal process within the wetland.

In comparison to figure 7-3, figure 7-4 presents the relationship between effluent Benzene concentrations and effluent water quality concentrations in the rig operated outdoor. The SOM map show that effluent Benzene concentrations associated with effluent water quality variables similarly. However, with exceptions of effluent turbidity concentrations that does not link to effluent Benzene concentrations outdoor. Moreover, SOM map of the outdoor filter 1 shows that temperature (<12 ^{o}C) and pH (<6.7) are apparently linked with low effluent Benzene concentrations (Figure 7-4).

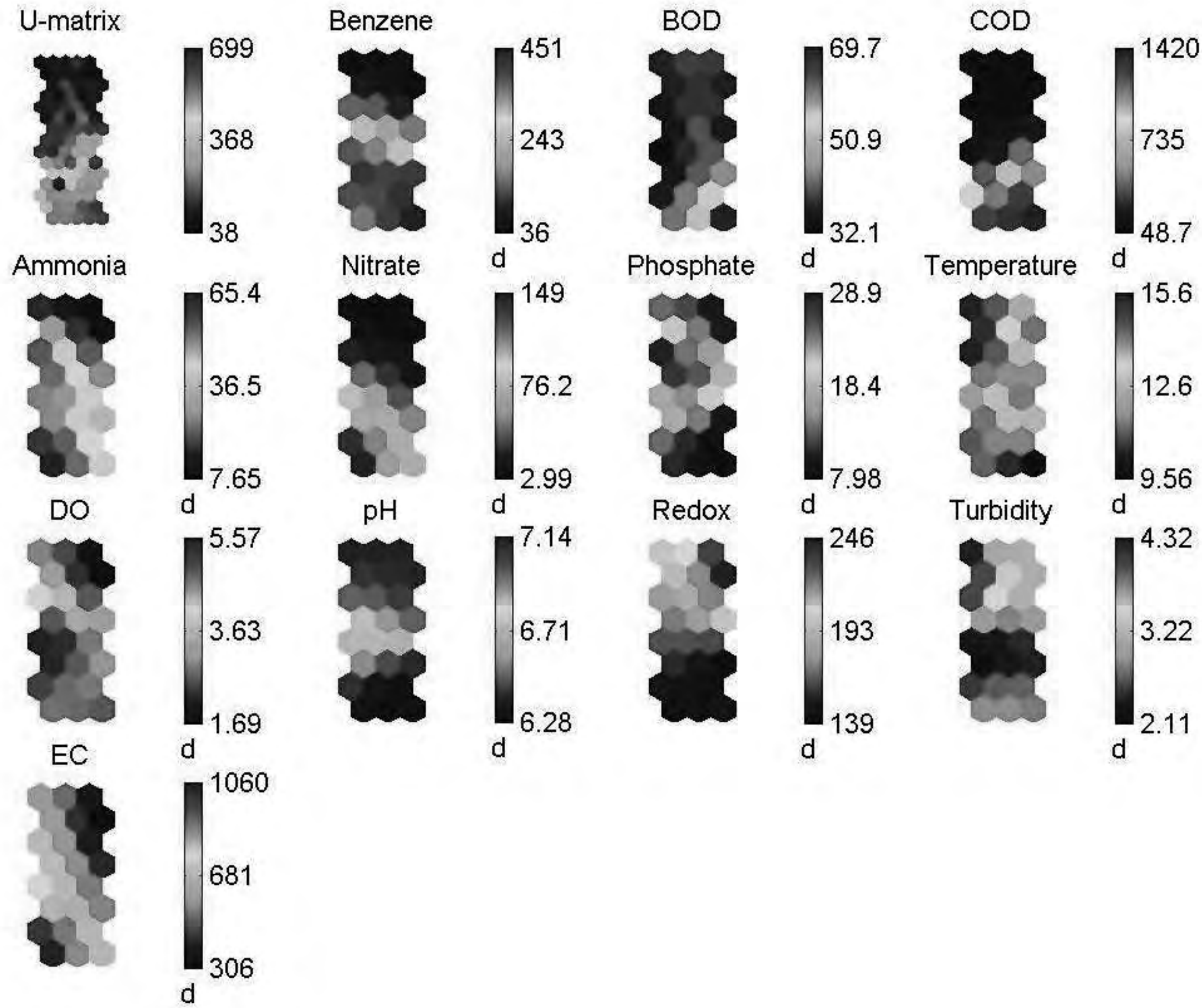


Figure 7.4. Self-Organizing Map showing relationship between effluent water quality variables and Benzene concentrations in wetland 1 outdoor (Map in original colour documented in appendix B).

7.6. Multivariable testing and simulation

Data obtained in this research have been subjected to descriptive and inferential statistical analysis (such as mean, standard deviation, ANOVA and time series presented in chapter 4) for the purpose of individual examination of the data. This subsection focus more on inferential statistics that is used to simulate and model patterns in the data (multiple variables) and drawing inferences about the internal working of the constructed treatment wetlands. These inferences integrated descriptions of association (correlation) with modeling of relationships (regression) to support constructed wetland operations and management.

Data collected from the indoor and outdoor constructed wetlands 1, 3 and 5 (all contaminated with benzene) were used for these statistical analysis and simulation. This is also an attempt to advance the understanding of the relationships between benzene removal and other water quality variables. Multiple regression analysis was carried out as statistical models. All statistic tests in this subsection were performed with the software SPSS (SPSS, 2003) including a one-way analysis of variance (ANOVA). According to the coefficients of determination (R) for benzene effluent concentration and other water quality variables, easily and inexpensive to measure variables (optimal regressors) such as DO, EC, temperature, pH and redox were chosen to predict benzene removal by multivariate linear regression analysis. Correlation coefficients (R^2) and p value were calculated to assess the linear relationships between variables. Differences were regarded as significant at $p \leq 0.05$ (Tao et al., 2007).

7.6.1. Correlation analysis and Multivariable regression

Correlation analysis measures the relationship between two items. Table 7-1 presented correlation matrix presented for all the variables in this study. Though correlation and causation are connected as observed in this analysis but "Correlation does not *imply* causation". However, correlation is needed for causation to be proved. It is pertinent to note that though the correlation coefficient provide useful summary statistic, it cannot replace the individual examination of the data (already done in chapters 4, 5 and 6).

A linear regression analysis was initiated to test the relationships between each variable and the effluent benzene concentration. The indoor wetland 1 was selected as an example. The coefficients of the corresponding correlation matrix for all variables are shown in Table 7-1. The effluent benzene concentrations were positively correlated with the effluent COD and DO concentrations with correlation coefficients of 0.557 and 0.590, respectively. However, the pH and temperature negatively correlated with the effluent benzene concentration and the correlation coefficients were-0.690 and -0.559, respectively.

In present study, BOD and COD removal were monitored in constructed wetlands treating benzene with influent concentrations of 1g/l. It was found that BOD removal was positively correlated with COD removal (R=0.601). This relationship was also observed in other wetland studies for domestic and municipal wastewater treatment (Merlin, 2002).

Anaerobic degradation of organic compounds occurs when oxygen is limiting at high organic loading rates (Cooper et al., 1996). Considering that the DO concentration of the indoor wetland 1 was 2.72±1.63mg/l, the low availability of oxygen suggests that anaerobic degradation is likely to be the most possible approach for COD removal. Previous studies indicated that the optimal pH range was between 6.5 and 7.5 for anaerobic degradation of organic compounds (Vymazal, 1999). The over-production of acid by acid formers (such as strictly anaerobic sulfate-reducing and methane-forming bacteria) can rapidly result in a low pH and in the reduction of the organic compounds removal efficiency. The pH range was 6.67±0.43 (Table 4-3) for the selected wetland 1, which corresponded well with the optimal range (6.5-7.5) for anaerobic degradation (Vymazal, 1999). An increase of the COD effluent concentrations was observed with a decrease of the pH values. A negative correlation coefficient (R=-0.802; Table 7-1) between the COD effluent concentrations and the pH values supported the above assumption.

Nitrogen and phosphorus removal processes within wetlands have been discussed previously (Kadlec and Knight, 1996; Scholz et al., 2002; Vymazal, 2007). In comparison, the impact of PO_4^{3-}, NH_4-N and NO_3-N on benzene removal was evaluated in this paper. The phosphorus removal is predominantly associated with the physical and chemical properties of the wetland aggregates. Phosphorus may precipitate within the aggregates and is often adsorbed by wetland media (Kadlec and Knight, 1996; Vymazal, 2007). Biological removal is another pathway for phosphorus reduction. Behrends et al. (2001) reported that the removal of phosphorus by microorganisms can reach between10 and 12%. Ammonia is usually used as a nitrogen source for microorganism growth and propagation, and microbial biomass may contain up tom 12% nitrogen (Cannon et al., 2000).

Table 7-1. Correlation matrix for all the variables (take indoor wetland 1 for example)

	Benzene	BOD	COD	PO_4^{3-}	NO_3-N	NH_4-N	T	DO	pH	EC	Redox	Turbidity
Benzene	1.000											
BOD	0.245	1.000										
COD	0.557	0.601	1.000									
PO_4^{3-}	0.015	0.105	-0.146	1.000								
NO_3-N	0.159	0.295	0.242	0.024	1.000							
NH_4-N	0.180	0.381	0.213	0.841	0.412	1.000						
T	-0.559	-0.359	-0.506	-0.295	-0.401	-0.499	1.000					
DO	0.590	0.365	0.265	0.444	0.386	0.603	-0.705	1.000				
pH	-0.690	-0.537	-0.802	-0.167	-0.416	-0.495	0.714	-0.635	1.000			
EC	0.368	0.438	0.187	0.194	0.528	0.387	-0.272	0.682	-0.398	1.000		
Redox	-0.487	-0.349	-0.560	-0.155	-0.318	-0.367	0.575	-0.634	0.648	-0.503	1.000	
Turbidity	-0.173	0.159	-0.223	0.020	-0.137	-0.065	0.555	-0.059	0.276	0.453	-0.036	1.000

Table 7-1 showed that PO_4^{3-} removal was positive correlated with NH_4-N (R=0.841). It follows that ammonia-nitrogen and ortho-phosphate-phosphorus can be simultaneously removed by microorganisms in wetlands. Nitrite is closely related to ammonia, as most of nitrite is produced through nitrification of ammonia (Sun et al., 2005; Vymazal, 2007). The NO_3-N effluent concentration increased with an increase in NH_4-N effluent concentration (R=0.412; Table 7-1), as shown in previous literature (Sun et al., 2005; Vymazal, 2007). Increased oxygen availability can stimulate nitrifying bacteria and enhance NH_4-N removal (Scholz et al., 2002). A correlation coefficient of 0.602 between NH_4-N and DO indicated that higher oxygen availability facilitates NH_4-N removal even if benzene is present.

Figure 7-1 shows correlations between benzene and other water quality variables. Positive and negative correlation coefficients have the following orders: COD>DO>EC>NO_3-N>BOD and pH> redox> temperature> turbidity, respectively. Very weak correlations between benzene and both PO_4^{3-} and NH_4-N were calculated.

Although PO_4^{3-} and NH_4-N had no significance on benzene removal (Table 7-1), positive correlation coefficients for BOD, COD and NO_3-N suggest that microbial activity and hydrocarbon removal efficiency were indirectly improved by the utilization of slowly released fertilizer. This support previous finding which states that the continuous supply of nutrients can maintain sufficient microbial activity and subsequently relatively high hydrocarbon removal efficiency (Riser-Roberts, 1992; Xu et al., 2003).

Mean pH values were between 6.38 and 6.70 (Table 4-3) Effluent benzene concentrations were negatively correlated with this pH range (Table 7-1). Lu et al. (2002) reported that the benzene removal efficiency increased with an increase of pH in the pH range between 5 and 8. Their finding was confirmed by this study.

Oxygen is another key factor impacting on benzene removal. Lu et al (2002) found out that DO concentrations decreased with decreasing effluent benzene concentrations. Their conclusion was supported by this study. The DO correlated positively with the effluent benzene; the correlation coefficient was 0.50 (Table 7-1).

Figure 7-5 summarizes the outcomes of a correlation analysis for the most important water quality variables. A regression analysis was conducted to determine time-consuming and expensive water quality variables such as benzene and BOD with easy and cheaply to determine variables such as DO, EC, redox, T (temperature) and pH.

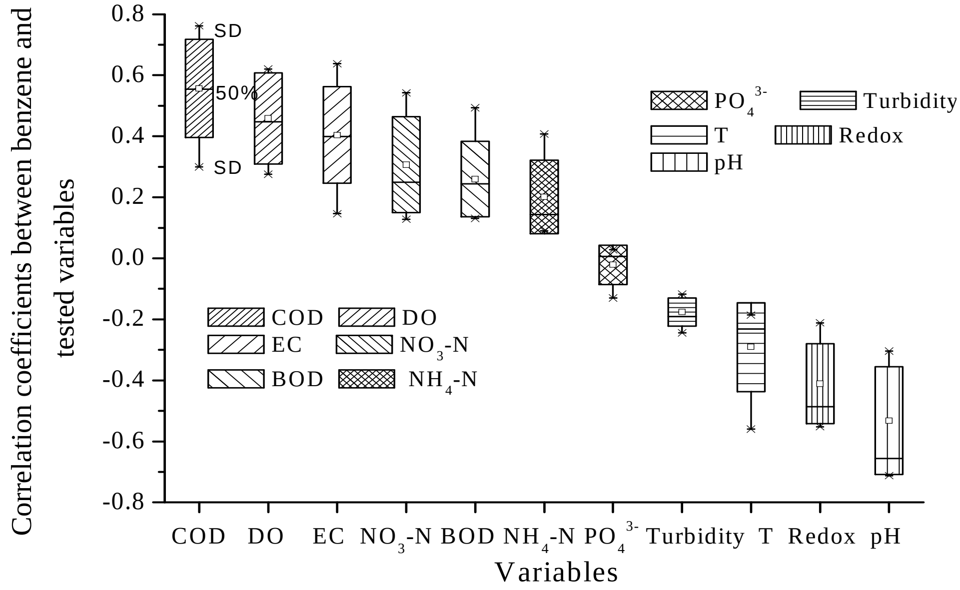


Figure 7-5. Distribution of personal correlation coefficient between benzene and water quality variables, S D, standard derivation, squares in each box center were the average values, and the line means 50% of the value range.

Monthly mean data were used, and the findings of the regression analysis are shown in Table 7-2. Weak correlation coefficients (R^2) indicated that both the indoor and outdoor wetlands 5 (controls) were unsuitable for the regression model. The effluent benzene concentrations for the indoor and outdoor planted wetlands were predicted (R^2 of 0.748 and 0.714, respectively; $p<0.05$; Table 7-2) with the five selected variables DO, EC, redox, T and pH. These results successful demonstrated the possibility of real time optimization and control of constructed wetland processes, which could be applied to field scale.

Table 7-2. Multivariable linear regression equations of selected constructed wetlands

n	linear regression equations	R^2	p
30	1In _Benzene=1413.57+16.33DO+0.04EC+0.28Redox-204.36pH-2.69T	0.748	0.001
30	3In _Benzene=764.76-2.51DO+0.30EC+0.23Redox-186.26pH+20.45T	0.663	0.009
30	5In _Benzene=957.03-1.55DO-0.014EC+0.25Redox-200.74pH-22.76T	0.482	0.021
30	1Out _Benzene=1878.16+32.41DO-0.03EC-0.33Redox-254.48pH-1.59T	0.714	0.002
30	3Out _Benzene=3544.26-12.62DO+0.04EC-0.53Redox-445.58pH-12.61T	0.724	0.001
30	5Out _Benzene=-764.49+32.13DO+0.21EC-1.21Redox+132.78pH-3.13T	0.538	0.019

In and Out represent indoor and outdoor selected constructed wetlands; N, sample number; DO, dissolved oxygen (mg/l); EC, electronic conductivity (μS); Redox, potential of reduction/oxidation reaction (mV); T, temperature (ºC); R^2, correlated coefficients; correlation and difference were considered significant at $P \leq 0.05$.

Mean values of benzene and other water quality variables were used to conduct a hierarchical cluster analysis to visualize and explore complex data sets as well as to quantify the overall performance of the selected wetlands. The basic aim of the cluster analysis is to represent the (dis)similarity between wetlands, so that similar wetlands are depicted near from each other and dissimilar wetland are found further apart from each other. The dendrogram in Fig. 7-6 indicates that both indoor and outdoor wetlands form two separate clusters. The control of environmental factors such as temperature, light and humidity did impact on the wetland performance. The formation of a separate cluster by the indoor wetlands 1 and 5 suggests that aggregates and wetland plants have no important impact on wetland processes under stable indoor conditions. In contrast, the outdoor wetlands 1 and 3 form another separate cluster implying that aggregates and plants play an indirect role for pollutant removal in constructed wetlands located in natural environments. This finding is well supported by previous researches focusing on substrates and plant studies (Kadlec and Knight, 1996; Lee and Scholz, 2006; Vymazal, 2007).

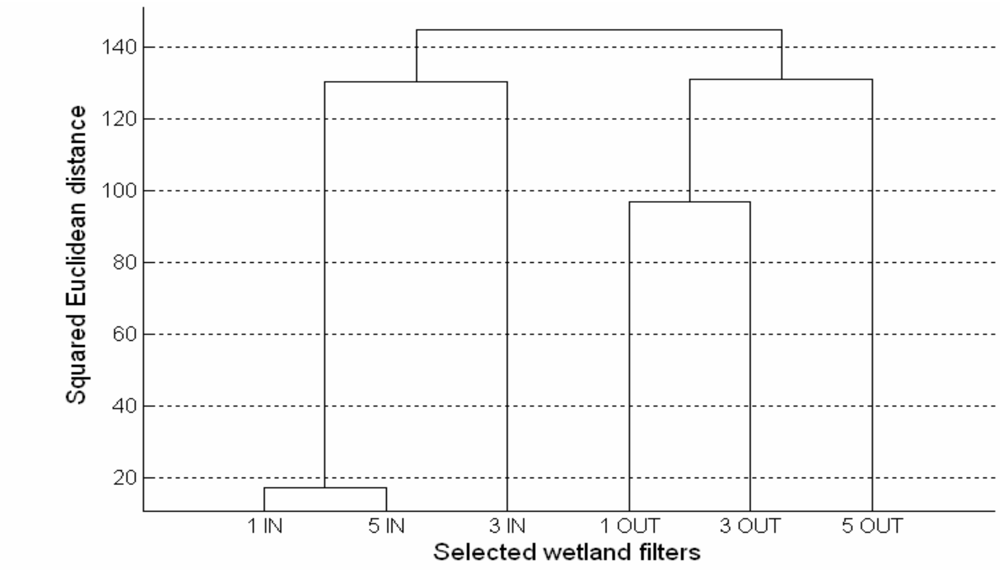


Figure 7-6. Hierarchical cluster dendrogram of all the selected constructed wetlands, IN and OUT are short for indoor and outdoor, respectively.

These performances are encouraging and support the potential for future use of these models as management tools for the day-to-day process control of constructed wetlands and other complex biochemical systems.

7.7. Large Scale Constructed Wetlands applied for Hydrocarbon Treatment: Case Studies

Feasibility of using of large scale constructed wetlands for hydrocarbon treatment case studies has been conducted by Wemple et al., (2000) and concluded that petroleum wastes naturally degrade in wetland environments. Despite the lack of widespread use of constructed wetlands to treat produced and wastewater from oil and gas facilities, many pilot scale and some large scale systems are in existence. Both surface-flow and subsurface-flow constructed wetlands have been used to treat petroleum wastewaters (American Petroleum Institute, 1998).

Some cases of working facilities are presented to show that the theory of constructed wetlands to treat hydrocarbons can be put into practice, supported by Lin et al., (2003), who stated that a small series of wetlands can be compared directly with a large wetland. A produced water treatment wetland in a Wyoming oilfield replaced disposal wells which treated up to 10,000 barrels of produced water per day at an annual cost of $185,000. The installation of the wetland system and associated facilities allowed the treatment of 35000 barrels of produced water per day at a cost of less than $10,000 per year, saving $175,000/year (Myers et al., 2001). The facility reported 95-100% hydrocarbon removal, which coincides with findings from this research (KB experimental Wetlands).

Having tried many unsuccessful biological treatment processes at the Williams Pipeline Company terminal in South Dakota, a planted constructed wetland facility was installed with aeration facilities to treat produced water separated from oil. BTEX compounds were the main issue for removal and the high BOD and Ammonia content of the water caused problems in other onsite treatment facilities. The study suggests that volatilization and biodegradation play key role in the hydrocarbon removal process (Wallace, 2001).

Presently, a wetland system implemented by British Petroleum (BP) in Casper, Wyoming (Wallace and Kadlec, 2005) was the largest and most recent remediation wetland in the United States. The treatment system had to handle up to three million gallons (3MGD) of gasoline-contaminated groundwater per day (Liner, 2006).

Recently, Atlantic Richfield Company (ARCO), Wellsville, New York designed (summer 2007) wetland for the removal of BTEX and iron contaminated wastewater (1000 m^3/d) from a former refinery site along the Genessee River (personal communication from Scott Wallace - my industrial advisor, ARCO wetland Process consultant and Executive Vice President of North American Wetland Engineering (NAWE)).

The above case studies demonstrate that up-scaling of the present research is feasible. However, constructed wetlands require large land which could limit the application in some area. Furthermore, improved understanding of internal processes for removal of hydrocarbon (BTEX) in constructed wetlands provided by this research will encourage more application of wetland technology in Petroleum industries. Presently, there are biases concerning constructed wetlands technology in the petroleum industry as it is ‘not yet ripe’, no in-depth special study has formed a complete system of knowledge as the critiques suggests. This in-depth research finding could reduce biases and limitations of several petroleum industries toward constructed wetland technology.

7.8. Limitations of the analysis

Environmental parameters and associated environmental heterogeneity in this research have been thoroughly quantified, all significant and measured variables have been considered. Exploratory multivariate analyses applied in this study also proved to be useful to reveal patterns of the large data sets in this study. However, it is essential to reiterate that multivariate statistical procedures may suggest causes or factors, but investigators should bear in mind that the synthetic variables, axes, or clusters derived do not necessarily correspond to biological or ecological entities in nature (James & McCulloch, 1990).

One should thus not over interpret the data by relying on unjustified causality, especially in the absence of real experimentation. In theory, it would be necessary to validate the inferences and models made about pattern formation and putative causes by analyzing new data, but this is rarely performed in practice. Moreover, whether the originally collected data are typical of the situation to be described should most of the time questioned.

Although exploratory analyses may help reveal interesting patterns in data sets as observed in this study, the interpretation and explanation of the observations ultimately rely on the researcher’s hypotheses and previous knowledge of the ecological situation. Researchers themselves need to formulate sound hypotheses and test them.

7.9. Summary

The findings in this chapter successfully established the potential use of SOM and multiple variable testing techniques as the management and optimization tools to enhance the understanding of 'black box' systems as well as reduce operation costs.

A statistical analysis indicated that the BOD, COD, NO_3-N, DO and EC values of the effluent were positively correlated with the effluent benzene concentrations following the order COD>DO>EC>NO_3-N>BOD, and negatively correlated according to the order pH> redox potential (redox)>temperature (T)>turbidity. No strong relationships between benzene and the variables ortho-phosphate-phosphorus (PO_4^{3-}) and ammonia-nitrogen (NH_4-N) were recorded.

A hierarchical cluster analysis indicated that the overall indoor wetland performance was significantly ($p<0.05$) better than the one for the outdoor wetlands. A positive correlation between the effluent nitrate-nitrogen (NO_3-N) and benzene concentrations suggested that an increase in NO_3-N removal improved benzene removal.
The order of positive and negative correlations between benzene and other water quality variables was as follows: chemical oxygen demand > dissolved oxygen (DO) > electrical conductivity (EC) > NO_3-N > five days at 20ºC N-allythiourea biochemical oxygen demand, and pH > redox potential (redox) > temperature (T) > turbidity. No strong relationships between benzene and both ortho-phosphate-phosphorus and ammonia-nitrogen were detected.

Finally, cost-effective and easily to measure online variables such as DO, EC, redox, T and pH were chosen for prediction of effluent benzene concentrations with a multivariable linear regression model. The artificial neural network such as self organizing map, correlation coefficients and multivariable regression results indicated that these models can be used as a promising tool for real time monitoring and prediction of benzene removal in planted constructed wetlands.

These performances are encouraging and support the potential for future use of these models as management tools for the day-to-day process control of constructed wetlands and other complex biochemical systems.

8

Conclusions

8.1. Overall conclusions

Hybrid (experimental vertical-flow with stabilization pond) experimental constructed wetland rigs were used to examine internal processes and effectiveness of wetlands in treating aromatic hydrocarbon. Furthermore, the artificial neural network and multivariable regression techniques were applied for operation, optimization and management of constructed wetlands and related complex biochemical processes. The overall results show that the hybrid wetlands operated in both environmental controlled laboratory and outdoors are highly efficient for the treatment of hydrocarbon and other water quality variables.

The key conclusions resulting from this study are summarized as follows:

(1) Intermittently flooded vertical-flow constructed wetlands treat petroleum hydrocarbons effectively in the presence of sufficient fertilizer, which provides nitrate as an alternative electron acceptor during anaerobic periods of full inundation. The successful removal of the aromatic hydrocarbon and other pollutants makes constructed

treatment wetland very attractive and sustainable technology capable of meeting zero discharge goal in the production, storage, refining and transportation sectors of the oil and gas industry (Chapters 4, 5 and 6) .

(2) The result of feasibility of improving (optimization) the performance of the hydrocarbon removal efficiency in constructed wetlands by increasing nutrient dosage investigated suggests that high rates of hydrocarbon treatment were indirectly linked with addition of an adequate dosage of fertilizer to the constructed wetlands. Too high fertilizer (30g/2wks of fertilizer for 1g of benzene) dosages were not associated with beneficial water treatment. The study proposed optimal design and operation guidelines for the use of fertilizers in the treatment of hydrocarbon (8g of the well balanced slow-releasing N-P-K Miracle-Gro fertilizer every second week to be the optimum dosage required for the treatment of 1g/l of benzene) (Chapters 5 and 6) .

(3) The results of investigations on variables and boundary conditions impacting on the operation and treatment performance (e.g. temperature, macrophytes and aggregates) were as follows:

The observed results show that benzene treatment in vertical-flow constructed wetlands did not always respond to temperature changes. Though temperature as a control variable was important because it influenced the concentration of benzene within the liquid phase, mobile (volatile) phase and the rate of microbial degradation, but generally overall suitable operating conditions were required. The results of the study also suggest that temperature though do not always correspond to removal efficiency trends but likely a significant control parameter for wetlands treating hydrocarbons.

Phragmites australis does not play a significant role in removing hydrocarbons. The results (tested with ANOVA) show that planted wetlands were not significantly better than unplanted ones in terms of benzene removal. This is likely to be linked to the very high corresponding inflow concentrations and sufficient nutrient availability. However, macrophytes played indirect role in the hydrocarbon removal by providing good filtration conditions, preventing the wetlands from clogging and providing surface for attachment of microbes. Filter media also played indirect role in the hydrocarbon removal.

(4) The results of investigations on role of environmental factors and seasonal variability on hydrocarbon were as follows:

The removal efficiencies for hydrocarbon in treatment wetlands operated in an environmental-controlled room are considerably higher than those efficiencies of corresponding systems operated outdoors. The result of microbes' examination show more hydrocarbon degrading microbes in the indoor rig which could be associated with improved removal efficiencies observed in indoor operated rig. This result also showcases the role of suitable environment for microbes responsible for degradation of hydrocarbon. This indicates that there is distinct environment for the microbes (as shown in the microbes HPC results in chapter 4) or seasonal changes required in conjunction with hydraulic retention time, temperature, dissolved oxygen, pH and nutrient enrichment to stimulate microorganisms to biodegrade hydrocarbon in constructed wetlands. However, the study in turn demonstrates that the extent of hydrocarbon biodegradation in constructed wetlands is critically dependent upon the creation of optimal environmental conditions to stimulate biodegradative activity.

The result of exclusive investigation on the seasonal internal interactions of benzene with other individual water quality variables in the constructed wetlands suggests the following conclusions:

The benzene removal efficiency was high (97-100%) during the first year of operation without visible seasonal variations. This suggests that the intermittently flooded vertical-flow constructed wetlands could be well adapted to the highly variable environmental conditions. However, seasonal variability in benzene removal was apparent after spring 2006. The highest benzene removal efficiency noted in spring and not in summer as expected indicates that the benzene removal did not solely depend on temperature (support finding conclusion number 5 above). No seasonal variability was detected in the effluent NH_4-N, NO_3-N and PO_4^{3-} concentrations.

(5) The result of additional study suggests that volatilization and biodegradation are major hydrocarbon removal mechanisms in the constructed wetlands.

(6) The monitored water quality variables show significant cumulative impact (hydrocarbon accumulations, stores and fluxes) associated with long time operation of experimental constructed wetlands treating hydrocarbons. This claim is visible on the results from second year of operation which shows the removal efficiencies subsequently reduced as hydrocarbon and its degradation products started to accumulate in the wetlands.

(7) The results of techniques and tools (Artificial Neural Network (SOM), Multivariable regression and hierarchical cluster analysis) demonstrated that these tools could be used for operation, optimization and management of constructed wetlands treating hydrocarbon, and could be applied to related complex biochemical processes. These tools were successfully used to assess variables and boundary conditions impacting on the operation and treatment performance (e.g. temperature, macrophytes and aggregates); and the efficiency of different filter set-ups in removing benzene, chemical oxygen demand, biochemical oxygen demand and nutrients.

SOM clearly visualized the relationship between benzene and other water quality variables. These findings successfully established the potential use of SOM and multiple variable testing techniques as the management and optimization tools to enhance the understanding of 'black box' systems as well as reduce operation costs.

Furthermore, the artificial neural network (SOM), correlation coefficients and multivariable regression results indicated that these models can be used as a promising tool for real time monitoring and prediction of hydrocarbon (benzene) removal in planted constructed wetlands. These also improved understanding of the physical and biochemical processes within vertical-flow constructed wetlands, particularly of the role of the different constituents of the constructed wetlands in removal of hydrocarbon. These techniques helped to provide answers to original research questions such as: What does the job? Physical design, filter media, macrophytes or micro-organisms?

These performances are encouraging and support the potential for future use of these models as management tools for the day-to-day process control of constructed wetlands and other complex biochemical systems (Chapter 7).

(8) The lessons learnt from the research cuts across various fields and were too numerous to mention but it is important to point out that:

The study provided various transferable skills especially in engineering design, process and operational control, environmental management and water quality (including microbiological) examinations. These skills were often demonstrated and shared with numerous visiting researchers from various parts of the world and final year project students that were part of the research team at various stages.

Finally, the study advanced our scientific knowledge as we gained a greater understanding of the governing processes (internal components, responses and interactions) that could be applied by the engineering community (especially those in petroleum industry) for optimum project design, operation and maintenance of constructed wetlands treating hydrocarbon in contaminated environments. Moreover, the study provides necessary information that will be useful to a wide range of users, regulators, various industries, consultants, researchers, and students.

8.2. Recommendations for future work

(1) While this project has demonstrated the potential for future use of constructed wetlands for treatment of hydrocarbon, there is an obvious need for numerical process modeling to predict removal rate in the field scale constructed wetlands treating hydrocarbon. This is strongly suggested for further studies.

(2) Findings indicate that both biodegradation and volatilization support hydrocarbon treatment in constructed wetlands. Further research should focus on the specification of biodegradation products and quantification of the proportion of hydrocarbons being lost through volatilization to the atmosphere under varying temperatures and other environmental conditions in field-scale constructed treatment wetlands.

(3) The causes of potential treatment efficiency decline of hydrocarbon removal during long-term experimental studies need to be assessed further more preferably in field scale wetlands for about eight years. The use of a multi-stage or integrated wetland system for benzene treatment is suggested as a better option that could address the decrease in treatment efficiency observed based on the use of a single constructed wetland from engineering point of view.

(4) Comprehensive field scale microbiological examination such as the microbial population dynamics is also recommended to quantify the microbiological potential in comparison to physical and chemical processes in constructed wetland. The establishment of healthy plant, hydrocarbon and microbial food chain network in the large scale constructed wetlands need to be investigated.

Reference

Adachi, A., Ikeda, C., Takagi, S., Fukao, N., Yoshie, E. and Okano, T. (2001). Efficiency of rice bran for removal of organochlorine compounds and benzene from industrial wastewater. Journal of Agricultural and Food Chemistry, 49 (3), 1309-1314.

Adachi, A., Yatani, Y. and Okano, T. (2003). Efficiency of wheat bran for removal of organochlorine compounds and benzene from solution. Bulletin of Environmental Contamination and Toxicology, 71(2), 375-378.

Admire, J. D., De Albuquerque, J. S., Cruze, J. A., Piontek, K. R. and Sale, T. C. (1995). Case Study: Natural Attenuation of Dissolved Hydrocarbons at a Former Gas Plant. Paper SPE 29755 presented in SPE/EPA Exploration and Production Environmental Conference held 27-29 March in Houston, Texas.

Aguilera, P. A., Frenich, A. G., Torres, J. A., Castro, H., Vidal J. L. M. and Canton, M. (2001). Application of the Kohonen Neural Network in Coastal Water Management: Methodological Development for the Assessment and Prediction of Water Quality. Water Res. 35(17), 4053-4062.

Ahn, C. H. 1999. The characteristics of crude oil biodegradation in sand columns under tidal cycles. M.S. Thesis, University of Cincinati, OH, USA.

Alexander, M. (1999). Biodegradation and Bioremediation. 2nd Edition, Academic Press, New York.

Allen, S. E. (1974). Chemical Analysis of Ecological Materials. Blackwell, Oxford, UK.

Allen, W. C., Hook, P. B., Biederman, J. A. and Stein, O. R. (2002). Wetland aquatic processes; Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. J. Environm. Qual., 31(3), 1010-1016.

Alper, H. (2003). Removal of oils and organic compounds from water and air with MYCELX HRM (Hydrocarbon Removal Matrix) technology. Federal Facilities Environmental Journal. 14(3), 79-101.

American Petroleum Institute, (1998). The Use of Treatment Wetlands for Petroleum Industry Effluents; Prepared for the API Biomonitoring Task Force by CH2M HILL. API Publication No. 4672.Washington, DC, USA.

Anderson, R. T. and Lovley, D. R. (1997). Ecology and biogeochemistry of in situ groundwater bioremediation. Adv. Microbial Ecol. 15, 289-350.

APHA, (1998). Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association/American Water Works Association/ Water Environment Federation, Washington DC, USA.

Aronson, D. and Howard, P. H. (1997). Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies. Environmental Science Center, North Syracuse, New York, USA.

Armstrong, W., Armstrong, J. and Beckett, P. M. (1990). Measurement and modeling of oxygen release from roots of *Phragmites australis*. In: P.F. Cooper and B.C. Findlater (Eds), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, p. 41–51.

Association of Oil and Gas Producers, (2002). Aromatics in produced water: occurrence, fate and effects, and treatment. Report No. 1.20/324, International Association of Oil and Gas Producers, London.

Atlas, R. M. (1993). Handbook of Microbiological Media. CRC Press, Florida.

Altlas, R. M. (1995). Principles of Microbiology. Mosby-Year Book, Missouri.

Atlas, R. M. and Bartha, R. (1992). Hydrocarbon biodegradation and oil spill bioremediation. Advances in Microbiology and Ecology 12, 287–338.

Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microb. Rev. 45(1), 180-209.

Atlas, R. M. and Cerniglia, C. E. (1995). Bioremediation of Petroleum Pollutants. Biosorption and Bioremediation, 45 (1-3), 332-338.

Babatunde, A. O. and Zhao, Y. Q. (2007). Constructive approaches towards water treatment works sludge management: An international review of beneficial reuses. Crit. Rev. in Env. Sci and Tech. 37(2), 129-164.

Balizon, M. E., Dolmus, R., Quintana, J., Navarro Y. and Donze M. (2002). Comparison of conventional and macrophyte-based systems for the treatment of domestic wastewater. Wat. Sci. Tech. 45(1), 111-116.

Baris, A. J., Eifert, W. H., Klotzer, K. and McGuckin, C. J. (2001). Use of a Subsurface Flow Constructed Wetland For Collection And Treatment of Water Containing BTEX. Roux Associates, Inc., Inslandia, New York.

Bastian, R. K. and Benforado, J. (1983). Waste Treatment: Doing What Comes Naturally. Technol. Rev. 86(2), 58.

Bastian, R. K. (1993). Constructed Wetlands for Wastewater Treatment and Wildlife Habitat: 17 Case Studies. USEPA 832-R-93-005, Municipal Technology Branch, Washington.

Bastian, R. K. and Hammer, D. A. (1993). The use of constructed wetlands for wastewater treatment and recycling. In: Moshiri, G.A. (Ed.), Constructed Wetlands for Water Quality Improvement. Lewis Publishers, Ann Arbor, pp. 59–68.

Bedient, P. B. (1994). Groundwater Contamination, Transport and Remediation, Prentice Hall PTR.

Behrends, L., Houke, L., Bailey, E., Jansen, P. and Brown, D. (2001). Reciprocating constructed wetlands for treating industrial, municipal and agricultural wastewater. Water Science and Technology 44(11/12), 399-405.

Bezbaruah, A. N. and Zhang, T. C. (2003). Performance of a Constructed Wetland with a Sulfur/Limestone Denitrification Section for Wastewater Nitrogen Removal. Environmental Science and Technology, 37(8), 1690-1697.

Bodelier, P. L. E., Libochant, J. A., Blom, C. W. P. M. and Laan-Broek, H. J. (1996). Dynamics of nitrification and denitrification in root- oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Appl. Environ. Microbiol. 62(11), 4100–4107.

Brinson, M. M. (1993a). A hydrogeomorphic classification for wetlands. Technical Report WRP-DE-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Britton, G. (1994). Wastewater Microbiology. Wiley-Liss, New York.

Brix, H. (1994a). Functions of macrophytes in constructed wetlands. Wat. Sci. Technol. 29(4), 71–78.

Brix, H. (1994b). Constructed wetlands for municipal wastewater treatment in Europe. In: Mitsch, W.J. (Ed.), Global Wetlands: Old World and New. Elsevier, Amsterdam, The Netherlands, pp. 325–333.

Brix, H. (1997). Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol., 35(5), 11-17

Brooks, A. S., Rozenwald, M. N., Geohring, L. D., Lion, L. W. and Steenhuis, T. S. (2000). Phosphorus Removal by Wollastonite: A Constructed Wetland Substrate. Ecological Engineering, 15(1), 121-132.

Burland, S. M. and Edwards, E. A. (1999). Anaerobic benzene biodegradation linked to nitrate reduction. Applied and Environmental Microbiology 65 (2), 529-533.

Bossert, I. and Bartha, R. (1984). The fate of petroleum in soil ecosystem, in: Atlas, R.M. (Ed.), Petroleum Microbiology. Macmillan Co., New York, pp. 435–476.

Callaway, R. M. and King, L. (1996). Temperature-driven variation in substrate oxygenation and the balance of competition and facilitation. Ecology, 77(4), 1189–1195.

Campbell, C. S. and Ogden, M. (1999). Constructed wetlands in the sustainable landscape. John Wiley & Sons, Inc.

Cannon, A. D., Gray, K. R., Biddlestone, A. J. and Thayanithy, K. (2000). Pilot-scale development of a bioreactor for the treatment of dairy dirty water. Journal of Agricultural Engineering Research, 77 (3), 327-334.

Carmichael, L. M. and Pfaender, F. K. (1997). The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. Biodegradation 8(1), 1-13.

Caswell, P. C., Gelb D., Marinello, S. A., Emerick, J. C. and Cohen, R. R. (1992). Evaluation of constructed surface-flow wetlands systems for the treatment of discharged waters from oil and gas operations in Wyoming, in SPE Rocky Mountain Regional Conference. Paper SPE 24331, Casper, Wyoming.

Chaillan, F., Chaıneau, C. H., Point, V., Saliot, A. and Oudot, J. (2006). Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. Environmental Pollution 144(1): 255-265.

Chaıˆneau, C. H., Morel, J. L. and Oudot, J. (2000). Biodegradation of fuel oil hydrocarbons in the rhizosphere of Maize (Zea mays L.). Journal of Environmental Quality 29(2), 569–578.

Chaıneau, C. H., Rougeux, G., Yepremian, C. and Oudot, J. (2005). Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. Soil Biol Biochem 37(8): 1490-1497.

Choi, S-C., Kwon, K. K., Sohn, J. H. and Kim, S-J. (2000). Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mescocosms. J Microbiol Biotechnol 12(3):431-436.

Christensen, J. S. and Elton, J. (1996). Augmented Bioremediation. Dept of Civil Engineering, Virginia Tech, CE 4594: Soil and Groundwater pollution from BTEX.

Chunming, S. and Puls, R. (1999). Kinetics of trichloroethene reduction by zerovalent Iron and Tin: Pretreatment effect, apparent activation energy, and intermediate products. Environm. Sci. Tech., 33(7), 163-168.

Coates, J. D., Bruce, R. A. and Haddock, J. D. (1998). Anoxic bioremediation of hydrocarbons. Nature, 396(6713), 730.

Coates, J. D., Chakraborty, R., Lack, J. G., O'Connor, S. M., Cole, K. A., Bender, K.S. and Achenbach, L. A. (2001). Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*. Nature, 411(6841), 1039-1043.

Coates, J. D., Chakraborty, R. and McInerney, M. J. (2002). Anaerobic benzene biodegradation—a new era. Res Microbiol 153(10):621– 628.

Cole, S. (1998). The Emergence of Treatment Wetlands. Environ. Sci. Technol., 218A-223A.

Cooney, J. (1984). The fate of petroleum pollutants in freshwater ecosystems, in: Atlas, R.M. (Ed.), Petroleum Microbiology. Macmillan, New York, pp 399-433.

Cooper, P. (1999). A Review of the Design and Performance of Vertical-flow and Hybrid Reed Bed Treatment Systems. Water Science and Technology, 40 (3), 1-9.

Cooper, P. E. (1993). The use of reed bed systems to treat domestic sewage: the European Design and Operations Guidelines for Reed Bed Treatment Systems. Constructed Wetlands for Water Quality Improvement. Florida: Lewis Publ., 203-217

Cooper, P.F. and Findlater, B.C. (1990). Constructed Wetlands in Water Pollution Control. Pergoman Press, New York.

Cooper, P.F., Job G.D., Green M.B. and Shutes R.B.E., (1996). Reed Beds and Constructed Wetlands for Wastewater Treatment. Water Research Centre, Swindon.

Corley, T. L., Farrell, J., Hong, B. and Conklin, M.H. (1996). VOC Accumulation and pore filling in unsaturated porous media. Environmental Science and Technology, 30 (10), 2884-2891.

Corley, T. L., Farrell, J., Hong, B. and Conklin, M. H. (1996). VOC Accumulation and pore filling in unsaturated porous media. Environmental Science and Technology 30 (10), 2884-2891.

Cowardin, L., Carter, V., Golet, F. and LaRoe, E. (1979). Classification of wetlands and deepwater habitats of the United States. Office of Biological Services, US Fish and Wildlife Service.

Crites, R. and Tchobanoglous, G. (1998). Small and Decentralized Wastewater Management Systems. WCB/McGraw-Hill, Boston.

DeBusk, T.A. and DeBusk, W.F. (2001). Wetlands for water treatment. In: D.M. Kent (Ed.), Applied Wetlands Science and Technology. 2nd ed., Lewis Publ., Boca Raton, Florida, pp. 241–279.

DeBusk, W. F. (1999). Wastewater Treatment Wetlands: Contaminant Removal Processes. Document SL155, a fact sheet of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 32611-0510. http://www.eco-pros.com/types_of_wetlands.htm (accessed 14/01/08).

Dennison, M. S., and Berry, J. F. (1993). Wetlands: Guide to Science, Law, and Technology. Park Ridge, NJ: Noyes.

Denny, P. (1997). Implementation of Constructed Wetlands in Developing Countries. Water Science and Technology, 35(5), 27-34.

Descousse, A., Monig, K. and Voldum, K. (2004). Evaluation study of various produced-water treatment technologies to remove dissolved aromatic components, in: Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition held 26-29 September 2004, in Houston, Texas. Paper SPE 90103 2004. (available on http://www.spe.org/elibrary).

De Wilde, W. and Geenens, D. (2003). Optimisation of green wastewater treatment technologies. Het Ingenieursblad, 10/11 (in Dutch).

Díaz, E. (2004). Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. Int Microbiol **7**(3), 173-180.

Dou, J., Liu, X., Hu, Z. and Deng, D. (2008). Anaerobic BTEX biodegradation linked to nitrate and sulfate reduction. Journal of Hazardous Materials, 151(2-3), 720-729.

Dagley S. (1986). Biochemistry of aromatic hydrocarbon degradation in *Pseudomona*s, in Sokatch, J. and Ornston, L.N. (ed.), The Bacteria Vol. 10:527-555. The biology of *Pseudomonas*. Academic Press, Orlando.

Eke, P.E. (2004). Critical Evaluation of Wastewater Treatment. MSc honours thesis, The Robert Gordon University Aberdeen.

Eke, P.E. and Scholz, M. (2008). Benzene removal with vertical-flow constructed treatment wetlands. Journal of Chemical Technology Biotechnology, 83 (1), 55-63.

Eke, P.E., Scholz, M. and Wallace, S.D. (2007a). Constructed treatment wetlands: sustainable technology for the petroleum industry, in: Starrett, S.K., Hong, J., Wilcock, R.J., Li, Q., Carson, J.H., Arnold, S. (Eds.), Proceedings of the 3rd International Conference on Environmental Science and Technology (06-09/08/2007), volume 2, 174-179, ISBN 978-0976885399, American Science Press, Houston, USA.

Eke, P.E., Scholz, M. and Wallace, S.D. (2007b). Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry. Paper presented at the 2007 Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition, and SPE International Student Paper Contest being held on 11-14 November in Anaheim, California, USA. Available online in Society of Petroleum Engineers elibrary (http://www.spe.org/elibrary), SPE 113644.

Eke, P. E. and Scholz, M. (2006). Hydrocarbon Removal with Constructed Treatment Wetlands for the Benefit of the Petroleum Industry. Proceedings of the 10th International Conference on Wetland Systems for Water Pollution Control (23-29/09/2006), ed by Dias V and Vymazal J. International Water Association, Lisbon, Portugal, Volume 3:1707-1714, ISBN: 989-20-0361-6.

Ellis, J.B., Shutes, R.B.E. and Revitt, D.M. (2003). Guidance Manual for Constructed Wetlands, Urban Pollution Research Centre, Middlesex University, London.

Etnier, C. and Guterstam, B. (1991). Ecology Engineering for Wastewater Treatment. Boksogen/Stensund Folk College, Sweden.

Foght, J.M., Westlake, D.W., Johnson, W.M. and Ridgway, H.F. (1996). Environmental gasoline-utilizing isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques. Microbiology, 142(9), 2333–2340.

Francis A.S. (1996). Augmented Bioremediation. CE 4594: Soil and Groundwater pollution. Virginia Tech, USA.

Fujita Research Archived Report, (1998). Constructed Wetlands for Wastewater Treatment. Archived Report 022, Fujita Research Archives, Tokyo, Japan. Available online at www.fujitaresearch.com/reports/wetlands.html (accessed on 05/01/08).

Garcia, H. L. and Gonzalez, I. M. (2004). Self-organizing Map and Clustering for Wastewater Treatment Monitoring. Engineering Applications of Artificial Intelligence 17(3), 215-225.

Geller, G. (1997). Horizontal Subsurface Flow Systems in the German Speaking Countries: Summary of Long-Term Scientific and Practical Experiences; Recommendations. Wat. Sci. Tech., 35(5), 157-166.

Gervin, L. and Brix, H. (2001). Removal of nutrients from combined sewer overflows and lake water in a vertical-flow constructed wetland system. Wat. Sci. Tech., 44(11-12), 171-176.

Gernaey, K. V., Van Loosdrecht M. C. M., Henze, M., Lind, M. and Jørgensen, S. B. (2004). Activated Sludge Wastewater Treatment Plant Modeling and Simulation: State of the Art. Environ. Modell. Softw. 19(9), 763-783.

Gessner, T. P., Kadlec, R. H. and Reaves, R. P. (2005). Wetland remediation of cyanide and hydrocarbons. Ecological Engineering 25(4), 457–469.

Gevrey, M., Rimet, F., Park, Y. S., Giraudel, J. L., Ector, L. and Lek, S. (2004). Water Quality Assessment Using Diatom Assemblages and Advanced Modelling Techniques. Freshwater biology, 49(2), 208-220.

Gibson, D.T. and Subramanian, V. (1984). Microbial degradation of aromatic hydrocarbons. In: Microbial Degradation of Organic Compounds, Gibson, D.T., (Ed.), pp 181-252. Marcel Dekker, New York.

Gopal, B. (1999). Natural and Constructed Wetlands for Wastewater Treatment: Potentials and Problems. Water Science and Technology, 40(3), 27-35.

Gosselink, J.G. and Turner, R.E. (1978). The role of hydrology in freshwater wetland ecosystems. In Freshwater wetlands: ecological processes and management potential, R.E. Good, D.F. Whigam and R.L. Simpson (ed.), pp. 63-78. New York, USA: Academic Press.

Granger, D.A., Butler, B.J. and Barker, J.F. (1999). A case of phosphorus limiting monoaromatic hydrocarbon biodegradation in groundwater. Bioremediation Journal 3 (3), 213-221.

Green, M., Friedler, E. and Safrai, I. (1998). Enhancing nitrification in vertical flow constructed wetland utilizing a passive air pump. Water Res. 32(12), 3513-3520.

Grieu, S., Traore, A., Polit, M. and Colprim, J. (2005). Prediction of Parameters Characterizing the State of a Pollution Removal Biologic Process. Eng. Appl. Artif. Intel. 18(5), 559-573.

Görgényi, M., Dewulf, J. and Langenhove, H. V. (2002). Temperature dependence of Henry's law constant in an extended temperature range. Chemosphere, 48 (7), 757-762.

Guirguis, M. (2004). Treatment of Waste Water: A Reed Bed Environmental Case History. Paper SPE86673 presented at the 2004 SPE International Conference on Health, Safety, and Environment in Oil and Gas Exploration and Production held 29-31 March 2004 in Calgary, Alberta Canada.

Haberl, R., Perfler, R. and Mayer, H. (1995). Constructed Wetlands in Europe. Water Science and Technology, 32(3), 305-315.

Haberl, R. (1999). Constructed Wetlands: A Chance to Solve Wastewater Problems in Developing Countries. Water Science and Technology, 40(3), 11-17.

Hamed, M. M., Khalafallah, M. G. and Hassanien, E. A. (2004). Prediction of Wastewater Treatment Plant Performance Using Artificial Neural Networks. Environ. Model. Softw. 19(10), 919-928.

Hammer, D.A. (1989). Constructed Wetlands for Waste water Treatment-Municipal, Industrial and Agricultural. Lewis Publishers, Chelsea, Michigan.

Hammer, D.E. and Kadlec, R.H. (1983). Design Principles for Wetland Treatment Systems. U.S. Environmental Protection Agency. Office of Research and Development. EPA-600/2-83-026.

Hamed, M. M., Khalafallah, M. G. and Hassanien, E. A. (2004). Prediction of Wastewater Treatment Plant Performance Using Artificial Neural Networks. Environ. Model. Softw. 19(10), 919-928.

Hartemink, A.E. (2006). The Future of Soil Science. International Union of Soil Sciences, Wageningen. 165 pp. ISBN 90 71556 16 6.

Harwood, C.S. and Gibson, J. (1997). Shedding light on anaerobic benzene ring degradation: a process unique to prokaryotes? Journal of Bacteriology, 179 (2), 301-309.

Heider, J. Spormann, A.M. Beller, H.R. and Widdel, F. (1998). Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol Rev 22(5), 459-473.

Hemond, F. H. and Fechner-Levy, E. J. (2000). Chemical Fate and Transport in the Environment. 2nd Edition, Academic Press, San Diego, California.

Hiegel, T. (2004). Analysis of Pilot Scale Constructed Wetland Treatment of Petroleum Contaminated Groundwater. MSc thesis, Department of Civil Engineering, University of Wyoming.

Hilton, B. L. (1993). Performance evaluation of a closed ecological life support system (CELSS) employing constructed wetlands. Pp 117-125 in Constructed Wetlands for Water Quality Improvement, G. A. Moshiri (ed.). CRC Press, Boca Raton, Florida.

Holder, A.W., Bedient, P.B. and Hughes, J.B. (1999). Modeling the impact of oxygen reaeration on natural attenuation. Bioremediation Journal, 3 (2), 137-149.

Hong, Y-S. T., Rosen, M. R. and Bhamidimarri, R. (2003). Analysis of a Municipal Wastewater Treatment Plant Using a Neural Network – Based Pattern Analysis, Water Res. 37(7), 1608-1618.

Hu, Z.F., Dou, J.F., Liu, X., Zheng, X.L. and Deng, D. (2007). Anaerobic biodegradation of benzene series compounds by mixed cultures based on optional electronic acceptors. Journal of Environmental Science, 19 (9), 1049-1054.

Ilker, U., Duan, Y. P. and Ogram, A. (2000). Characterization of the naphthalene-degradingbacterium, Rhodococus opacus M213. FEMS Microbiol. Lett., 185(2), 231–238.

International Association of Oil and Gas Producers, (2002). Aromatics in produced water: occurrence, fate and effects, and treatment. Report No. 1.20/324 (January 2002), International Association of Oil and Gas Producers, London.

ITRC, (2003). Technical and regulatory guidance document for constructed treatment wetlands. The Interstate Technology and Regulatory Council Wetlands Team. 128 pp.

IWA Specialist Group on Use of Macrophytes in Water Pollution Control. (2000).

Constructed Wetlands for Pollution Control. Scientific and Technical Report n°8. IWA Publishing, London.

James, F. C. and McCulloch, C. E. (1990). Multivariate analysis in ecology and systematics: panacea or pandora's box? Annu Rev Ecolog Syst 21, 129–166.

Janks, J. S. and Cadena, F. (1991). Identification and properties of Modified Zeolites for the Removal of Benzene, Toluene and Xylene from Aqueous Solutions. Paper SPE 22833 presented in 1991 Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition held 6-9 October, Dallas, Texas, U.S.A.

Johansson, L. (1996). Use of Leca (Light Expanded Clay Aggregates) for the Removal of Phosphorus from Wastewater. Water Science and Technology, 35(5), 87- 94.

Johnson, S.J., Woolhouse, K.J., Prommer, H., Barry, D.A. and Christofi, N. (2003). Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater. Engineering Geology 70 (3-4), 343-349.

Jung, I.G. and Park, C.H. (2004). Characteristics of Rhodococcus pyridinovorans PYJ-1 for the biodegradation of benzene, toluene, *m*-xylene (BTX), and their mixtures. Journal of Bioscience and Bioengineering, 97 (6), 429-431.

Kadlec, J.A. (1983). Water budgets for small diked marshes. Water Resour. Bull. 19, 223–229.

Kadlec, R.H., 1994. Detention and mixing in free water wetlands. Ecol. Eng. 3(4), 345–380.

Kadlec, R.H. and Brix, H. (1995). Wetland Systems for Water Pollution Control. Water Science and Technology, 32 (3), 21-29.

Kadlec, R. H. and Knight, R. L. (1996). Treatment Wetlands; CRC Press, Inc.: Boca Raton, Florida, USA.

Kadlec, R. H. (1997). Deterministic and Stochastic Aspects of Constructed Wetlands Performance and Design. Wat. Sci. Tech., 35(5), 149-156.

Kadlec, R. H. (2001). Feasibility of Wetland Treatment BP – Amoco Casper Refinery Remediation. Internal BP Amoco Draft Document.

Kadlec, R. H. (2006). Water temperature and evapotranspiration in surface flow wetlands in hot arid climate. Ecol. Eng., 26(4), 328-340.

Kadlec, R. H., Knight, L. R., Vymazal, J., Brix, H., Cooper, P. and Haberl, R. (2000). Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation. International Water Association (IWA) Specialist Group on Use of Macrophytes in Water Pollution Control, Scientific and Technical Report No. 8, IWA Publishing, London**.**

Kadlec, R. H. (2002). Effects of Pollutant Speciation in Treatment Wetlands Design; Wetland Management Services, Chelsea, Michigan, USA.

Karathanasis, A. D., Potter C. L. and Coyne, M. S. (2003). Vegetation effects on fecal bacteria, BOD, and suspended solids removal in constructed wetland treating domestic wastewater. Ecol. Eng., 20(2), 157-169.

Kadlec, R. and Reddy, R. (2000). Temperature effects in treatment wetlands. Wat. Environ. Res., 73(5), 543-555.

Kikuth, R. (1977). Degradation and incorporation of nutrients from rural wastewaters by plant rhizosphere under liminic conditions. Utilization of Manure by Land Spreading, Comm. of the Europ. Communitite, EUR 5672e, London, 235-243.

Kivaisi, A. K. (2001). The Potential for Constructed Wetlands for Wastewater Treatment and Reuse in Developing Countries: A Review, Ecological Engineering, 16(4), 545-560.

Kim, S., Choi, D. H., Sim, D. S. and Oh, Y. (2005). Evaluation of bioremediation effectiveness on crude oil-contaminated sand. Chemosphere, 59(6), 845- 852.

Knight, R.L., Kadlec, R.H. and Ohlendorf, H.M. (1999). The Use of treatment wetlands for petroleum industry effluents. Environmental Science and Technology, 33 (7), 973-980.

Korkusuz, E. A., Berklioglu, M. and Demirer, G. N. (2005). Comparison of the treatment performances of blast furnace slag-based and gravel-based vertical flow wetlands operated identically for domestic wastewater treatment in Turkey. Ecol. Eng. 24(3), 187-200.

Kuehn, E. and Moore, J. A. (1995). Variability of treatment performance in constructed wetlands. Wat. Sci. Tech. 32(3), 241-250.

Kniemeyer, O., Fischer, T., Wilkes, H., Glockner, F.O. and Widdel, F. (2003). Anaerobic degradation of ethylbenzene by a new type of marine sulfate-reducing bacterium, Applied and Environmental Microbiology, 69 (2), 760-768.

Kohonen, T. (2001). Self-organizing Maps, 3rd edition. Springer: Berlin, Germany, ISBN 3-540-67921-9, pp 501.

Lahvis, M. A., Baehr, A. L. and Baker, R. J. (1999). Quantification of aerobic biodegradation and volatilization rates of gasoline hydrocarbons near the water table under natural attenuation conditions. Wat Resour Res 35(3), 753-765.

Lee, B-H. and Scholz, M. (2006). A Comparative study: Prediction of constructed treatment wetland performance with k-nearest neighbors and neural networks. Water, Air, & Soil Pollution, 174(1-4), 279-301).

Lee, B.-H., Scholz, M. and Horn, A. (2005). Constructed Wetlands for the Treatment of Concentrated Stormwater Runoff (Part A). Environ. Eng. Sci. 23(2), 191-202.

Lee, J. F., Chao, H. P., Chiou, C. T. and Manes, M. (2004).Turbulence effects on volatilization rates of liquids and solutes. Environmental Science and Technology 38 (16), 4327-4333.

Leonard, K.M. (2000). Analysis of residential subsurface flow constructed wetlands performance in Northern Alabama. Small Flows Quart. 1(3), 34–39.

Li, H., Liu, Y. H., Luo, N., Zhang, X. Y., Luan, T. G., Hu, J. M., Wang, Z. Y., Wu, P. C., Chen, M. J. and Lu, J. Q. (2006). Biodegradation of benzene and its derivatives by a psychrotolerant and moderately haloalkaliphilic *Planococcus* sp. strain ZD22. Research in Microbiology, 157 (7), 629-636.

Ji, G.D., Sun, T.H. and Ni, J.R. (2007). Surface flow constructed wetland for heavy oil-produced water treatment. *Biores. Tech*., 98(2), 436-441.

Liner, M. 2006. No Big Pipe? No Problem. Pollution Engineering. September 5, Pp 20-23.

Liu, H.-X., Zhang, R.-S., Yao, X.-J., Liu, M.-C., Hu, Z.-D. and Fan, B.-T. (2004). Prediction of Electrophoretic Mobility of Substituted Aromatic Acids in Different

Aqueous-alcoholic Solvents by Capillary Zone Electrophoresis Based on Support Vector Machine. Analytica Chimica Acta, 525(1), 31-41.

Lu, C., Lin, M. R. and Chu, C. (2002). Effects of pH, moisture, and flow pattern on trickle-bed air biofilter performance for BTEX removal. Advance in Environmental Research 6 (2), 99-106.

Lu, R.-S. and Lo, S.-L. (2002). Diagnosing Reservoir Water Quality Using Self organizing Maps and Fuzzy Theory. Water Res. 36(9), 2265-2274.

Lu, W.-Z. and Wang, W.-J. (2005). Potential Assessment of the Support Vector Machine Method in Forecasting Ambient Air Pollutant Trends. Chemosphere 59(5), 693-701.

Luederitz, V., Eckert, E., Lange-Weber, M., Lange, A. and Gersberg, R. (2001). Nutrient Removal Efficiency and Resource Economics of Vertical Flow and Horizontal Flow Constructed Wetlands. Ecological Engineering, 18(2), 157-171.

Magmedov, V. G., Zakharchenko, M. A., Yakovleva, L. I. and Ince, M. E. (1996). The use of constructed wetlands for the treatment of run-off and drainage waters: The UK and Ukraine experience. Water Sci. Technol. 33(4), 315-323.

Mann, R. A. and Bavor, H. J. (1993). Phosphorus Removal in Constructed Wetlands Using Gravel and Industrial Waste Substrate. Water Science and Technology, 27(1), 107-113.

Margesin, A., Zimmerbauer, A. and Schinner, F. (1999). Monitoring of bioremediation by soil biological activities. Chemosphere 40(4), 339-346.

Mausbach, M. J., and Richardson, J. L. (1994). Biogeochemical processes in hydric soil formation. Pp. 68-127 in Current Topics in Wetland Biogeochemistry, Vol. 1. Louisiana State University: Wetland Biogeochemistry Institute.

Merlin, G., Pajean, J.L. and Lissolo, T. (2002). Performances of constructed wetlands for municipal wastewater treatment in rural mountainous area. Hydrobiologia 469(1-3), 87-98.

Mitsch, W. J. and Gosselink, J. G. (1986). Wetlands. New York: Van Nostrand Reinhold.

Mitsch, W.J. and Gosselink, J.G. (1993). Wetlands. 2nd ed. John Wiley publ, New York.

Mitsch, W.J. and Gosselink, J.G. (2000). Wetlands. 3rd ed. John Wiley publ, New York.

Monjeau, C. (1901). United States patent 681,884. December 18, 1900.

Moshiri, G. A. (2000). Constructed Wetland for Water Quality Improvement. CRC Press, Florida, USA.

Moshiri, G. A. (1993). Constructed Wetlands for Water Quality Improvement. Lewis Publishers, CRC Press, Boca Raton, Florida, USA.

Mukherjee, A. (1997). Self-organizing Neural Network for Identification of Natural Models. J. Comput. Civil. Eng. 11(1), 74-77.

Maier, H. R., Morgan, N. and Chow, C. W. K. (2004). Use of Artificial Neural Networks for Predicting Optimal Alum Doses and Treated Water Quality Parameters. Environ. Model. Softw. 19(5), 485-494.

Morgan, P. and Watkinson, R. J. (1989). Microbiological methods for the cleanup of soil and ground water contaminated with halogenated organic compounds. FEMS

Microbiology Reviews 63(4), 277-300.

Mueller, J. G., Resnick, S. M., Shelton, M. E. and Pritchard, P.H. (1992). Effect of inoculation on the biodegradation of weathered Prudhoe Bay crude oil. Journal of Industrial Microbiology and Biotechnology, 10 (2), 95-102.

Myers J. E. and Jackson L. M. (2001). An evaluation of the Department of Energy Naval Petroleum Reserve No. 3 - Produced water bio-treatment facility, Paper SPE 66522 Presented in SPE/EPA/DOE Exploration and Production Environmental Conference held 26-28 Feb., San Antonio, Texas.

National Research Council (NRC), (1993). In Situ Bioremediation: When does it work? National Academy Press, Washington, D.C.

Neralla, S., Weaver, R. W., Leikar, B. J. and Rersys, R. A. (2000). Improvement of domestic wastewater quality by subsurface flow constructed wetlands. Bioresource Technol. 75(1), 19-25.

Nguyen, L. M. (2000). Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters. Ecol. Eng. 16(2), 199-221.

Njau, K. N., Minja, R. J. A. and Katima, J. H. Y. (2003). Pumice Soil: A Potential Wetland Substrate for Treatment of Domestic Wastewater. Water Science and Technology, 48(5), 85-92.

Novontny, V. and Olem, V. (1994). Water Quality: Prevention, Identification and Management of Diffuse Pollution. Van Nostrand Reinhold, New York, USA.

Obarska-Pempkowiak, H. and Klimkowska, K. (1999). Distribution of nutrients and heavy metals in a constructed wetland system. Chemosphere, 39(2), 303-312.

Omari K., Revitt, M., Shutes, B. and Garelick, H. (2003). Hydrocarbon removal in an experimental gravel bed constructed wetland. *Wat Sci Tech* 48(5) 275-81.

Onkal-Engin, G., Demir, I. and Engin, S. N. (2005). Determination of the Relationship between Sewage Odor and BOD by Neural Networks. Environ. Model. Softw. 20(4), 843-850.

Oudot J, Merlin, F. X. and Pinvidic, P. (1998). Weathering rates of oil components in a bioremediation experiment in estuarine sediments. Mar Environ Res 45(2)**:** 113-125.

Pardue, J. H., Kassenga, G. and Shin, W.S. (2000). Design approaches for chlorinated VOC treatment wetlands. In: J.L. Means and R.E. Hinchee (Eds.), Wetlands and Remediation, an International Conference (16-17 November 1999), Salt Lake City, Utah, Battelle Press, Columbus, Ohio, pp. 301-308.

Patten, B.C. 1990. Introduction and Overview. In: Patten, B.C. (ed), Wetlands and Shallow Continental Water Bodies, Vol: 1, SBP Academic Publishing , The Hague.

Pinney, M.L., Westerhoff, P.K. and Baker, L. (2000). Transformations in dissolved orgarnic carbon through constructed wetlands. Water Res. 34(6), 1897-1911.

Prince, R. C., Clark, J. R. and Lee, L. (2002). Bioremediation effectiveness: Removing hydrocarbons while minimizing environmental impact. 9th International Petroleum Environmental Conference, IPEC (Integrated Petroleum Environmental Consortium), Albuquerque, NM.

Ramsar, (1971). What are wetlands? Ramsar Information Paper no. 1. Available online: http://www.ramsar.org/about/info2007-01-e.pdf (accessed 070108).

Reddy, K. R. and D'Angelo, E. M. (1994). Soil processes regulating water quality in wetlands. p. 309-324. In Mitsch, W. J. (ed.) Global wetlands: old world and new. Elsevier Science, Amsterdam.

Reddy, K.R., Angelo, E.M.D. and DeBusk, T.A. (1989). Oxygen transport through aquatic macrophytes: The role in wastewater treatment. J. Environ. Qual., 19(2), 261-267.

Reddy, K. R. and Smith, W. H., (1987). Aquatic Plants for Water Treatment and Resource Recovery, Magnolia Pub., Orlando, FL.

Reed, S. C. and Brown, D. S. (1992). Constructed wetland design—the first generation. Water Environ. Res. 64 (6), 776-781.

Reed, S. C. and Brown, D. S. (1995). Subsurface flow wetlands—a performance evaluation. Water Environ. Res. 67 (2), 244–248.

Reed, S. C., Middlebrooks, E. J. and Crites, R. W. (1988). Natural Systems for Waste Management & Treatment. McGraw Hill, New York, NY.

Reinelt, L. E., Surowiec, M. S. and Horner, R. R. (1993). Urbanization effects on palustrine wetland hydrology as determined by a comprehensive water balance. King County Resource Planning. King County, WA.

Rew, S. and Mulamoottil, G. A. (1999). A cost comparison of leachate treatment alternatives, in Constructed Wetlands for the Treatment of Landfill Leachates, ed by Mulamoottil G, McBean EA and Rovers F, Lewis, Boca Raton.

Ridgeway, H.F., Safarik, J., Phipps, D., Carl, P. and Clark, D. (1990). Identification and catabolic activity of well-derived gasoline-degrading bacteria and a contaminated aquifer. Appl Environ Microbiol 56(11), 3565–3575.

Riser-Roberts, E. (1992). Bioremediation of Petroleum Contaminated Sites, CRC Press, Boca Raton, Florida.

Rosenberg, E. and Ron, E.Z. (1996). Bioremediation of petroleum contamination. In: R.L. Crawford and D.L. Crawford, Editors, Bioremediation: Principles and Applications, Cambridge University Press, UK , pp. 100–124.

Rosso, L., Lobry, J., Bajard, S. and Flandrois, J. (1995). Convenient model to describe the combined effects of temperature and pH on microbial growth. Appl. Environm. Microbiol., 61(2), 610-616.

Salanitro, J. P. (1993). The role of bioattenuation in the management of aromatic hydrocarbon plumes in aquifers. Ground Water Monitoring and Remediation 13(4), 150-161.

Salmon, C., Crabos, J. L., Sambuco, J.P., Bessiere, J.M., Brasseres, A., Caumette, P. And Baccou, J.C. (1998). Artificial wetland performances in the purification efficiency of hydrocarbon wastewater. Water, Air, and Soil Pollution, 104 (3-4), 313-329.

Sather, J.H. and Smith, R.D., (1984). An Overviewof Mafor Wetland Functions and Values. FWS/OBS84/18. Western Energy and Land Use Team, U.S. Fish and Wildlife Service, Washington.

Schnoor, L.J. (1996). Environmental Modeling - Fate and Transport of Pollutants in Water, Air and Soil, Wiley, New York.

Scholz, M. (2004). Treatment of gully pot effluent containing nickel and copper with constructed wetlands in a cold climate. J Chem Technol Biotech 79 (2), 153-162.

Scholz, M. and Xu, J. (2002). Performance comparison of experimental constructed wetlands with different filter media and macrophytes treating industrial wastewater contaminated with lead and copper. Bioresource Technology, 83(2), 71–79.

Scholz, M. (2006). Wetland systems to control urban runoff. Elesevier, Amsterdam, The Netherlands.

Scholz, M. and Lee, B.-H. (2005). Constructed Wetlands: A Review. Int. J. Environ. Stud. 62(4), 421-447.

Scholz, M., Harrington, R. Carroll, P. and Mustafa, A. (2007). The Integrated Constructed Wetlands (ICW) concept. Wetlands, 27(2), 337-354.

Scholz, M., Höhn, P. and Minall, R. (2002). Mature experimental constructed wetlands treating urban water receiving high metal loads. Biotechnology Progress 18 (6), 1257-1264.

Schreiber, M.E. and Bahr, J.M. (2002).Nitrate-enhanced bioremediation of BTEX-contaminated groundwater: parameter estimation from natural-gradient tracer experiments. Journal of Contaminant Hydrology 55 (1-2), 29-56.

Seidel, K. (1973). System for Purification of Polluted Water. United States Patent No. 3770623, Washington D.C.

Seidel, K. (1955). Die Flechtbinse Scirpus lacustris, in Okologie, Morphologie und Entwicklung, ihre Stellung bei den Volkern und ihre wirtschaftliche Bedeutung. *Schweizerbartsche Verlagsbuchhdlg*, Stuttgart.

Seidel, K. (1961). Zur Problematik der Keim-und Pflanzengewasser, *Verh. International Limnology*, 14, 1035.

Seidel, K. (1976). Macrophytes and Water Purification. Biological Control of Water Pollution, Pennsylvania University Press, Philadelphia.

Seidel, K. (1966). Reinigung von Gewassern durch hohere Pflanzen *Deutsche Naturwissenschaft*, 12, 298-297.

Sheoran, A.S. and Sheoran, V. (2006). Heavy metal removal mechanism of acid mine drainage in wetlands: A critical review. 19(2), 105-116.

Shutes, R.B.E., Revitt, D.M., Lagerberg, I.M. and Barraud, V.C.E. (1999). The design of vegetative constructed wetlands for the treatment of highway runoff. Sci. total Environ. 235(1-3), 189-197.

Skaggs, R. W., Gilliam, J. W. and Evans, R. O. (1991). A computer simulation study of pocosin hydrology. Wetlands 11(sp), 399-416.

Smith, R. L. (1980). Ecology and field biology, 3rd ed. Harper & Rowe, New York.

Sorrell, B.K. and Armstrong, W. (1994). On the difficulties of measuring oxygen release by root systems of wetland plants. J. Ecol., 82(1), 177–183.

Spence, J.M., Bottrell, S.H. Thornton, S.F. Richnow, H.H. and Spence, K.H. (2005). Hydrochemical and isotopic effects associated with petroleum fuel biodegradation pathways in a chalk aquifer. Journal of Contaminant Hydrology, 79 (1-2), 67-88.

SPSS, Analytical Software. Statistical Package for the Social Sciences (SPSS) Headquarters, 233 South Wacker Drive, Chicago, Illinois, USA (2003).

Steinberg, S.L. and Coonrod, H.S. (1994). Oxidation of the root zone by aquatic plants growing in gravel–nutrient solution culture. J. Environ. Qual. 23(5), 907–913.

Stephenson, M.T. (1992). A Survey of Produced Water Studies," in Produced Water, J.P. Ray and F.R. Englehart (eds.), Plenum Press, New York.

Stottmeister, U., Wiesner, A., Kuschk, P., Kappelmeyer, M. and Kaster, M. (2003). Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotech. Advan. 22(1), 93-117.

Su, J.L. and Ouyang, C.F. (1996). Nutrient removal using a combined process with activated sludge and fixed biofilm.Water Sci. Technol. 34 (1–2), 477–486.

Sugai, S. F., Lindstrom, J. E. and Braddock, J. F. (1997). Environmental influences on the microbial degradation of Exxon Valdez oil on the shorelines of Prince William Sound, Alaska. Env Sci Tech 31(5):1564-1572.

Sun, G., Gray, K. R., Biddlestone, A. J., Allen, S. J. and Cooper, D. J. (2003). Effect of effluent recirculation on the performance of a reed bed system treating agricultural wastewater. Process Biochemistry, 39(3), 351–357.

Sun, G., Zhao, Y., & Allen, S. (2005). Enhanced removal of organic matter and ammoniacal-nitrogen in a column experiment of tidal flow constructed wetland system. Journal of Biotechnology, 115(2), 189–197.

Tao, W.D., Hall, K.J. and Duff, S.J.B. (2007). Microbial biomass and heterotrophic production of surface flow mesocosm wetlands treating woodwaste leachate: Responses to hydraulic and organic loading and relations with mass reduction. Ecological Engineering 31(2), 132-139.

Tchobanoglous, G. and Burton, F. L. (1991). Wastewater Engineering: Treatment, Disposal and Reuse, 3rd edition. New York: McGraw-Hill.

Trinidade, P., Sobral, L. G. Rizzo, A. C. Leite, S. G. F. Lemos, J. L. S. Milloili, V. S. and Soriano, A.U. (2002). Evaluation of the biostimulation and bioaugmentation techniques in the bioremediation process of petroleum hydrocarbon contaminated soils. 9th International Petroleum Environmental Conference, IPEC (Integrated Petroleum Environmental Consortium), Albuquerque, NM.

US EPA, (1993). Subsurface Flow Constructed Wetlands for Wastewater Treatment: a Technology Assessment. United States Environmental Protection Agency Report 542-R-01-004. Washington, D.C.

US EPA, (2000). A Handbook of Constructed Wetlands: A Guide to Creating Wetlands for: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage Stormwater in the Mid - Atlantic Region: Volume 1: General Considerations. United States Environmental Protection Agency, EPA Report Number 843B00005, ISBN 0-16-052999-9. Washington, D.C.

Van Acker, J., Buts, L., Thoeye, C. and De Gueldre, G. (2005). Floating plant beds: BAT for CSO treatment? Book of abstracts International Symposium on Wetland Pollutant Dynamics and Control, Ghent, Belgium, 4 – 8 September 2005.

Venosa, A.D. and Zhu, X. (2003). Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. Spill Science & Technology Bulletin, 8 (2), 163-178.

Verdenius, F. and Broeze, J. (1999). Generalized and Instance-specific Modeling for Biological Systems. Environ. Model. Softw. 14(5), 339-348.

Vesanto, J., Himberg, J., Alhoniemi, E. and Parhankangas, J. (1999). Self-organizing Map in Matlab: the SOM Toolbox, Proceedings of the Matlab DSP Conference, Espoo, Finland, Nov. pp. 35-40. Software available at http://www.cis.hut.fi/projects/somtoolbox/.

Vieira, J.J., Dias, F.M. and Mota, A. (2004). Artificial neural networks and neuro-fuzzy systems for modelling and controlling real systems: a comparative study. Engineering Applications of Artificial Intelligence, 17(3), 265-273.

Vrhovsek, D., Kukanja, V. and Bulc, T. (1996). Constructed wetland (CW) for industrial wastewater treatment. Wat Res 30 (10), 2287-2292.

Vymazal, J., 2005. Constructed wetlands for wastewater treatment. Ecol. Eng., 25(5), 475–477.

Vymazal, J. (1995). Algae and Nutrient Cycling in Wetlands. CRC Press/Lewis Publisher, Boca Raton, Florida.

Vymazal, J., Brix, H., Cooper, P. F., Green, B. and Haberl, R. (1998a). Constructed wetlands for wastewater treatment in Europe. Backhuys Publishers, Leiden, 366 p.

Vymazal, J., Brix, H., Cooper, P. F., Green, M. B. and Haberl, R. (1998). Constructed wetlands for wastewater treatment in Europe (pp. 17–66). The Netherlands: Backhuys.

Vymazal, J., Brix, H., Cooper, P.F., Haberl, R., Perfler, R. and Laber, J. (1998b). Removal mechanisms and types of constructed wetlands. In: Vymazal, J., Brix, H., Cooper, P.F., Green, M.B. and Haberl, R. (Eds.) (1998). Constructed wetlands for wastewater treatment in Europe. Backhuys Publishers, Leiden, 366 pp.

Vymazal, J. (1999). Removal of BOD in constructed wetland with horizontal subsurface flow: Czech experience. Water Sci. Technol. 40(3), 133-138

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. Science of the Total Environment, 380 (1-3), 48-65.

Vymazal, J. (2001). Types of constructed wetlands for wastewater treatment; their potential for nutrient removal. In: Vymazal J. (ed.), Transformations of Nutrients in Natural and Constructed Wetlands. Backhuys Publishers, Leiden.

Vymazal, J. (2002). The Use of Sub-Surface Constructed Wetlands for Wastewater Treatment in the Czech Republic: 10 Years Experience. Ecological Engineering, 18(5), 633-646.

Wallace, S. and Kadlec, R. (2005). BTEX degradation in a cold-climate wetland system. Wat. Sci. Tech., 51(9), 165–171.

Wallace, S., Parkin, G. and Cross, C. (2000). Cold climate wetlands: Design and performance. International Water Association 7th International Conference on Wetland Systems for Water Pollution Control, Nov. 11-16, Lake Buena Vista, Florida.

Wallace, S. D. (2001). Onsite remediation of petroleum contact wastes using subsurface flow wetlands. Proceedings of Wetlands and Remediation: The Second International Conference, 5-6 September 2001; Battelle Institute: Columbus, Ohio.

Wallace, S. D. and Knight, R. L. (2006). Small-scale constructed treatment systems: feasibility, design criteria, and O&M requirements. Final report, Project 01-CTS-5. Water Environment Research Foundation, Alexandria, Virginia.

Wallace S. D. (2004). Engineered wetlands lead the way. Land and Water 48(5), 13-16.

Ward, D.M., Brock, T.D. 1976.Environmental factors influencing the rate of hydrocarbon oxidation in temperate lakes. Applied and Environmental Microbiology, 31(5), 764-772.

Wemple, C. and Hendricks, L. (2000). Documenting the Recovery of Hydrocarbon-impacted Wetlands: a Multi-disciplinary Approach. In: Wetlands and Remediation: An International Conference, Battelle Press, Columbus, Ohio, pp. 73-78

Wemple, C. and Hendricks, L. (2000). Documenting the recovery of hydrocarbon-impacted wetlands: A multi-disciplinary approach, in Wetlands and Remediation: An International Conference, ed by Means JL and Hinchee RE. Battelle Press, Columbus, Ohio, USA, 73-78.

Werker, A.G., Dougherty, J.M., McHenry, J.L. and Van Loon, W.A. (2002). Treatment variability for wetland wastewater treatment design in cold climates. Ecol. Eng., 19(1), 1-11.

Werner, H. and Obach, M. (2001). New Neural Network Types Estimating the Accuracy of Response for Ecological Modeling. Ecol. Model. 146 (1-3), 289-298.

Werner, T.M. and Kadlec, R.H. (2000). Wetland residence time distribution modeling. Ecological Engineering 15(1-2), 77–90.

Wetzel, R.G. (1993). Constructed wetlands: scientific foundations are critical. pp 3-7 in Constructed Wetlands for Water Quality Improvement, G.-A. Moshiri (ed.). CRC Press, Boca Raton, FL.

Winter, T.C. (1992). A physiographic and climatic framework for hydrologic studies of wetlands. Pp. 127-148 in Aquatic Ecosystems in Semi-Arid Regions: Implications for Resource Management, R. D. Roberts and M. L. Bothwell, eds. N.H.R.I. Symposium Series 7. Saskatoon: Environment Canada.

World Health Organization. (2005). Water for Life, Making It Happen (On line). WHO and UNICEF. ISBN 92 4 156293 5. Available in: http://whqlibdoc.who.int/publications/2005/9241562935.pdf (accessed on 10/01/08).

Wrenn, B.A., Haines, J.R., Venosa, A.D., Kadkhodayan, M. and Suidan, M.T. (1994). Effects of nitrogen source on crude oil biodegradation. Journal of Industrial Microbiology and Biotechnology 13 (5), 279-286.

Wright, J. O. (1907). Swamp and overflowed lands in the United States. U.S. Department of Agriculture, Circular 76. Washington, DC: U.S. Government Printing Office.

Yang, L., Chang, H. and Huang, M. (2001). Nutrient removal in gravel- and soil-based wetland microcosms with and without vegetation. Ecol. Eng., 18 (1), 91-105.

Yang, S., Yoshida, N., Baba, D. and Katayama, A. (2008). Anaerobic biodegradation of biphenyl in various paddy soils and river sediment. Chemosphere 71 (2), 328-336.

Ye, S. H., Huang, L. C., Li, Y. O., Ding, M., Hu Y. Y. and Ding, D.W. (2006). Investigation on bioremediation of oil-polluted wetland at Liaodong Bay in northeast China. Applied Microbiology and Biotechnology 71 (4), 543-548.

Yeom, S. H. and Yoo, Y. J. (1999). Removal of benzene in a hybrid bioreactor. Process Biochemistry 34 (3), 281-288.

Yerushalmi, L. and Guiot, S.R. (2001). Biodegradation of benzene in a laboratory-scale biobarrier at low dissolved oxygen concentrations. Bioremediation Journal 5 (1), 63-77.

Zhao, Y. Q., Sun, G. and Allen, S. J. (2004). Purification capacity of a highly loaded laboratory scale tidal flow reed bed system with effluent recirculation. Science of the Total Environment, 330(1-3), 1–8.

Zhao, Y. Q., Sun, G. and Allen, S. J. (2004b). Anti-sized reed bed system for animal wastewater treatment: a comparative study. Wat. Res., 38(12), 2907-2917.

Zhou, E. and Crawford, R. L. (1995). Effects of oxygen, nitrogen, and temperature on gasoline biodegradation in soil. Biodegradation, 6(2), 127-40.

Zhu, T., Maehlum, T., Jenssen, P.D. and Krogstad, T. (2002). Phosphorus Sorption Characteristics of a Light-Weight Aggregate. In: Proceedings of the IWA 8*th*
International Conference on Wetland Systems for Water Pollution Control, Arusha, Tanzania. pp. 556-566.

Zhu, T., Maehlum, T., Jenssen, P.D. and Krogstad, T. (2003). Phosphorus sorption characteristics of a light-weight aggregate. Wat. Sci & Tech., 48(5), 93-100.

Appendix A

Selected Publications:

Eke, P. E. and **Scholz, M.** (2008). Benzene removal with vertical-flow constructed treatment wetlands. *Journal of Chemical Technology & Biotechnology* 83(1), 55-63 DOI: 10.1002/jctb.1778.

Eke, P. E., Scholz, M., and **Wallace, S. D.** (2007b). Constructed Treatment Wetlands: Innovative Technology for the Petroleum Industry. Proceedings of the 2007 Society of Petroleum Engineers (SPE) Annual Technical Conference and Exhibition, and Paper presented at the European Student Paper Contest during Offshore Europe Oil & Gas Conference & Exhibition held on 4 -7 September 2007 in Aberdeen, Scotland, United Kingdom, and also presented at the SPE International Student Paper Contest held on 11-14 November in Anaheim, California, USA. Available online in Society of Petroleum Engineers elibrary (http://www.spe.org/elibrary), SPE 113644, DOI: 10.2118/113644-STU.

Tang, T., Eke, P. E., Scholz, M. and **Huang, S.** (2008). Sustainable management of the seasonal variability in benzene removal by planted vertical-flow constructed wetlands to prevent pollution. *Journal of Environmental Management,* submitted.

Appendix B

SOM in original colour:

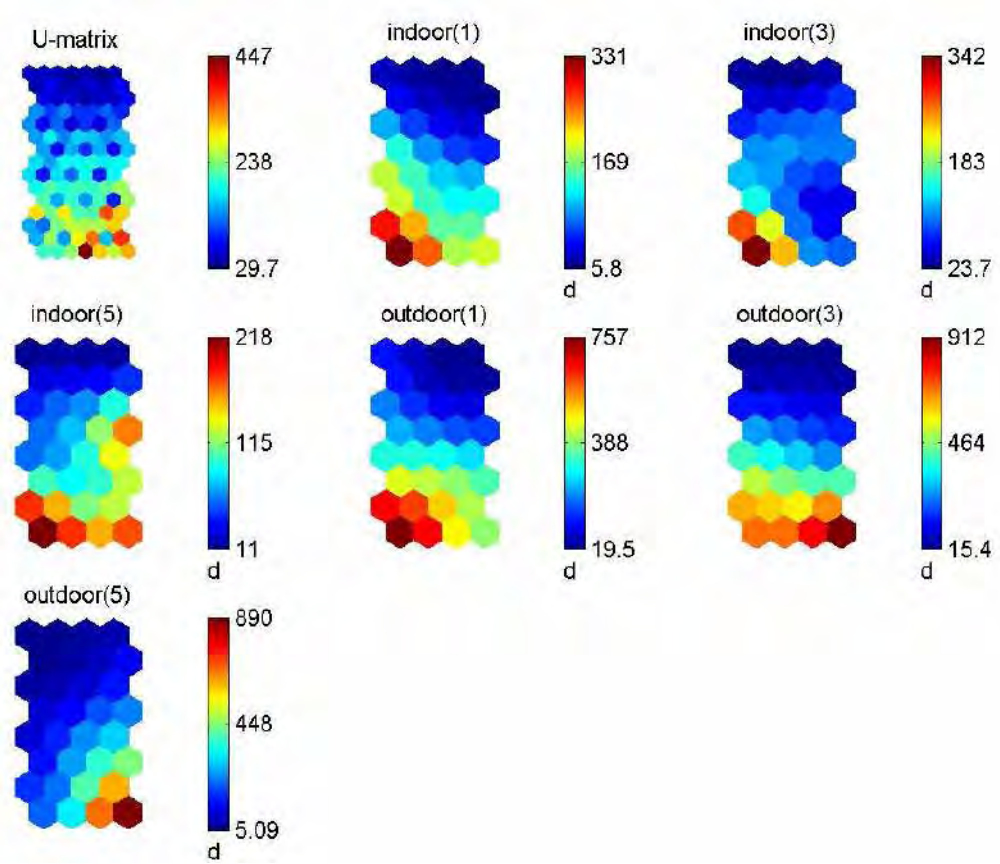


Figure 7-2. Self-Organizing Map visualizing relationship between effluent Benzene concentrations in indoor and outdoor wetlands. U-matrix on top left, then component planes. The seven figures are linked by position: in each figure, the hexagon in a certain position corresponds to the same map unit.

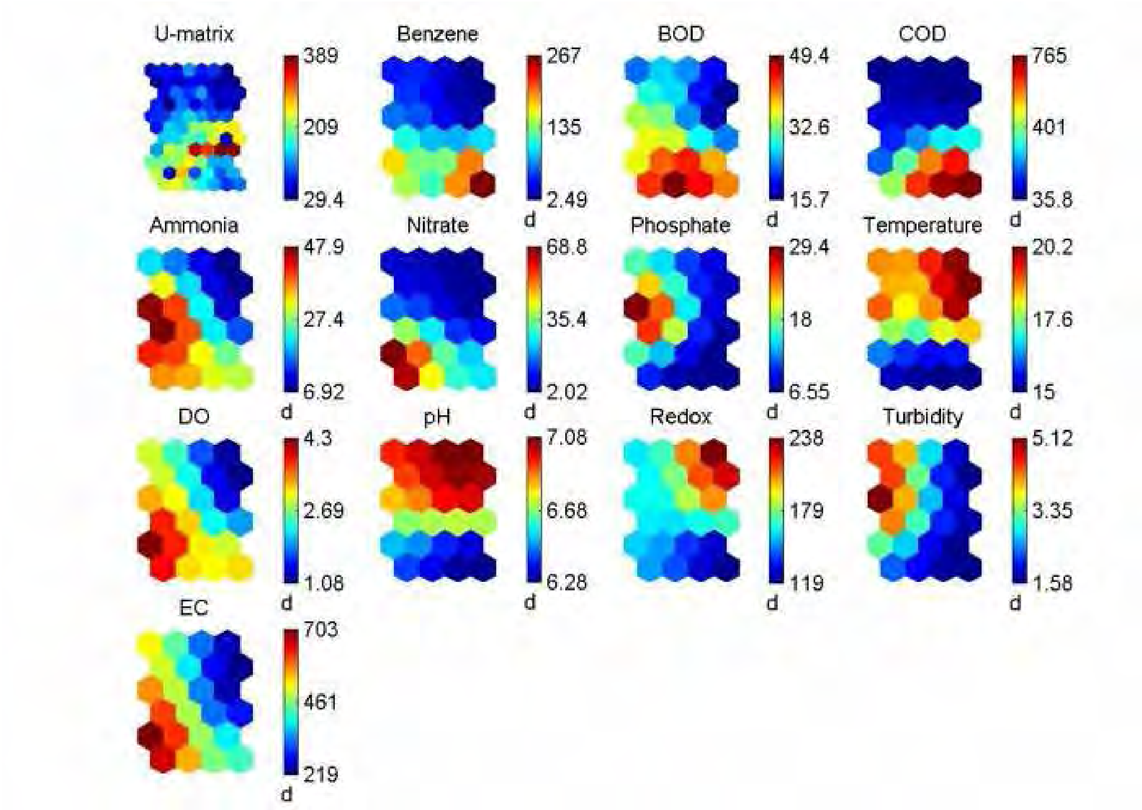


Figure 7-3. Self-Organizing Map visualizing relationship between effluent water quality variables and effluent Benzene concentrations in wetland 1 indoor.

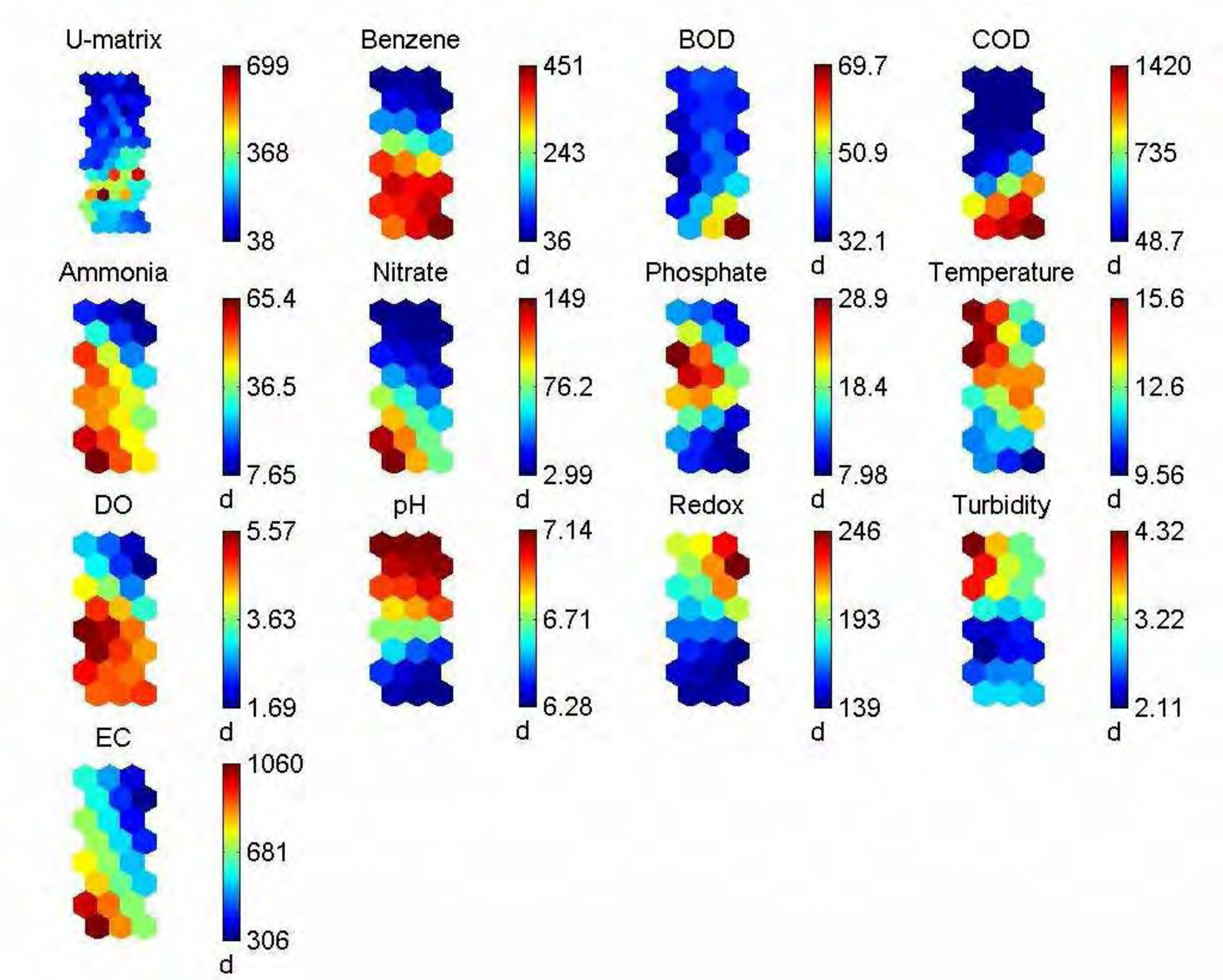


Figure 7-4. Self-Organizing Map showing relationship between effluent water quality variables and Benzene concentrations in wetland 1 outdoor.