REMOVAL OF ORGANIC AND INORGANIC NUTRIENTS IN A CONSTRUCTED RHIZOFILTRATION SYSTEM USING MACROPHYTES AND MICROBIAL BIOFILMS

MATHEWS SIMON MTHEMBU

2015

DURBAN UNIVERSITY OF TECHNOLOGY

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This work is submitted in complete fulfilment for the degree of Doctor of Philosophy (Biotechnology) in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences at the Durban University of Technology, Durban, South Africa

Mathews Simon Mthembu
(B.Sc. Medical Science; B.Sc. Honours, M.Sc. Microbiology)

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Supervisor : Dr Feroz Mahomed Swalaha
Co-supervisor : Prof. Faizal Bux

DECLARATION

"I declare that the thesis herewith submitted for the degree Doctor of Philosophy: Biotechnology at the Durban University of Technology is my original work and has not been previously submitted for a degree at any other institution of higher education, and that its only prior publication was in the form of conference papers, book chapter and/or journal articles. I further declare that all the sources cited or quoted are acknowledged and indicated by means of a comprehensive list of references".

21 October 2015

……. **……**

MS Mthembu **Date**

I hereby approve the final submission of the following thesis.

……. **……**

Dr F. M. Swalaha **Prof. F. Bux**

D. Tech (DUT) D. Tech (DUT)

This……day…….. of 2015, at the Durban University of Technology.

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ABSTRACT

Many households in developing countries are still without proper sanitation systems. The problems are even more prevalent in rural communities where there are no septic systems in place for the treatment of wastewater. This has resulted in the urgent need for the development and implementation of innovative wastewater treatment systems that are inexpensive, environmental friendly and are able to reduce contaminants to levels that pose no harm to the communities. Constructed rhizofiltration systems have been explored for this purpose. They have been used for many decades in many countries with varying degrees of success at the primary, secondary and tertiary levels of wastewater treatment. Poor optimization of this technology has been due to limited information available about the roles played by the whole system as well as by each component involved in the treatment technology. The current work elucidates the role played by macrophytes and microbial biofilms in the removal of nutrients in the rhizofiltration system. Factors affecting waste removal as well as environmental friendliness of the system were also investigated.

The rhizofiltration system was constructed in Durban and was divided into planted (planted with *Phragmites australis* and *Kyllinga nemoralis*) and unplanted (reference) section. Dissolved oxygen (DO), pH, water temperature, total dissolved solids (TDS), electrical conductivity (EC) and salinity were monitored. The removal efficiency of nutrients was measured using spectrophotometric methods by measuring the concentration of ammonia, nitrate, nitrite, phosphate and orthophosphate in the wastewater pre- and post-treatment. The total organic carbon, chemical oxygen demand (COD), total Kehldjahl nitrogen, biological oxygen demand (BOD), ammonia, nitrate and the flow rate of wastewater into the system from the settling tank were used for the estimation of carbon dioxide, methane and nitrous oxide emitted from the rhizofilter using the 2009 EPA formulae.

Both the planted and reference sections of the system removed nutrients with varying efficiencies. The reduction of nutrients in the rhizofilter was found to be seasonal, with most nutrients removed during the warm seasons. The system also retained more nutrients when wastewater containing

low levels of nutrients was used. The unpaired *t*-test was used to determine the differences between nutrient removals between planted and reference sections. Higher reduction efficiencies of nutrients were obtained in the planted section. Up to 65% nitrite and 99% nitrate were removed while up to 86% total phosphorus was removed in a form of orthophosphate (86%). Removal of total nitrogen was shown to increase under high temperature conditions, while the same conditions decreased the total phosphorus removal. High temperatures also increased the performance of the system. The reduction of nutrients in the system corresponded to reduction of the chemical oxygen demand which also positively correlated to the dissolved oxygen concentration. Considering the discharge limits for all nutrients, the discharges in the effluent of the planted section were within the allowable limits as per South Africa's Department of Water affairs and Forestry in 2012 but not in 2013. The results obtained in 2013 were due to increased nutrient loading introduced into the system.

Diverse microbial communities occurred in the treatment system, with more diversity in the planted section. These organisms were supported by macrophytes in the planted section, and were responsible for nitrogen and phosphorus transformation. This explains why total nitrogen and phosphorus reduction was higher in the planted compared to the reference section.

Both the planted and the reference sections of the rhizofiltration system produced the greenhouse gases. When the two sections were compared, the planted section produced more gases. Gases emitted by both sections were lower when compared to emission from sludge treatment reed beds and other conventional systems of wastewater treatments. These findings indicated that constructed rhizofiltration is a cleaner form of waste treatment, producing significantly less greenhouse gases and affecting less of a climate change. Findings of this work have revealed that rhizofiltration technology can be used as a low-cost alternative technology for the treatment of wastewater, using the combination of macrophytes and microbial biofilms. Macrophytes accumulated nitrogen and phosphorus as well as supported diverse microorganisms that metabolized and reduced nutrients in the rhizofiltration unit.

DEDICATION

This work is dedicated to my family, especially my children. Ntombizamagudu you are my biggest inspiration. I hope that when you grow up you can learn that when you approach resolutions and goals in the same manner, you will end up with a much better chance of achieving success. I would not have done this without a smile from all of you BaThembu.

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To solve a problem there are three questions one need to ask him/herself. These are what could I do, what could I read, and what could I ask? No one could have better guided me through answering these questions in this journey other than Dr. Feroz Mahomed Swalaha. Thanks Feroz for guiding, assisting and venturing with me throughout the entire journey. Thank you for showing me that the pursuit of happiness is a chase of a life time. Mostly, thank you for teaching me that by recording a dream and goals on paper, you set in motion the process of becoming the person you most want to be! I couldn't ask for a better mentor. In you I had the best and indeed, I learned from the Master! May GOD the Lord richly bless you abundantly.

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To everyone who contributed positively to the success of this work I say to them that from today I shall look upon every obstacle as a down payment towards my success. I will use the obstacles to strengthen me instead of weakening me. Thank you to everyone.

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PREFACE

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

In South Africa, water quality is deteriorating at an alarming level and is escalating into a national crisis equivalent to the energy crisis currently being experienced in South Africa. Sewage is running directly into the rivers; the environment is suffering and ultimately the people living in the respective river's catchment area. Fueling the problem is infrastructure inadequacy and poor maintenance of the existing structures. At some water plants no chlorine gas is available for the treatment of wastewater (Oneale, 2014). South Africa's water and sanitation infrastructure is crumbling because of a chronic lack of investment. The Department of Water Affairs says it needs more that R600-billion to fix the problem yet the fiscus can only provide half of this (Kings, 2014). In her budget speech in 2014, the Minister of Water and Sanitation said that 10% of existing wastewater treatment systems were dysfunctional. A further 26% were not reliable and would require an estimated R670-billion as a capital investment and infrastructure (Oneale, 2014).

It is therefore clear that wastewater treatment will always pose problems if there are no new or alternative technologies in place to replace and/or to be used in conjunction with the currently available technologies. Deterioration of the sources of water and water quality itself calls for implementation of new strategies aimed at improving wastewater. Current technologies are increasingly becoming unaffordable by many rural communities. Increased housing in the communities as well as changing communities accompanied by their changing needs for wastewater treatment also calls for the implementation of new strategies in the water sector for sustainability. This combined with poor performance of the current systems means that there is a need for the development and establishment of efficient treatment systems that may supplement the current available technologies in wastewater treatment.

South Africa is made up of approximately 850 municipal wastewater treatment plants, yet according to research by the South African Department of Water Affairs less than 50 percent of the 449 wastewater treatment systems which have been assessed, meet the regulatory national and international water quality standards for wastewater treatment. These findings are proof that South Africa's wastewater treatment and management systems are inadequate;

therefore the need for new innovative technologies and strategies to meet the required discharge limits. This has resulted in the urgent need for the development and implementation of innovative systems to resolve the current constrains to wastewater treatment (Kalbar *et al*., 2012). It is for these reasons that interest has grown regarding investigation of alternative wastewater treatment technologies for the treatment of wastewater. Constructed rhizofiltration systems are examples of alternative wastewater technologies that have the potential to meet the required influent treatment standards as compared to conventional methods. The technology dates back to 1952 (Siedel, 1973) and has been in operation from 1974 but with no scientific basis for its applications (Kickuth, 1977). Since wastewater treatment systems are inadequate for informal settings and require high resources for operation, constructed wetlands with their low operational requirements, cost and easy building and setting, could provide solution to informal wastewater development problems. This type of technology has the potential to spear receiving waters such as rivers the full effect of directly discharge sewage. Constructed wetland technology was developed through the simulation of natural wetlands affected by an increase in anthropogenic activities and environmental changes. However their success was limited due to the lack of knowledge in understanding their operations (Chong-Bang *et al*., 2010; Litter *et al.*, 2012).

Recently, it has been estimated that developing countries will run out of water by 2050 (Netherlands Environmental Assessment Agency, 2012; OECD, 2012). This is a cause for concern not only to communities but also a challenge to the scientist in the water sector to come up with alternative means of wastewater recycling. Water losses can be avoided through implementation of easy and inexpensive technologies for wastewater treatment and recycling. Environmental concerns over poor performing septic systems and high expenses in the construction of sewer systems as well as their operations within centralized water purification systems have spurred investigation into the appropriateness of the use of wetland technology for wastewater treatment. Constructed rhizofiltration systems efficiency in contaminant reduction and potential application in wastewater treatment has been reported in the past (Vymazal, 2009; Chong-Bang *et al*., 2010; Yongjun *et al*., 2010; Litter *et al.*, 2012). However, detailed functioning of the system and how this technology may be put into practical applications has never been elucidated in details. Research has shown that these systems can achieve high treatment efficiencies with regards to both organic and inorganic nutrients as well as pathogen removal if properly managed and efficiently utilized. This can have a profound

effect in the management and conservation of the world's scarce and depleting water resources (Fadel and Massould, 2001).

Operation of a full scale rhizofiltration system may have its challenges though. Construction of the systems adjacent to an existing stream or creek which is heavily polluted from years of receiving contaminated water may result in poor performance of the rhizofilter. Seasonal weather conditions may also reduce treatment reliability of the rhizofiltration. Temperature fluctuations and wastewater flow rates in the system may lead to inconsistent contaminant removal efficiencies in the rhizofilter. Heavy flow of incoming water may lead to the overloading of the removal mechanisms. Some wetland systems for acid mine drainage treatment use a compost or peat lining. Metal precipitates can build up in the compost, peat, or sediment, causing these layers to become non-permeable. Thus, it becomes a necessity to dredge the contaminated substrate after it has reached saturation in order to improve the treatment efficiency (Pedescoll *et al*., 2011). A combination of two or more macrophytes used in concerted action with microbial biofilms has an ability to reduce from wastewater into an acceptable level for safe disposal. The roles of both macrophytes and microbial biofilms need to be investigated and be understood in order to elucidate the extent at which both macrophytes and microbial may be used in wastewater treatment.

1.2 AIM AND OBJECTIVES OF THE STUDY

The aim of the study was to investigate organic and inorganic nutrient removal in a rhizofiltration system using macrophytes and microbial biofilms in order to improve domestic wastewater quality.

The objectives were:

a) To establish and monitor the development of a macrophyte rhizofiltration system in a pilot-scale rhizofiltration plant.
b) To determine the effects of extraneous physical parameters on organic and inorganic nutrient removal by a constructed rhizofiltration plant.
c) To determine the role of microbial biofilm and microbial population structure and dynamics in the removal of organic and inorganic nutrient by the system.
d) To determine the effects of macrophytes on organic and inorganic nutrient removal by the system.
e) To determine the carbon footprint of the rhizofiltration system.

1.3 LITERATURE REVIEW

1.3.1 Introduction

Constructed rhizofiltration systems are amongst the most recently proven efficient technologies for wastewater treatment. These have been shown to be a low cost, easy to maintain and to operate technology with a potential to treat wastewater. The technology has shown a potential for application in developing countries, particularly in small rural communities (Kivaisi, 2001). It has been used in Latin American rural isolated communities to remove contaminants from wastewater for populations with low economic resources (Chong-Bang *et al*., 2010; Yongjun *et al*., 2010; Litter *et al.*, 2012). The systems have been reported to be engineered and constructed to mimic processes found in natural wastewater treatment (Yeh *et al*., 2009). They are known to exploit natural processes in order to remove pollutants from municipal, industrial wastewater or from mine drainage (Stefanakis *et al*., 2011). Natural processes employed include vegetation, soil and microbial activities to treat contaminated water. The relationship and interactions between plants and microbial assembles highlights the importance of the performance of the wetland systems (Vymazal, 2005). However, other characteristics that define the ability and the potential of the constructed wetland such as construction and combination of different systems, flow characteristics, loading rate, effect of different operational parameters and the use of different plants need to be considered in the success of any constructed wetland technology (Stefanakis *et al.*, 2011). Rhizofiltration systems have been studied for years but the above-mentioned synergistic characteristics have never been dealt with in details. Dealing with these issues is imperative if these systems are to be introduced as an alternative wastewater treatment technology.

Plants and microorganisms have been reported to be major contributors to the processes occurring in rhizofilter systems (Kadlec and Wallace, 2009). Rhizofiltration systems have earned much of their focus in the research field, not only because of their low operational costs but also due to their potential use by small house-holds for wastewater remediation (Brix, 1987). They have been used to treat wastewater from point and non-point pollution sources including stormwater runoff, domestic wastewater, agricultural wastewater, and coal mine drainage. However, the mode of action and detailed mechanisms of contaminants removal from these systems has not been thoroughly investigated. This is due to the lack of detailed studies as well as understanding of the complex chemical and biological processes involved in the rhizofilter treatment systems, leading to the hindering of their introduction for local municipal

and industrial wastewater treatment. Studies that have been done to date therefore can at this point neither permit nor allow the introduction of constructed rhizofiltration technology for large scale operations as well as long term wastewater treatment (Mthembu *et al*., 2013).

An understanding of these processes is fundamental not only to designing rhizofiltration systems but also to the understanding of the fate of contaminants once they have entered the rhizofiltration system. This could aid in understanding their potential use for commercial/large scale applications. This work contributes to the possibility of the applications of the constructed rhizofiltration systems as an alternative technology for wastewater treatment by local municipalities. The focus of this research lies in the role played by microorganisms, plants as well as the effect of physical parameters in nutrient removal. The constructed rhizofiltration system efficiency and dynamics as well as processes involved in the technology are also discussed.

1.3.2 Operations and Design Characteristics of Constructed Rhizofiltration Systems

There are three main types of constructed rhizofiltration systems characterized by configuration design and operation. They are surface flow (SF), subsurface flow (SSF) and vertical flow (VF) constructed systems. These systems are constructed in a closed basin with a substrate and the bottom covered by a rubber lining (geotextile) to ensure that the processes occurring in them are completely waterproof. This is essential in any environment where leakage of water from the system can have adverse effects e.g. contamination of ground water. The substrates of the systems are gravel and sand or lava stones that contribute to nutrient retention in these systems (Farroqi *et al.,* 2008). Advances in engineering and technology have now permitted construction of a multi-designed system functioning as vertical, horizontal as well as subsurface system. This type of the technology represents a new trend and an emerging tool in wastewater treatment relying on macrophytes and sand as a medium. Although the design of these systems may be expensive, their success in nutrient reduction in wastewater may offer more advantages than conventional systems.

These multi-engineered systems (Figure 1.1) are being investigated for their maximal contaminant removal efficiency in municipal wastewater for their potential applications in large scale. These rhizofiltration systems were constructed to permit feeding and collection of effluent from different positions alongside the filter. The systems have vertical, surface flow as well as subsurface influent loading channels. Filters at the collection point/taps are used to

determine the flow out of the filter at different points and collect the effluent for measurement purposes (Figure 1.2).

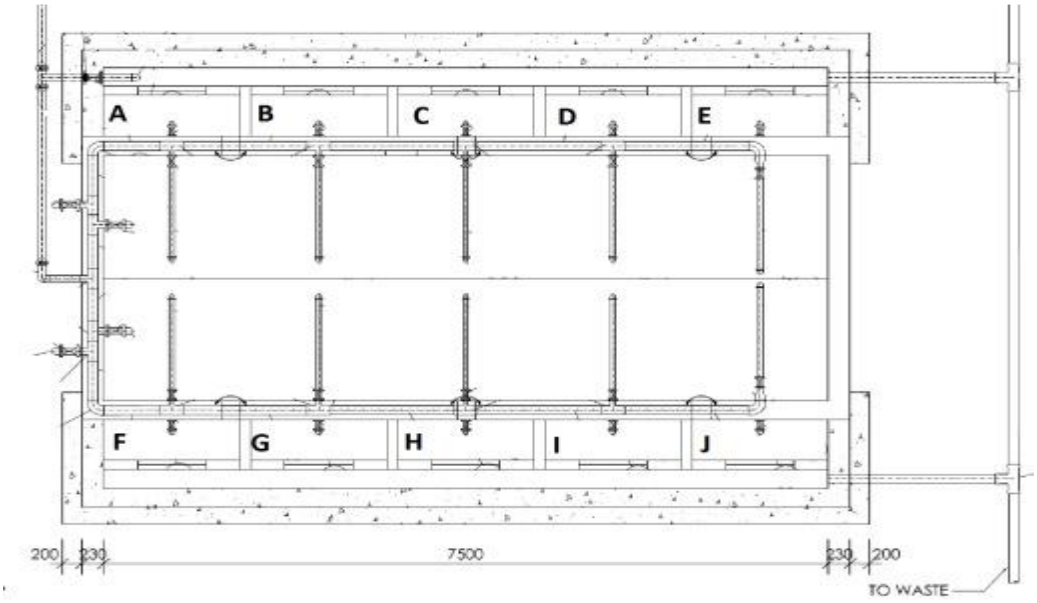

Figure 1.1: Schematic representation of the engineered rhizofiltration system, constructed at Kingburg wastewater treatment plant in Durban, South Africa. The system is along the center to provide space for a planted and reference section.

Figure 1.2: Basins and waste channel with a bypass outlet option in the left hand bottom corner of the multi-engineerd system constructed at Kingburg wastewater treatment plant in Durban, South Africa.

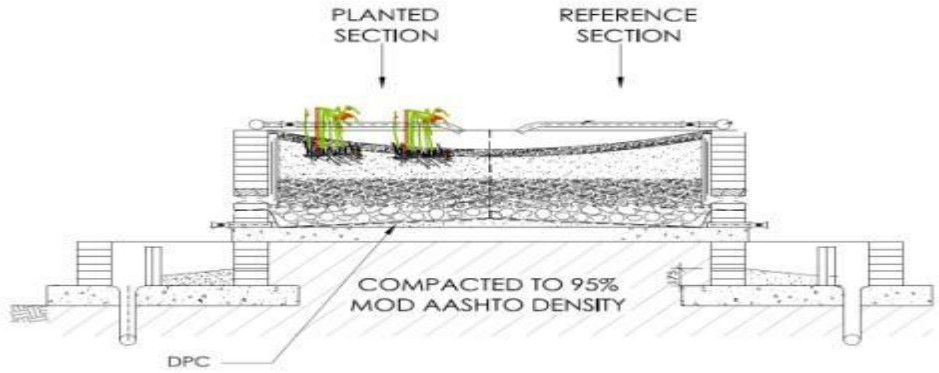

Figure 1.3: Cross section of the multi-engineerd system. The plants are planted in such a way that they are evenly distributed across the test section of the wetland. Species of the plants used are *Phragmites australis* and *Kyllinga nemoralis.*

The rhizofilter of the system is made up of different layers of rocks (pebble) and sand ranging from coarse rocks (100-200 mm) at the bottom to crushed pebbles (19-25 mm) at the top, which is topped off with fine sand on which a thin layer of the crushed rock is placed to protect the sand (Figure 1.3).

Figure 1.4: Front view of the engineered system. The middle vertical pipe comes from the tank. The vertical pipes on the left and right are the pipes to the bottom inlet for subsurface flow. Small pipes on top of the bed along the rhizofilter are for vertical flow, while those of surface flow are at the inside front of the system.

Multi-engineered systems (Figures 1.2 and 1.3) should be investigated and encouraged for use commercially. If properly constructed, monitored, controlled and well understood these systems may remove up to 100% of the contaminants from wastewater. For highest removal

efficiencies, wastewater has to flow from one type of flow system to the next within the wetland and for this to be possible, wetland segment 'separation' is required. For sustainability, ideal systems designed for municipal or industrial applications should use less or no energy at all (gravity flow). A well-designed wetland should transfer water by gravity through the system. If site topography limits the use of gravity, pumps should be used which would increase the cost of operation. Unlike modern sewage treatment plants, constructed wetlands will reduce or completely eliminate odor. Odor can become a serious problem when handling and treating animal or domestic wastewater, especially if the operation is located in close proximity to residential housing (Farroqi *et al.*, 2008).

1.3.3 Typical Concentrations of Contaminants from Domestic Wastewater Discharges

The organic material in municipal wastewater has been shown to be a combination of different compounds ranging from proteins and carbohydrates to lipids (Culp *et al.*, 1986). These are domestically produced through anthropogenic effects, and depending on concentration of their organic matter content, may be classified as weak, medium or strong depending (Metcalf and Eddy, 1991). The typical discharges from house-hold into wastewater treatment plants are shown in Table 1.1. The main priority of treatment is to reduce the level of these compounds into acceptable levels in wastewater and thereby assist in the recycling process of the water. This can be equally achieved by using a rhizofiltration technology. The appropriateness of constructed rhizofiltration systems for wastewater treatment had been reported from all over the world (Miller, 1996; Verma *et al*., 1997; Vymazal, 2009; Yeh *et al*., 2009; Kalbar *et al*., 2012). A properly operating rhizofiltration system treating wastewater has been shown to produce a secondary effluent with a soluble chemical oxygen demand of between 30 to 50 mg/l and a soluble biological oxygen demand of between 1 to 2 mg/l (Vymazal *et al.*, 1998). The total organic carbon levels in wastewater produced were acceptable between the ranges of 30 to 50 mg/l, and acceptable values for nitrogen and phosphorus for municipal effluent should be about 1 mg/l and 0, 1 mg/l respectively (Culp *et al.*, 1986).

Table 1.1: Classification of a typical influent discharges into the municipalities (Metcalf and Eddy, 1991; Department of Public Works, RSA, 2012)**.**

Contaminants		**Concentration**		
		Weak	**Medium**	**Strong**
Dissolved solids (DS) (mg/l)	Fixed	145	300	525
	Volatile	105	200	325
	Total	**250**	**500**	**850**
Suspended solids (SS) (mg/l)	Fixed	20	55	75
	Volatile	80	165	275
	Total	**100**	**220**	**350**
Total solids (TS) (mg/l)		**350**	**720**	**1200**
Settleable solids (mg/l)		5	10	20
BOD_5 at 20˚C (mg/l)		110	220	400
Total organic carbon (TOC) (mg/l)		80	160	290
Chemical oxygen demand (COD) (mg/l)		250	500	1000
	Organic	8	15	35
Nitrogen (mg/l)	Free ammonia	12	25	50
	Nitrites	0	0	0
	Nitrates	0	0	0
	Total	**20**	**40**	**85**
Phosphorus (as total P) (mg/l)	Organic	1	3	5
	Inorganic	3	5	10
	Total	**4**	**8**	**15**
Chlorides (mg/l)		30	50	100
Sulphate (mg/l)		20	30	50
Total coliform CFU/100ml		10^6-10^7	10^7-10^8	10^8-10^9
Volatile organic compounds (VOCs) (mg/l)		<100	100-400	>400

1.3.4 Aquatic Macrophytes and Rhizofiltration Technology

Aquatic macrophytes are plants that grow in or near water. They may be classified as either emergent, submerged or floating based on their location within the watercourse (Brix and Schierup, 1989; Crank and Fennessy, 2001; Wetzel, 2001).

- Emergent macrophytes: These are macrophytes that grow and emerge out of water and are most common in most constructed rhizofiltration systems. They grow within the water and range from 50 cm below the soil surface to the water depth of 150 cm or more. As emergent macrophytes grow, they produce aerial stems, leaves, and extension root and rhizome systems. Their morphologies are adapted to growing in a submersed substrate by virtue of large internal air spaces for transportation of oxygen to the roots and rhizomes. Most common form of emergent macrophytes include: *Phragmites*

australis, Glyceria spp., *Eleochons* spp., *Typha* spp., *Scirpus* spp., *Kylinga nemoralis* and *Iris* spp.

- Floating-leaved macrophytes: These macrophytes include both species which are rooted in the substrate and species which are freely floating in the water surface. They are highly diverse in form and habitat ranging from large macrophytes with rosettes of aerial, floating leaves and well developed submerged roots to maintain surface floating macrophytes with few or no roots. Most common forms of floating macrophytes available include *Eichhornia, Trapa, Hydrochans, Lemnoceae, Azalla* and *Salainia* spp.

- Submerged macrophytes: These macrophytes consist of photosynthetic tissue entirely submerged but usually the flower is exposed to the atmosphere. Two types of submerged macrophytes are usually recognized. These are *Elodea, Myriophyllum, Ceratophyllum, Isoctes, Littorella* and *Labelia* spp.

In lakes, aquatic macrophytes have been found to provide cover for fish, substrates for aquatic invertebrate, produce oxygen and act as food for some fish and wildlife (Farroqi *et al*., 2008). Aquatic macrophytes have also been shown to be efficient indicators of water quality and their presence may change with water quality due to their ability to absorb excessive loads of contaminants (Vymazal, 2009). These properties of macrophytes have been used in wastewater treatment as well as in biomanipulation of water bodies for enhancing fish production. Emerging and floating aquatic macrophytes seldom grow in water exceeding a depth of 3 m. The presence of large stands of aquatic macrophytes results in variations in the dissolved oxygen content in water diurnally (Confield and Hoger, 1992). The distribution of aquatic macrophytes in water could be influenced in a number of ways and may fall into three broad categories:

- Factors related to the change in chemical and physical conditions in water and sediments.
- Metabolic effects related to production and processing of organic matter and nutrient cycling.
- Effect on biotic interactions and community structure related to the role of macrophytes in producing a structural habitant (Gasith and Hoger, 1998).

Macrophytes have demonstrated the ability to remove contaminants in wastewater which makes them useful in the removal of agricultural effluents. These assist in reducing contaminants to acceptable levels in natural water bodies and reservoirs (Rakacy and Allison, 1981). The functions of aquatic macrophytes in rhizhosystems have been found to generally to support components of aquatic environments that improve wastewater treatment capabilities and reliability of the environment (Stowell *et al*., 1980). Roots and stems in the water column may provide surfaces for bacterial growth, media for filtration and adsorption of solids while stems and leaves on or above water surface attenuate sunlight and also prevent the growth of algae and reduce the effects of wind on water. Stems and leaves may also function in the transfer of gases to and from the submerged part of macrophytes (Fukuda *et al*., 1999).

1.3.5 Growth of Macrophytes and their Benefits to Aquatic Environments

Macrophytes may only grow in water or in soil that is permanently saturated with water, which make them an important component of the rhizofiltration process in wastewater treatment. The growth of aquatic macrophytes requires the presence of nutrients such as nitrogen and phosphorus for their growth and reproduction. A constructed rhizofilter receives wastewater which is rich in various types of nutrients required by aquatic macrophytes for growth (Keddy, 2000). Most of the natural aquatic macrophytes reproduce by flowering and seed settling while many other types are capable of extensive asexual reproduction by means of rhizomes and fragments (Hutchinson, 1975). Aquatic macrophytes require warm weather for growth. They usually grow at a much higher rate during the period between October and April. Major factors such as nutrient availability and salinity need to be monitored during growth of aquatic macrophytes as high concentrations may retard growth (Keddy, 2000).

The growth of many macrophytes requires the presence of a root system growing within an organic rich soil substrate. Macrophyte roots hanging within a rhizosphere or beneath have been demonstrated to provide an extensive surface area for the attachment of biofilm growth and entrapment of fine suspended particles (Bourn, 1932; Hochereutiner, 1986). Macrophytes use their root systems to release oxygen into the rhizosphere required by aerobic microorganisms in order to degrade contaminants in the rhizosphere. The amount of oxygen released in the sub-tropical region is higher and decreases with distance from the root apex (Armstrong, 1979). Another important role of roots is the absorption of carbon dioxide from the substrate which serves as rich source of this dissolved gas. Some macrophytes have shown

rapid growth in the absence of any substrate when carbon dioxide enriched air is bubbled through nutrient medium (Bristow, 1974).

1.3.6 Plants and their Role in Constructed Rhizofiltration Technology

The general mechanisms of nutrient removal from wastewater in constructed rhizofiltration systems have been attributed to microbial processes such as nitrification and denitrification as well as physicochemical processes such as fixation and precipitation by heavy metals. Moreover, plants are able to tolerate high concentrations of nutrients and heavy metals and in some cases even accumulate them in their tissues (Stottmeister *et al*., 2003). Plants may also be involved in the uptake of nitrogen, phosphates and heavy metals in water thereby decreasing nutrient content in wastewater (Kalbar *et al.,* 2012). The most reactive zones of the plant in constructed rhizofiltration are in the rhizosphere where all physicochemical and biological processes take place. These processes are induced by interactions of plants, microorganisms, soil matrix and contaminants.

Macrophytes have also been shown to be responsible for approximately 90% of oxygen transport available in the rhizosphere (Vymazal, 2011). Oxygen and nitrogen transport stimulates aerobic and anoxic decomposition of organic matter respectively as well as promoting the growth of nitrifying bacteria and periphytons in the soil medium (Zhang *et al*., 2007). Table 1.2 summarizes some of the major roles of macrophytes in a rhizofiltration system for wastewater treatment.

Table 1.2: Major roles of macrophytes in constructed wetland treatment systems (Vymazal, 2011).

Macrophyte property	Role in treatment process
Aerial plant tissue	Light attenuation—reduced growth of photosynthesis. Influence of microclimate—insulation during winter. Reduced wind velocity—reduced risk of re-suspension. Aesthetically pleasing appearance of the system. Storage of nutrients.
Plant tissue in water	Filtering effect—filter out large debris. Reduced current velocity—increased rate of sedimentation, reduced risk of re-suspension. Excretion of photosynthesis oxygen—increased aerobic degradation Uptake of nutrients. Provision of surface for periphyton attachment.
Roots and rhizomes in the sediment	Stabilizing the sediment surface—less erosion. Prevention of the medium clogging in vertical flow systems. Provision of surface for bacterial growth. Release of oxygen increases degradation (and nitrification). Uptake of nutrients. Release of antibiotics.

For nitrogen removal from the rhizofilter by macrophytes, nitrogen assimilation processes convert inorganic nitrogen into organic forms that serve as building blocks for plant cells and tissues (Brix, 1997). Ammonia and nitrate are the two main forms of nitrogen assimilation with ammonia being the most preferred source because it is readily utilizable (Vymazal, 2007). These nitrogenous compounds have been shown to be assimilated by rooted floating-leaved macrophytes in the sediments and by free-floating macrophytes in water (Dhote and Dixit, 2009). There are many different plant species available for use as potential macrophytes and they differ in their preferred forms of nitrogen (Zhang *et al.*, 2007; Dhote and Dixit, 2009). Many plant species are able to take up any soluble form of nitrogen.

The ability of the plants to absorb nutrients, particularly nitrogen, has been demonstrated to be seasonal (Vymazal and Kopfelova, 2011). Nitrogen uptake by macrophytes has been found to be mostly high during spring and summer in temperate climates. Species of plants such as *Typha* and *P. australis* have an annual cycle above ground biomass, which means new shoots start from zero biomass in early spring then grow at a maximum rate in spring and early

summer. During late summer, growth is reduced which later is followed by a complete shoot die off (Vymazal, 2007). This phenomenon of plant growth and nutrient uptake is possible because nutrient concentration of the plant is increased at an early age of plant development (due to high nitrogen demand by the plant) and reduces at later stage. If rhizofiltration technology is to be introduced as an alternative technology for wastewater treatment, seasonal variations affecting nutrient uptake by the plants and microbial activities should be considered. Systems should always be optimized for the best performances throughout a year-long cycle.

During plant shoot die off, plant biomass may be decomposed to release carbon and nitrogen and the release is important in the rhizofilter nitrogen cycle because it may impair total nitrogen removal. Some portion of nitrogen may be released back into the rhizofilter unit, some, subjected to aerobic process while some may be translocated to rhizomes (Vymazal, 2007). The potential rate of nutrient uptake by plants is ultimately determined by plant growth rate and the concentration of nutrients in the plant tissue, thus nutrient storage of the plant is dependent on plant tissue nutrient concentrations and on plant biomass accumulation. Ideal characteristics for plants to be used as ideal macrophytes in rhizofiltration systems are fast growth rate, high tissue nutrient content and the ability to attain a high standing crop (plant sustainability). If constructed rhizofiltration systems are to be used as efficiently as possible for commercial treatment of wastewater, knowledge of effectiveness of various plant species, colonization characteristics of certain groups of microorganisms and information on how biogenic compounds and particular contaminants interact with the soil matrix are essential. This information is also critical to the design strategy and construction of the system for its usefulness. Effectiveness of the combination of different macrophytes should also be considered (Graves and Haystead, 2002).

1.3.7 Microbial Biofilms and their Role in Nutrient Removal in Rhizofiltration Technology

Microbial biofilms have been reported to play a prominent role in the transformation, mineralization and subsequent removal of organic and inorganic contaminants in the rhizofiltration system (Moeseneder *et al.*, 1993; Moura *et al.*, 2007). These nutrients are metabolized in various ways. In subsurface flow constructed rhizofiltration systems aerobic microbial-driven processes occurs predominantly near plant roots as well as on root surfaces. These are mainly responsible for nitrogen removal in the rhizofiltration technology. In the areas that are largely oxygen free, anaerobic processes such as denitrification, sulphate reduction and methanogenesis occur, which removes nitrogen, sulphates, phosphates and carbon

respectively. The combination of compost degradation and microbial biofilms in heterotrophic microbial growth is responsible for oxygen removal from the rhizofilter units, and thereby promotes the formation of hydrogen sulphide. Sulphate reducing bacteria degrade and reduce nutrients that contain sulphates and produce hydrogen sulphide in the process (Lee and Yang, 2010).

Both autotrophs and heterotrophs have been found to incorporate ammonia and convert it to amino acids and proteins (Vymazal, 2007). However, this removal mechanism is less significant compared to microbial transformation. Nitrification-denitrification is the main microbial nitrogen removal mechanism (Stottmeister *et al.,* 2003). Nitrogen compounds are continually transformed from inorganic to organic compounds and back from organic to inorganic through processes like volatilization, ammonification, nitrification, nitrate-ammonification, denitrification and nitrogen fixation. All these transformations are necessary for the rhizofiltration ecosystem to function successfully and all chemical changes are controlled by enzymes produced by microorganisms. Thus microorganisms are essential in the rhizofiltration system if nutrients are to be completely degraded and removed from wastewater in constructed systems (Lizotter *et al.,* 2001).

1.3.8 Water Flow Rates and Biofilm Growth and Development

High wastewater flow rates may reduce the rate of biofilm attachment and growth in the rhizofiltration system. Microorganisms have been shown to be washed away and their ability to attach on surfaces decreased (Farroqi *et al.,* 2008). With high flows, nutrients availability and uptake by microorganisms become limited and growth as well as development of biofilms is decreased. In a rhizofiltration system that is dependent mainly on biofilms for nutrient removal in wastewater, this may significantly reduce the performance of the system.

Biofilm formation needs a large surface area for microbial attachment. Suspended material in industrial and/or wastewater offer large surface areas for attachment (Singh and Kapoor, 2010). Smooth surfaces offers low biofilm formation rates compared to rough surfaces. High water flow rate alters biofilm growth and development but does not prevent the attachment of microorganisms to surfaces. This is due to the fact that water flow rates can be high at the center of the running/flowing water but drops to zero at the surfaces/walls. In water systems with continuous high flows, organisms that accumulate to form biofilm are usually a variety of filamentous bacteria suited for attachments with filaments. These organisms anchor and attach

themselves to the surfaces with their filaments. Although high water flow rate does not prevent the attachment of microorganisms to the surfaces, it has the effect on the biofilm sizes and population dynamics in water. High flow rates result in the formation of a low thickness biofilm on the surfaces of the filter and results to limited nutrient availability in water for biofilms. Nutrient limitations may lead to limited microbial growth, which in turn may limits biofilm thickness (Martins *et al*., 2011).

1.3.9 Mechanisms of Contaminants Removals Using Rhizofiltration Technology

Combinations of biological, chemical and physical processes have been shown to be responsible for the removal of contaminants from wastewater (Table 1.3). Biologically, plants and microorganisms play a major role in removal of contaminants by transforming and/or accumulating them and converting them into biomass. Wastewater treatment within a constructed rhizofilter occurs as the water passes through the rhizofiltration media and macrophytes. Interactions between water and macrophyte roots lead to rhizofiltration and sedimentation while that of microorganisms and contaminants lead to biodegradation (Figure 1.5). Root hairs and rootlets provide an aerobic environment which supports the activities of aerobic microorganisms. Aerobic and anaerobic microorganisms facilitate the decomposition of organic matter and inorganic substances in water through degradation and nutrient uptake. Figure 1.5 illustrates some of the possible interactions between rhizofilter medium (sediment), rhizomes (roots) and microorganism in the removal/transformation of contaminants. During these interactions, nitrogen is liberated from the system through microbial nitrification and subsequent denitrification processes. Organic nitrogen, nitrate, nitrite, ammonia, ammonium and nitrogen gases are the most common forms of nitrogenous compounds available/liberated during treatment process (Cooper *et al.*, 1996). These compounds are essential for plant growth and development; however, it is important that they are removed as some may be toxic to aquatic life (Brisson and Chazarenc, 2009). Suspended solids are removed by settling in the water column in surface flow systems or are physically filtered out by the medium within subsurface flow filters. Pathogens are removed by filtration and adsorption onto biofilms or plant roots. Heavy metals and phosphates are removed by either plant uptake or through sedimentation (Hunter *et al.*, 2001). Macrophytes themselves also need to be removed from time to time from the system in order to prevent die-off in the system and re-introduction or recycling of the contaminants in the system.

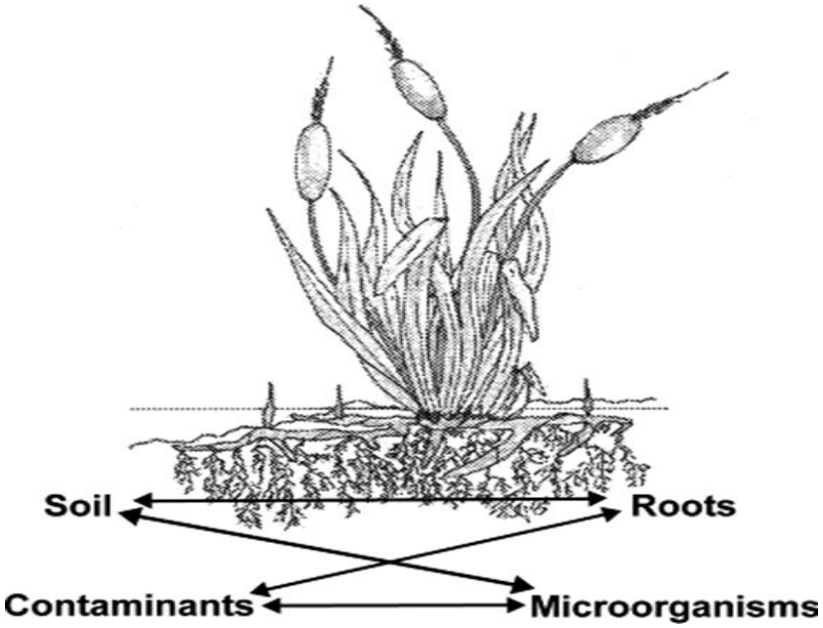

Figure 1.5: Some possible interactions occurring in wetlands (Stottmeister *et al.,* 2003).

In order to understand more about the complexities of what happens when contaminants are degraded in a constructed rhizofiltration system during treatment, it is important to know more about plants and their activities as well as microbial community structure and their abundance in the system. This can be done through studying the following properties:

a) Different macrophytes.
b) Characterizing microbial population present in the rhizofiltration system.
c) Media used in the rhizofilter system.

Table 1.3: Mechanisms of wastewater treatment using wetland technology (Cooper *et al*., 1996; Stottmeister *et al.*, 2003; Vymazal, 2009).

Wastewater Constituents	**Removal Mechanism**
Suspended solids	Sedimentation Filtration
Soluble organics	Aerobic microbial degradation Anaerobic microbial degradation
Phosphorus	Media sorption Plant uptake
Nitrogen	Ammonification followed by microbial nitrification Denitrification Plant uptake Matrix sorption Ammonia volatilization by microbial processes
Metals	Adsorption and cation exchange with other elements like calcium Complexation to other metals and media Plant uptake Precipitation Microbial oxidation/reduction
Pathogens	Sedimentation Filtration by media Natural die-off Predation by microorganisms UV irradiation Secretion of antimicrobial agents by macrophytes and microorganisms

1.3.10 Non-biological Mechanisms of Contaminant Removal

Contaminants such as nutrients and heavy metals may be removed from the constructed rhizofilter by means other than biological processes/activities. These include ammonia adsorption and physical burying the nitrogen-bearing organics. Ammonia may be adsorbed from solution through cationic exchange reaction with inorganic sediments or soil when it is ionized. It becomes loosely bound and can be released easily when pH and/or temperature changes. Such conditions decrease the concentration of ammonia in the water column. Ammonium ions are generally adsorbed as exchangeable ion clays and fixed within the clay lattice (Vymazal, 2007). Some fractions of organic nitrogen incorporated into detritus in rhizofiltration systems eventually become unavailable for additional nutrient cycling through the process of peat formation and burial (Simeral, 1999; Yeh *et al*., 2009; Yadav *et al*., 2010). Peat formation and burial in the rhizofilter significantly removes and reduces nutrients in water.

Phosphates may also be removed from wastewater through adsorption onto the walls of the filter system, and burial by the rhizofiltration media. Heavy metals may form complexes with other metals and precipitate out of water.

1.3.11 Removal of Heavy Metals in a Constructed Rhizofilter System

Heavy metals are usually found in industrial wastewater and mine drainage. However, small quantities may be detected in municipal wastewaters. The main heavy metals associated with wastewater and produced by mines and industries are chromium, iron, mercury, copper, lead, cadmium and zinc. These heavy metals may be removed from wastewater by the rhizofiltration system through a variety of methods including filtration and sedimentation, adsorption, uptake into plant material and precipitation by geochemical processes (Stottmeister *et al.,* 2003). Removal efficiencies of heavy metals by constructed rhizofiltration have been reported to be up to 100% (Romero *et al.,* 2011). Other possible removal efficiencies through the system as reported by Sheoran and Sheoran (2006) are 75-99% for cadmium, 26% for lead, 76% for silver, and 67% for zinc, while COD, BOD and TSS were removed at an efficiency of between 75 and 80%. Metals were demonstrated to accumulate in the leaves, shoots, rhizomes with roots and lateral roots having the highest content, while the lowest concentrations were found within the shoots. This was demonstrated through sampling the abovementioned parts of the plant and concentrations of the metals determined using spectrophotometric methods.

In surface flow systems used to treat mine drainage, Fe (II) is oxidized to Fe (III) by abiotic and microbial oxidation. In such a system, other inorganic substances such as arsenic may also precipitate. Iron may also be immobilized in the anoxic soil matrix by microbial dissimilatory sulphate reduction, producing hydrogen sulphide. Most heavy metals are taken up and accumulated within the plants. After being taken up, metals concentrate in the plant roots and less commonly in the stems. Only few heavy metals like mercury are able to translocate to the leaves (Romero *et al.,* 2011). Different plant species have differing abilities to take up heavy metals (Mitsch and Jorgensen, 2004). Some species of plants have high biomass which enhances their phytoremediation capacity. Plants like *Polygonum punctatum* have been proposed as copper and zinc biomonitors and phytoremediators and could be useful in constructed rhizofilters for the treatment of industrial wastewater and mine drainages.

Previous studies by Stottmeister *et al.* (2003); Sheoran and Sheoran (2006) and Romero *et al.* (2011) indicated that from technological point of view, heavy metal accumulation by plants

was insignificant when considering treatment of industrial wastewater and mine drainages. This was because the amount of heavy metals that could be accumulated by plants was found to be far too small when compared to the total load in wastewater. In a rhizofiltration system filter sediments and precipitation are the leading means of heavy metal removal.

1.3.12 Pathogens Removal in Constructed Rhizofiltration System

For successful applications in wastewater treatment, rhizofiltration systems should have the ability to remove pathogens from wastewater. Past research indicates that rhizofiltration systems have an ability to reduce pathogens with varying but significant degrees of effectiveness (Karim *et al.,* 2004). Microbial water quality improvements using rhizofilter units have been reported, with some studies documenting up to 57% reduction of total coliforms, 62% of fecal coliforms, 98% reduction of most species of *Giardia,* 87% of most *Cryptosriduim* spp. and 38% of coliphage (Stottmeister *et al*., 2003; Karim *et al*., 2004). Human pathogenic viruses were also found to be removed from wastewater by the systems. Viruses associated with large particles leave the water column and settle into the bottom sediments while some that are adsorbed on colloidal particles tend to stay suspended in water before filtered (Karim *et al.,* 2004).

Pathogen removal through rhizofiltration systems was also reported by Greenway (2005) and Boutilier *et al*. (2011). They documented enteropathogenic *Escherichia coli* removal efficiencies to be 52% and 99.9% respectively. To mitigate elimination variability, it is necessary to better understand what pathogen removal mechanisms dominate within the system and how these mechanisms may be intensified through the manipulation of the rhizofilter operational parameters at optimum levels. Previous studies on pathogen removal by constructed rhizofiltration treatment have been a grey zone and were mainly aimed at comparing the influent and effluent pathogen levels. However, many mechanisms have been associated with the removal of pathogen from constructed wetland systems. These include physical (filtration, sedimentation, adsorption, and aggregation), biological (consumed by protozoa, lytic bacteria, bacteriophages, natural death) and chemical (oxidative damage, influence of toxins from other microorganisms and plants) processes. Sedimentation remains a leading mechanism responsible for pathogen removal from wetland system (Karim *et al.,* 2004). This has been demonstrated by many studies which found total coliforms, fecal coliforms and *Salmonella* had concentrated in sediments of contaminated surface water in wetland systems (Jonson *et al.,* 1997; Greenway, 2005; Boutilier *et al*., 2011). They also

demonstrated that revival of such organisms from sediments was easier than in water column itself. Jonson *et al.* (1997) and Chauret *et al.* (1998) observed higher numbers of fecal coliforms in marine sediments than in overlying water. They also found that about 90% of *Salmonella* isolates from sediments showed high recovery in sediments than in water. *Escherichia coli* was also demonstrated to survive longer in sediments than in overlying water.

Accumulation of microorganisms, pathogens in particular, in sediments of constructed rhizofiltration systems designed for wastewater treatment means that these systems could be used for elimination/reduction of pathogens from influents. However, removal of pathogens using sedimentation process could also pose some serious threats as the bottom sediments of the system could serve as a potential reservoir of human pathogens. These reservoirs might be released back into the water column by events such as storm and thereby released with effluent to the river during floods or through increased runoff (Pedescoll *et al.*, 2011).

Plants have also been found to reduce pathogens in constructed rhizofiltration systems. Plants like *Mentha aquatica, Phragmites australis* and *Scorchi lacustric* were found to inhibit the growth of *E. coli* (Stottmeister *et al.*, 2003). Other than a bactericidal effect of the plants, which requires direct effect of the plants in wastewater, other mechanisms and indirect mechanisms of plant pathogen removal such as adsorption, aggregation and filtration were also shown to be involved in the reduction and subsequent removal of pathogens. It could be concluded from the literature study that the combined action of physical, chemical and biological processes are required to achieve high removal efficiencies of pathogen from constructed rhizofiltration technology. However, the removal mechanisms are still not well understood. For efficient removal of pathogens from these systems for their utilization, more research is needed to define these mechanisms as well as their synergistic effects in the removal efficiency.

1.3.13 Modeling Contaminant Removal in the Rhizofiltration System

Contaminant removal using rhizofiltration systems may be predicted using mathematical models. In the past few years, different prediction tools for processes occurring in a constructed rhizofiltration systems have been described (Shrestha *et al*., 2003; Langergraber, 2011; Kumar *et al*., 2011). These models may be numerical, statistical, or even computationally based. They are described based on rhizofiltration design and type (Odeja *et al.*, 2008; Freire *et al.*, 2009; Langergraber, 2011). The importance of rhizofiltration system modeling is that it allowed a better understanding of the processes involved in the system and to explain or describe their

functioning in more simplified terms. They aid in predicting the system's ability to remove contaminants and are thus useful in controlling or maintaining the system's operational dynamics. Recently, a numeric dynamic simulation model was developed for the removal of soluble reactive phosphorus from the vertical flow constructed systems using structural experiential learning laboratory with animation (STELLA). This model is a dynamic software model whose development was aimed at simulating the environment and showed succession of relationships between interdependent components and processes occurring in a vertical flow constructed rhizofiltration system (Kumar *et al.*, 2011). In this model, alum sludge was used as a main substrate and the model indicated the alum sludge to have high phosphorus removal by both plants and microbial activities.

Ideally, each different rhizofilter unit configuration should have its own model. Therefore, horizontal and vertical flow systems are modeled differently. Horizontal flow systems are simulated when water flow saturations saturated with contaminants are considered, and also uses a network of continuous-stirred tank reactor to describe the hydraulics. Reactions are modeled with various complexities in horizontal flow systems. Transient variable-saturated flow models are required for the modeling of vertical flow constructed systems with intermittent loading. Modeling with these systems has been found to be more complex because they are usually highly dynamic due to intermittent loading. Models applicable for use in vertical flow constructed rhizofilter systems have used either the Richards equation or a simplified approach to describe variable-saturated flows (Langergraber, 2011).

The most commonly used models in describing subsurface constructed rhizofiltration systems are numerical models and have been explained in detail by Langergraber (2011). They have been shown to be complex flow models but single-solute transport only, reactive transport models for variable-saturated flow and reactive transport models for saturated flows. These different models offer description of biochemical transformation and degradation processes for both organic and inorganic substances in subsurface flow constructed rhizofiltration system. They have been introduced and published with an aim of providing a widely accepted model formulation for biochemical transformation and degradation processes in a constructed rhizofilter system that can thus be implemented in various simulation tools. They have been used to describe aerobic, anoxic and anaerobic processes occurring in a horizontal and vertical flow constructed wetland systems requiring prediction of the effluent concentration of organic and inorganic substances (Shrestha *et al.*, 2003; Kumar and Kumar, 2005; Langergraber, 2011).

Constructed rhizofiltration modeling is one of the most powerful tools that can be used to predict the removal efficiency of contaminants from wastewater. However microorganisms, organic and inorganic substance's fate and transport modeling within rhizofilter systems requires further development if they are to become a reliable predictive forms of wastewater treatment ability, particularly in municipal wastewater treatment (Shrestha *et al*., 2003).

1.3.14 Pollution Treatment Efficiency of Constructed Rhizofiltration Technologies

Previous studies have shown a high treatment efficiency of constructed rhizofiltration systems with regards to organic and inorganic nutrient removal as well as removal of other general contaminants such as, total suspended solids (TSS) and microbial contaminants from wastewater (Cooper *et al*., 1996; Shrestha, 2005; Yadav *et al.*, 2010; Vymazal, 2011). Regular monitoring of systems had shown close to 100% removal of total coliforms and organic pollutants (Shrestha *et al*., 2003). Although average removal efficiencies of nitrogen and phosphate have been reported, significant difference in removal efficiency is observed among plant species as well as among different type of rhizofiltration system configurations (Yeh *et al.,* 2009). The main mechanisms leading to contaminant removal in these systems are microbial activities. However plants also have a huge role in contaminant removal in wastewater. They take up nutrients and incorporate them into plant tissue and thus increase in plant biomass (Zhang *et al*., 2007). Plants also create suitable conditions for the processes leading to nutrient removal to occur (Dunabin and Browmer, 1992; Wu *et al*., 2011).

Various types of wastewater are also treated with varying degrees of efficiencies. Vymazal and Kopfelova (2009) have used subsurface flow constructed rhizofilter to treat wastewater from municipal sewage, agriculture, industry and from landfill leachate. From 400 constructed rhizofilter systems in 36 countries with varying types of wastewater it was found that municipal wastewater had, overall, the highest contaminant removal efficiencies while the lowest removal efficiency were observed from landfill leachates. These observations suggested that most systems had been designed to treat municipal sewage and also the fact that most municipal wastewater contained predominantly labile organics while landfill leachates often contained recalcitrant organics which were difficult to degrade (Simeral, 1999). Systems designed for municipal applications should be able to achieve and sustain the highest maximum possible removal of contaminants from wastewater at high efficiencies if they are to be introduced as alternative technology for wastewater treatment. This will enable them to meet high municipal demands with high volumes and flow rates.

1.3.15 Physical and Chemical Parameters Affecting Treatment Efficiency of Wastewater

1.3.15.1 Potential of hydrogen (pH)

Potential of hydrogen is the hydrogen ion activity of water. It determines the concentration of hydrogen ions in water. Indigenous and foreign microorganisms involved in the removal of nutrients in the rhizofiltration system have different definite pH growth rates and pH growth optima. Nitrifying bacteria involved in nitrification are neutrophiles and have their growth optimum between pH of 5.5 and 8.0. However, when the pH is below 5.5 it favors the growth of acidophiles and above 8.0 is suitable for alkalophiles which are denitrifying bacteria (Willey *et al.,* 2008). Although microorganisms can grow under wide ranges of pH and far from their optima, they have limitations to their tolerance of pH (Willey *et al.,* 2008). Variations in pH can harm microorganisms by disrupting the plasma membrane, inhibiting the enzyme activity and altering the ionization of nutrient molecules, which reduce microorganism's nutrient uptake capability (Willey *et al.*, 2008). This may negatively affect the removal efficiency of nutrients in the system. Microorganisms' die and nutrient removal decreases resulting in large accumulation of biomass.

At extremely high or low pH levels wastewater becomes unsuitable for the growth of most microorganisms. A pH greater than 7.5 promotes nitrification, an essential process in the removal of nitrogen by constructed rhizofiltration system, thus causing loss of nitrogen from the soil. At acidic pH values, the amount of conversion of ammonium ion to nitrate by *Nitrosomas* spp. and *Nitrobacter* spp. is decreased. Nitrogen removal decreases at a pH of 6.0 and lower (Cortes-Lorenzo *et al.,* 2012). Acidic pH decreases the conversion of ammonium ions to nitrates. The pH in domestic wastewater is limited to the neutral range of between 6.5 to 8.5 units (Culp *et al.*, 1986). At pH values between 6.5 and 8.5 the removal efficiency is favored as the pH values favors the growth of the microbial community and avoids precipitation of the salts (Culp *et al.*, 1986).

1.3.15.2 Temperature

Water temperature is the most important parameter to be considered in wastewater treatment in a constructed rhizofiltration system. Temperature affects the rates of chemical reactions involved in nutrient removal in the system. It is highly affected by environmental factors such as change in seasons, time of the day, cloud cover and the flow rate of the influent (Culp *et al.*, 1986). Water temperature profoundly affects microbial growth and activities (Willey *et al.*,

2008). Growth and activity of the microbes have fairly characteristic temperature dependence with distinct cardinal temperatures. Therefore, different types of microorganisms present in the system are affected by temperatures which further affect the removal of nutrients. Unsuitable temperature for certain microbial species results in cessation of the species, and thereby reducing nutrient removal potential of the rhizofiltration systems (Willey *et al.*, 2008).

Cold temperatures decrease nutrient transformation and subsequent reduction in the rhizofiltration system (Renee and Knight, 2001). The total ammonia utilization rates increases with increased temperature. High temperatures support the degradation of nutrients in wastewater treatment systems. Biological nitrogen removal is most efficient at 20 to 25˚C, the microbial activities related to nitrification and denitrification can drop at temperatures below 5˚C or above 30˚C (Cheng *et al.*, 2009). It has been confirmed that microorganisms living in constructed rhizofiltration systems usually reach their optimal activity at temperatures between 15 and 25˚C, especially nitrifying bacteria (Cheng *et al.*, 2009). Temperature ranges between 16.5 and 32˚C are favourable for nitrification in constructed rhizofiltration systems (Fisher *et al*., 2009).

1.3.15.3 Salinity

Salinity is measured as either total dissolved solids or electrical conductivity. High salinity in wastewater results in:

a) Poor biodegraded organic nutrients due to toxic effect of the sodium content on biofilm that has not adapted to the saline concentration.
b) Cell plasmolysis due to the dramatic increase in osmotic pressure and changes on microbial metabolism (Cortes-Lorenzo *et al.*, 2012).
c) Reduced hydrolytic rates of biofilm.
d) Reduced COD/BOD_5 (Cortes-Lorenzo *et al.*, 2012).
e) Inhibition of nutrients removal from the wastewater treatment systems through retardation of the denitrification potential in medium and macrophytes in rhizofiltration systems.

The optimum salinity level that support nutrient removal in rhizofilter systems is about 15 ppt (Brown *et al.*, 1999). The negative effect of salinity on treatment efficiencies is due to its inhibition of microbial activities in the medium; hence the reduction in the rate of denitrification. Denitrification converts nitrate to nitrogen gas under anaerobic conditions and

is inhibited at levels between 15 and 30 ppt salinity (Brown *et al.*, 1999). Salinity levels beyond the normal range for any microbial species causes stress or even death to microorganisms and also affects the availability of nutrients to macrophytes roots, thus results to decrease in nutrient removal efficiency (Cortes-Lorenzo *et al.*, 2012).

1.3.15.4 Electrical conductivity

Electrical conductivity reflects the capacity of water to conduct electrical current and ionic activity, and is directly proportional to salinity. High electrical conductivity signal high amounts of dissolved salts. High electrical conductivity retards the rate of the removal efficiency of nitrogen as it reduces denitrification. The high salt content from organic waste in wastewater inhibits nitrogen removal. The acceptable discharge limits after treatment should be 70 mS/cm (Lin *et al*., 2004).

1.3.15.5 Turbidity

Turbidity results from suspended solids in water. Particles in water absorb heat from sunlight and cause water temperature increase. Warmer temperatures hold less dissolved oxygen than colder temperatures and less dissolved oxygen is unsuitable for some microorganisms, thus affecting the removal efficiency of nutrients in the system as microbes use oxygen to break down organic matter. They also prevent sunlight from reaching roots in the rhizosphere which results to limited plant growth, which also reduces nutrient removal. However, nutrients may bind with suspended solids and settle in bottom sediments where they become concentrated and this result in clogging which reduces the removal efficiency of nutrients. For optimum performance of the system, turbidity must be less than or equal to 0.3 nephelometric turbidity unit (NTU) in wastewater treatment systems (EPA, 2009).

1.3.15.6 Dissolved oxygen (DO)

The organic matter present in wastewater decreases the dissolved oxygen which reduces the breakdown of nutrients by microorganisms. The higher the concentration of the microbial community, the more oxygen is required for contaminants degradation. Dissolved oxygen may be decreased due to warm temperatures. The amount of oxygen in water indicates the extent of treatment. High amounts of dissolved oxygen means contamination in wastewater is relatively low and while low levels indicate high oxygen demand and the high level of contamination in water. Oxygen level is one of the most important factors that determine the activity of biofilm

in wastewater treatment (Tyroller *et al.*, 2010). The optimum range of dissolved oxygen is 60-100%. High levels of oxygen in rhizofiltration system facilitate nitrification. Oxygen is also important for the reduction of phosphorus. It is essential for keeping phosphorus complexes in the sediment. When oxygen levels are low these complexes may dissolve and phosphorus is released into water reducing the removal efficiency in the system (El-Hoz and Apperler, 1996).

1.3.15.7 Total dissolved solids (TDS)

Dissolved solids refer to any minerals, organic solids, salts, metals and anions dissolved in water. Total dissolved solids are one of the most important indicators of water quality. Salinity is the measurement of dissolved salts in wastewater. There is a relationship between salinity and total dissolved solids. The acceptable values of TDS range between 500-750 mg/l (Culp *et al.*, 1986). It is essential to monitor TDS levels in water as they indicate the amount of contamination. Total dissolved solids highly affect the oxygen diffusion required by microorganisms.

1.3.15.8 Chemical/ biological oxygen demand (COD/BOD)

Chemical oxygen demand/biological oxygen demand represents the amount of organic matter in wastewater. The amounts of organic matter in wastewater, and its relative biodegradability may be estimated by analyzing samples for BOD_5 (Menson and Roberts, 2000). It is the measure of the amount of biochemically degradable organic matter in water (Chapman, 1992). Biological oxygen demand directly affects the amount of dissolved oxygen. The greater the BOD the more rapidly oxygen is depleted in water. Low dissolved oxygen results in stress, and suffocation of microorganisms and can even be lethal to microbial cells (Chapman, 1992). The acceptable BOD removal percentile range in wastewater treatment is between 90 to 95 percent (Brix and Arias, 2005), with discharge limits to range between 1.0 mg/l and 10.0 mg/l for COD (Culp *et al.*, 1986).

1.3.16 Rhizofiltration Systems as an Alternative Technology in Wastewater Treatment

Constructed rhizofiltration systems have been proposed to be better alternatives in wastewater treatment due to their advantages including provision of high wastewater treatment efficiencies (Kalbar *et al.*, 2012). Contaminants in wastewater have been demonstrated to be reduced to acceptable levels using this technology (Vymazal, 2011). Water produced after treatment has been reported to be within the required discharge limits by the Department of Water Affairs

and posing no negative impacts on the environment. These systems are inexpensive, with little energy requirements, equipment needs are minimal and have low-construction cost. This technology needs full establishment and widespread implementation before it can be considered for full or maximal contaminant removal. In this case, establishment means full development or growth of macrophytes and biofilms responsible for contaminant breakdown. Once established, properly designed and constructed rhizofilter can be largely self-maintained (Cooper *et al*., 1996).

For effective and efficient wastewater treatment using this technology, detailed knowledge about the effectiveness of various plant species, colonization characteristics of certain groups of microorganisms as well as their interaction with the soil material has been found to be essential. Previously, most research into this technology was concerned mainly with technological design issues, with the active reaction zones being ignored. The main issues of concern were the influent and effluent loadings. This was mostly because of the lack of suitable testing systems and study methods. However, small-scale process modeling experiments have been developed for studying the process of contaminant removal from these system. For optimum performance of the rhizofilter, research into microbial colonization in the system and their activity, biofilm population structure, dynamic and their role in pollutant removal, macrophyte activity and their interaction with media and biofilms as well as the role of physical parameters in nutrient removal efficiencies is needed to achieve a better understanding of the complex interactions and processes involved in the technology itself. The understanding of the underlying processes will enable the basic scientific aspects to be optimally combined with the technical possibilities available and thus enable rhizofiltration technology to be efficiently used on a broader scale in wastewater treatments. Maintenance and monitoring from time to time of a running large scale unit should also be factored in the development of this technology (van der Graaf *et al*., 1996).

The applicability of rhizofiltration technology for municipal wastewater treatment could play a huge role towards establishment of ‘green technology’ in the treatment of wastewater if this technology could also prove to be environmental friendly and sustainable. It may eliminate the use of chemicals currently used in conventional wastewater treatment as well as minimize the amount of carbon dioxide and methane released into the atmosphere. Carbon dioxide released through microbial decomposition in this technology may be re-used by macrophytes in the process of photosynthesis.

1.3.17 Rhizofiltration Systems and Greenhouse Gas Production

Rhizofiltration systems may be considered as both greenhouse gas sources as well as sinks because of their ability to produce methane and at the same time, acts as sinks of carbon dioxide (Whiting and Chanton, 2001). Carbon fixation under rhizofilter anaerobic soil conditions may provide unique conditions for long term storage of carbon. However, this carbon sequestration process is intimately linked to methane emission from wetlands. The potential contribution of this emitted methane to the greenhouse effect can be mitigated by the removal of atmospheric CO_2 and storage into peat. The balance of CH_4 and CO_2 exchange may provide an index of a rhizofilters' greenhouse gas contribution to the atmosphere. The emission of CO_2 and CH_4 in rhizofiltration systems may depend on interactions between several factors including soil characteristics, environmental conditions and plant properties contributing to organic matter quantity and quality (Inglett *et al*., 2012). While wastewater treatment systems have been reported to produce greenhouse gases, (Ugetti *et al*., 2012a; Liikanen *et al*., 2006), any wastewater treatment technology that may contribute minimum greenhouse gases may be essential in combating the effects of the climate change and global warming. It is for this reason that rhizofiltration systems are being explored for their potential for wastewater treatment with possible greenhouses emissions.

1.4 CONCLUSIONS

Alternative and effective ways of managing an increasing amounts of watewater generated by population increase is imperative. Currecntly experienced drought accompanied by fast depletion as well as escalating of fresh water sources may be compensated by rhizofiltration technology in wastewater circling to ensure the availability fresh water that would meet the demand of the population. Rhizofiltration systems are a promising, cheap and effective wastewater treatment tool using local resources in small and mainly rural communities. These systems have been in operation for a while as watewater treatment methods. However, the lack of knowledge associated with their applications has derailed the progress for the use of this technique as an alternative technogy in watewater treatment. The role played by diferent components during treatment need to be understood if these systems were to be applied for industrial and domestic wastewater treatment. With more research, recent emegernt or contruction of hybrib systems may contribute to achieving higher contaminant reductions with little invesments. While this technology may be of great benetif to everyone who may use it, developing countries may greatly benefic from this technology because of its low capical cost

and energy requirements compared conventioal systems of watewater treatment. The system may tolerate both great and small volumes of water and varying contaminant levels. These include municipal and domestic wastewater, urban storm runoff, agricultural wastewater, industrial effluents and polluted surface waters in rivers and lakes. The system could be promoted to various potential users for water quality improvement and pollutant removal. These potential users may include the tourism industry, governmental departments, private entrepreneurs, private residences, aquaculture industries and agro-industries.

CHAPTER 2

MONITORING OF A MACROPHYTE RHIZOFILTRATION SYSTEM IN A PILOT-SCALE RHIZOFILTER PLANT

2.1 INTRODUCTION

Rhizofiltration system establishment means system set-up and bringing it into a state of being used or tested. Testing for system functioning as well as setting up a steady state of the system was critical before the experiments could be conducted to ensure the reliability and repeatability of the results that would latter obtained from the system. System set-up and monitoring was achieved through construction of the system, and then monitoring for parameters that would represent both the physicochemical and nutrients. These included pH, temperature, DO, COD, ammonia and phosphate. The creation of a rhizofiltration treatment system may be divided into a rhizofilter construction and macrophytes establishment (Hua, 2015). Rhizofiltration systems have the potential to treat wastewater if fully established (Kivaisi, 2001). Differences in system design and configuration endow systems with different capabilities for absorbing heavy metals, removing nutrients, organic toxins, chemical oxygen demand (COD) as well as supporting different microorganisms in their respective rhizosphere niches (Otte and Jacob, 2006; Mthembu *et al*., 2014). The rhizofiltration system was designed to operate in multiple modes. Within the system, wastewater could move through or over the filter, either in vertical or horizontal flow modes depending on system operation. Differences in the system's operation may influence its performance in wastewater treatment (Luederitz, 2001). These aforementioned features of constructed rhizofiltration systems have formed the basis of the concept on which an experimental rhizofilter was designed and constructed for this study.

2.2 METHODS

2.2.1 Design Specification of the Rhizofilter System

A 6 m x 4 m x 1.5 m rhizofiltration unit was constructed from concrete in Durban, South Africa, situated in Kingsburgh at the eThekwini wastewater treatment plant. Durban is an area with subtropical climate conditions. The area is associated with high rainfall and temperatures, with a humid environment. The overall system was composed of an above-ground rhizofiltration structure and two settling tanks. The tanks were a small 5 kl tank settling a raw sewage

immediately after collection and 10 kl large storage tank. The process involved the pumping of raw sewage into the small tank where it was allowed to settle before flowing over to the large tank as settled sewage through the overflow outlet which was fitted at the top of both tanks. From the large tank, the settled sewage was channeled to flow into the rhizofilter by gravity into the vertical feed system of the rhizofilter. The second tank (large) was connected to the rhizofiltration system. This tank was also connected to the post-secondary-treatment but pre-chlorinated wastewater basin. This was to facilitate mixing of raw sewage with treated wastewater in order to control the concentration of the nutrients when necessary.

Figure 2.1: Rhizofiltration system used in the study. The system was divided into planted and reference section. Taps on either side along the system were used for sample collection.

In order to meet the main aim of the investigation, the rhizofiltration system design was based on the following criteria:

- To produce different flow types (vertical, horizontal and subsurface flow) by having different controllable input points in the rhizofilter unit.
- To have two separate sections, planted vs. non-planted (reference), the sections being physically separated from each other.

- To have different influent flow rates via storage tanks with adjustable valves ensuring that the system flow rate was not the limiting factor.
- To have unrestricted exit flow rates with all outlets, both in the planted and the reference sections designed for a flow rate equal or greater than the maximum flow rate from the storage tank.

This rhizofiltration system was designed and constructed to process a certain flow rate, higher than the normal design guidelines for constructed rhizofiltration systems (0.04-0.5 m^3/d) or trickling filters (0.7-10 m^3/d). The system was designed to have the flow rate of between 25-50 m^3/d from the tank to the rhizofilter. Based on the horizontal flow area of 11.25 m^2, the maximum flow was found to be about 560 m^3/d or 6.5 l/s. The filters were designed to test different flow rate scenarios from the two outlets. Different flow rates through the filter unit were obtained using valves. With different outlets on the rhizofilter, different flows (surface, horizontal, subsurface flow) through the system could be tested. For the purpose of this study only vertical flow was used.

The rhizofiltration media (sediment matrix) of the system was made up of different layers of rocks and sand ranging from coarse rocks (100-200 mm) at the bottom to crushed rocks (19-25 mm) at the top, which was topped off with fine sand on which a thin layer of the crushed rock was placed to protect the sand from being washed away. The composition of the soil medium used in the construction of the system is shown in Table 2.1. The entire system was divided lengthwise, in which one side contained only the matrix (reference section) and the other side (planted section), was planted with *Phragmites australis* and *Kyllinga nemoralis*. The two sections were physically separated from one another by a concrete and a heavy duty plastic layer in order to prevent mixing of wastewater from the two sections after system feeding. *Phragmites australis* is a common reed found abundantly growing around Durban while *Kyllinga nemoralis* was also found to grow near and around where the system was constructed. It is on the basis of their availability and their ability to accumulate nutrients that these macrophytes were chosen. Macrophytes in the planted section were planted such that they were evenly distributed across the system (30 cm from the walls of the system and 20 cm from each other), forming two planted rows. Apart from the planted and reference divisions of the rhizofilter, the system was further divided into basins A, B, C, D, E in the planted and F, G, H, I, and J in the reference section. These divisions across the system enabled us to investigate the performance of the system in different basins. Each basin had an inflow (Figure

1.3) and an outflow valve (Figure 1.4). The inflows into the filter were for system feeding while the outflow valves (taps) served for sample collection in the basins after treatment in the respective planted and reference sections. The design of the rhizofiltration system is shown in Figure 1.2. After construction, the rhizofiltration system was calibrated through flow rate testing in order to accurately determine the various flow rates through the filters. These hydraulic characteristics were needed for future testing and application of the system for its ability to remove contaminants from the wastewater.

Table 2.1: Composition of the rhizofiltration medium used in the system

Layer	**Average diameter (mm)**	**Thickness layer (mm)**
Small stones (to protect the sand layer)	19-25	± 50
Coarse river sand	-	200
Crusher run	6	100
Small stone / rock	19-25	100
Crushed rock	63	150
Coarse rock	100-120	250

2.2.2 Flow Measurements and Calibration of the Rhizofiltration System

The flow rate of wastewater into the settling tank from raw wastewater pump was measured. Through measuring the time it took the tank to be filled with raw sewage, after which wastewater was transferred into the storage tank. The storage tank was equipped with sight glass to indicate tank filling level. The sight glass had markings every 10 cm measured from the top of the outlet pipe (75 mm). The flow into the storage tank was determined by measuring the rise in water level using the sight glass over a period of time. When emptying the tank through outlets, the time it took to drop between the markings (every 10 cm) was recorded. The flow rate from the storage tank to the outlets into the rhizofilter was calculated from this data.

2.2.3 Determination of the Flow Rates in the Rhizofiltration System

With the volumes of the basins known, the flow rate through the filter into the basins was determined. The wastewater was run into the filter. The flow rate at each basin, and thus a certain position in the filter, was determined by measuring the time it took for each basin to fill. The time was measured from the moment the wastewater transferred into the basin up to the time it ran over the sluice (openings on the sides of the filter unit). With the known volume of the basin the average flow rate was calculated. For the determination of the volume of the basins, a hosepipe with clean water was used. The hosepipe was used to determine the volumes of the basins after it was determined that the flow rate was constant, which was determined by taking and filling up the 5 litre bucket over several times, and time it took to fill the bucket was recorded.

2.2.4 Preliminary Setup and Monitoring

Planting of the completed constructed pilot plant was started on 12th June 2011. *Phragmites australis* was planted in two rows of 10 plants each for a total of 20 plants. After two months of system monitoring another twenty plant species of *Kyllinga nemoralis* were introduced following the same protocol used in the planting of *P. australis*. The planting scheme was according to the experimental design previously explained in the last paragraph of section 2.2.1. Fresh water (tap water) was applied to the plants in the system once a week for a period of 1 month in order to stimulate growth. This was followed by application of settled pretreated pre-chlorinated wastewater from the municipal treatment system for a period of 3 months in order to establish the treatment process (microorganisms and biofilm establishment) to achieve a steady state operation.

On the 1st of July 2011 (after 18 days of operation) during the establishment process, leakage was noticed from the small Jojo tank (settling tank) as well as on the walls of the rhizofiltration system. After careful investigation through visual inspection coupled with COD and physical parameter analysis, it was concluded that the leakage would not significantly affect the research operations. During this period samples were taken to test the state of the filter unit. In August 2011, the system was assumed to be established for initial sampling and analysis. This conclusion was arrived at after observing the initial shoots of the leaves from the macrophytes as well as based on the monitoring results obtained. A 3000 l volume of raw sewage was added and the samples were collected.

During further investigations on the pilot plant on 1st September 2011, more cracks were observed on the walls (all four sides) of the constructed rhizofiltration pilot plant that were allowing significant amounts of water to leak through. This situation called for immediate suspension of the plant operations to allow for repair of the unit and the Jojo tank. The repair work was completed in mid-September 2011 and the system was fixed to apply pre-treated pre-chlorinated wastewater directly into the second Jojo (storage) tank with a capacity of 10 kl. Macrophytes were replanted and the system was allowed to re-establish for a period of three months during which time, the plants were watered weekly using tap water through a hose pipe and once a month with pre-treated pre-chlorinated wastewater.

2.2.5 Re-Establishment of the System

After macrophytes were replanted in September 2011, it was noticed that the macrophytes on the planted section were not healthy. The cause was believed to be the lack of nutrients and thus 10 kg of commercially available compost was added to encourage plant growth. Pre-treated pre-chlorinated wastewater was run into the system at a flow rate of 30 l/hr through each inlet into the rhizofiltration unit.

2.2.6 Parameters Measured During Establishment and Monitoring Phase of the System

Combinations of parameters were selected for monitoring and determination of the steady state operation for the rhizofiltration system. This was imperative before the study could commence in order to establish a 'ready-to-use' state of the rhizofiltration system. Parameters measured in the monitoring phase were chosen to represent physical and chemical parameters as well as nutrients. These analytes were pH, temperature, electrical conductivity, dissolved oxygen, chemical oxygen demand, ammonia and phosphate. The procedure followed in the measurement of these parameters is explained in Chapter 3, section 3.2. Samples were collected once a month until the results obtained indicated that the rhizofiltration system was fully established which was found to be in December 2011. The first samples collected in June 2011 were collected before macrophytes were planted in the system.

2.3 RESULTS

2.3.1 Rhizofiltration System Flow Rate Measurements

The results from two measurements of the flow rate of wastewater from the pump into the settling and storage tanks are shown in Table 2.2. The first measurement was taken right after the pump was installed, while a second measurement was taken two days later. The void volume of the system was about 3 000 l, with a flow rate ranging between 0.2-2 l/s. The flow rate into the settling tank was found to be 0.61 l/s while from the settling tank into the storage tank was 1.12 l/s. The flow rate through the rhizofilter (0.02 l/s) was found to be lower than the flow rate out of the tank (0.52 l/s) as well as from the outlets into the filter unit (0.81 l/s). This led to the influent forming a head of ± 100 mm at the top of the filter. This was thought to have several consequences. One being that the flow rate was a limiting step in the rhizofiltration process. High pressure was required to force wastewater through smaller pores (filter) in order to force water through narrow openings compared to open flow in the filter unit. When the flow from the pump supplying the wastewater and the head of the wastewater above the filter was constant, the flow rate through the filter was also found be constant. In addition, constant flow from the pump resulted in an overall constant flow throughout the filter, which was independent of the position of effluent taps. However, treated effluent flow rates from the basins differed depending on how the rock media in different layers constricted the flow into the outlet collection basins. This resulted in a different flow rate through the outlet, regardless of the flow through the overlying layers. Therefore the average flow rates through the filter (i.e. the sum of the flow rates) were compared instead of the flow rate at each individual basin/tap. Since the flow rate from the sewage pump was constant, the flow rate from the tank was used to determine the flow rate through the basins.

Table 2.2: Flow rate obtained from the rhizofiltration system measured immediately after system construction.

Time (h:m:s)	Height liquid level (mm)	Flow (l/sec)
2:18:42	2385	1.12
1:40:52	975	0.61

2.3.2 Monitoring of pH

The result of the pH readings obtained during the establishment period was between 6.7 and 7.9 (Figure 2.2). The highest pH was obtained in July 2011 while the lowest was obtained in October. The pH obtained in June was almost the same between influent, planted and the reference section. Thus there was no statistically significant difference in pH values obtained when influent was compared to both effluent planted and reference sections (p = 0.49). These samples were taken before macrophytes were planted on the system. Between June and September 2011, the pH increased in the planted section and was more or less constant between October and December 2011, the same could not be said about the influent and the reference section. They both varied from very high to very low from month to month.

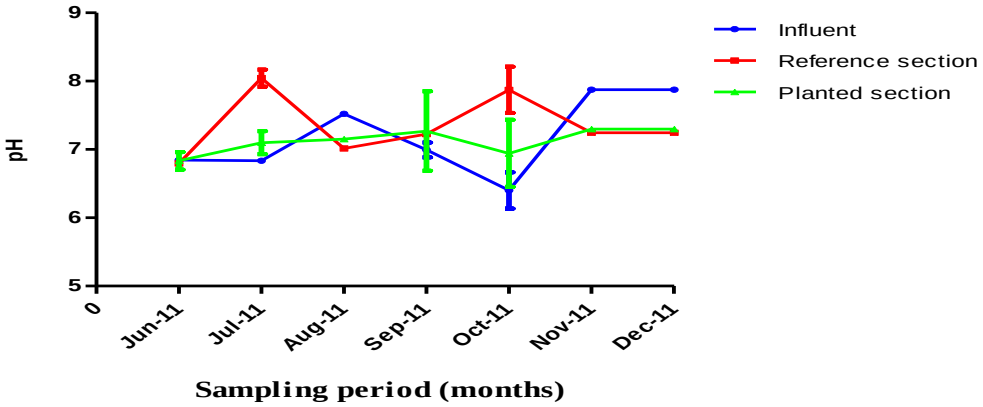

Figure 2.2: pH variation from influent and effluent wastewater in a rhizofiltration during monitoring and establishment from June to December 2011. Mean values per month were used in the analysis. Error bars are indicated.

2.3.3 Temperature

Temperature showed an overall increase between June and December in the influent and from reference and planted sections during the monitoring phase (Figure 2.3). Influent and reference section recorded low average temperatures compared to the planted sections. While temperature was the same in June 2011, in all the samples, variations were observed between July and December 2011. No significant difference in temperature was obtained when influent was compared to effluent (p = 0.69) as well as when planted section was compared to the reference section (p = 0.73) from June to December 2011. Between November and December 2011, constant temperatures of 24.2^{o}C, 23.8^{o}C and 23.7^{o}C on the reference, influent and planted sections were recorded respectively.

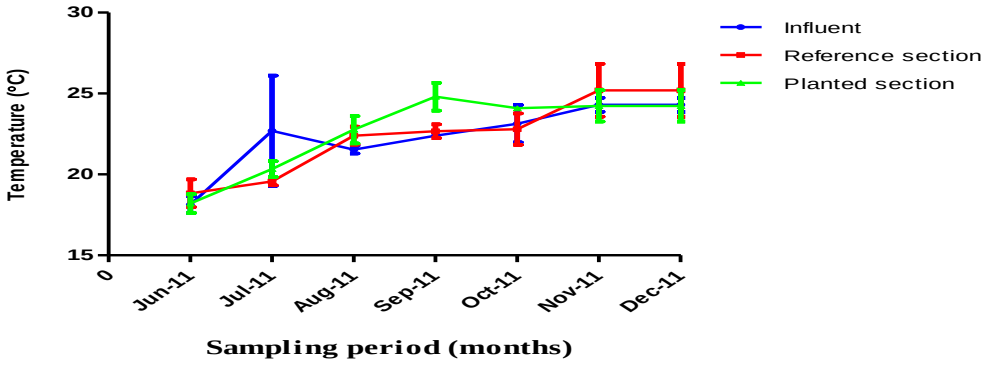

Figure 2.3: Temperatures obtained in the system during monitoring and establishment from June to December 2011. Mean values per month were used in the analysis. Error bars are indicated.

2.3.4 Electrical Conductivity

The results of electrical conductivity during the establishment and monitoring phase may be seen in Figure 2.4. While lowest salinity was obtained in August, the highest was recorded in September 2011. September was when commercial compost was added to the system in order to encourage macrophyte growth in the rhizofiltration system. Compost contained nutrients and minerals which did not only supported macrophyte growth but also increased contamination levels of the wastewater. Thus increased electrical conductivity during this period is attributed to increased contamination due to the added compost.

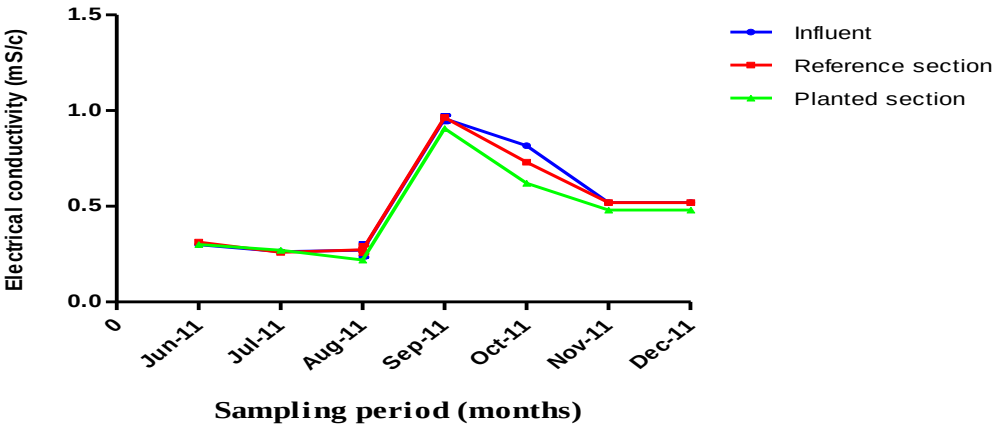

Figure 2.4: Electrical conductivity obtained in the system during monitoring and establishment from June to December 2011. Standard errors of means are indicated as whiskers.

2.3.5 Dissolved Oxygen

Dissolved oxygen concentration (DO) is a better general environmental monitoring indicator that is also applicable to assessing organic pollution. It measures the quality of water or the extent of water contamination. Dissolved oxygen was low between June and July as well as in September 2011 in both the influent and effluent samples (Figure 2.5). While a decrease between June and July could be due to drop in temperatures as well as acclimatization of the system to operation, September may have been due to contaminants introduced in the system in a form of compost. From September to December 2011, a general increase in the dissolved oxygen was observed in all the samples collected from the system, with the planted section recording highest dissolved oxygen concentrations followed by the reference section.

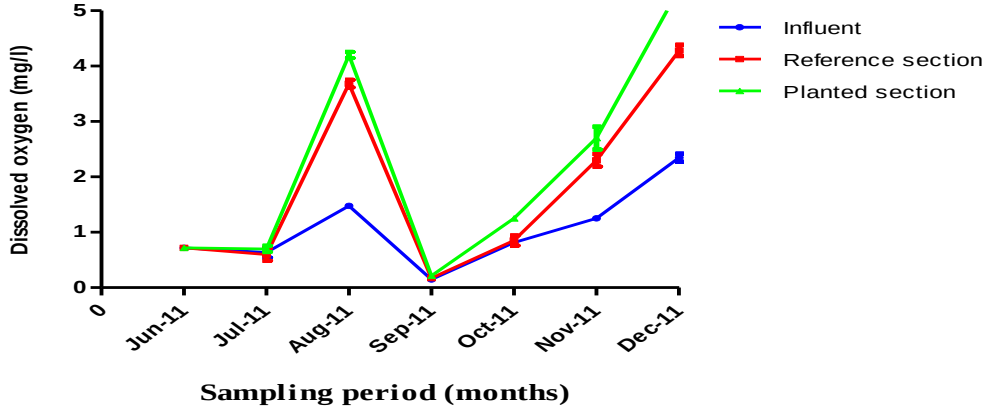

Figure 2.5: Dissolved oxygen concentration obtained in the rhizofiltration system during monitoring period from June to December 2011. Mean values were used in the analysis. Standard errors of means are indicated as whiskers.

2.3.6 Chemical Oxygen Demand Removal

Chemical oxygen demand is an alternative measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical exigent. Chemical oxygen demand in all the samples was very high in June (Figure 2.6). Between July and August a decrease was observed which was up to 46 mg/l in the planted section, which was within the allowable discharge limits. Lowest COD removal efficiencies were recorded in September due to the addition of compost in the system while a steady state was achieved between October and December 2011. It could be seen from the results that the planted section had higher removal efficiencies of the COD in all sampling points compared to the reference section and the difference was statistically significant ($p < 0.05$).

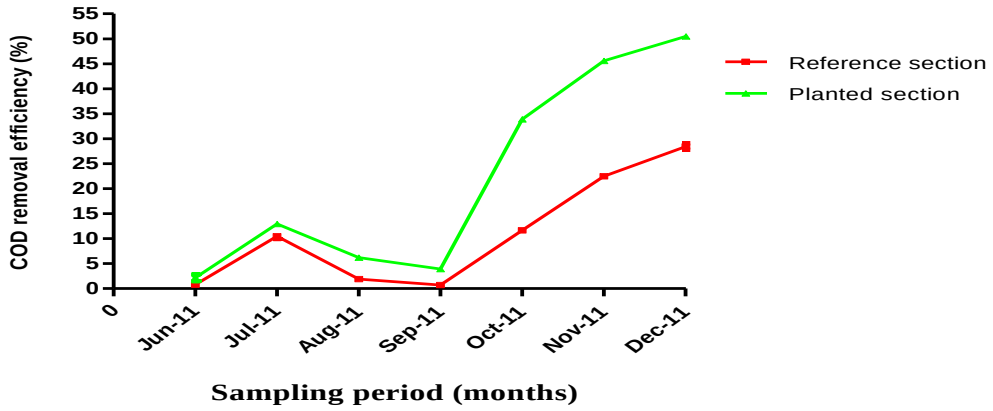

Figure 2.6: Chemical oxygen demand removal efficiency in a rhizofilter obtained in the system during the period of establishment from June to December 2011. Mean values were used in the analysis. Standard errors of means are indicated as whiskers.

2.3.7 Ammonia

Results for ammonia removal in the rhizofiltration system during the establishment period may be seen in Figure 2.7. Highest concentrations were recorded in June (560 mg/l) while the lowest was obtained December 2011 (79 mg/l). The planted section of the rhizofiltration system recorded the highest removal efficiencies of ammonia. While the removal efficiencies of ammonia were low in September, improvements were observed between October and November, with a steady state obtained between November and December 2011. When the effluent of the planted section was compared to the reference section no statistically significant difference was observed during the establishment period of the rhizofiltration system (p = 0.11). However, when the results from October 2011 to December 2011 were compared, a statistically significant difference was obtained ($p < 0.05$), with more ammonia either transformed to nitrite and then to nitrate absorbed by macrophytes in the planted section. Low concentration of ammonia in the rhizofiltration system obtained in August may have been an indication that the system was then beginning to function well. However, the cracks in system observed which led to rhizofilter re-construction and macrophytes replanting and subsequent adding of compost led to increased ammonia concentrations in September 2011.

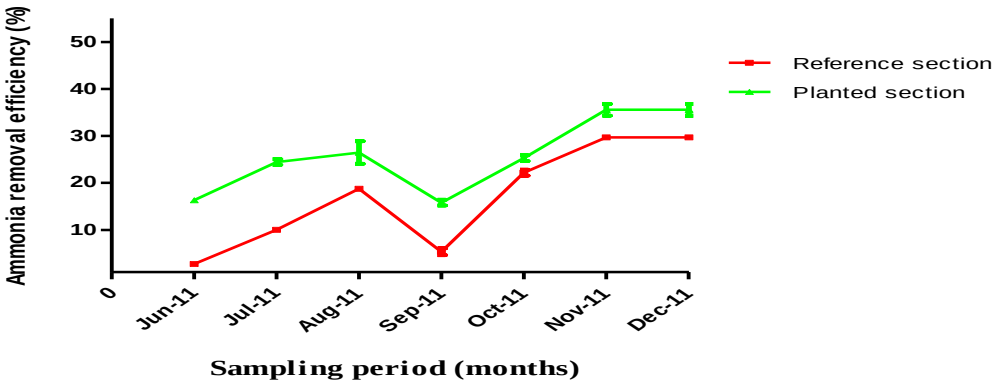

Figure 2.7: Ammonia removal efficiency obtained in the system during the monitoring and establishment period from June to December 2011. Mean values were used in the analysis. Standard errors of means are indicated as whiskers.

2.3.8 Phosphate

A trend of increase in phosphate removal efficiency between June 2011 and November 2011 could be seen in Figure 2.8. Phosphate concentrations obtained in June were more or less the same in all the samples in the system. Macrophytes had not been planted in the system in June when the first samples were taken and this could have not affected the treatment of wastewater in the system. Thus the influent and the effluent were similar. Between September and November 2011, significant differences was obtained when the influent was compared to the reference as well as to the planted section (p = 0.02), while when the reference was compared to the planted section the statistically significant difference was (p = 0.007), with more phosphate (mean = 1.9 mg/l) removed by the planted section.

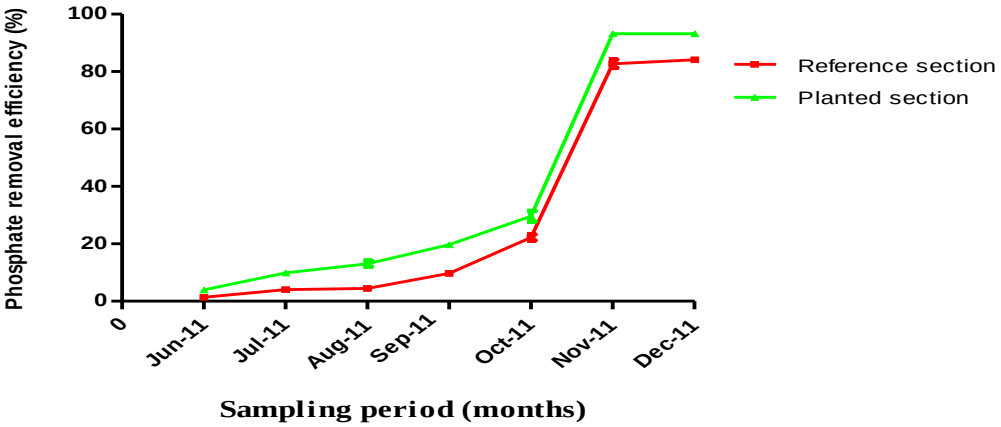

Figure 2.8: Phosphate removal efficiency from influent and effluent wastewater in a rhizofilter from June to December 2011 establishment period. Standard errors of means are indicated as whiskers.

2.4 DISCUSSION

Variations in wastewater contents, and therefore the flow rates in the system (Table 2.2) were due to the fact that the pump was placed on the bottom of the sump (domestic wastewater receiver) where raw wastewater was collected. Flow rate variations were actually due to varying solidity and viscosity of the effluent rather than the pump itself. The system received wastewater from people around Kingsburgh with an estimated population of about 200 000 people. All the pumps installed into the system were found to be in good working condition and produced an adequate flow rate for running the system. Measurements taken were for system calibration to ensure the repeatability and standardization of the procedures for results replication when necessary. These measurements were also important as they enabled to track and monitor the changes in the system flow rates over time due to change with continual usage. Wastewater treatment in the constructed rhizofiltration system is known to be affected by the system's flow rate (Vymazal, 2005). Thus it was first imperative to analyse the causes for the different flow rates in the rhizofilter unit before it could be operated.

Monitoring and regulation of water flow rate was a critical aspect in the success of the constructed rhizofiltration treatment process. The system consisted of three sections, which were inflow, settling basin (disposal section) and outflow. Rhizofiltration systems are constructed with the reservoirs from which the inflow is controlled. In his study, Huang *et al*. (2010) further used perforated board to divide inflow and disposal sections in order to ensure flow uniformity. Of equal importance was the prevention of the clogging and short-circuiting of the rhizofiltration system flow as well as initial filtration of wastewater. This was prevented in the system by filling the inflow section with gravel, which consisted of different layers of different sizes of gravel particles.

In the constructed rhizofiltration system, the bed was wide and shallow. This maximized the flow path and ensured maximum water flows. Pre-treatment through wastewater settling in order to remove suspended solids was essential in the system to prevent clogging as well as to ensure efficient treatment. Therefore this technology is not appropriate for untreated domestic wastewater. It may be a good treatment method for communities that have primary treatment but are looking to achieve a higher quality effluent (Vymazal, 2011). The filter media in a rhizofiltration system acted as both a filter for removing solids, a fixed surface upon which biofilms might attach, and a base for the macrophytes. Macrophyte roots played an important role in maintaining the permeability of the filter. Macrophytes planted in the system had deep,

wide roots that could grow in a waterlogged nutrient-rich environment. *Phragmites australis* and *Kyllinga nemoralis* were common choices because they formed horizontal rhizomes that penetrated the entire filter depth (Vymazal and Kopfelova, 2011).

The rhizofiltration system built for this research was purpose-based and had been constructed to simulate rhizofilters used in the past to treat raw municipal wastewater. Pre-settling of the raw wastewater in order to efficiently remove suspended solids in constructed wetlands has been reported in the past (Solano *et al*., 2004; Vymazal, 2011). It is with this consideration that a rhizofiltration system was constructed in Kingsburgh municipal wastewater treatment works in Durban to treat wastewater, which enabled pre-treatment of wastewater for a better performance of the system.

A significant difference was observed in the analytes with the age of the rhizofilter, with high concentrations obtained at an early stage of system monitoring. The removal efficiencies of COD (Figure 2.6), ammonia (Figure 2.7) and phosphate (Figure 2.8) also increased with the age of the system in both the planted and reference sections of the system. In July 2011 the difference obtained was very low when compared to the difference obtained in December 2011. This is because of the growth and development to full functionality of both microbial biofilms and macrophytes in the rhizofilter. For ammonia, phosphate and COD to be removed in the filter the concerted action of both macrophytes and biofilms was essential.

Between November and December 2011 the analytes monitored (Figures 2.2 - 2.8) were shown to achieve a steady state concentration in both the planted and reference sections compared to the early stages of system operation. During this period macrophytes showed highest population as well as highest biomass growth. According to Vymazal, 2011; Vera *et al*., 2011 and Lim *et al*., 2001, a system is said to achieve steady state of operation when there is sufficient plant population and significant reduction in organic loading and suspended matter aimed at achieving the laid down wastewater discharge standards. As with these previous studies, a steady state in nutrient reduction was achieved between three to six months of the system operation. When the first three months (June 2011 to August 2011) of system establishment were compared to the last three months (October 2011 and December 2011), the removal efficiencies were found to be statistically significantly different (ammonia, $p = 0.025$; phosphorus, $p = 0.03$; COD, $p = 0.026$), with a steady state achieved between November 2011 and December 2011. It is on this basis (monitoring results obtained and observation of

macrophytes growth) that the rhizofilter was declared fully established that in December 2011 that the experimental work was started in January 2012.

2.5 CONCLUSIONS

During the establishment and monitoring period, it could be concluded that:

- Monitoring and maintenance of a rhizofilter is key in maintaining wetland's functioning. Monitoring is required to obtain sufficient data to determine the performance of the rhizofilter in fulfilling the set objectives.
- Effective rhizofilter performance depends on adequate pre-treatment, conservative constituent and hydraulic loading rates, collection of monitoring information to assess system performance, and knowledge of successful operation strategies. The system could be rather easy to design and construct, however it needs to be closely monitored and maintained for efficient performance.
- Sustaining a dense stand of desirable vegetation within the wetland is crucial to ensure treatment efficiency.
- The system achieved higher reductions in amounts of ammonia, phosphorus and COD when the last three months of the system establishment were compared to the first three months, with high removal further obtained in the planted section, when planted and reference sections were compared.
- The dissolved oxygen concentrations also increased in the last three months of the system establishment.
- Results obtained at later stages of monitoring were consistent and thus the system was declared fully established and ready for operation.

CHAPTER 3

EFFECTS OF PHYSICAL AND CHEMICAL PARAMETERS ON ORGANIC AND INORGANIC NUTRIENT REMOVAL BY A CONSTRUCTED RHIZOFILTRATION PLANT

3.1 INTRODUCTION

A worldwide increase in the use of rhizofiltration systems to reduce nutrient concentrations and degrade organic compounds in wastewaters from different sources has occurred over the past two decades (Lui *et al.*, 2009). Though these wastewater treatment systems may reduce nutrients from wastewater, their effectiveness may be affected by a variety of physical and chemical parameters and in turn, influence the removal efficiency of nitrogen and phosphorus in wastewater (Krogstad *et al*., 2005). Understanding of the impact of physicochemical parameters in nutrient removal from these systems may be imperative in the improvement of wastewater treatment efficiency using the rhizofiltration technology.

Rhizofiltration systems are used to remove nitrogen and phosphorus in order to treat and recycle wastewater. The contaminants are removed or transformed to atmospheric nitrogen by microorganisms that are attached to the filter media and root system of the macrophytes. Removal of the suspended solids through settling prior to running the system with wastewater counteracts the clogging of the media (Brix and Arias, 2005) and allows for flow within the system. Microorganisms also utilize nutrients for their growth through several mechanisms while removing nutrients. Rhizofilter system carry out wastewater treatment as the influent passes through the filter medium and the rhizosphere of the macrophytes. The rhizosphere provides aerobic conditions which supports the activities of aerobic microorganisms. Microorganisms in the system facilitate decomposition of organic matter as well as inorganic substances. Filtration, uptake, adsorption, absorption, precipitation, nitrification and denitrification are all responsible process for nutrient reduction. While rhizofiltration medium plays a vital role in the removal of phosphorus through sorption, its action may be greatly influenced by external factors which may decrease its ability. Therefore, it is essential to understand what and how those factors may affect the performance of the treatment efficiency of the rhizofilter unit (Yoon *et al*., 2001).

Parameters such as temperature can either have beneficial or negative effects on macrophytes, microbial growth and other processes in the system. *Phragmites australis* and *Kyllinga*

nemoralis require temperatures between 12 and 25ºC for optimal growth. When temperatures are outside these ranges, the absorption ability of macrophytes to nutrients decrease and thus reduce treatment efficiency. The contaminants have been found to be removed by microorganisms attached to the filter media and root system of the macrophytes (Moeseneder *et al.*, 1993). Constructed rhizofiltration systems have shown reduction efficiencies ranging between 21% for phosphorus and 85% for nitrogen (expressed as plant biomass which accounts for 53% PO_4-P removal and 50% NO_x-N removal) (Greenway and Woolley, 2001). Denitrification process may remove between 60 and 70% total nitrogen, with 20 to 30% of it derived from plant uptake (Lee, 2011). The main priority is to reduce the level of excessive concentrations of organic nutrients that may cause oxygen deprivation when consumed (Culp *et al.*, 1986). Ammonia, nitrate, nitrite, phosphates and orthophosphates are the main organic nutrient present in wastewater that needs to be reduced. Removal of these compounds in wastewater may be microbially driven, adsorption by the rhizofiltration medium or by macrophytes. Microbially, ammonia is converted to nitrate and then to nitrite, and subsequently to nitrogen gas. For these processes to occur optimally, they require environmental parameters to be at an optimum level. In this study the effect of pH, temperature, salinity, electrical conductivity, dissolved oxygen and total dissolved solids of the influent on ammonia, nitrate, nitrite, and phosphate and orthophosphate removal efficiency in a rhizofiltration system was investigated.

3.1.1 Aim

The aim of this chapter was to determine the effects of physical and chemical parameters on organic and inorganic nutrients removal from a constructed rhizofiltration system.

3.1.2 Objectives

a) To measure physical and chemical parameters as well as nutrients in wastewater influent and effluent.
b) To establish fundamental relationships between extraneous physical and chemical parameters and different processes occurring during nutrient removal in the rhizofiltration system.
c) To investigate nutrient removal efficiency in the constructed rhizofiltration system and relate it to extraneous parameters.

d) To compare rhizofiltration discharge measurements with internationally and nationally acceptable water quality standards and conventional wastewater treatment discharge limits.

3.2 METHODS

3.2.1 Sample Collection

Wastewater samples were collected for the analysis of physical and chemical parameters. They were collected once a week over a period of 18 months from January 2012 to June 2013. Samples in 2013 were as demonstrated in Table 3.1, where pre-treated samples were samples that had undergone both aerobic and anaerobic treatment but without chlorination at the final stage. Influent and effluents of both the planted and reference sections were collected. Samples were collected using autoclaved one litre Schott bottles and were then transported on ice cold cooler box to the laboratory for analysis. The samples were analyzed immediately after collection for temperature, pH, dissolved oxygen, total dissolved solids, salinity and electrical conductivity on site using a portable Inolab multi-meter (Merck). Chemical oxygen demand, total organic carbon, ammonia, nitrite, nitrate, phosphate and orthophosphate were analyzed in the laboratory by spectrophotometric methods using a Spectroquant Pharo 300 spectrophotometer (Merck). The spectrophotometer was used with cell and reagent test kits obtained from Merck, following the instructional manual for each analysis. All tests were conducted in triplicates as explained below:

3.2.2 Chemical Oxygen Demand

One hundred microlitres of sample was carefully transferred using a pipette into the reaction cell containing the reagent, carefully held at the neck and the screw cap was then tightened according to COD 14560 cell test. The content of the cell was vigorously mixed. The cell was heated at 148˚C for 120 minutes in the preheated Aqualytic thermoreactor ET108 (Merck). After two hours of heating, cells were removed from the thermoreactor and allowed to cool down in a test tube rack for 30 minutes at room temperature. The absorbance was measured in a Spectroquant Pharo 300 (Merck) at 620 nm and results were recorded.

3.2.3 Total Organic Carbon

Twenty five millilitres of the sample was transferred into a 50 ml glass vessel using a pipette. The pH was adjusted by addition of sulphuric acid to be below 2.4 according to the instruction manual (Merck), for TOC cell 14879. Three drops of reagent TOC-1K was added into a glass vessel and the reaction cell was placed on a shaking platform for ten minutes at a medium speed of seven revolutions per minutes. Three millilitres from the stirred sample was transferred into a reaction cell using a pipette. One level grey micro-spoon of reagent TOC-2K was added into a stirred sample. The cell was closed with an aluminium cap. The cell was placed standing on its head in the preheated thermoreactor and placed at 120˚C for 120 minutes. After 120 minutes the cell was taken out of the thermoreactor and placed in a test tube rack to cool for 60 minutes. The absorbance was then measured using a Spectroquant Pharo 300 spectrophotometer (Merck) at 620 nm.

3.2.4 Ammonia

Five hundred microlitres of the reagent NH_4-1 was transferred into a 10 ml test tube and 0.20 ml of the sample was added using a pipette following the instruction manual for the ammonia cell test 14559 (Merck). The content was then mixed through shaking. One micro-spoon of the reagent NH_4-2 was added and then vigorously mixed until the reagent was completely dissolved. The test tube was left to stand for 15 minutes. The sample was then transferred into a 10 mm cell and measured in a Spectroquant Pharo 300 spectrophotometer (Merck) at 620 nm and results were recorded.

3.2.5 Nitrate

The pH of the sample was maintained within 2-4 using sulphuric acid following the instruction manual for the Merck Spectrophotometer 14556 nitrate cell test (Merck). Four millilitres of the reagent NO_3-1 was transferred into a test tube and then 0.50 ml of the sample was added. Care was taken not to mix before the cap was closed. After the test tube was closed with a stopper, it was then mixed until the reagent was dissolved. The hot test tube was carefully held at the neck and placed in a test tube rack for 10 minutes. After 10 minutes the content was transferred into a 10 mm cuvette and nitrate concentration was measured using a calibrated Spectroquant Pharo 300 spectrophotometer at 620 nm and the results were recorded.

3.2.6 Nitrite

The pH was maintained within 0-2 using sulphuric acid following instruction manual for the Merck Spectroquant 114547 nitrite cell test (Merck). Five hundred microlitres of the sample was transferred into a test tube using a pipette and then one micro-spoon of the reagent NO_2-1 was added and the mixture was vigorously mixed until the reagent was completely dissolved. It was allowed to stand for 10 minutes and then transfered into a 10 mm cuvette and measured with a Spectroquant Pharo 300 spectrophotometer (Merck) at 620 nm and results were recorded.

3.2.7 Phosphate

The pH of the samples was measured and maintained within 2-4 using sulphuric acid following the instruction manual for the Merck Spectroquant 14729 phosphate cell test (Merck). Five millilitres of the sample was transferred into a reaction cell and one dose of green dose metering cap of P-1K was added. The content was mixed, the reaction cell was placed in a thermoreactor preheated to 148˚C for 30 minutes and then cooled down to room temperature. Five drops of P-2K was added and the cell was tightly closed and vigorously mixed until the solid substance was dissolved. The reaction cell was allowed to stand for five minutes and then placed into the cell compartment aligned on the mark on the cell with that on a Spectroquant Pharo 300 spectrophotometer (Merck). The absorbance of the contents in the cell was then read at 620 nm.

3.2.8 Orthophosphate

The pH of the samples was maintained within 2-4 using sulphuric acid following instruction manual 14729 phosphate cell test (Merck). One milliliter of the sample was transferred into the reaction cell using a pipette. Five drops of reagent P-2K was added and then the cell was tightly closed and mixed. One dose of P-3K was then added using the blue dose-metering cap and the cell was then closed with the screw cap and vigorously mixed until the solid substance was completely dissolved. The reaction cell was allowed to stand for five minutes and then placed into the cell compartment aligned to the mark on the cell with that on a Spectroquant Pharo 300 spectrophotometer (Merck). The absorbance was then read at 620 nm.

3.3 RESULTS

This section presents the results of physical and chemical parameters measured in the system from January 2012 to June 2013 (Appendix 2 and 3). Nutrients in January 2013 were not measured because of the equipment failure. In 2012, pretreated samples were collected while in 2013 diluted pretreated but pre-chlorinated wastewater with raw wastewater was collected. Dilutions were made to increase the amount of contaminants introduced to the rhizofilter unit as well as to assess the performance of the rhizofiltration unit under different wastewater nutrient loading levels. Wastewater dilutions used in 2013 are presented in Table 3.1.

Table 3.1: Ratios of pre-treated and raw water used as feed wastewater for the constructed rhizofiltration over a five month period in 2013

Sample	Month collected	Dilution (Pre-treated %: Raw wastewater %)
1	February	100:0
2	March	90:10
3	April	80:20
4	May	50:50
5	June	20:80

3.3.1. Physical and Chemical Parameters

3.3.1.1 Potential of hydrogen (pH) monitoring

Results for both influent and effluent pH measurements in the rhizofiltration system are represented in Figure 3.1. The influent pH ranged between 4.8 and 9.7, while the effluent of the reference section ranged between 4.9 and 10.2 and was between 5.2 and 9.7 for the planted section. It could also be seen from the Figure that in January 2012 while the pH was higher in the reference section, the planted section had the similar pH values with the influent section (9.7). There was no statistically significant difference in pH concentrations obtained between the planted and the reference sections as well as when the results for 2012 were compared to 2013 ($p > 0.05$). Maintenance of pH at optimum levels in water is crucial as pH affect chemical and biological processes occurring in water. Acidic pH was obtained between February and

May 2012 as well as in February, May and June 2013 in all the section. Low pH may have been caused by respiration by macrophytes or from bacterial decay of organic matter in the rhizofilter producing high amounts of carbon dioxide. Low pH in water may allow nutrients and other substances to be available for uptake by macrophytes in wastewater. This was especially observed in March 2012 where the removal of nitrogen, phosphorus and COD was high. In 2013 the pH was within the range of 6 to 8. Culp *et al*. (1986) reported surface water pH shall not be less than 6.5 nor be more than 8.5. From the results, the values outside these limits were obtained in January 2012 (basic), between February and May 2012 (acidic) as well as in January 2013 (basic) and in February, May and June (acidic) in all sections. Culp *et al.* (1986) reported pH of less than 7.5 to promote nitrification while Cortes-Lorenzo *et al.* (2012) reported pH of 6 and lower to decrease the conversion of ammonia to nitrate by *Nitrobacter* and *Nitrosomonous* spp.

3.3.1.2 Temperature

Figure 3.2 shows the results of temperatures obtained in the rhizofiltration system. The influent temperature ranged between 18.6°C to 35°C, the reference ranged between 18.2°C to 32.92°C, while the planted section ranged between 18.6°C to 32°C. Samples collected in warm seasons both in 2012 (January, February, November and December) and 2013 (January, February) showed a statistically significant difference in temperature when compared with a *t*-test between planted and reference sections ($p < 0.05$), with lowest temperatures obtained in the planted section.

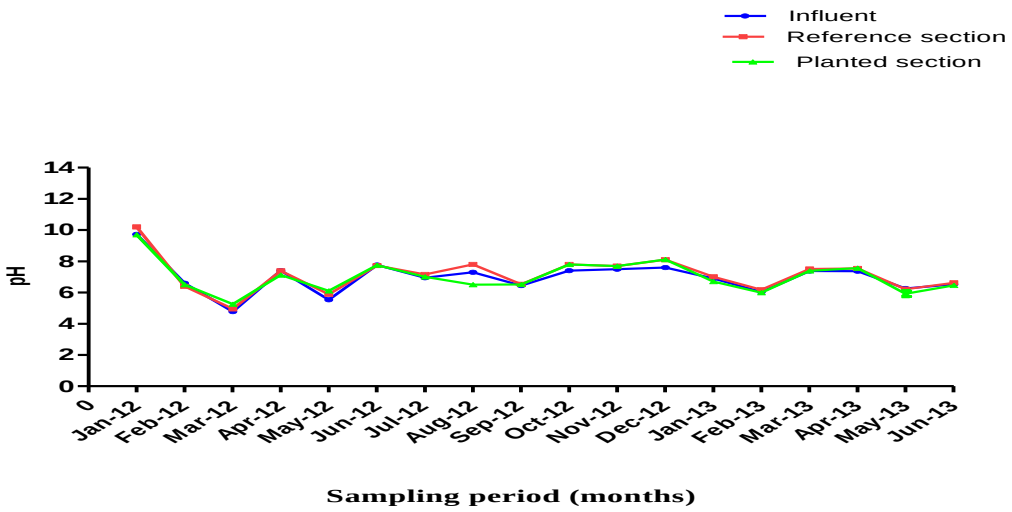

Figure 3.1: pH variation from influent and effluent wastewater in the rhizofilter from January 2012 to June 2013. Mean values of the water samples are shown for each month. Whiskers represent standard errors of means.

This difference was also observed when samples collected in warm seasons were compared to samples collected in cold seasons. In January 2012 and 2013 as well as December 2012, highest temperatures in the system were obtained. These were summer (warm) season in subtropical hemisphere. The temperature of water is controlled primarily by ambient temperatures. Water temperature is characteristic that determines water suitability for use or for survival of organisms and aquatic system functioning. It may affect dissolved oxygen concentrations, an important water parameter that affects microbial biofilms in water. In 2013, temperatures also increased during warm seasons and decreased during cool seasons. Comparing the temperature of the influent, the planted and the reference section in 2013, the influent had highest means (28^{o}C), followed by the reference section (25.3°C), with the planted section having the least (24.5°C). Temperatures obtained in the system were not less than the minimum of 15°C, while above 25°C were obtained in warm seasons (January 2012 and between December 2012 and March 2013) in both the influent and the effluent of the reference and planted sections.

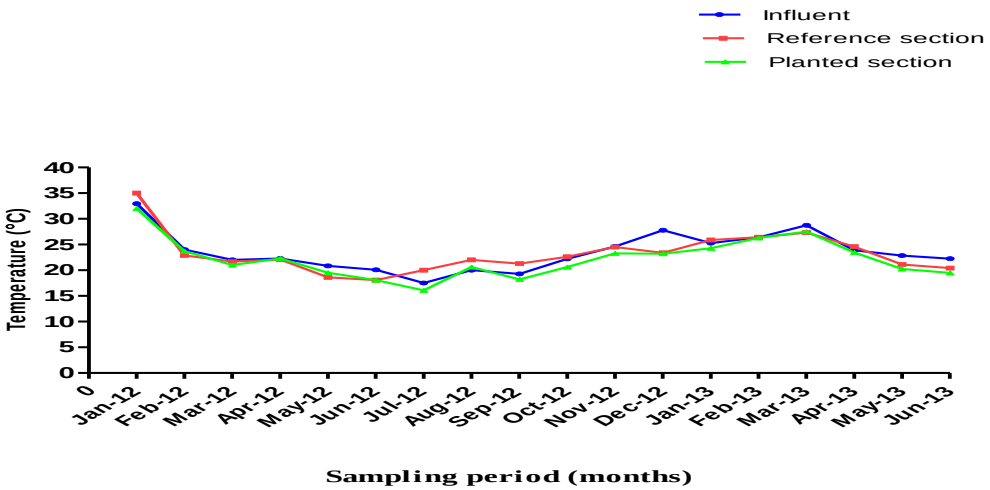

Figure 3.2: Temperature variations from influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means of three readings are represented by each point with whiskers representing standard errors of means.

3.3.1.3 Salinity

It could be seen from Figure 3.3 that variable results of salinity were obtained throughout the study period. Salinity ranged between 0.2 mg/l to 0.4 mg/l in the influent, 0.15 mg/l and 0.39 mg/l in the reference section and ranged between 0.1 mg/l and 0.3 mg/l in the planted section. These salinities were high when compared to the standard limits of between 0.015 mg/l to 0.03 for surface waters (EPA, 2009). Freshwater emergent wetlands also are characterized by interstitial water salinity that is normally less than 0.002 mg/l (Brown *et al.*, 1999).

A significant difference was obtained when warm season (mean = 0.19 mg/l) was compared to cold season (mean = 0.23 mg/l), as well as when planted (mean = 0.15 mg/l) was compared to the reference section (mean = 0.19 mg/l) ($p < 0.05$), with the low salinity (mean = 0.19 mg/l) found in the planted section and in the warm season. This also coincided with high nutrient concentrations in the system (ammonia = 0.4 mg/l difference, nitrate = 0.3 mg/l difference, nitrite = 0.42 mg/l difference, phosphate = 0.9 mg/l difference and orthophosphate = 0.5 mg/l difference respectively between planted and reference sections). While between September and October 2012 the salinity concentrations were constant (0.2 mg/l) and were the same in all three sources of wastewater, i.e. influent, planted and reference sections, a trend of increase in was observed December 2012 to June 2013.

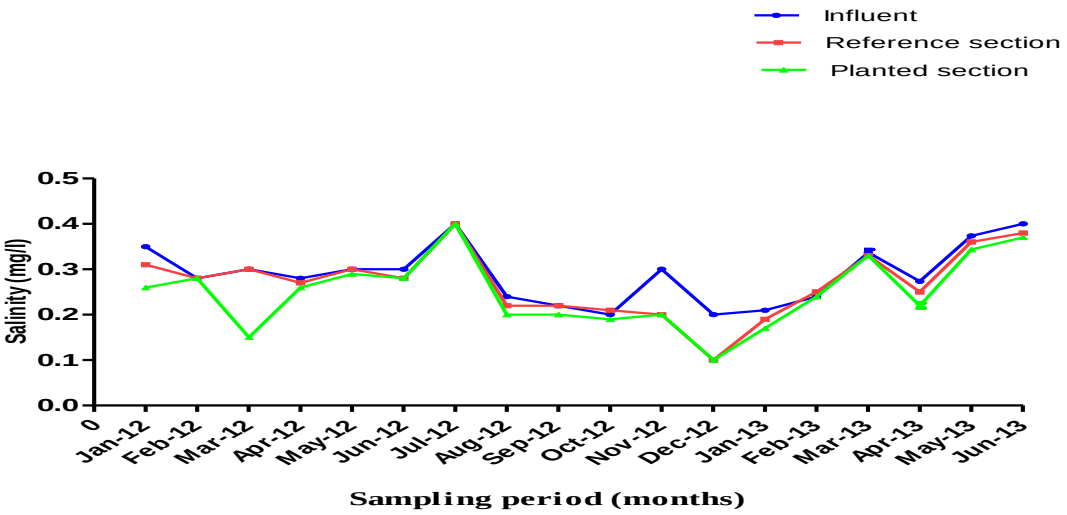

Figure 3.3: Salinity content of influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means of three readings are represented by each point with whiskers representing standard errors of means.

There was also a statistically significant difference in salinity obtained when the 2012 results (mean = 0.31 mg/l) were compared to 2013 (mean = 0.601 mg/l) ($p < 0.05$), with low salinities obtained in 2012. Low salinity concentrations in the planted section may be due to macrophytes which may have filtered and utilized the electrolytes in the planted section. On average, high salinity concentrations were obtained in 2013 compared to 2012 due to increased contamination levels introduced in wastewater. Salinity affects the abundance, the distribution and composition of biological resources in water. Thus it could be seen from the results that samples collected in 2013 were more contaminated than samples collected in 2012. This was also confirmed by the removal efficiency of nutrients and COD obtained in 2013 than in 2012 as well as variations in removal efficiencies obtained with variations in salinity.

3.3.1.4 Electrical conductivity

Conductivity is the ability of a substance to conduct electricity. It is the measure of ionic activities in water and is directly proportional to salinity (Chapman, 1992). Conductivity itself is not a human or aquatic health concern but because it is easily measured, it serves as an indicator of other water quality problems. If the conductivity of a stream or surface water suddenly increases, it indicates that there is a source of dissolved ions in the vicinity. Therefore, conductivity measurements are used as a quick way to locate potential water quality problems.

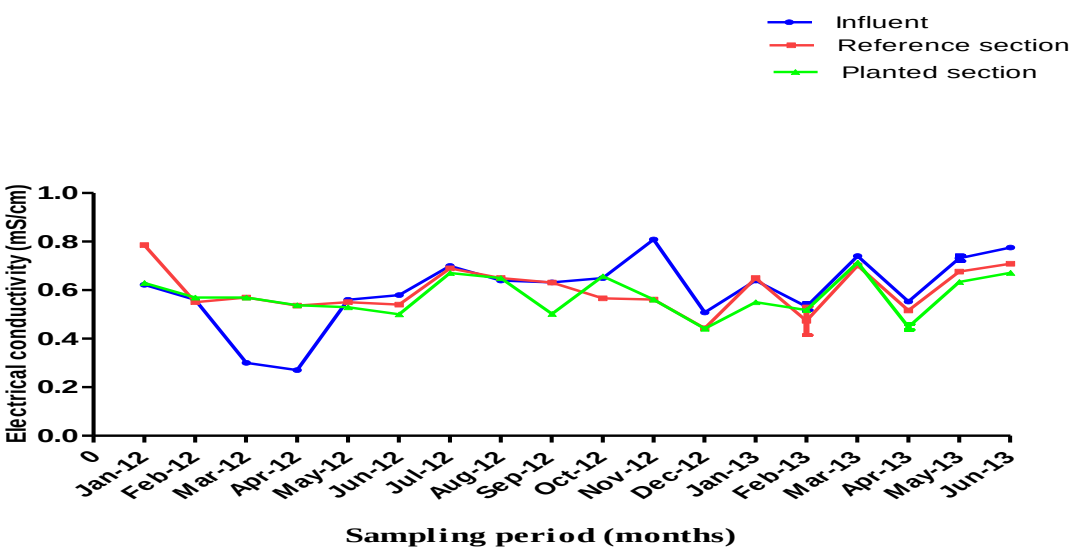

Figure 3.4: Electrical conductivity variations measured from influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Mean values of three readings are represented by each point with whiskers representing standard errors of means.

Influent conductivity ranged between 0.27-0.784 mg/l, while the effluent of the reference and planted section ranged between 0.44-0.71 and 0.441-0.673 mg/l respectively (Figure 3.4). Warm seasons had less (mean = 0.41 mScm) conductivity than cold seasons (0.57 mScm), as well as less in 2012 (0.36 mScm) than 2013 (0.56 mScm). The conductivity obtained in cold seasons was influenced by increased salinity readings obtained in cold seasons, as it reflected the amount of dissolved salts present in wastewater. In all the three sources of the samples, influent was found to have highest electrical conductivity followed by the effluent of the reference section while planted section had the least. The conductivity results obtained in both the influent and the effluent of the reference were found to be higher than the standard normal limits in surface waters of 0.25 mScm (Lin *et al*, 2004).

3.3.1.5 Total dissolved solids (TDS)

Total dissolved solid is one of the most essential water quality indicators. It measures dissolved organic solids, salts and minerals in wastewater and highly influences microbial activities. Total dissolved solids indicate the amount of contaminants present in wastewater. It has been reported to greatly affect the amount of oxygen required by microorganisms to break down nitrogen and phosphorus compounds (Tao *et al*., 2011). The results and the trends of total dissolved solids during the course of the study could be seen in Figure 3.5. More total dissolved solids were obtained in 2013 (means = 489 mg/l and 304 mg/l in the reference and planted

sections respectively) than in 2012 (means = 390 mg/l, 250 mg/l in the reference and planted section respectively), and may be attributed to increased contamination levels in 2013, as well as in the reference section. The general trend of TDS removal efficiency in the system showed to be high in warm seasons as well as at low contamination levels. When comparing planted to the reference section, sampling points between January and March 2012 it was found that the statistically significant difference was ($p < 0.001$), with more TDS retained by the planted section. Between October and December 2012 both planted and reference section means over three month-period were significantly different to the influent. The TDS in the influent ranged between 490 mg/l to 869 mg/l. In the effluent of the reference, it ranged between 380 mg/l to 590 and (the removal efficiency was between 10 and 46%) while in the planted section it ranged between 30 and 480 mg/l (the removal efficiency was between 20 and 69%). Culp *et al.* (1986) reported TDS to not exceed 200 mg/l in surface waters and should be kept as low as practicable to maintain the best usage of waters but in no case should it exceed 500 mg/l. In the rhizofilter, while the planted section conformed to these standards (the highest was 480 mg/l), most of the sampling points in the reference section did not, particularly in cold seasons.

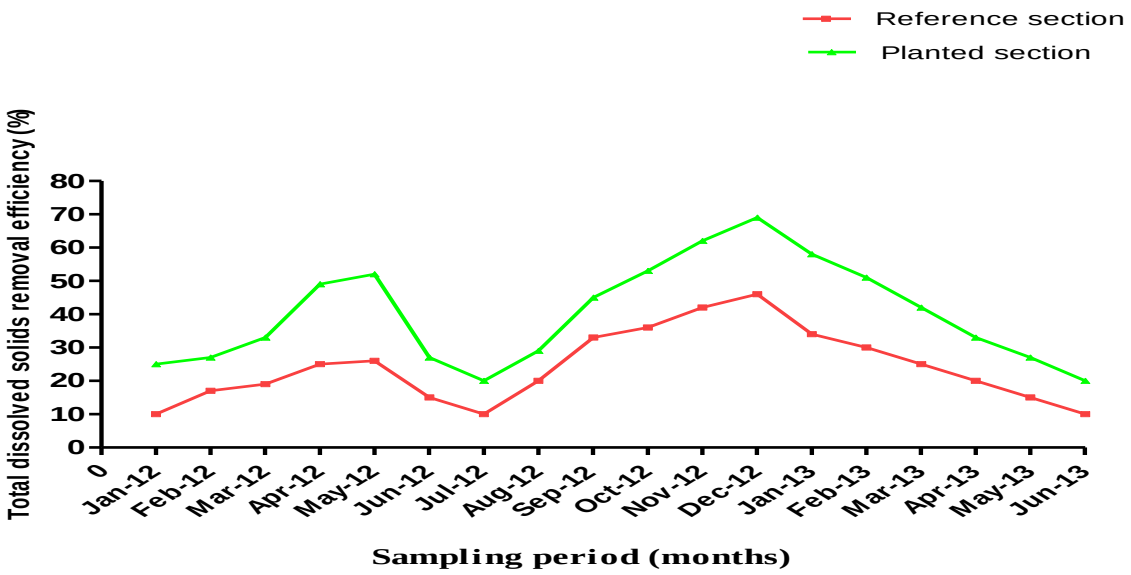

Figure 3.5: Total dissolved solids removal efficiencies from influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means of three readings per month are represented by each point with whiskers representing standard errors of means. In 2012 pre-treated pre-chlorinated wastewater was used for sampling.

3.3.1.6 Dissolved oxygen (DO)

Figure 3.6 shows that the DO was lowest in the influent (3.8 mg/l) and the effluent of the reference section (4.1 mg/l) in January 2012. The DO results ranged between 1.1 mg/l to 7.7 mg/l in the influent, 3 mg/l to 8.8 mg/l in the reference section and 3.7 mg/l to 8.8 mg/l in the planted section. The results obtained indicated that both the influent and the reference section were more than ten times more contaminated compared to the planted section. In January 2012 the DO results were 3 mg/l in the reference section and 3.7 mg/l in the influent while it was 6.08 mg/l in the planted section. High DO in the planted than in the reference section also explained low removal of COD and nitrogen obtained in the reference section compared to the planted section where DO was always relatively higher. While most sampling periods/points in 2012 indicated DO to be higher in the planted section compared to the reference section, the opposite was found in 2013. The main contributory factor to these observations was the difference in nutrient loading in the influent between the two periods. While a trend of the DO showed to increase at the early stages of system operation, the DO decreased substantially in 2013. The means of the DO for the planted section in 2012 were 7.8 mg/l while in 2013 they were 6.8 mg/l. In the reference section, they were 5.2 mg/l and 4.9 mg/l in 2012 and 2013 respectively. Tyroller *et al.* (2010) reported that DO should not be less than 6 mg/l and at no time should it be less than 5 mg/l.

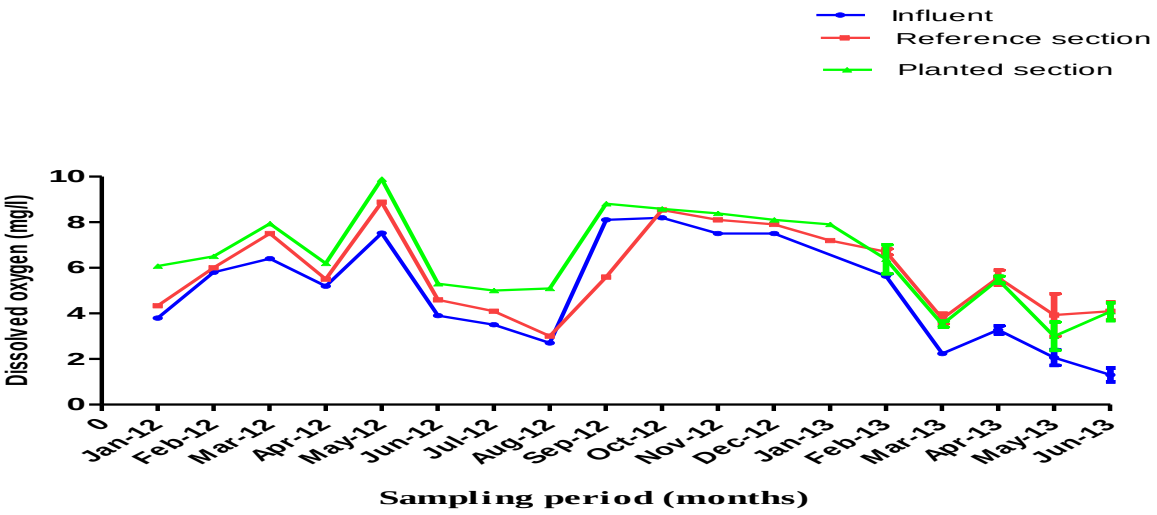

Figure 3.6: Dissolved oxygen variations in influent and effluent wastewater in a rhizofilter unit from January 2012 to June 2013. Means of three readings monthly are represented by each point with whiskers representing standard errors of means.

3.3.1.7 Chemical oxygen demand (COD)

Figure 3.7 shows the COD results obtained in the rhizofiltration system. The trends of COD measurement observed were similar to those of the dissolved oxygen. While there was high variability of COD during 2012, the trend in 2013 showed an increase. High COD concentrations were obtained in 2013 compared to 2012. There was no statistically significant difference in COD in all sampling periods/points when the planted section was compared to the reference section ($p > 0.05$). However, when both the effluent of the planted and reference sections were compared to the influent, statistically significant differences were obtained in warm seasons ($p < 0.05$), with warm seasons retaining more COD. In the reference section COD ranged between 39 mg/l and 344 mg/l, while in the planted section it ranged between 33 mg/l and 216 mg/l. The removal efficiency was up to 50% in the reference section and up to 62.3% in the planted section. Lin *et al*. (2004) reported rivers not to have more than 75 mg/l of COD in surface waters. Based on these limits, the planted section was found to be within the limit in the warm seasons (mean = 55 mg/l), while 39 mg/l was only obtained in October 2012.

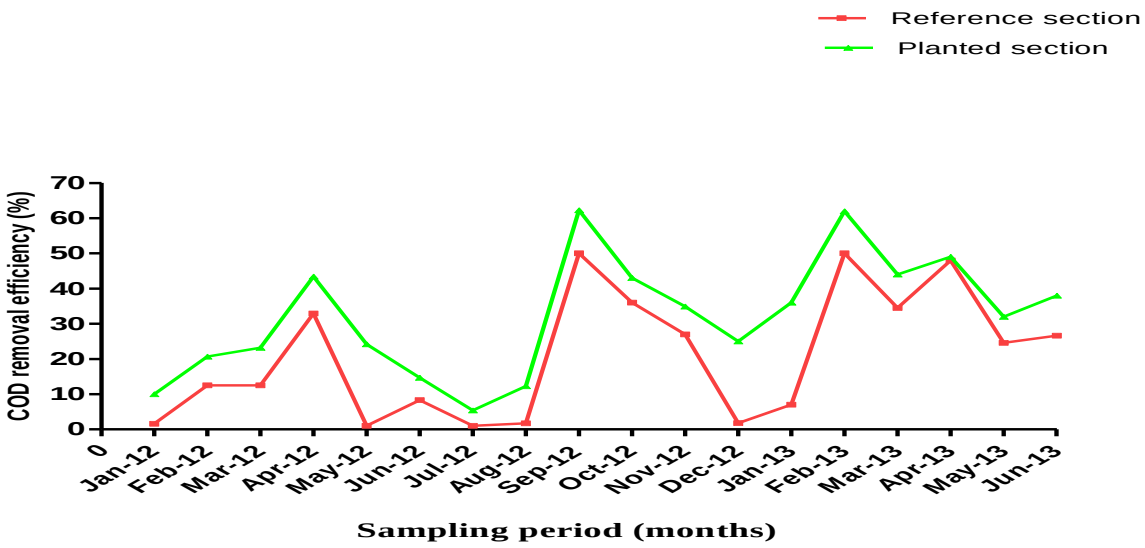

Figure 3.7: Chemical oxygen demand removal efficiencies measured in influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means of three monthly readings are represented by each point with whiskers representing standard errors of means.

3.3.2 Nutrients

3.3.2.1 Ammonia

Ammonia (NH_3) is one of the main contaminants present in the municipal wastewater. Most of quality standards of the treated wastewater specified ammonia concentration in the treated-recharged wastewater to be less than 5.0 mg-N/l (Wang *et al*., 2008; EPA, 2009). The natural level of ammonia in surface water is typically low (less than 1 mg/l). In the effluent of wastewater treatment plants it can range up to 30 mg/l. From the results it could be seen that ammonia was present in acceptable amounts in 2012 than in 2013 (Figure 3.8). Influent concentrations were also found in required standards in 2012. This is because samples were pre-treated in 2012 than in 2013. In 2012 the limits were only exceeded in July in all sources of water (33.5 mg/l in the influent, 27.8 mg/l in the reference section and 15.8 in the planted section). Ammonia was represented by up to 75% and 85% removal efficiency in the reference and planted sections respectively. In 2012 while the *t*-test indicated that there was only a statistically significant difference in the samples collected in the cold season (May to August) in the planted section as compared to the reference section ($p < 0.05$), with more ammonia retained in the planted section of the rhizofiltration system (1.17 mg/l mean retention difference between planted and reference section), in 2013 it was obtained between January and April. While the planted section was dependant on both macrophytes and soil media for ammonia removal, the reference section was dependant only on soil media. Ammonia removal efficiency was found to be significantly higher in the warm seasons (mean = 46% and 57% removal in the reference and planted sections respectively) than in winter (mean = 16% and 45% removal in the reference and planted sections respectively).

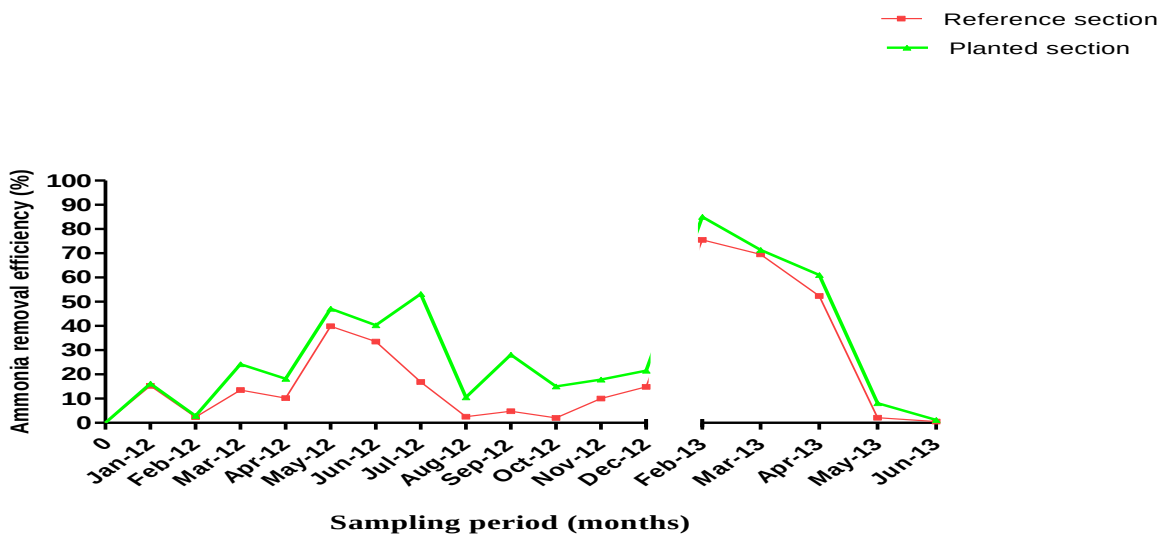

Figure 3.8: Ammonia removal efficiencies measured in influent and effluent wastewater in a rhizofilter unit from January 2012 to June 2013. The break between December 2012 and February 2013 indicate the period where the samples were not analyzed due to equipment failure.

3.3.2.2. Nitrate

Nitrate is a form of inorganic nitrogen required for microbial growth and is also needed by macrophyte cell growth. Though nitrate is an essential plant nutrient, in excess amounts, it may cause significant water quality deterioration. Together with phosphorus, nitrate in excess amounts can accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in the types of plants and animals that live in the water. Excess nitrate may cause low levels of dissolved oxygen and can become toxic to aquatic organisms at higher concentrations (10 mg/l or higher) under certain conditions. The natural level of nitrate in surface water is typically low (less than 1 mg/l). In the effluent of wastewater treatment plants it can range up to 30 mg/l (Wang *et al*., 2008).

Nitrate concentrations ranged between 0.8- 22.1 mg/l in the influent, 0.7-16.9 mg/l in the reference section and 0.6-13.4 mg/l in the planted section. The removal efficiency was up to 98% removal in the reference section while it was removed at an efficiency of up to 99% in the planted section (Figure 3.9). These results were within the limits in 2012. The *t*-test indicated statistically significant differences in samples collected between January and May 2012 as well as between October and December 2012 (both of these occasions were warm seasons; $p < 0.05$) when the planted was compared to the reference section. Highest removal efficiency was obtained in June 2012 in both the planted and the reference sections. This is because the influent concentration during this period was lowest (0.8 mg/l).

High nitrate removal in June 2012 coincided with high dissolved oxygen in both planted and reference sections (6.2 and 6.8 mg/l respectively). Oxygen was available in sufficient quantities for nitrifying microbes for converting ammonia to nitrite and then to nitrate. When oxygen is sufficiently high in wastewater, nitrate formation was aerobically enhanced as oppose to the anammox process, which facilitated the removal of nitrogen. Ciudad *et al.* (2005) also observed increased nitrogen removal in both the planted and unplanted models of wetlands due to increases formation of nitrate from ammonia, resulting from increased oxygen concentration in the system. Similar to the present study, the improvement in ammonia removal was enhanced by an increase in nitrite and nitrate removals under aerobic conditions (Chung *et al.*, 2006). It could therefore be concluded that the improvement in ammonia removal was due to increased nitrification activity. Dong and Sun (2007) also found that aerating soil and water columns increased nitrification and mineralization. Nitrate appeared to accumulate within the rhizofiltration system with increased dissolved oxygen.

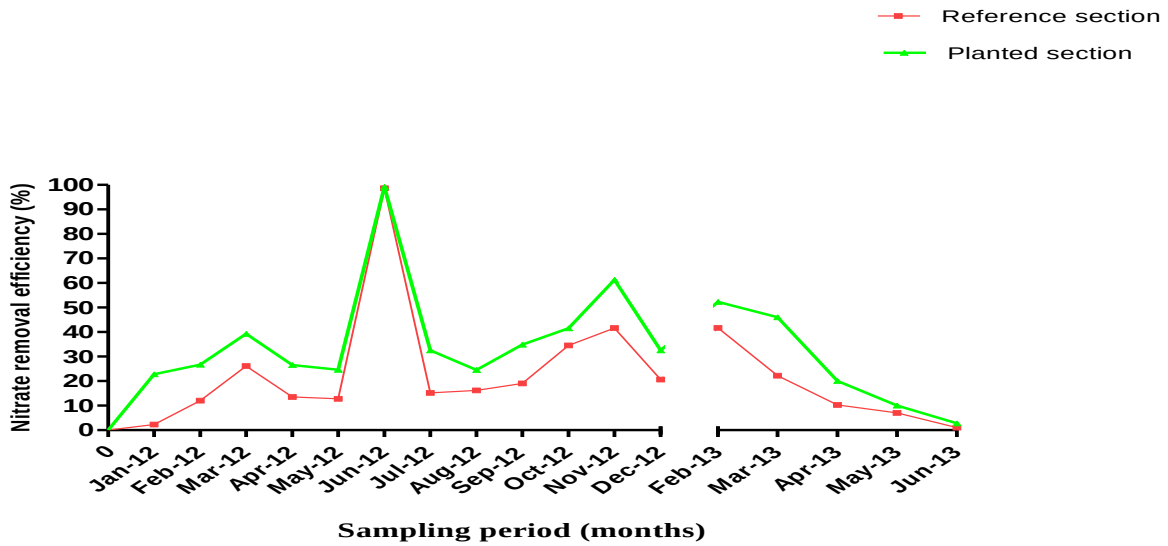

Figure 3.9: Nitrate removal efficiencies measured in influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Mean values of three readings are represented by each point with whiskers representing standard errors of means. The break between December 2012 and February 2013 indicate the period where the samples were not analyzed due to equipment failure.

3.3.2.3 Nitrite

Nitrite is another form of inorganic nitrogen found in wastewater. It can be seen from Figure 3.10 that nitrite removal efficiency in 2012 was up to 68% in the reference section and 99% in the planted section and the difference was statistically significant (p = 0.04), with more nitrite removed in the planted section. The trends of nitrite removal in the system in 2012 were similar

to that of nitrate removal; however the opposite was observed in 2013. The removal of nitrite was influenced by influent concentrations. The influent nitrite ranged between 0.1-2.3 mg/l, while it was between 0.01-2.1 mg/l in the reference section and 0.008-1.3 mg/l in the planted section. Concentrations obtained in 2012 (means = 1.8 mg/l influent, 1.1 mg/l planted and 0.89 reference) were within the limits when compared to the standard discharge limits in surface waters after wastewater treatment (5 mg/l). Low nitrite obtained in 2012 was attributed to low influent concentration.

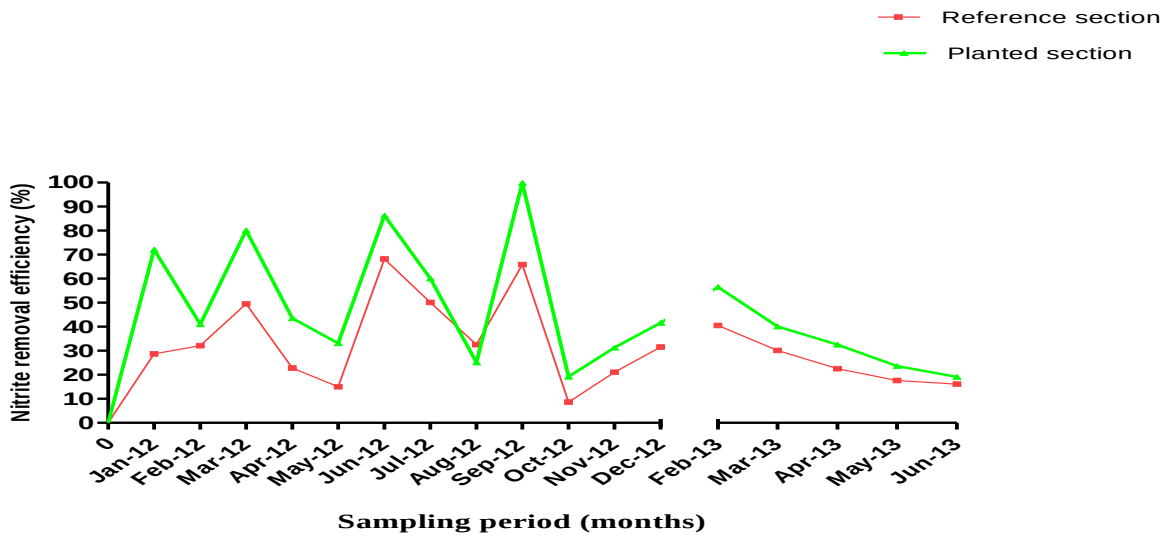

Figure 3.10: Nitrite removal efficiencies measured in influent and effluent wastewater in a rhizofilter from January to December 2012. Means of three readings are represented by each point with whiskers representing standard errors of means. The break between December 2012 and February 2013 indicate the period where the samples were not analyzed due to equipment failure.

3.3.2.4 Phosphate

Phosphate removal trends showed great variability in 2012 while a decrease in 2013 was observed (Figure 3.11). The results of phosphate concentrations ranged between 0.44-6.3 mg/l in the influent, 0.3-5.3 mg/l in the reference section and 0.3-4.5 mg/l in the planted section. These concentrations were lower both in the planted and reference sections and were within the discharge limits (5 mg/l) (EPA, 2009), except in June 2013. Removal of phosphorus was lower in cold seasons. Cold seasons are non-growing season of the plants in cold subtropical climatic conditions. Phosphate was removed at an efficiency of between 5.2 and 65.9% in the reference sections and ranged between 13.6 and 99.5% in the planted section. The difference was statistically significant (p = 0.028), with more phosphate removed in the planted section. The removal was high at the early stages of system operation and showed an overall gradual

decrease. A sharp increase in removal was observed in September 2012 due to low influent phosphorus concentration obtained in the system (0.4 mg/l).

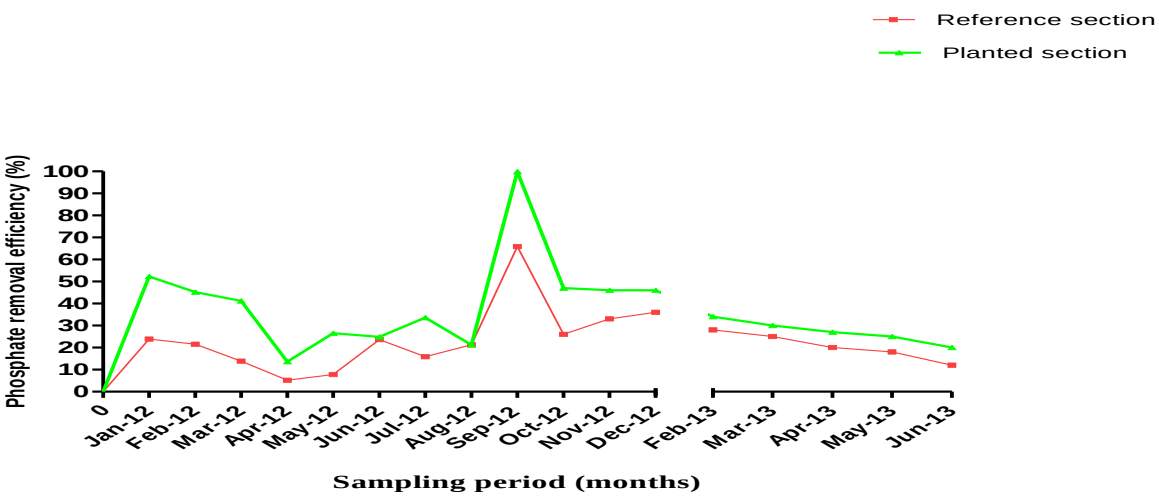

Figure 3.11: Phosphate removal efficiencies measured in influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means values of three readings are represented by each point with whiskers representing standard errors of means. The break between December 2012 and February 2013 indicate the period where the samples were not analyzed due to equipment failure.

3.3.2.5 Orthophosphate

Orthophosphate is another form of phosphorus found in wastewater. It was removed at an efficiency of between 1 and 36.8% in the reference section and between 10 and 63.9% in the planted section (Figure 3.12). Orthophosphate in the influent ranged between 0.6 mg/l-7.54 mg/l while it was between 0.45-4.48 mg/l and 0.1 - 4.5 mg/l in the reference and planted sections respectively. The removal of orthophosphate increased at the early stages of the system operation, which was followed by a decrease at later stages of the system operation. The *t*-test indicated the difference to be statistically significant in orthophosphate removal during all sampling periods in 2013 ($p < 0.0001$). In 2012 when the similar test was conducted, significant difference was obtained in the samples collected between January and May as well as between September and December (warm seasons), with more orthophosphate retained in the planted section.

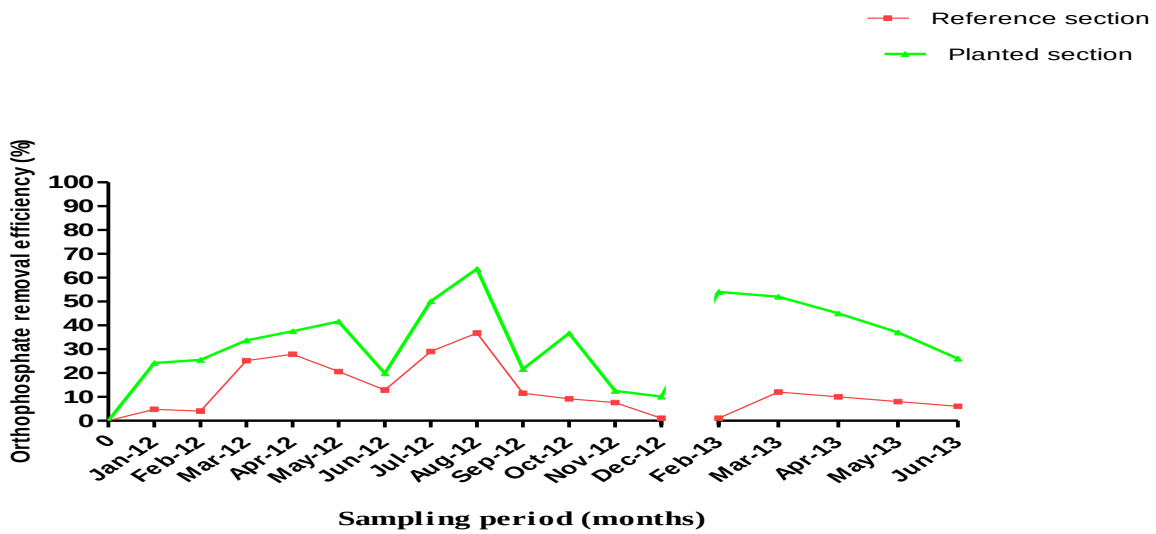

Figure 3.12: Orthophosphate removal efficiencies measured in influent and effluent wastewater in a rhizofilter from January 2012 to June 2013. Means of three readings are represented by each point with whiskers representing standard errors of means. The break between December 2012 and February 2013 indicate the period where the samples were not analyzed due to equipment failure.

3.3.3 Effect of Physicochemical Parameters on Nitrogen and Phosphorus Removal in the Rhizofiltration System

The effect of physical and chemical parameters on nutrient removal in the rhizofilter unit was determined using linear and non-linear regression models. The choice of the model used in each case (where either linear or non-linear model used) was based on the physical observation and judgement made based on the plots/coordinates. Their choice was also based on their use in the previous studies, where they were able to predict with up to 75% confidence the removal of nitrogen and phosphorus in wetland systems (Shrestha *et al*., 2003; Kumar *et al*., 2011).

3.3.3.1 pH

3.3.3.1.1 Effect of pH on nitrogen removal

The effect of pH on nutrient removal from the rhizofiltration system was evaluated by comparing pH with the removal efficiencies of ammonia, nitrate and nitrite obtained in the system. Effect of pH on ammonia, nitrate and nitrite is shown in Figures 3.13 to 3.15 respectively. While in 2012 (low ammonia concentrations in the system) a weak negative correlation between pH and ammonia removal efficiency was observed in both the planted and reference sections (r = -0.34 and -0.16 respectively), in 2013 (high ammonia concentration in the system) a poor positive correlation was observed (r = 0.32 and 0.38 in planted and reference sections respectively) (Figure 3.13). In 2012, 28.1% and 12.3% of ammonia was removed per

unit of pH decrease in the planted and reference sections respectively, while in 2013 the removal rate was 38 and 32% respectively. Nitrate (Figure 3.14) and nitrite (Figure 3.15) removals decreased with an increase in pH under both low (2012) and high (2013) concentrations (Figures 3.14 and 3.15 respectively). These observations were, however, with exception with nitrate in the planted section in 2013, where a very weak positive correlation was observed, and indicated 6.2% nitrite removal per unit of pH increase. In 2012 the pH of 9.7 was recorded in January. During this period and at this pH, ammonia and nitrate reduction from the rhizofilter were reduced significantly by 15.4% and 22.5% respectively in the planted section. In the reference section during the same period the pH was 10.2 and the removal of ammonia and nitrate was 15.4% and 2.3% respectively.

Hao *et al*. (2002) measured ammonia volatilization in subsurface flow wetlands treating dairy wastewater at pH 7.5 in warm season. The influent ammonia concentration (average mean 29.9 mg/l) was similar to the present study. The monthly average ammonia volatilization fluxes found by Hellinga *et al*. (1998) were 0.015–0.099 N gm^2d, which is equivalent to 0.03–0.22 N gm^3d in the present study. It was therefore estimated that ammonia volatilization contributed <3% to the measured ammonia removal efficiency in the reference section and <8% in the planted section which had 2.8 times free ammonia at its higher pH values in the planted section. Accordingly, the relative abundance of ammonia oxidizing bacteria and anammox together was higher in the planted than in the reference section. Anammox consumes nitrite and produces nitrate. The higher nitrite accumulation and lower nitrate removal efficiencies in the planted suggested that the higher pH in planted enhanced nitritation–anammox, but anammox was still the limiting stage. Anammox in the planted was possibly inhibited by the high free ammonia concentrations. Free ammonia concentrations at 1.7–8.3 mg/l have been reported to be inhibitory to anammox activity in anammox reactors (Ciudad *et al*., 2005). Therefore, both pH and free ammonia concentration need to be controlled for enhancement of nitritation–anammox.

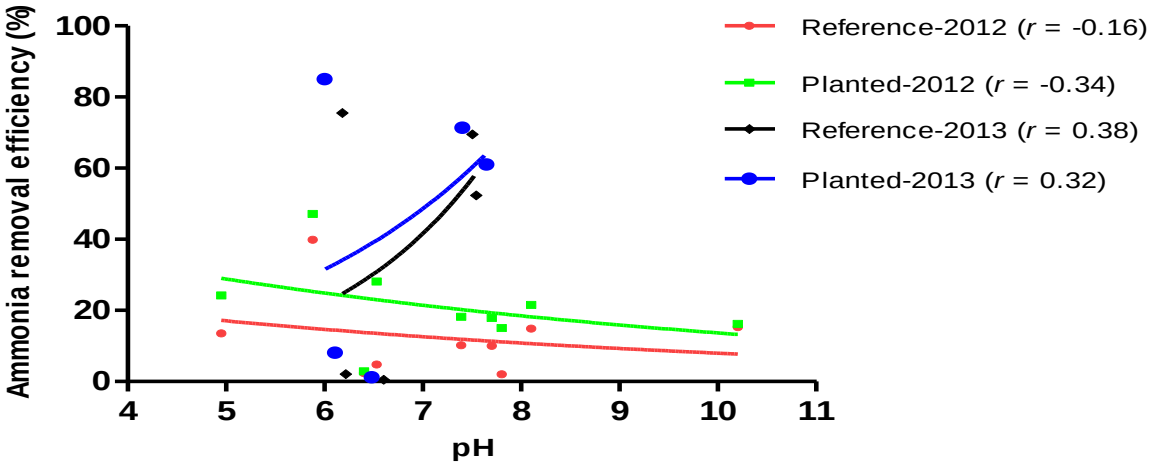

Figure 3.13: Effect of pH on ammonia removal efficiency in both the planted and reference sections of the rhizofiltration plant in 2012 and 2013 study period. Means of ammonia removal efficiency were compared against their corresponding pH means. Linear (2012) and non-linear (2013) curve fit was used to demonstrate the relationship between the pH and ammonia removal efficiency in the system.

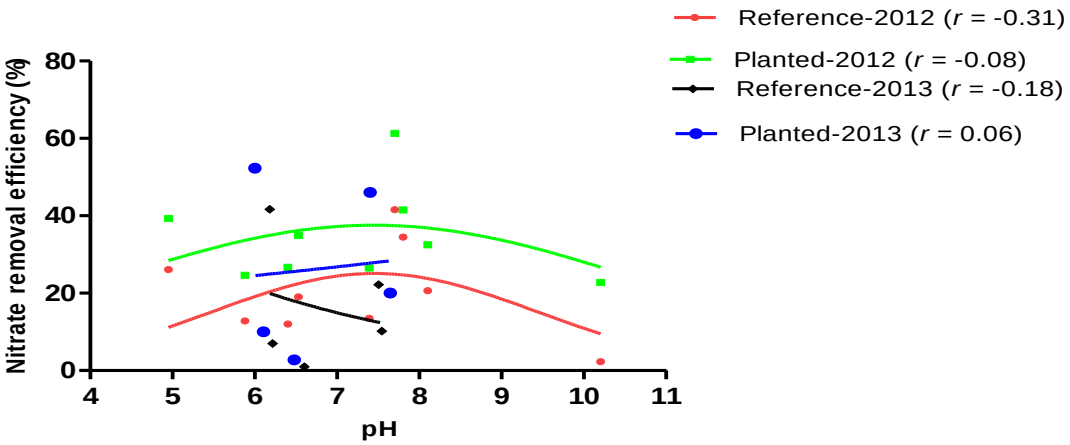

Figure 3.14: Effect of pH on nitrate removal efficiency in the planted section of the constructed rhizofiltration system between January 2012 and June 2013. Means of nitrate removal was drawn against their corresponding pH means over time from February to June 2013. A non-linear curve fit was used to demonstrate the relationship between the pH and nitrate removal efficiency in the system.

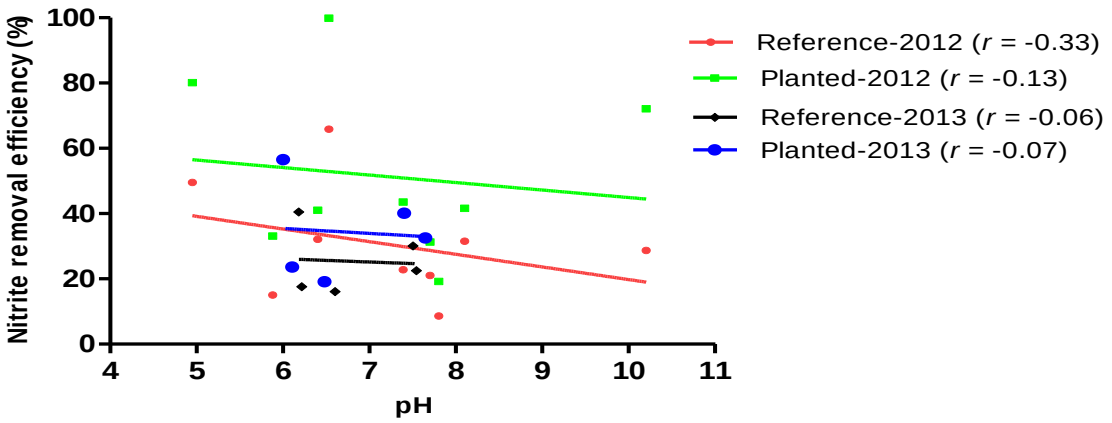

Figure 3.15: Effect of pH on nitrite removal efficiency in both the planted and reference sections of a rhizofiltration system during the 2012 and 2013 sampling period. Mean values of nitrite removal were compared to their corresponding pH means in the system. A linear curve fit was used to demonstrate the relationship between the pH and nitrite removal.

3.3.3.1.2 Effect of pH on phosphorus removal

The effect of pH on phosphorus removal in the system was evaluated by comparing pH variations with the removal efficiencies of phosphate and orthophosphate. While a very weak positive correlation between phosphate removal efficiency and pH was observed in 2012 in the planted and reference sections, 2013 had very weak positive and negative correlations in the reference and planted sections (Figure 3.16). In 2012, 16.5% and 78% phosphorus was removed per unit of pH increase, while in 2013 the removal rate was 7.2% per pH increase in the planted section and 4% in the reference section. A moderate negative correlation in orthophosphate removal at low concentrations in 2012 was observed (36.4 and 28.6% removal per unit decrease in pH in planted and reference section respectively), while a weak positive correlation was obtained at high concentration levels in 2013 (51.2 and 34.5% removal per unit increase in pH in planted and reference section respectively) (Figure 3.17). Phosphorus was mostly bio-available at slightly acidic to neutral pH levels. In acidic situations phosphorus tends to be bound to aluminum and iron. In basic conditions it forms complexes with calcium and magnesium. Thus, increased removal was obtained in acidic pH, which promoted phosphate availability to macrophytes for uptake.

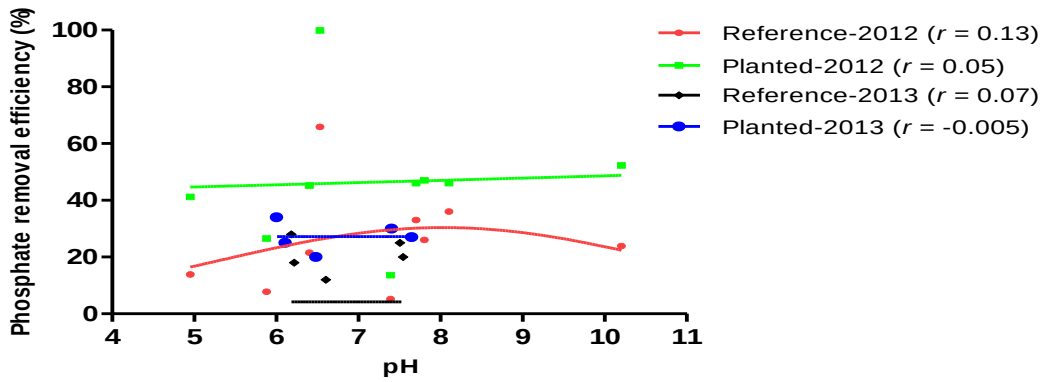

Figure 3.16: Effect of pH on phosphate removal in the system. Means of phosphate removal efficiency were used to draw the graphs. A linear curve fit was used to demonstrate the relationship between the pH and phosphorus removal efficiency in the system.

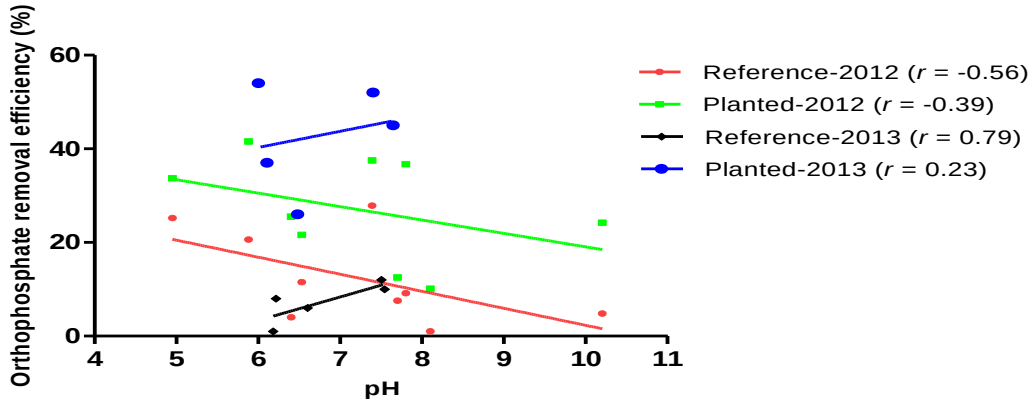

Figure 3.17: Effect of pH on orthophosphate removal efficiency in both the planted and reference sections of a constructed rhizofiltration system during the 2012 and 2013 sampling period. Mean values of orthophosphate were used in the analysis. A linear curve fit was used to demonstrate the relationship between the pH and orthophosphate removal efficiency in the system.

3.3.3.2 Temperature

3.3.3.2.1 Effect of temperature on nitrogen removal

The effect of temperature on nitrogen removal in a rhizofiltration system is shown in Figures 3.18 to 3.20. It has been demonstrated that at low concentrations in 2012, ammonia was reduced by 4.2% and 13.6% in reference and planted sections respectively per unit change in temperature (Figure 3.18) and nitrate (7.2 and 9.2% per unit change in reference and planted sections respectively) (Figure 3.19) removal are decreased with temperature increase in both the planted and the reference sections. While a very weak negative correlation between nitrite

removal and temperature was observed at low concentrations in 2012 in the reference section, at high concentration (2013), a strong positive correlation was obtained in both the planted and reference sections (Figure 3.20). Nitrogen removal was negatively affected by either very high or low temperatures. Successful nitrification and denitrification enhancement have been observed at higher temperatures between 25-30°C (Tao *et al.*, 2012). According to Cheng *et al*. (2009) nitrifying bacteria convert ammonia to nitrate between 15-25°C. Similarly, Vymazal, (2007) reported maximum specific rate of ammonia oxidation to be increased by 3.7 times as temperature increased from 10 to 30°C. Temperature varied remarkably in the system between January 2012 and June 2013. In 2013, highest ammonia removal was obtained at temperatures between 27-29°C in the planted section. These were observed in February 2012 (75% and 85% removal in the reference and planted section respectively) and March 2012 (69 and 71% removal in the reference and planted section respectively). During these periods temperatures were 27.3˚C and 28.9˚C respectively. The highest temperature obtained in 2013 was 24.3˚C and it was during this period where nutrients were removed at highest efficiencies. Between April and June 2013 temperature fluctuations were obtained, notable below 19˚C. Results obtained were consistent with findings by Yaun *et al*. (2013) who reported temperature to affect microbial activities responsible for nitrogen and phosphorus removal, thereby influencing their removal from wastewater. Dong and Sun (2007) found temperatures between 16.5-32°C to be favourable for nitrification and denitrification in the wetland systems. These results were also confirmed by Metcalf and Eddy (1991), who find that nitrification was favoured at temperatures between 18-35°C.

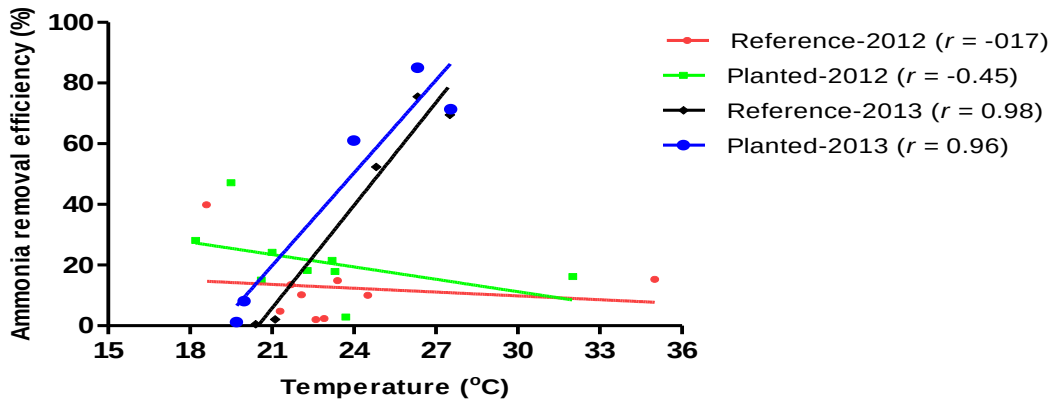

Figure 3.18: Effect of temperature on ammonia removal efficiency in the planted and reference sections of the rhizofiltration system between 2012 and 2013 study period. Mean values were used in the analysis. A linear curve fit was used to demonstrate the relationship between the temperature and ammonia removal efficiency in the system.

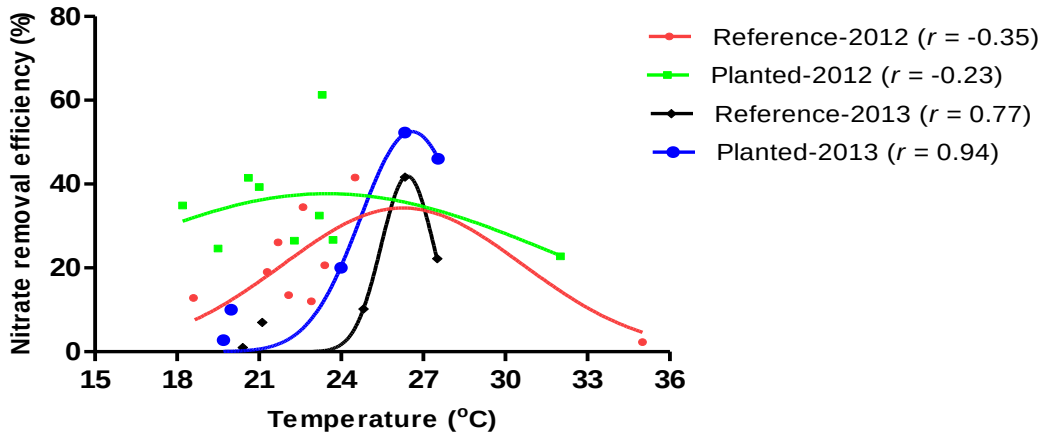

Figure 3.19: Effect of temperature on nitrate removal efficiency in the planted and reference sections of a rhizofiltration system between January2012 and June 2013 sampling period. Means of nitrate removals and their corresponding means of temperature were used in the analysis of the results from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the temperature and nitrate removal efficiency in the system.

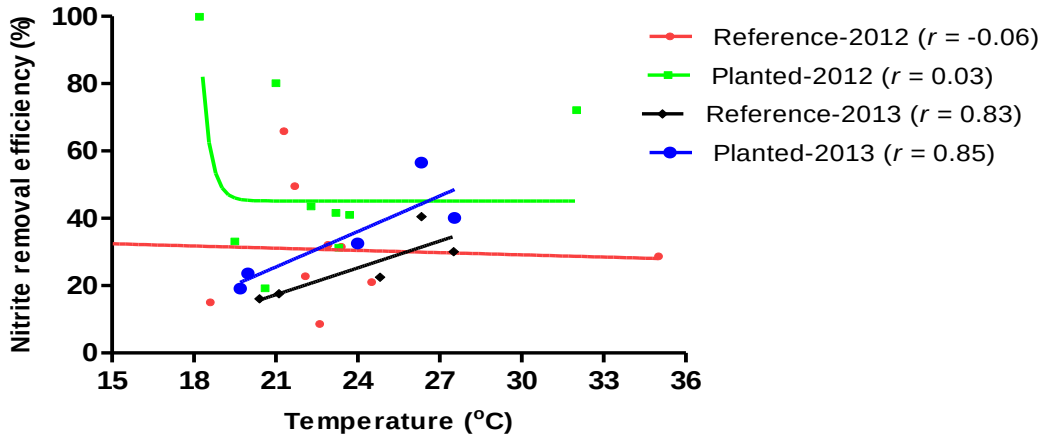

Figure 3.20: Effect of temperature on nitrite removal efficiency in both the planted and reference sections of a rhizofiltration system between January 2012 and June 2013. Means of nitrite and temperature were used in the analysis of the results. Non-linear (2012) and linear (2013) curve fit was used to demonstrate the relationship between the temperature and nitrite removal efficiency in the system.

3.3.3.2.2 Effect of temperature on total phosphorus removal

Temperature is one of the factors that influenced the removal of phosphorus. The effect of temperature on phosphate and orthophosphate is shown in Figures 3.21 and 3.22 respectively. In 2013 phosphate removal showed a very strong positive correlation with temperature in both the planted (r = 0.9) and the reference section (r = 0.86) of the rhizofiltration system, while a very weak positive (reference) and negative correlations were obtained in 2012 (Figure 3.21). Orthophosphate had a negative correlation in 2012 (low nutrient concentration in the system) and a positive correlation in 2013 with temperature (Figure 3.22), with 9.9 and 8.1% as well as 1.2 and 9.3% removal per change in a unit of temperature in the reference and planted section respectively. Phosphorus removal rate of 22 and 17.9% per unit change of temperature was observed in the reference section in both 2012 and 2013 respectively, while in the planted section it was 7.8 and 12.7°C respectively (Figure 3.21). Phosphate removal was more efficient at temperatures around 24°C while the removal of orthophosphate was efficient around 20°C. Phosphorus tends to accumulates in rhizofiltration system due to its lack of gaseous form mechanism.

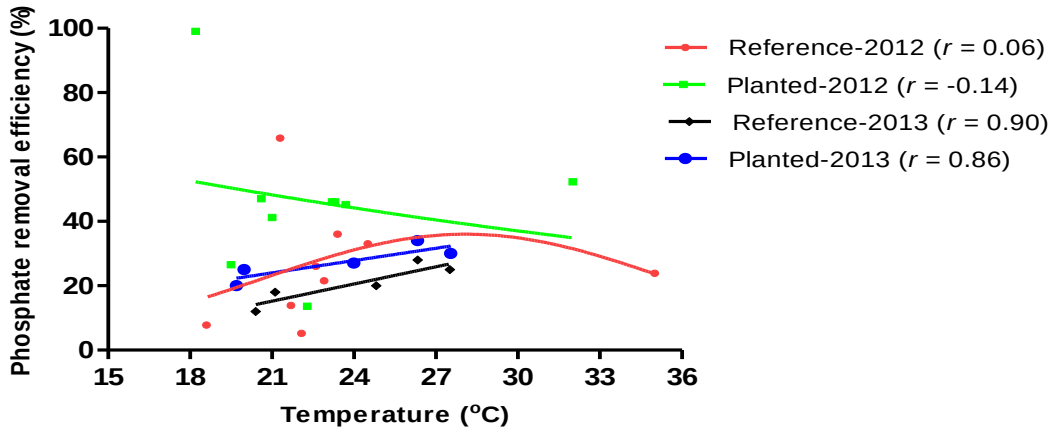

Figure 3.21: Effect of temperature on phosphate removal efficiency in the planted and reference sections of a constructed rhizofiltration between January 2012 and June 2013. Means of phosphate removal and temperature from January 2012 to June 2013 were used in for the analysis. Non-linear (2012) and linear (2013) curve fit was used to demonstrate the relationship between the temperature and phosphate removal efficiency in the system.

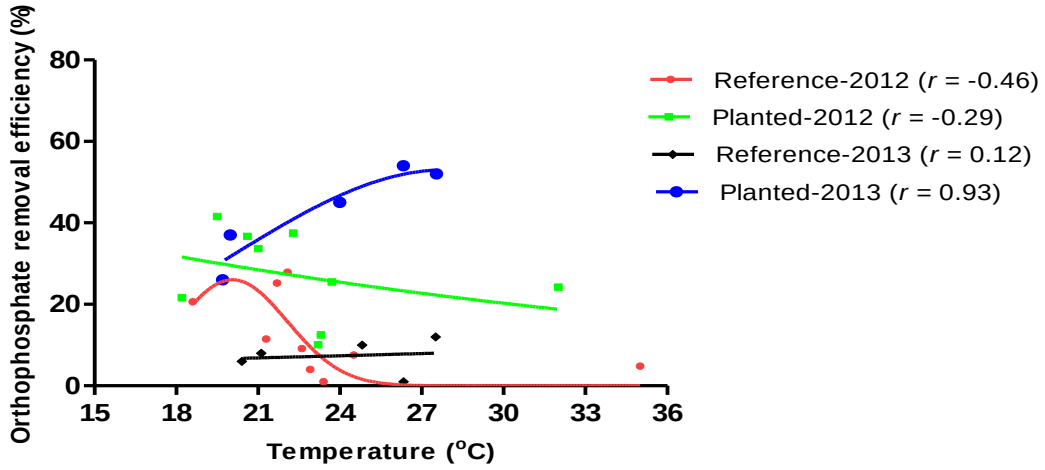

Figure 3.22: Effect of temperature on orthophosphate removal efficiency in the planted and reference sections of a constructed rhizofiltration between January 2012 and June 2013. Means of orthophosphate removal and temperature from January 2012 to June 2013 were used in for the analysis. A non-linear curve fit was used to demonstrate the relationship between the temperature and orthophosphate removal efficiency in the system.

3.3.3.3 Salinity

3.3.3.3.1 Nitrogen

The effect of salinity on nitrogen removal in the system can be seen in Figures 3.23 to 3.25 respectively. It can be seen from the Figures that at low concentrations, ammonia removal efficiency decreased under high salinity concentrations in 2013, and increased at low concentrations in 2012 (Figure 3.23). Nitrate (Figure 3.24) and nitrite (Figure 3.25) removal efficiencies decreased with increased salinity levels. Salinity results obtained varied with nutrient influent loading in the rhizofiltration system. Sodium content and dissolved solids affected microbial activities. Microbial diversity and abundance within the rhizofiltration system was low at high salinity concentrations and high electrical conductivity. This reduced the reduction potential of nutrients in the rhizofilter system. High salinity caused lysis of microorganisms through plasmolysis. This resulted in decreased nutrient removal. Low levels of salinity, electrical conductivity and TDS favoured the removal of total nitrogen, with ranges between 02-0.3 mg/l of salinity found to be optimum for the removal of ammonia compared to nitrate and nitrite.

In the rhizofiltration system, salinity did not influence the oxygen concentrations, which was very low for all the measured salinities. Salinity was particularly lowest in the planted section in 2012 (mean = 0.18 mg/l). The low concentrations of oxygen resulted in the redox potential

being independent of salinity and therefore the registered variations were the same in different cycles and for different salinities. Conversely, the redox potential changed under conditions of high dissolved oxygen when the amount of oxygen increased in the planted section and consequently the redox potential. Macrophytes enhanced the dissolved oxygen concentrations and electrolytes reduction in the planted section.

Ciudad *et al*. (2005) reported salinity to affect the growth of microorganisms resulting in a switch of the microbial community in a pilot-scale. Generally, anaerobic conditions in a pilot-scale in wetlands cause low removal of ammonium because the oxidation of ammonia to nitrite and then to nitrate occurs under aerobic conditions by autotrophic bacteria. Contrary to Hellinga *et al.* (1998) who found inhibition of the nitrifiers in the case of a rapid increase in the chloride concentration with conventional microorganisms, the results obtained in this study indicate the removal of ammonia not to be influenced by increased salt concentrations in the case of halophiles. These results are in accordance with Tao *et al*. (2011) who found the adverse effects of high salt concentrations to be significantly alleviated by the use of salt-tolerant microorganisms. Vymazal, (2007) concluded that the ability of a horizontal flow constructed wetlands to nitrify ammonia was very limited because anaerobic conditions usually existed. This is in accordance with the present results where, at the same salinity, high removal of ammonia did not occur even with high dissolved oxygen because the conditions were still anoxic. The lower concentration of ammonia found in the planted section with increment in salts were also in accordance with the study of Vymazal, 2007 who reported some degradation processes to require energy (typically derived from an organic carbon source) to proceed, and others release energy, which can be used by organisms for growth and survival. This suggests that increased concentration of salts and electrolyte stimulated the growth of the microbial community and also the nitrification process and that dissolved solids acted as a source of energy for the organisms.

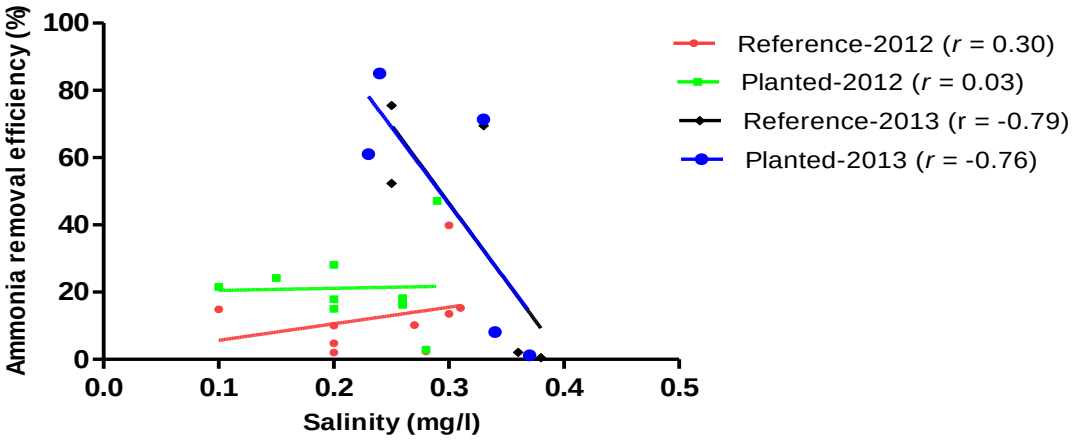

Figure 3.23: Effect of salinity on ammonia removal in the planted and reference section of a rhizofiltration system between January 2012 and June 2013. Means were used in the analysis of the results. A linear curve fit was used to demonstrate the relationship between salinity and ammonia removal efficiency in the system.

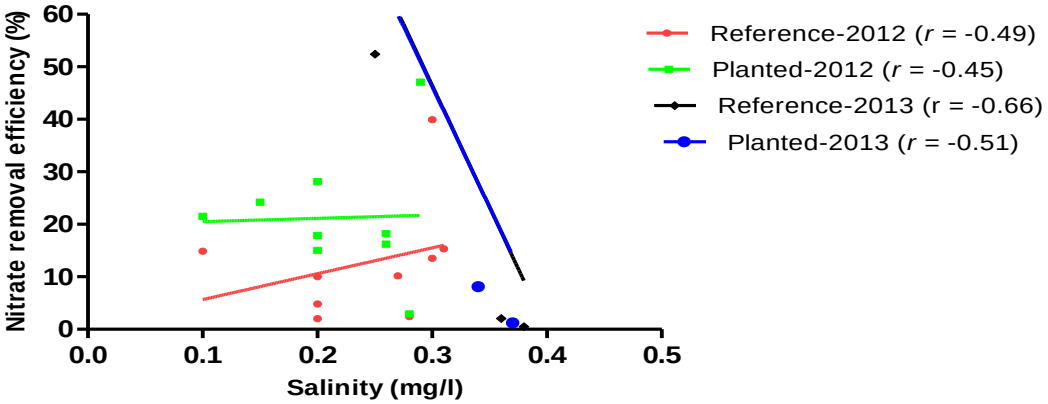

Figure 3.24: Effect of salinity on nitrate removal efficiency measured in a planted and reference section of a rhizofiltration system between January 2012 and June 2013. Mean values of both nitrate and their corresponding salinity were used in the analysis of the results. A linear curve fit was used to demonstrate the relationship between salinity and nitrate removal efficiency in the system.

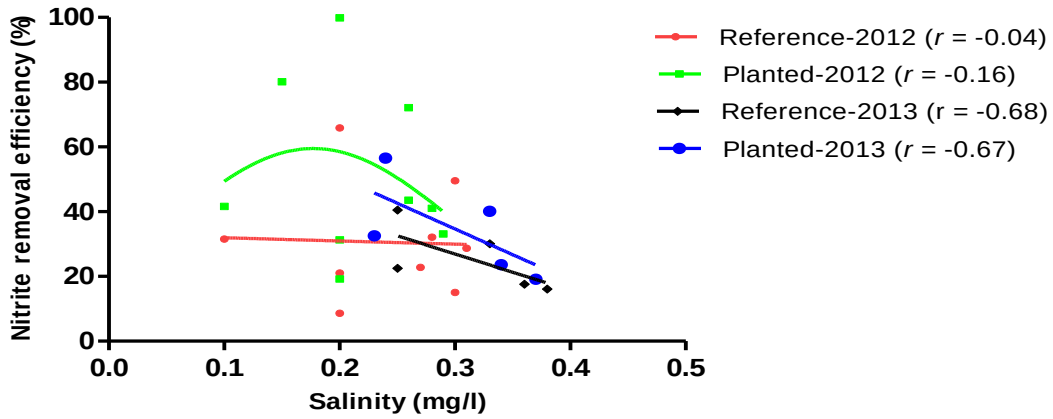

Figure 3.25: Effect of salinity on nitrite removal efficiency in a planted and reference section of a rhizofiltration system in the 2012 and 2013 study period. Mean values of nitrite and salinity were used in the analysis. Non-linear (2012) and linear (2013) curve fit were used to demonstrate the relationship between salinity and nitrite removal efficiency in the system.

3.3.3.3.2 Phosphorus

The effect of salinity on phosphate and orthophosphate can be seen on Figures 3.26 and 3.27 respectively. Phosphate removal decreased with increased salinity levels both in the planted and reference sections (Figure 3.26), while orthophosphate removal increased with salinity increase under low salinity concentrations in 2012 (Figure 3.27). The removal rate per decrease in one unit of salinity were 15.5 and 10% in the planted and reference section respectively in 2012, while in 2013 it was 7.05 and 5.68% respectively. When comparing different salt concentrations, the mean values for phosphate and orthophosphate removal showed an efficiency of 4 and 33% in the reference and planted sections at 0.2 and 0.1 mg/l concentrations of phosphate and orthophosphate respectively. The COD removals at the same conditions were 24 and 49% respectively. The trends showed a very slightly change in percentages of phosphorus and COD removal as salinity increased, suggesting that organisms present and processes that led to phosphorus removals were slightly affected by salinity and could therefore have improved the saline wastewater treatment process in the system. In the case of lower concentrations of saline, it was noticed that higher removal efficiencies were achieved with increased dissolved oxygen compared to removals in wastewater containing low dissolved oxygen. This suggests high dissolved oxygen improved organic matter decomposition processes and that the processes were hindered by lack of oxygen.

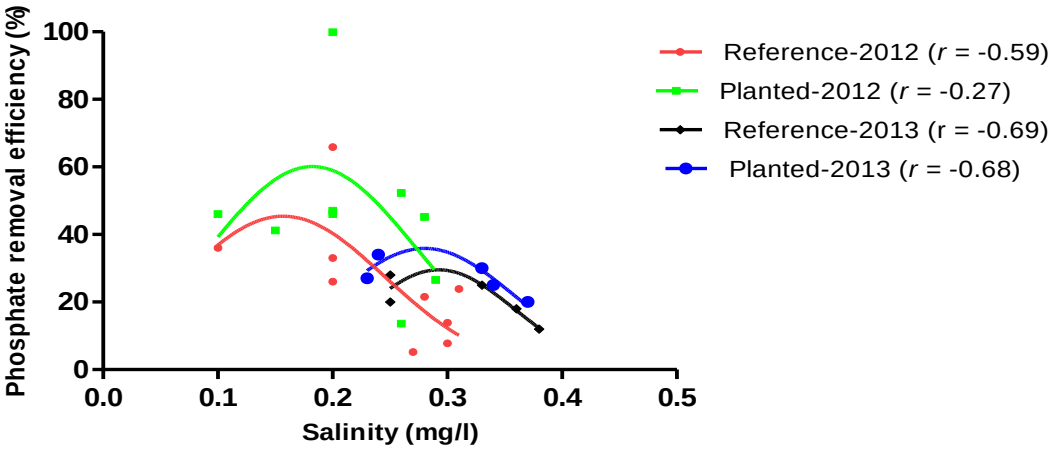

Figure 3.26: Effect of salinity on phosphate removal in a planted and reference section of a rhizofiltration system from January 2012 to June 2013. Means of phosphate and salinity were used in the analysis. A non-linear curve fit was used to demonstrate the relationship between salinity and phosphate removal efficiency in the system.

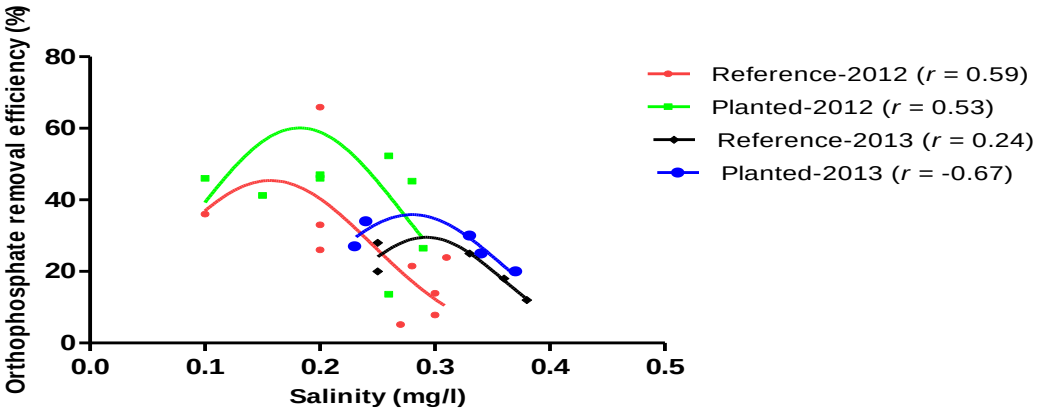

Figure 3.27: Effect of salinity on orthophosphate removal efficiency in the planted and reference section of a rhizofiltration system from January 2012 to June 2013. Means of orthophosphate were drawn against their corresponding means of salinity over time. A non-linear curve fit was used to demonstrate the relationship between salinity and orthophosphate removal efficiency.

3.3.3.4 Dissolved oxygen

3.3.3.4.1 Nitrogen

Nitrogen removal from the rhizofiltration system was depended on the levels of dissolved oxygen. Ammonia (Figure 3.28), nitrate (Figure 3.29) and nitrite (Figure 3.30) removal efficiencies all positively correlated with the DO both in the planted and reference sections, with exceptions being in 2012 at low concentrations, where very weak inverse correlations were obtained in the reference and planted sections for ammonia and nitrite respectively. In

2012 Ammonia removal decreased at a rate of 1.7% per unit of DO increase in the reference section, while a 4% increase in DO was obtained in the planted section. In 2013, both the planted and the reference sections had 18.9 and 17.27% ammonia removal efficiency increase rate per increase in DO in the system. When levels of DO were high, aerobic conditions that enhance the enzymatic activities of microorganisms were created (Quan *et al*., 2012). High DO did not only support oxygen respiring microorganism in the rhizofiltration system but also created breeding ground for such organisms for fast growth, and thus led to nutrient degradation and subsequent removal in wastewater (Quan *et al*., 2012). High removal of organic matter in a form of COD was most favourable at high DO levels in the system. Results indicated DO to increase at lower temperatures (18-24°C) compared to warmer temperatures (26-34°C). Low DO concentrations in wastewater represented high levels of contaminants in the system (El-Hoz and Apperler, 1996). Ciudad *et al*. (2005) investigated the use of aeration to promote nitrification activity in a laboratory scale wetland but used subsurface flow gravel bed models and compared planted to an unplanted conditions. The models investigated received primary domestic sewage. Prior to aeration, the mean ammonia concentration decreased by 5 and 18% in their unplanted and planted sections, respectively. After aeration was introduced, ammonia removal increased to 38 and 68% for the unplanted and planted sections respectively. Since ammonia was being nitrified to nitrate within the system, effluent nitrate increased. The accumulation of nitrate, particularly in the reference section in the rhizofilter indicated that after ammonia was nitrified, however, subsequent denitrification was limited. Possible factors that could limit denitrification may have included inadequate retention time for denitrification to remove nitrate in the system, the presence of low dissolved oxygen, or even lack of available carbon source in the reference section. Denitrification activity is reduced if available carbon supply is low (Strous *et al.,* 1997; Shipin *et al.,* 2005) and proceeded only when the oxygen supply was inadequate for microbial demand (Smith *et al*., 1997). However, limited denitrification activity has been observed in the presence of dissolved oxygen (Tao *et al*., 2011). In constructed wetlands, after nitrate is formed under aerobic conditions, it diffuses down into the anaerobic portion of the soil where it is denitrified (Ciudad *et al*., 2005). Ciudad *et al*. (2005) also experienced nitrate accumulation within an aerated wetland model and suggested that the addition of a carbon-source in the final section of the model may have improved denitrification activity. In the present study carbon availability may have been inadequate to support high levels of denitrification due to the lack of an established litter layer in this relatively young rhizofiltration system, particularly in the reference section, a problem

also experienced by van der Graaf *et al*. (1996). As wetlands age, organic matter accumulates in the litter layer provided by decaying macrophytes. Van der Star *et al*. (2007) estimated that 5 to 10 years of constructed wetland development may be necessary for the accumulation of organic matter to become sufficient to support maximum denitrification. The rhizofilter was given only six months to establish itself. If, on the other hand, the influent wastewater itself was an adequate source of carbon, the lack of denitrification may be attributed to the short hydraulic retention time of the system.

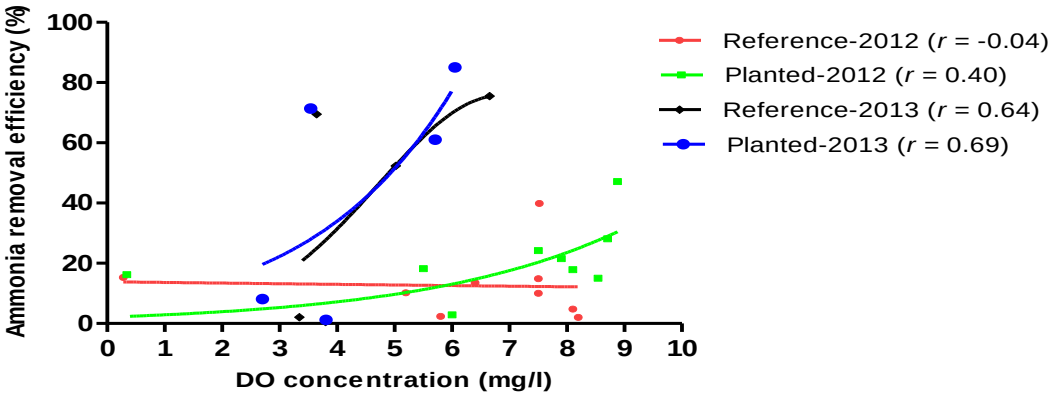

Figure 3.28: Effect of the DO on ammonia removal efficiency in wastewater treatment using rhizofiltration technology. Means of ammonia removal efficiency in the planted and reference sections were used against their corresponding means of the dissolved oxygen from January 2012 to July 2013 in the rhizofilter system. A non-linear curve fit was used to demonstrate the relationship between the DO and ammonia removal efficiency in the system.

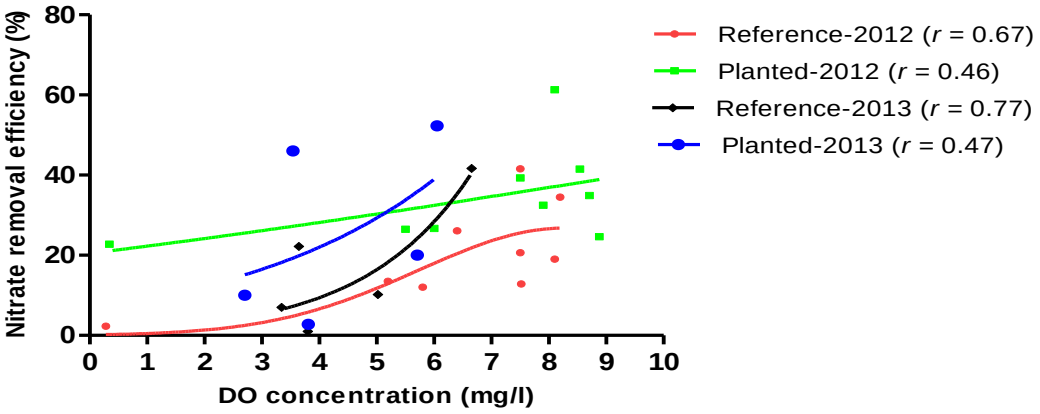

Figure 3.29: Effect of the DO on nitrate removal in a rhizofiltration system. Means of nitrite removal efficiency were used against their corresponding means of the dissolved oxygen in the planted and reference sections of the rhizofiltration system from January 2012 to June 2013 in the rhizofilter system. A non-linear curve fit was used to demonstrate the relationship between the DO and nitrate removal efficiency in the system.

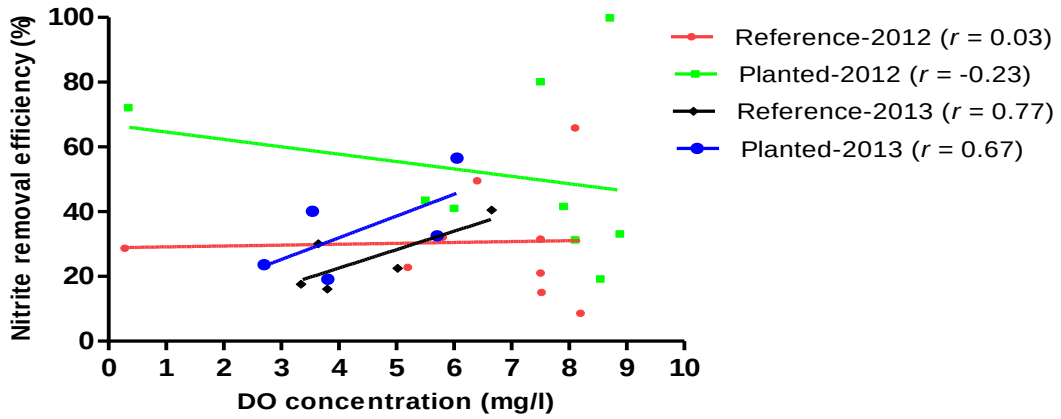

Figure 3.30: Effect of the DO on nitrite removal efficiency between January 2012 and June 2013 in a rhizofiltration system. Means of nitrite removal efficiency were used against their corresponding means of the dissolved oxygen in the analysis of the results. A linear curve fit was used to demonstrate the relationship between the DO and nitrite removal.

3.3.3.4.2 Phosphorus

Phosphate and orthophosphate removal in the rhizofiltration system increased with increased dissolved oxygen concentration, though orthophosphate showed a strong inverse correlation at high orthophosphate concentrations in 2013 (Figures 3.31 and 3.32 respectively). The rate of phosphate removal efficiency was 3 and 1.2% and 6.1 and 5.4% in 2012 and 2013 respectively both in the reference section as planted section as DO increase in the system. Under aerobic conditions, phosphorus formed a complex and precipitated with various chemicals such as iron, calcium, and aluminum (Smith *et al*., 1997). Unlike nitrogen, phosphorus does not serve as an oxidizing agent; however, it is linked to the reduction/oxidation of iron and under reducing conditions is freed from iron complexes, and bound under oxidizing conditions (Smith *et al*., 1997). Phosphorus may also become unavailable to biota through binding to rhizofiltration media and by being incorporated into living matter through uptake by microbes and macrophytes. These factors are all favored by high oxygen concentrations. The formation of complexes of phosphorus and iron in rhizofiltration facilitates the uptake by macrophytes (Smith *et al*., 1997). This could have been evident in the rhizofilter since investigation of macrophytes showed the accumulation of total phosphorus in the system.

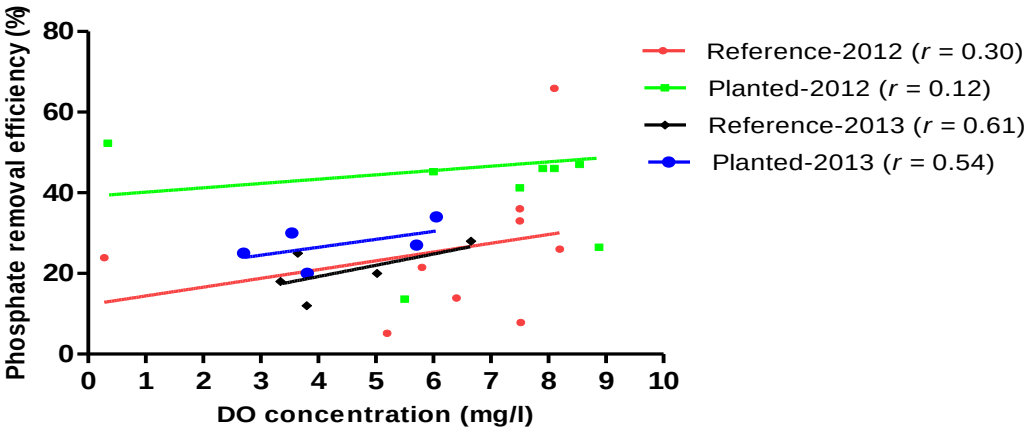

Figure 3.31: Effect of the DO on phosphate removal efficiency in a rhizofilter system. Means of phosphate removal efficiency in the planted and reference sections were used against their corresponding means of the dissolved oxygen from January 2012 to June 2013 in the rhizofiltration system. A linear curve fit was used to demonstrate the relationship between the DO and phosphate removal efficiency in the system.

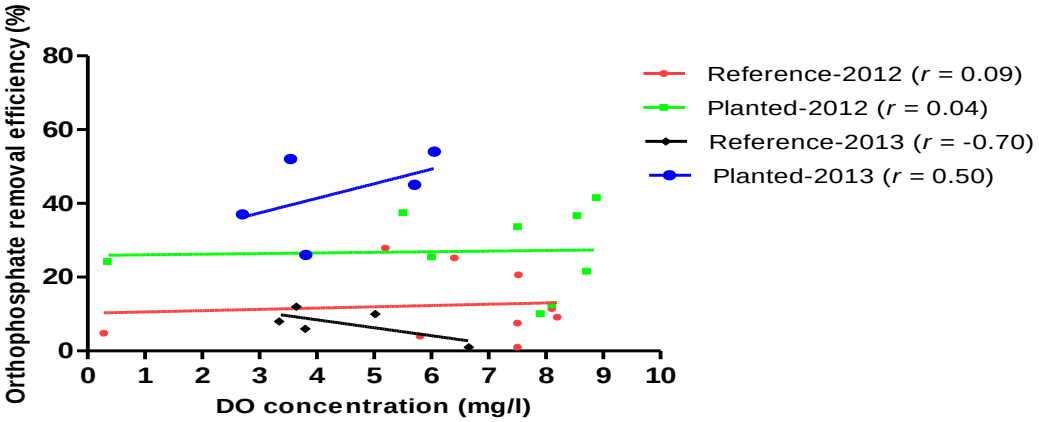

Figure 3.32: Effect of the DO on orthophosphate removal efficiency in a rhizofiltration system in a constructed rhizofiltration between January 2012 and June 2013. Means of orthophosphate removal efficiency in the planted and reference section of the rhizofiltration system were used against their corresponding means of the dissolved oxygen in the analysis of the results. A linear curve fit was used to demonstrate the relationship between the DO and orthophosphate removal efficiency in the system.

3.3.3.5 Chemical oxygen demand

3.3.3.5.1 Nitrogen

At high nitrogen concentration in 2013, removal of ammonia (Figure 3.33), nitrate (Figure 3.34) and nitrite (Figure 3.35) decreased with increased COD levels in the system (very strong negative correlation), while at low nitrogen concentrations in 2012; a moderate positive

correlations were obtained, with high COD removal in the rhizofiltration system coinciding with nitrogen reduction in some sampling points. This is because COD represent the contamination in wastewater (Shipin *et al*. (2005). It is the measure of the extent of wastewater treatment or purification. When COD was high, the level of water contamination was also found to be high (Barnes *et al*., 1998). As a result when the nutrients were removed in wastewater, the COD level also dropped.

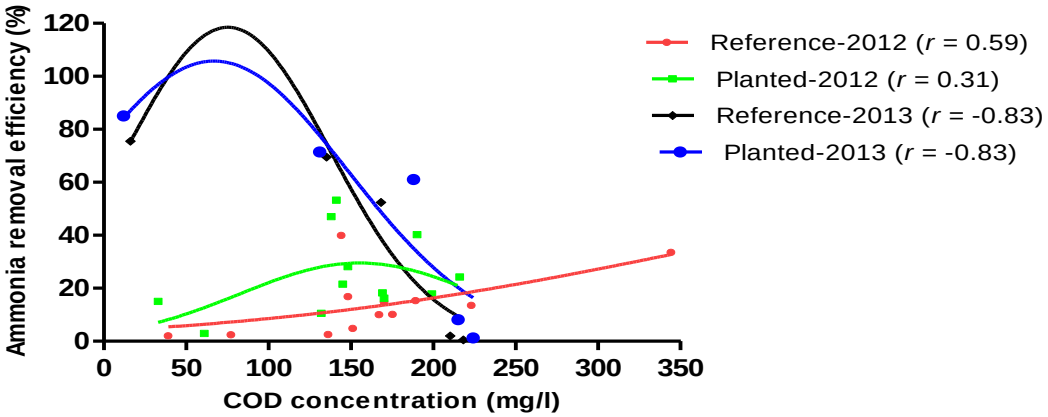

Figure 3.33: Effect of COD on ammonia removal efficiency in the planted and reference section of the rhizofiltration system. Means of ammonia removal efficiency were used against their corresponding means chemical oxygen demand from January 2012 to June 2013 in a rhizofilter system. A non-linear curve fit was used to demonstrate the relationship between the COD and ammonia removal efficiency in the system.

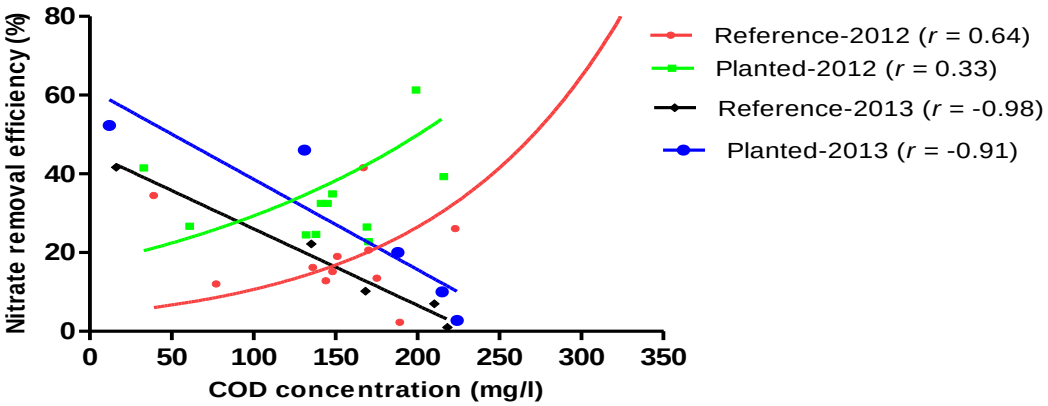

Figure 3.34: Effect of COD on nitrate removal efficiency in a rhizofiltration system between January 2012 and June 2013. Means of nitrate removal efficiency were used against their corresponding means chemical oxygen demand in the planted and reference section of the rhizofilter system. Non-linear (2012) and linear (2013) curve fit were used to demonstrate the relationship between the COD and nitrate removal efficiency in the system.

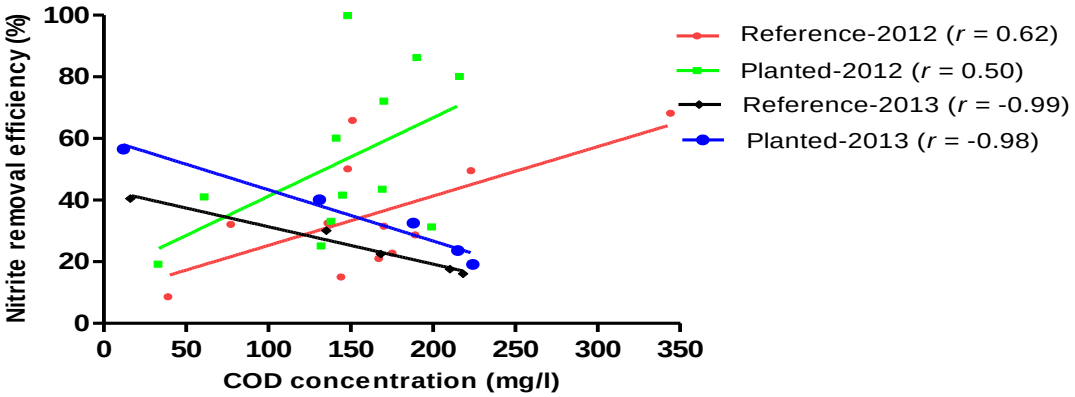

Figure 3.35: Effect of COD on nitrite removal efficiency in a rhizofiltration system during the study period. Means of nitrite removal efficiency were used against their corresponding means chemical oxygen demand from January 2012 to June 2013 in the in the planted and reference sections of the rhizofiltration system. A linear curve fit was used to demonstrate the relationship between the COD and nitrite removal efficiency in the system.

3.3.3.5.2 Phosphorus

While a negative correlation (very strong in 2013; 7% and 6% removal rates in reference and planted sections respectively, and very weak in 2012; 1 and 4% removal rates in reference and planted sections respectively) in phosphate removal was obtained when compared to COD both in the planted and the reference section (Figures 3.36), both negative and positive correlations were obtained between orthophosphate removal and COD at different COD concentrations (Figures 3.37). Low phosphate removal efficiencies and lack of oxygen in 2013 suggested that phosphate was mainly removed by adsorption on the filter media and that reducing conditions could have led to solubilization of minerals and release of dissolved phosphorus. Phosphorus does not have a gaseous form thus its removal by microbial process was limited (Smith *et al.*, 1997).

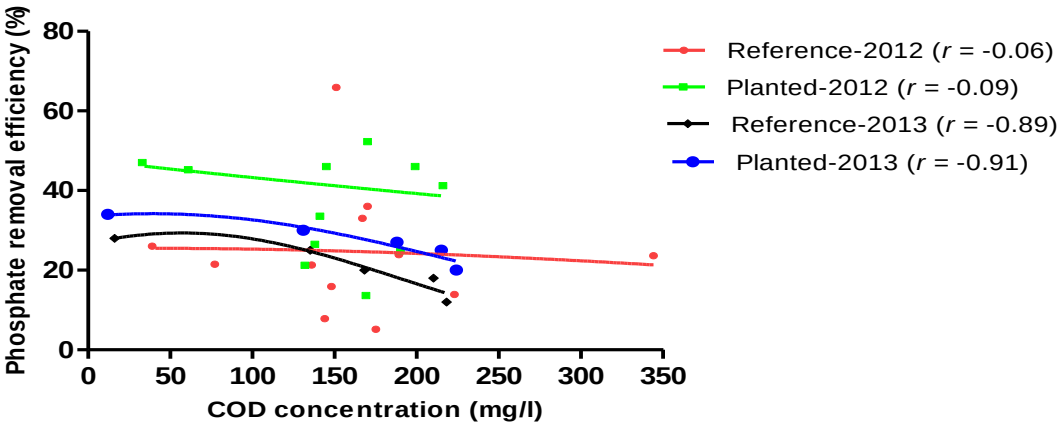

Figure 3.36: Effect of COD on phosphate removal efficiency in a constructed rhizofiltration during the period of the study. Means of phosphate removal efficiency were used against their corresponding means of chemical oxygen demand from January 2012 to June 2013 in the planted and reference section of the rhizofiltration system. A non-linear curve fit was used to demonstrate the relationship between the COD and phosphate removal efficiency in the system.

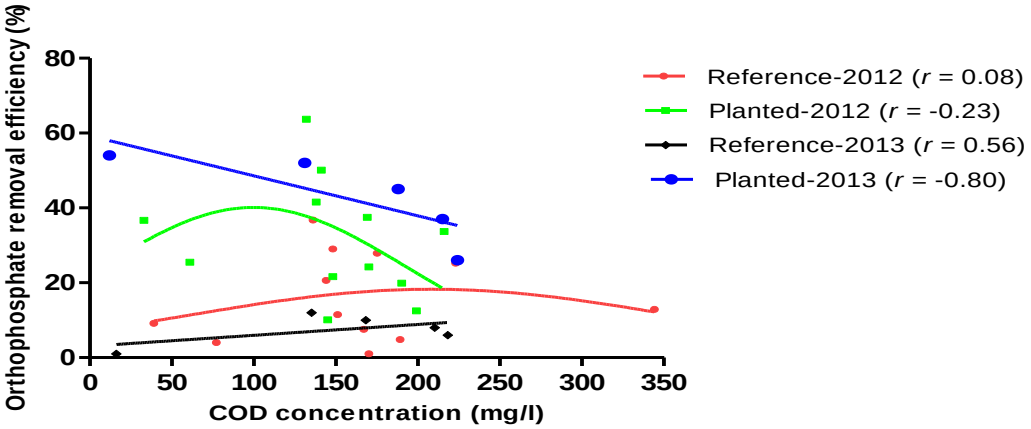

Figure 3.37: Effect of COD on orthophosphate removal efficiency in a constructed rhizofiltration system between January 2012 and June 2013. Means of orthophosphate removal efficiency were used against their corresponding means chemical oxygen demand in the analysis of results of the planted and reference sections of the rhizofiltration system. Non-linear (2012) and linear (2013) curve fit was used to demonstrate the relationship between the COD and orthophosphate removal efficiency in the system.

3.4 DISCUSSION

Different parameters differentially affected the removal of nutrients in the rhizofiltration system. It can be seen from the comparison of purifying effect or treatment efficiency in different seasons that the change of seasons has a great effect on removal of organic matter and nitrogen. The removal shows a declining trend with a decrease in temperature under the common influence of temperature and the decrease in temperature restrains the proliferation

rate and activity and affects macrophytes growth or nutrient accumulation ability. The pH of water also affected the solubility of many toxic and nutritive chemicals which in turn affected organism's water. Culp *et al.* (1986) reported most streams draining coniferous woodlands to be slightly acidic (6.8 to 6.5) due to organic acids produced by the decaying of organic matter. As acidity increases, most metals become more water soluble and more toxic. Ammonia is known for becoming more toxic with only a slight increase in pH (Culp *et al*., 1986). Based on the results obtained, the rhizofiltration system was within the pH limits, except in February 2012, where very acidic pH was obtained (Figure 3.1). This also coincided with less nitrogen and phosphorus removal in the system. While low nitrogen removal was due to decreased conversion of ammonia to nitrite, and then to nitrate, low phosphorus removal was due to low phosphorus availability to aquatic organisms for uptake or utilization at low pH (Cortes-Lorenzo *et al.*, 2012).

Growth and activity of microorganisms are temperature dependent. Thus, variations in temperature had a significant impact on the removal of contaminants in the rhizofiltration system. High temperatures obtained in the influent both in 2012 and 2013 (Figure 3.2; Appendix 2) may have been caused by exposure of wastewater to sunlight during storage and settling in the Jojo tank before distributing to the system. Temperatures decreased during the filtering process in the rhizofiltration unit as well as when in contact with the cooler media (soil) in the rhizofilter, which resulted to a decrease in the effluent (planted and reference sections) temperatures. While the growth and survival of macrophytes may be seasonal, microbial growth is not. Thus microorganisms were adapted to specific temperature ranges for survival throughout the year. If temperatures are outside the normal range for a prolonged period of time aquatic organisms may become stressed and die (Vymazal, 2009). This may impact negatively on the removal of nutrients and COD in the rhizofiltration system. Temperatures between 20 and 25°C was suitable for biological nitrogen removal. The results are also supported by Cheng *et al*. (2009) who found nitrification to be optimal at temperatures between 15 and 25°C. Warm seasons had higher nitrogen and phosphorus removal in the system. Vymazal (2008) also reported increased nitrogen and phosphorus reduction in wetlands due to microbial transformation as well as plant accumulation in summer seasons.

Salinity affects microorganism's activities due to its inhibitory effect on cellular functions when it is present in large amounts in water (Brown *et al.*, 1999). High salinity levels (Figure

3.3) resulted in denitrification retardation. Cortes-Lorenzo *et al.* (2012) also observed the reduction of denitrification process due to high salinity, which negatively affected the removal of nitrogen and phosphorus.

Microorganisms require oxygen to degrade contaminants, thus oxygen is required in high concentrations in water. In the reference section where the DO was lower, the removal of nitrogen was also lower. It was also lower in both sections where the DO was less than five (Figures 3.28 – 3.30). Chapman (1992) found the DO of less than 2 mg/l to cause severe physiological stress to marine organisms, leading to their death, while Tyroller *et al.* (2010) reported 4 mg/l and less to be considered unhealthy to many aquatic community habitants. The amount of DO in wastewater is indicative of the extent of water purification. Dissolved oxygen levels were seasonal (Figure 3.6) and affected microbial activities. High DO was obtained at temperatures between 20˚C-32˚C and it is at these temperatures where highest nitrogen and phosphorus removal was obtained.

Development of economical and sustainable techniques for reducing the nitrogen content from wastewaters had attracted a great deal of attention. Several methods may be applied to remove nitrogen from wastewater, such as biological denitrification (van der Graaf, *et al.*, 1996), ammonia-stripping (Tanner, 1996), chemical precipitation (Quan, *et al.*, 2012) and ion exchange (Poach *et al.*, 2004). These methods vary in their pros and cons. Nitrification-denitrification was the common application in the rhizofiltration system. Nitrification reaction was coupled with denitrification and low concentrations of nitrite in the system suggested that it proceeded rapidly to form nitrate. Nitrifiers are strict aerobes, thus must have free dissolved oxygen to perform their activities (Abeling and Seyfrid, 1992; Aslam *et al.*, 2007). Nitrification in the system occurred only under aerobic conditions at higher dissolved oxygen level (Wang, *et al.*, 2008). Thus, less dissolved oxygen in the reference compared to the planted section led to a decreased nitrite oxidation The EPA (2009) reported nitrification to have required 4.3 mg/l O_2 per mgN oxidized; therefore increased levels of dissolved oxygen increased nitrification in the system. Oxygen demand increased levels of nutrient content consumed the available oxygen and decreased the opportunity for nitrification to occur both in the reference section and in 2013 (Yongjun *et al.*, 2010).

Kadlec and Wallace (2009) reviewed that less than 10% of horizontal flow subsurface wetlands removed ammonium greater than 1.57 g N/m^2d and nitrate greater than 0.44 g N/m^2d. Vymazal (2009) reported the mean ammonium and nitrate removal rates of horizontal flow subsurface

wetlands to be 0.36 g N/m^2d and 0.09 g N/m^2d respectively. Compared to these studies, the rhizofiltration system achieved high removal efficiencies of 2.375 g N/m^2d of ammonia and 0.663 g N/m^2d of total nitrogen. Up to 99% of nitrate removal was obtained in May 2012 in the planted section.

Wang *et al.* (2008) reported nitrification to require adequate buffering system for efficient nitrogen removal in wastewater. Zhang *et al.* (2007) also reported alkalinity of no less than 50 mg/l in the aeration tank to be required for adequate buffering of wastewater during treatment. The nitrification process produced acid. Acid formation lowered the pH of the biological population in the system, and because it is toxic, caused a reduction of the growth rate of nitrifying bacteria in February 2012. The optimum pH for *Nitrosomonas* and *Nitrobacter* is between 7.5 and 8.5. However, most treatment plants are able to effectively nitrify with a pH of 6.5 to 7.0 (Nicominat *et al*., 2006). Hellinga *et al*. (1998) also observed nitrification cessation at a pH below 6.0.

Nitrification is known to reach its maximum rate at temperatures between 30 and 35℃ and proceeds slower at temperatures below 20℃ (Zhang *et al.*, 2007; Tao *et al*., 2012). Higher temperatures obtained in warm seasons ensured high nitrogen removal. Biological nitrogen removal in the system did not respond well to increased nitrogen loading in the system. It was suitable only for relatively low nitrogen concentrations. Forbes *et al*. (2011) also observed that when concentrations of the contaminants were low, removal efficiencies tends to increase. In the rhizofilter, influent concentrations were low in 2012 because pre-treated wastewater was used while in 2013 nutrient concentrations obtained were higher. The extent of nitrogen removal was depended on the amount of nitrogen present in the wastewater. If influent nitrogen content were low, macrophytes directly competed with nitrifying and denitrifying bacteria for ammonia and nitrate, while in high nitrogen content, particularly ammonia, nitrification and denitrification processes were stimulated (Hua, 2015).

Wastewater is relatively rich in phosphorus compounds. Phosphorus is used by organisms for growth. Sources of phosphates may include agricultural fertilizers, domestic wastewater, detergents, industrial process wastes and geological formations (Dzakpasu *et al*., 2011). Wastewater discharges containing phosphorus may cause algal growth in quantities sufficient to cause adverse effect in water (Browning and Greenway, 2007). Algal growth may subsequently lead to oxygen depletion problems which, in turn, could destroy aquatic life (Massmann *et al.*, 2006). For this reason, phosphorus removal is an essential requirement in

wastewater treatment. Levels of phosphorus removal in the rhizofiltration decreased with increased nutrient loading (Figures 3.11 and 3.12). High phosphorus loading rate introduced into the system due to increased nutrient loading in 2013 as well as phosphorus saturation in the macrophytes and the media used were the cause of the decrease. The media removed phosphorus through absorption and adsorption (Lantzke *et al*., 1999).

Orthophosphates was the main form of soluble inorganic phosphorus in the rhizofiltration system, as well as the main form associated with anthropogenic effects. While orthophosphate was readily utilizable by macrophytes, all other forms of phosphorus (i.e., soluble and insoluble organic and insoluble inorganic) had to be transformed to soluble inorganic forms in order for them to serve as nutrients for macrophytes in the system (Mitsch and Gosselink, 2000). This made orthophosphate to be available in high concentration for utilization by macrophytes than phosphate in the rhizofilter. Utilization of orthophosphate led to its removal in wastewater. Patterns of phosphorus removal ability by the system were similar to those reported by Gill *et al*. (2011), who demonstrated a strong linear correlation between phosphate surface loading and removal efficiency. While contribution through assimilation into microbial biomass, filtration and sedimentation may have been insignificant, removal of phosphorus through plant uptake, microbiological pathways and adsorption played a significant role in phosphorus removal in the rhizofilter (Bialowiec *et al*., 2011). The rhizofilter system ageing together with increased influent concentrations resulted in low removal efficiencies of the nutrients.

3.5 CONCLUSIONS

The chemical, physical, and biological aspects of water quality are inter-related and must be considered together in wastewater treatment. In the present study, it has been indicated that constructed rhizofiltration had the potential to reduce nitrogen and phosphorus from wastewater. The major findings obtained were:

- Nutrient reduction/removal in the system was depended on macrophyte's nutrient accumulation rates, absorption efficiency and nutrient composition of wastewater as well as the physical and chemical condition of water.
- Higher reduction efficiencies of nutrients were obtained in the planted section. Nitrite and nitrate (form of nitrogen) showed the highest removal efficiencies while highest total phosphorus removal was in the form of orthophosphate.

- Wastewater containing low concentrations of contaminants supported high nitrogen and phosphorus removal.
- Nutrient removal was seasonal, with more removal obtained in warm seasons. Higher water temperatures also reduced the solubility of dissolved oxygen, causing a dissolved oxygen shortage in the system. Increased COD levels also decreased nitrification rates due to competition for available dissolved oxygen.
- A very weak negative to a very strong positive correlation between nitrogen and phosphorus removal in the system was obtained with varying physical and chemical parameters.
- While nutrient discharge limits after treatment were achieved in 2012 and in February 2013, they were not achieved between March 2013 and June 2013. This was attributed to increased nutrient loading in the systems' influent.

CHAPTER 4

THE ROLE OF MICROORGANISMS ON THE REMOVAL OF ORGANIC AND INORGANIC NUTRIENTS FROM THE RHIZOFILTER SYSTEM

4.1 INTRODUCTION

Constructed rhizofiltration system is a relatively new practical technology of wastewater treatment and has attracted research about its ability to reduce nutrients in wastewater to acceptable levels. Microorganisms are one of the main important components in the success of this system and may affect the purification or removal of nutrients in rhizofilter system (Song *et al*., 2011). While contaminants may be removed from the system through physical, chemical and biological processes, with nitrogen being removed by adsorption, assimilation into macrophytes, nitrification and denitrifying processes are strictly microbially driven and contribute a significant portion of nitrogen removal in rhizofiltration technology (Sleytr *et al*., 2009; Ning *et al*., 2014). Thus, the determination of microbial communities and their abundance in rhizofiltration technology is of great significance in studying the purification mechanism by microbial biofilms.

Of several processes involved in nitrogen transformation and subsequent removal in rhizofiltration system, not all may efficiently remove nitrogen in wastewater (Fukuda *et al*., 1999; Vymazal, 2007). The main mechanisms of nitrogen removal in wastewater include ammonia volatilization, denitrification, ammonium adsorption, anammox and organic nitrogen burial. The removal of nitrogen through volatilization becomes significant mainly at pH 5 and the removal rate is usually about 2.2 g $Nm^{-2}d^{-1}$ (Stowell *et al*., 1980; Vymazal, 2007). The decrease in pH also promotes algae, plankton and periphytic algae growth (Suresh and Ravishanker, 2004). Denitrification coupled with nitrification is the major nitrogen removal mechanism that has so far been identified in constructed rhizofiltration system. Since nitrification is a limiting process for the removal of nitrogen, nitrate levels are usually low (Vymazal, 2009). Nitrification is similar to ammonification in that both do not remove nitrogen but are converted to ammonia which then becomes utilized or incorporated into nitrogen removing mechanisms such as volatilization and ammonium adsorption (Vymazal, 2007). Nitrification takes place in the presence of high oxygen levels which supports the growth of strictly aerobic nitrifying bacteria and its operation is therefore determined by the availability

of oxygen. The variance of oxygen requirement for nitrification and denitrification is a major challenge towards achieving higher levels of nitrogen removal (Vymazal, 2007).

Various other studies have also reported the significance of microorganisms in the degradation of contaminants in rhizofiltration systems (Yanan, 1978; Todd and Josephson, 1996; Arroyo *et al*., 2010). However, microbial community structures, their distribution and their specific roles in constructed rhizofiltration systems have not been adequately addressed (Lie *et al*., 2006; Nicominat *et al*., 2006; Arroyo *et al*., 2010; Ning *et al*., 2014). Microorganisms play a crucial role in the treatment of wastewater (Sawaya *et al.*, 2006). Microbes grow in the rhizosphere of macrophytes and on growth media forming biofilm layers which play a role in removal of nutrients and degradation of contaminants (Bigambo and Mayo, 2005; Arroyo *et al*., 2010).

Microbial community structures in wastewater treatment were initially evaluated using culture-based methods (Bohgat *et al*., 1999; Dorte, 2003). These method were based on culturing microorganism in the medium such as Nutrient agar, Salmonella-Shigella agar etc. However, only about 1-10% of microbes can be evaluated using these techniques since other microbes are viable but non cultivable (Besemer *et al*., 2005; Dong and Reddy, 2010). Therefore, these culture-dependent methods underestimate the real abundance of organisms found in natural environments (Truu *et al*., 2009). Along with culture-based techniques, microscopic techniques have also been used to study different group of microbial community but these techniques are not widely used because they are laborious and provide restricted information (Decamp and Warren, 2001). To overcome limitations posed by these culture-dependent techniques, amplification of the 16S rDNA may be desirable (Moeseneder *et al*., 1993). This technique has been applied to identify unknown microbes and diversity among microbial populations (Boyer *et al*., 1999; Arroyo *et al.*, 2010). The 16S rDNA is amplified using PCR (Paul and Clarck, 1996; Kern, 2003). It involves manipulation of genes consisting of the location which have repeated base pairs or substitution of base pairs referred to as hypervariable regions with strongly conserved sequences which are amplified by primers during PCR amplification of the gene (Boyer *et al*., 1999; Vymazal, 2008). This technique has been widely applied in conventional system like activated sludge (Stowel, 1998; Lui *et al*., 2009) and in an aerobic bioreactor (Sawaya *et al*., 2006). However, their applications in non-conventional systems like constructed rhizofiltration and lagoons are still limited (Moura *et al*., 2007; Arroyo *et al*., 2010; Stout and Nusselein, 2010). Using the 16S rDNA gene, the phylogeny of organisms can be

constructed. This may also enable the differentiation at a taxonomical level due to degree of conservation of the sequences of the gene (Scharmm and Amann, 1999).

The amplification of the 16S rDNA which encodes for 16S rRNA may broaden the knowledge about microbial community and its diversity in the rhizofiltration system used for wastewater treatment. This study aimed to contribute to the pool of knowledge and broadening the understanding of the role of microorganisms in the treatment of wastewater in rhizofiltration system. It elucidates microbial population structures in the rhizofiltration system as well as their contribution in constructed rhizofiltration systems for wastewater treatment.

4.1.1 Aim

The aim of this chapter was to investigate microbial community structures and their distribution using 16S rDNA which was extracted from soil and water samples of the constructed rhizofiltration system and to relate them to their spatial nutrient removal functions.

4.1.2 Objectives

a) To extract the DNA from water and soil samples from the rhizofiltration system and quantify it in order to estimate microbial abundance.

b) To amplify rDNA which encode for 16S rRNA using PCR amplification and sequencing of the amplicons for bacterial identification and quantification as well as compare the sequenced DNA data with Gene Bank database using the nucleotide BLAST method.

c) To establish the relationships between nitrogen and phosphorus removal with the organisms identified in the system.

4.2 METHODS

4.2.1 Sample Collection

For the analysis of the biofilms from the system, 1000 ml water and 200 g soil samples were collected as shown in Figure 4.1. The samples were collected once a month and transported on ice cold cooler bags and were analyzed within two hours of collection. Influent and effluent from both the planted and reference sections of the rhizofiltration system were collected. Soil samples were collected on the surface of the system and at a depth of 110 mm on both the

planted and reference sections of the system. This was performed in order to investigate the diversity and distribution of microbial biofilms in the rhizofiltration system and the change in population dynamics between the planted and reference sections.

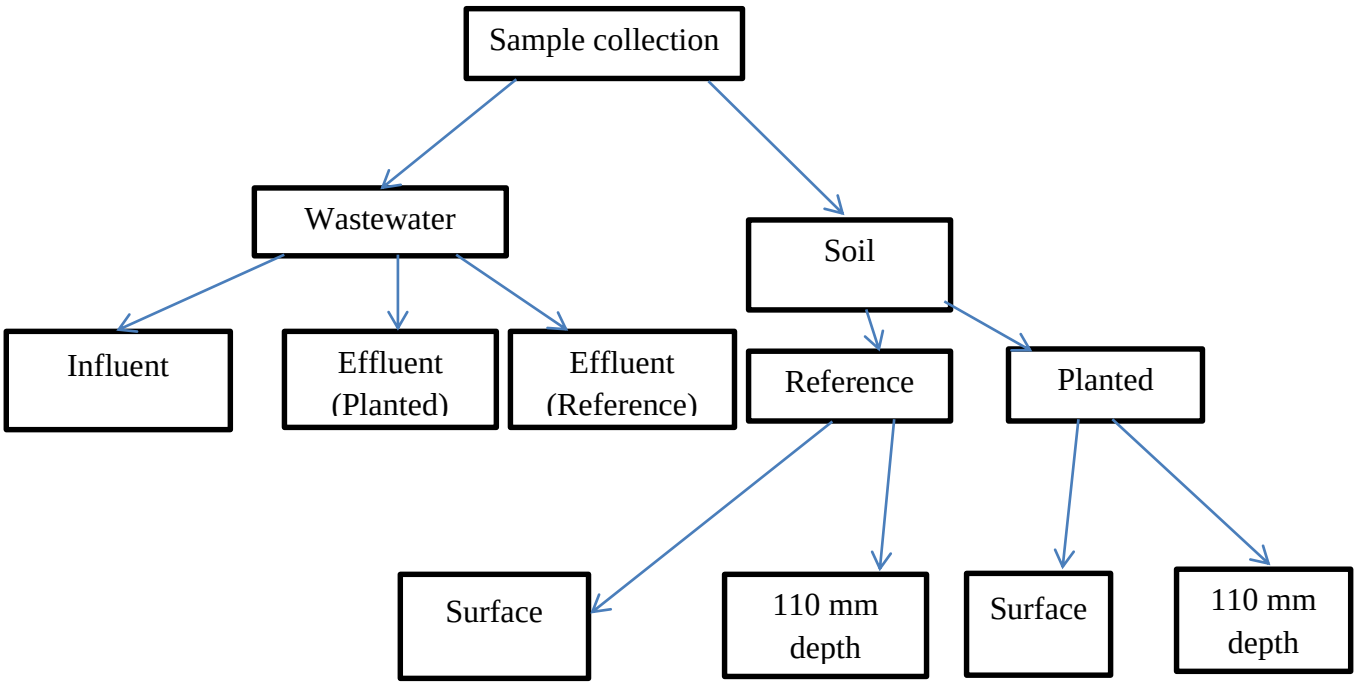

Figure 4.1: Sampling scheme used for biofilm analysis from the rhizofiltration system.

4.2.2 Sample Preparation

To concentrate microorganisms present in wastewater, 200 ml of wastewater was filtered using membrane filtration, employing Whatman filter papers (Sigma-Aldrich) with a 20-25 µm pore size (50 mm diameter). The filter papers, after filtration, were cut into approximately 5 cm squares using sterile scissors. These squares were used for DNA extraction following prescribed method (Laboratory Manual for Environmental Pathogen Research, 2004). This was performed to ensure that microorganisms were available at high concentration so that high quantity of DNA could be extracted.

4.2.3 DNA Extraction from Wastewater Sample

DNA from water samples was extracted using the Zymo Research (ZR) Fungal/Bacterial DNA mini-Prep kit (Inqaba Biotech) following the manufacturer's protocol. Five square-centimeter pieces of the filter papers used to filter wastewater were introduced into 5 ml bashing bead lysis

tubes. Two hundred microlitres of phosphate buffered saline (PBS) (137 mM NaCl, 27 mM KCl, 4.3 mM $NaHPO_4$ and 2.8 mM KH_2PO_4) was then added followed by the addition of 750 µl of the lysis solution. The sample was vortexed for 10 minutes and then centrifuged (Eppendorf 5804 R) at 10 000 x *g* for a minute. After centrifugation 400 µl of supernatant was transferred into the Zymo-Spin IV tube which was inside the collector tube and then centrifuged (Eppendorf 5804 R) again at 7 000 x *g* for another minute. One thousand two hundred microlitres of DNA binding buffer was added to the filtrate inside the collector tube and 800 µl of the DNA binding mixture was transferred into the Zymo III column which was inside a new collector tube and centrifuged (Eppendorf 5804 R) at 1 000 x *g* for one minute. After centrifugation the flow through in a collector tube was discarded and 800 µl of a DNA binding mixture was added into the same Zymo III column and centrifuged for a further minute at the same speed as above. Two hundred microlitres of DNA pre-wash buffer was added to the Zymo-Spin column in a collector tube and centrifuged (Eppendorf 5804 R) at 10 000 x *g* for a minute. After centrifugation 500 µl of Fungal/Bacterial DNA wash buffer was added to the Zymo-Spin IIC column and further centrifuged (Eppendorf 5804 R) at 1 000 x *g* for a minute. After centrifugation the Zymo- Spin IIC column was then transferred into 1.5 ml Eppendorf tubes and 30 µl of DNA elution buffer was added and centrifuged for 30 seconds. After extraction the DNA was stored at - 4^0C to prevent DNA degradation.

4.2.4 DNA Extraction from Soil Samples

One hundred and fifty grams of soil samples were used for the extraction of the DNA. DNA in soil samples was extracted using Soil/Fecal DNA Mini-Prep kits (Inqaba Biotech) following the manufacturer's protocol. In short, 0.25 g of the soil was added into the ZR bashing bead lysis tube followed by the addition of 750 µl of lysis/stabilizing solution and vortexed for 10 minutes. From here, protocol explained above in section 4.2.3 in the isolation of the DNA was followed.

4.2.5 DNA Analysis

The DNA was visualized on 0.8% agarose in the presence of ethiduim bromide. Agarose was suspended in 0.5 x TAE buffer (2 mM Tris base, 10 mM glacial acetic acid, 5 mM EDTA, pH 8.0). It was then boiled for approximately 60-90 seconds to dissolve and then cooled and 15 µl of ethidium bromide was added to make a final concentration of 5 µl. The agarose was poured into a casting tank in the presence of a 20 well-comb for making the gel wells. The comb was

then removed after the gel had set. The gel was placed into an electrophoresis tank and 0.5 X TAE buffer added to a level sufficient to cover the gel. The electrophoresis tank was connected to a power source to ensure that the DNA moved toward the anode. The voltage was adjusted to 100 volts and the gel was allowed to run for 25 minutes. The DNA was run along with the GeneRuler 1kb DNA ladder (Thermo Scientific) for size determination of the extracted DNA. The gel was then analyzed using the Syngene Bioimage system (Equipnet) for DNA visualization.

4.2.6 DNA Amplification

The extracted DNA was amplified by PCR using the forward primer A341F (5' CCTAGGGDGGCWCAG3') with an AC341F GC-clamp (5'CGCCCGCCCCCGCCCGTCCCGCCGCCCCCGCCTACGGC3') (Ishii and Sadowsky, 2009) and the reverse primers AC907R (5'CCGYCWATTCMTTTGAGAGTTT3'). The PCR process was set up using the Eppendorf thermal cycler (Equipnet) under the following conditions: Denaturation was at 95^0C for three minutes followed by the 40 cycles of denaturation at 95^0C for 30 seconds. This was followed by annealing at 55^0C for one minute and extension at 72^0C for 30 seconds. Final extension was at 72^0C for 15 minutes (Laboratory for Microbial Ecology, 2004; Fraser, 2008). The primers used were chosen because they were small, unique, and specific to 16S rDNA and they were not complementary to each other. The conditions under which PCR was set are known to amplify the target fragment efficiently (Song *et al*., 2011). The last nucleotide of the primers was Guanine. This enabled the primer to strongly hybridize and to ensure recognition by the enzyme *Taq* polymerase. This enzyme adds nucleotide in the primers to synthesize new DNA strand complementary to the template strand. After amplification the DNA was purified.

4.2.7 Purification of DNA from Agarose Gels

The first step in the purification was running the amplified DNA in a 0.8% agarose gel. After running agarose gel electrophoresis as described above, the Zymoclean gel DNA recovery kits (Inqaba Biotech) was used for the DNA purification following the manufacturer's protocol. In short, the band was excised from the gel to remove DNA fragments using a sterile razor blade. DNA fragments were then transferred into 1.5 ml microcentrifuge tubes and 150 µl of agarose dissolving gel (ADG) solution was added into 50 µl of the excised agarose. After the addition of ADG solution the samples were incubated at 37ºC for 10 minutes until the gel was

completely dissolved. The dissolved agarose solution was then transferred into a Zymo-spin I column in a 5 ml collection tube and centrifuged (Eppendorf 5804 R) for one minute at 1 000 x *g*. The flow through in the collection tube was discarded and 200 µl of wash buffer was added into the column. This step was repeated twice. The DNA was eluted with 10 µl of nuclease free water which was directly added into the column matrix. The purified DNA was ready for sequencing.

4.2.8 DNA Sequencing

The purified DNA was sequenced using the ABI Prism® BigDye™ terminator cycle sequencing kits at the Stellenbosch University, South Africa. The DNA concentration was adjusted to 10 µg/µl since the amplicons were less than a 1 000 bp. Primer AC341F was prepared to 1.1 µg/µl and used for sequencing reactions (Graham and Hill, 2001). Sequence analysis was carried out on Applied Biosystems 3730 capillary instruments. The sequencing reactions utilize fluorescent-labeled dideoxynucleotides (Big Dye Terminators) and Taq FS DNA polymerase in a thermal cycling. Using template and primer in section 4.2.6, Taq/dye-terminator cycle sequencing provided 500-600 bases of sequence with a 98-99% accuracy. Sequences generated were analyzed using Bioedit v7.0.9 (ibis Biosciences, USA). It is important to note that microbial biofilms analysis did not involve the biofilm studies in their actual forms *in-situ* but rather involved conducting microbial population dynamics.

4.3 RESULTS

Soil and water samples were collected in order to study microbial biofilms in the rhizofiltration system. DNA was isolated from the samples in order to study microbial diversity and their population shift during treatment as well as correlating the organisms with nutrient removal in the system. For the determination of the distribution of microorganisms within the rhizofilter unit, care was taken to ensure that samples were collected randomly throughout the system.

4.3.1 DNA in the Wastewater Samples

Figure 4.2 shows the gel electrophoresis results of the DNA extracted from the influent, planted and the reference sections of the rhizofilter system, with their amplicons indicated in Figure 4.3. The highest quantity of DNA was obtained from the planted section (lanes 6-9) compared to the reference section (lanes 1-4). The size of the extracted DNA was measured using a DNA marker (lane M) which was used as a ruler. The genomic DNA isolated was found to be around

10 000 kb in size. Lane 5 represented the DNA obtained from the influent of the system and this DNA was extracted in low quantities compared to the planted and reference sections. The influent came from the Jojo tank which collected wastewater from the receiving pump from effluent directly from receiving water. Biofims had not developed in the influent and thus contributed to less DNA (organisms). In the planted and the reference sections the biofilms had developed which resulted in more DNA being extracted from wastewater post treatment by these sections. Highest amplification (DNA producing more amplicons) was obtained in the planted section (lanes 6-9), with lane 7 having the least amplicons in the planted section. The amplicons were found to be approximately 500 kb while the bands below 250 kb represented primer dimers.

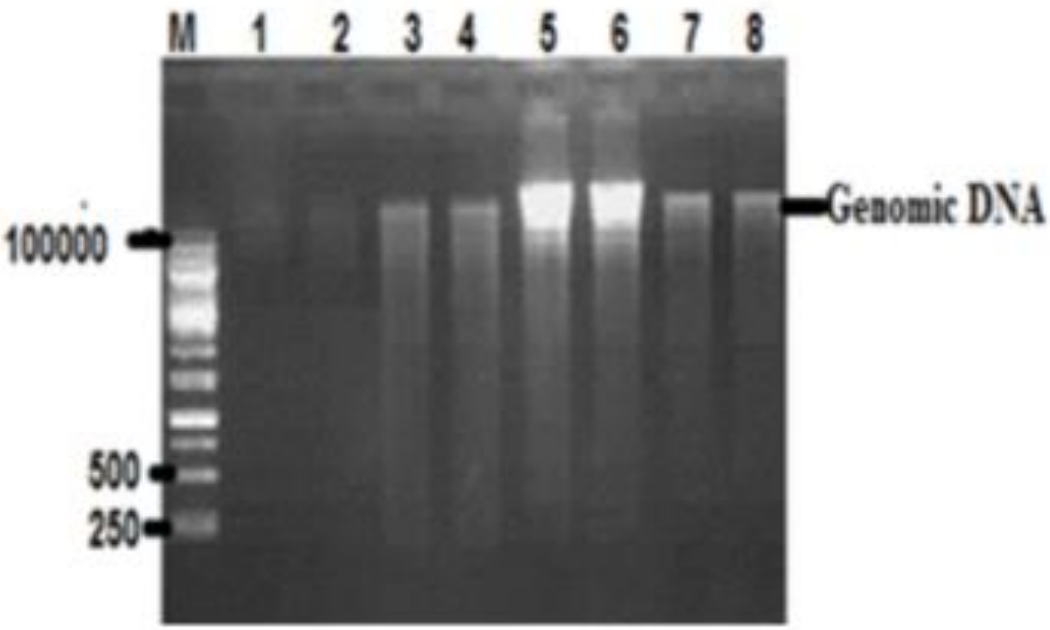

Figure 4.2: Agarose gel electrophoresis of the genomic DNA extracted from wastewater samples. Lane M represents the DNA marker (100 000 kb). Lanes 1-4 represent DNA extracted from the reference section. Lane 5 represent DNA isolated from influent while lanes 6-8 represent DNA extracted from the planted section of the system.

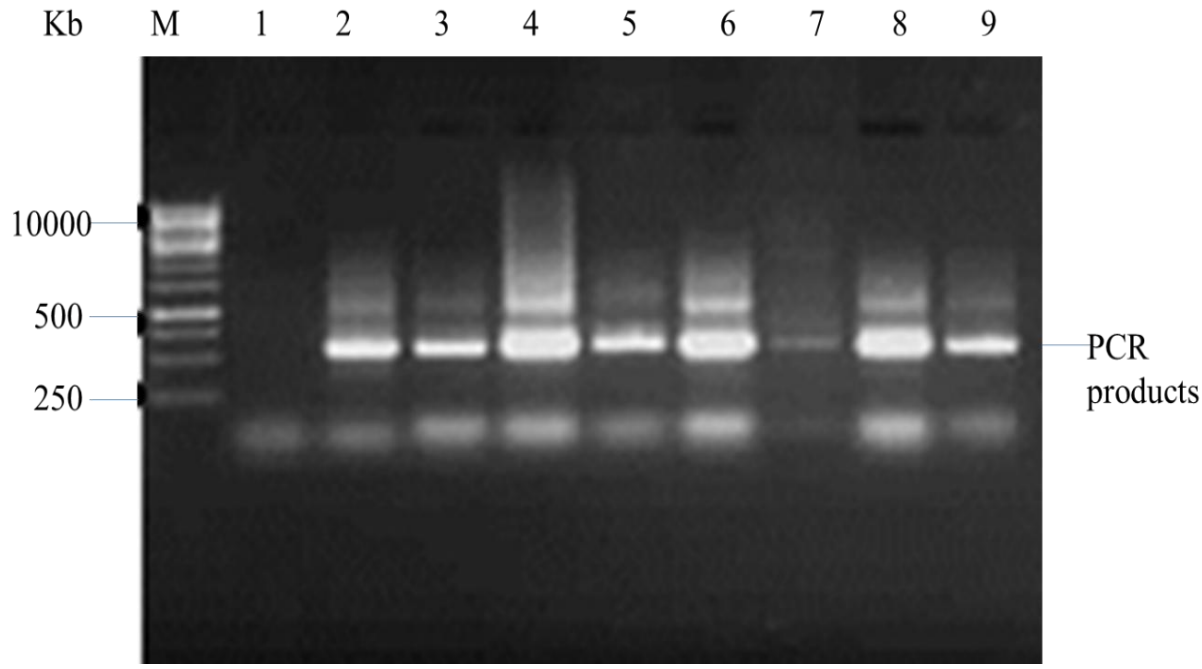

Figure 4.3: PCR products (amplicons) of the 16S rDNA gene. Lane M is a DNA marker. Lane 1 represents amplicons of the influent. Lane 2-5 reference section and lanes 6-9 are the amplicons of the planted section.

Based on fluorescence intensity, DNA concentration was estimated (Barbas *et al*., 2001). Differences in DNA band intensity observed between the samples obtained in the planted and reference sections are suggestive of community structure differences as well as diversity of microorganisms existing in the two section. Based on the DNA isolated, it can be said that high diversity and abundance of microbial communities were in the planted section of the rhizofilter. High microbial diversity and abundance in wastewater in the planted section was due to the presence of macrophytes in the planted section. Macrophytes provided an aerobic and anaerobic condition that supported the growth of a wide variety of biofilms. The findings also coincided with dissolved oxygen concentrations which were higher in the planted section.

While there were many organisms in wastewater which were found to be uncultured, Figure 4.4 represents the groups of cultured organisms in the water samples. More organisms were identified in the effluent of the planted section in the system. Gammaproteobacteria, Bacilli and Clostridia were found in all the samples, i.e. in influents and effluents of both planted and reference sections. Gammaproteobacteria, including Clostridia and Bacilli were predominately obtained in the effluent of the planted section. Several uncultured bacterial clones were observed in all samples. While Deniococcales and Gleobacteria were obtained in the effluent of the reference section only, influent and effluent reference sections were both dominated by

Gammaproteobacteria while the planted section was dominated by Bacilli and Gammaproteobacteria. The main organisms in Gammaproteobacteria were *Pseudomonas* species in the effluent of the planted section while effluent reference was dominated by *Enterobacter* and *Actinobacter* spp. Bacilli, found mainly in the planted section was dominated by *Bacillus cereus*.

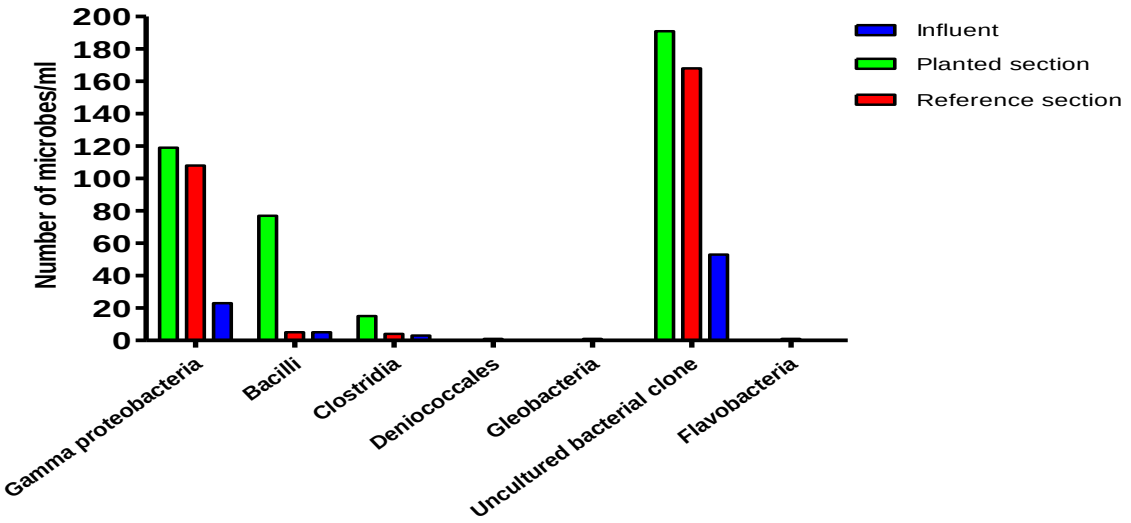

Figure 4.4: Groups of microorganisms identified in wastewater samples in the rhizofiltration system. The groups were obtained through grouping of the related organisms after sequencing.

4.3.2 DNA in the Soil Sample

Figure 4.5 shows the results of the DNA in both the planted and reference sections, the amplicons are shown in Figure 4.6, with maximum sizes approximating 250 kb. While more concentrations of the DNA was extracted from the planted compared to the reference section, less DNA was extracted at 110 mm depth in the rhizosphere compared to the DNA obtained on the surfaces. These results indicated less microbial communities on the bottom soil of the system compared to the surfaces on both sections. Macrophytes and the surfaces of the system offered more biofilm attachment areas and thus had more biofilms. The genomic DNA isolated was measured using DNA marker (lane M) and was found to be about 10 000 kb.

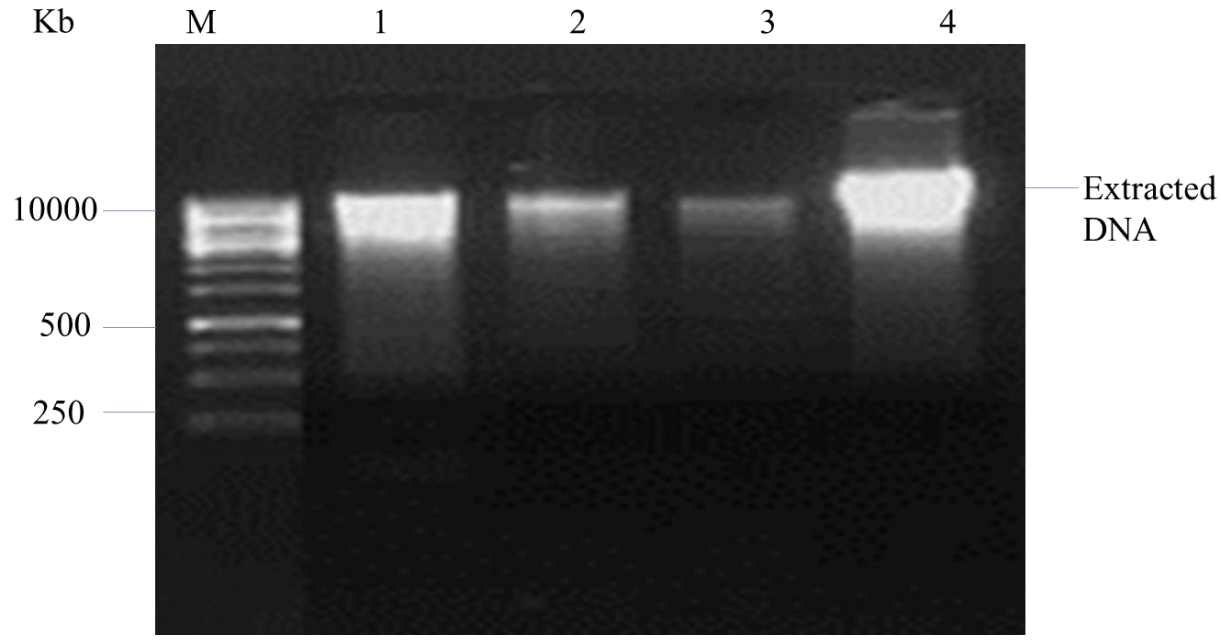

Figure 4.5: Agarose gel electrophoresis of DNA extracted from the surface and 110 mm deep in the planted and reference section of rhizofiltration system. Lane M is a DNA marker. Lane 1 is DNA extracted on the surface of the planted section. Lane 2 is DNA extracted at 110 mm depth from the planted section of the system. Lane 3 is the DNA extracted at 110 mm depth from the referenced section. Lane 4 is the DNA collected on the surface in the reference section.

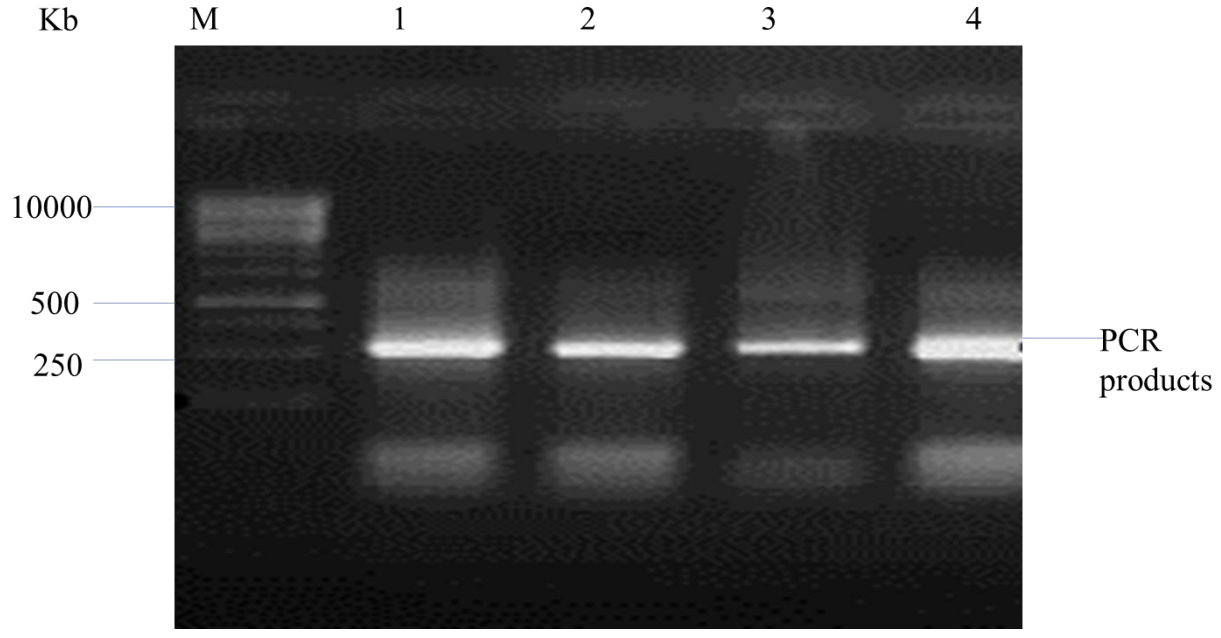

Figure 4.6: PCR amplification product of the DNA extracted from soil sample taken from both planted and referenced section. Lane M represents the DNA marker. Lanes 1 and 4 represent DNA extracted from soil sample collected at 110 mm in the planted and reference sections respectively. Lanes 2 and 3 present DNA extracted at the surface of reference and planted section respectively.

Microorganisms identified at the surface of the planted and reference sections of the system were mostly aerobic microorganisms. Most of these organisms have been found to be involved in the removal of nitrogen while those isolated at the bottom and in the rhizosphere of the system were anaerobic and facultative microbes. The aerobic microorganisms found on the surface and on the rhizosphere of both the planted and reference sections were nitrifying bacteria associated with Bradyrhizobium, Alpha, Beta and Gammaproteobacteria (Bigambo and Mayo, 2005).

4.3.3 Organisms Identified in Soil Samples

The sequences of nucleotide obtained were subjected to the GeneBank database for obtaining microorganisms present in soil samples. The microbes with the similar scoring were grouped according to their structure, function and relatedness (Figure 4.7). More microorganisms affiliated with Gammaproteobacteria were obtained on the surfaces of the planted than referenced sections. Bacilli and Betaproteobacteria were predominately found in the surface of the reference section while Alphaproteobacteria were dominant in the surface of the planted section. Several uncultured bacterial clone were found in both the surfaces of the planted and reference section.

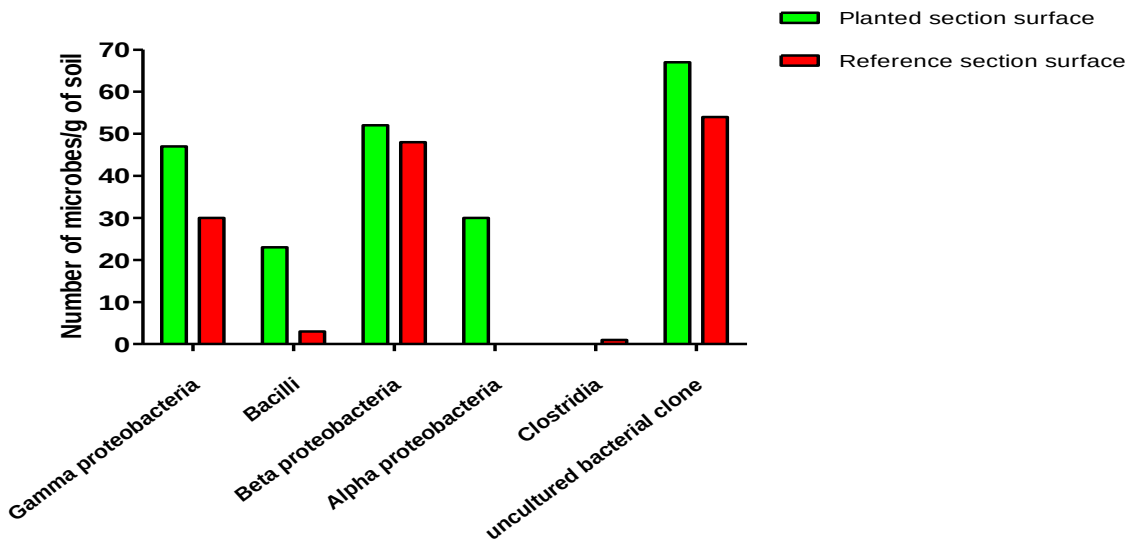

Figure 4.7: Groups of microorganisms identified in soil samples collected from the surface of both the planted and reference sections of the rhizofiltration system. The groups were obtained through grouping related organisms identified from the system by using nucleotide sequences obtained after sequencing.

Groups of microorganisms found to be dominant in soil samples collected at 110 mm depth in the rhizosphere of both planted and reference section of the system are illustrated in Figure 4.8. Gamma- and Alpha-proteobacteria as well as uncultured bacterial clones were obtained in both planted and reference sections. While Mollicutes were abundant in the reference section only, Bacilli, Delta and Betaproteobacteria were only found in the planted section.

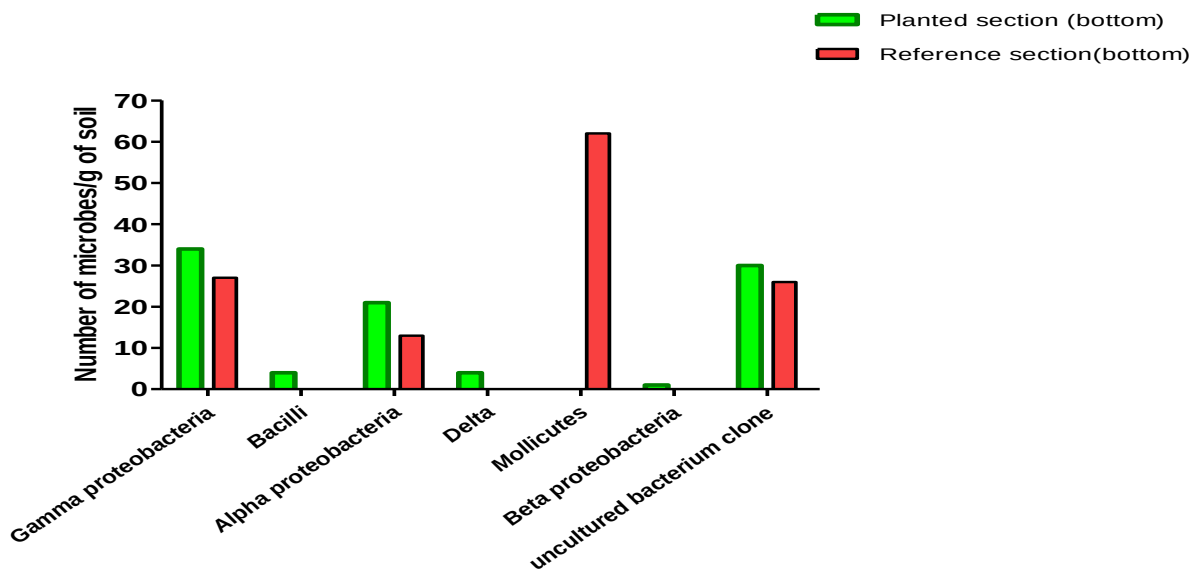

Figure 4.8: Groups of microorganisms identified and found dominant 110 mm deep in the planted and reference sections of the rhizofiltration system. The groups were obtained through grouping related organisms identified, using nucleotide sequences obtained after sequencing.

A general phylogenetic tree based on the sources of the samples was constructed from the 16S DNA using Bioedit (Figure 4.9). The tree included microorganisms identified from both the soil and water samples collected at both the planted and reference section of the rhizofilter. The tree gave the relationship (based on the sources) between microbial communities obtained in the rhizofilter unit.

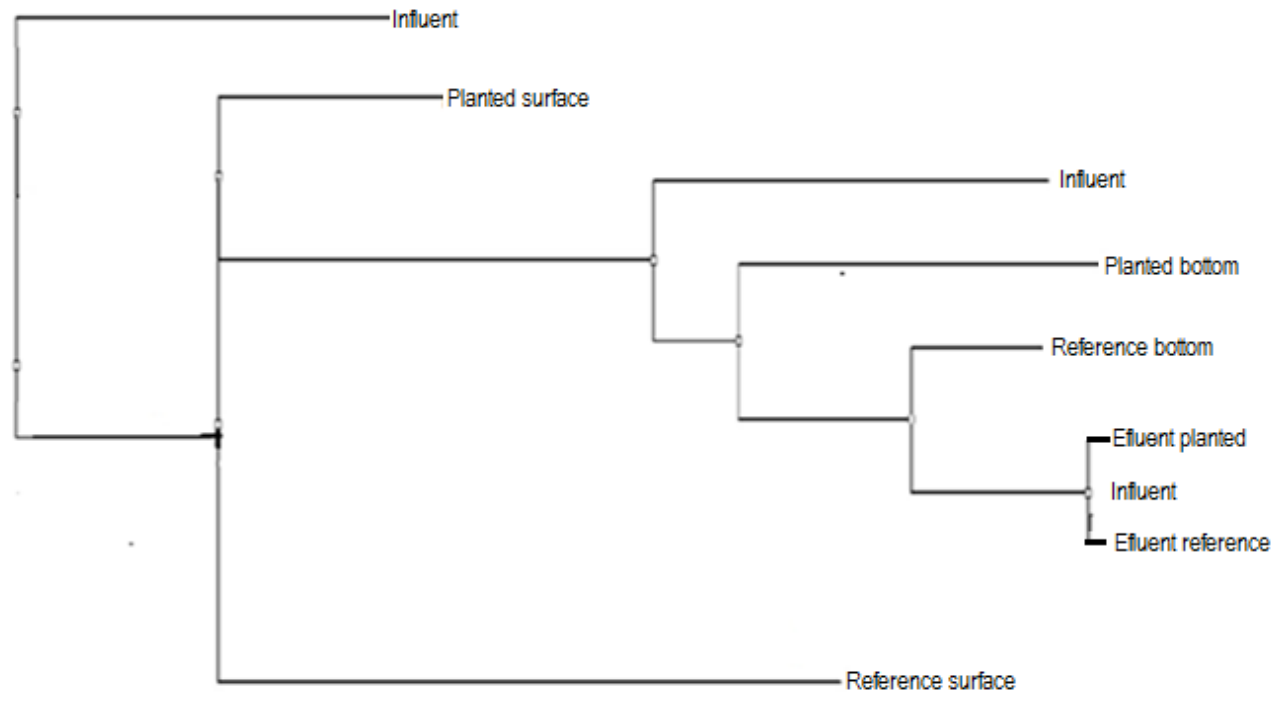

Figure 4.9: The phylogenetic tree of 16S rDNA excised from agarose gel electrophoresis showing the relatedness of organisms per sources of isolation.

From the phylogenetic tree generated organisms obtained in each group were manually aligned to detect the organism relationship or relatedness. Figures 4.10-4.16 shows the results of the relationship between organisms in the rhizofilter system after manual alignment of the organisms' groups. Manual alignment of these organisms was due to the fact that the Bioedit software used could not allow the automatic alignment of these organisms.

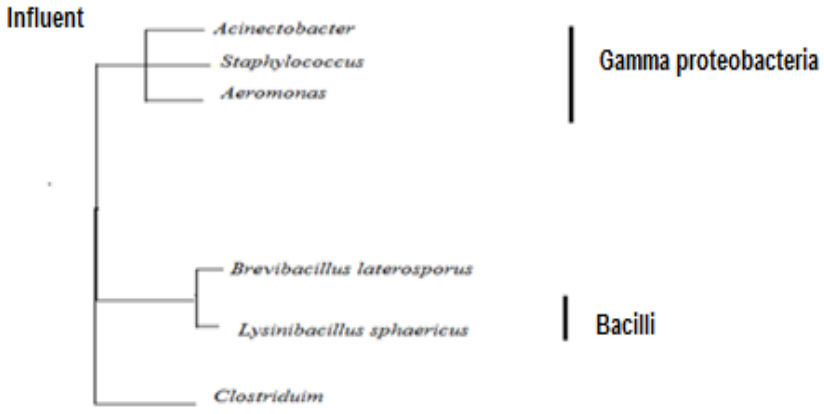

Figure 4.10: The phylogenetic tree of organisms showing the relatedness of microorganism isolated from wastewater influent.

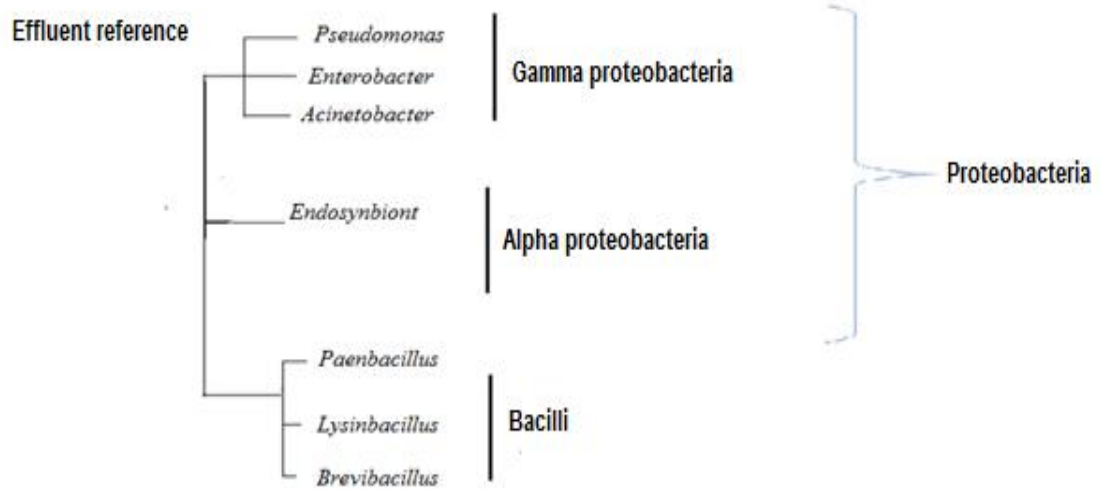

Figure 4.11: The phylogenetic tree showing the relatedness of microorganism isolated from wastewater effluent of the reference section.

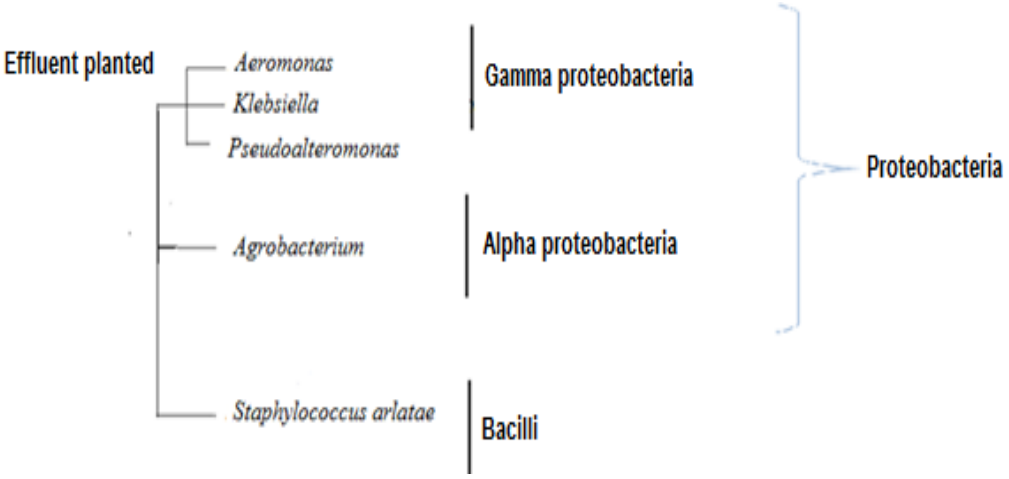

Figure 4.12: The phylogenetic tree showing the relatedness of microorganism isolated from wastewater effluent of the planted section.

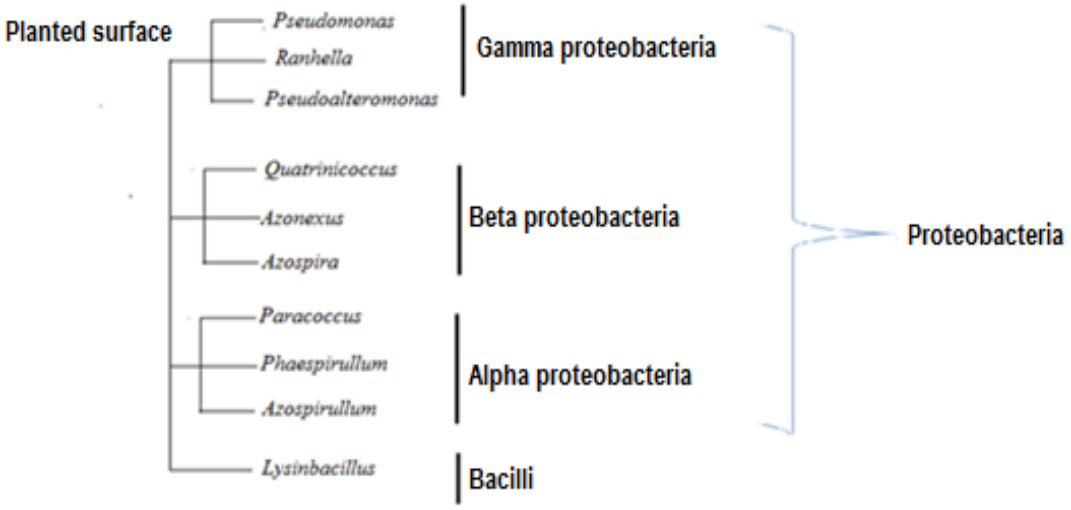

Figure 4.13: The phylogenetic tree showing the relatedness of microorganism isolated from the soil on the surface of the planted section.

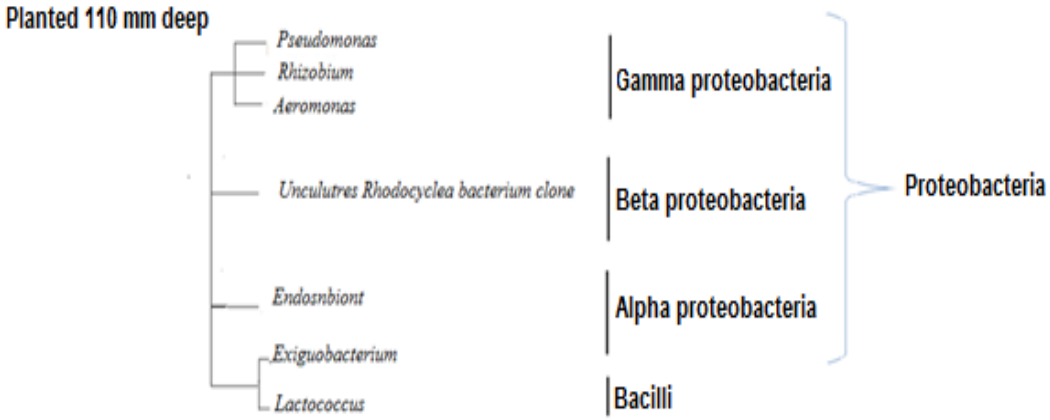

Figure 4.14: The phylogenetic tree showing the relatedness of microorganism isolated 110 mm deep from the soil in the planted section of the system.

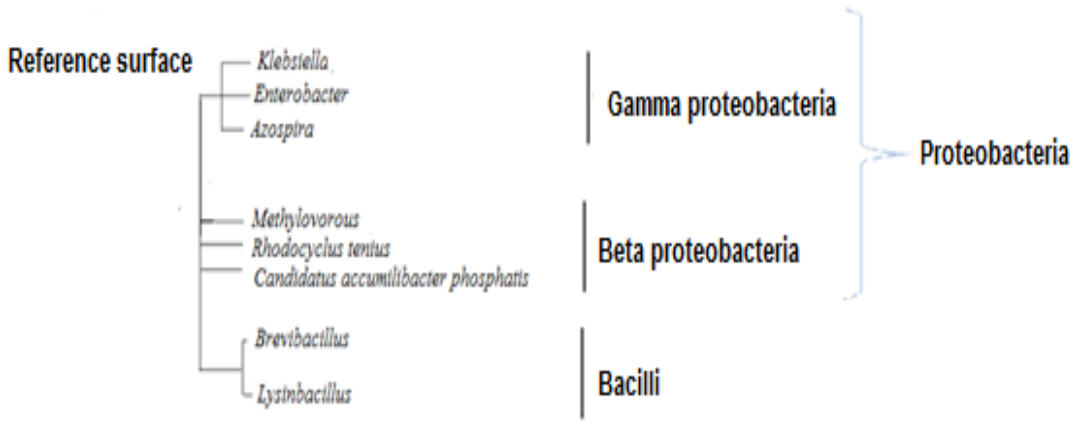

Figure 4.15: The phylogenetic tree showing the relatedness of microorganism isolated from the soil surface in the referenced section of the rhizofiltration system.

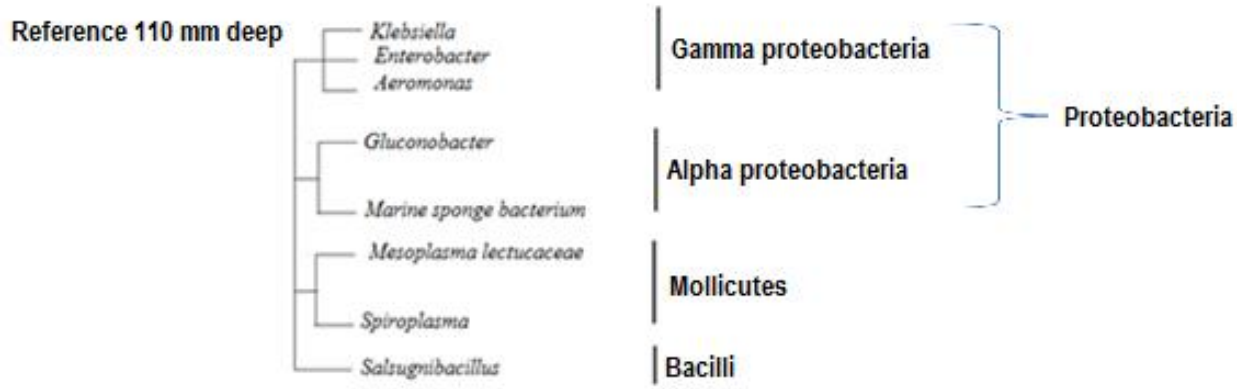

Figure 4.16: The phylogenetic tree showing the relatedness of microorganism isolated 110 mm deep from the soil in the reference section of the system.

4.4 DISCUSSION

Microbial community structure and population dynamics was carried out using the amplification and sequencing of the 16S rDNA. The 16S rDNA gene was targeted because it encodes the rRNA with conserved regions and that it is the smallest subunit of the rRNA. These features of the 16S rDNA make it easy to amplify and identify as well as trace the evolutional relation among microbes (Boyer *et al*., 1999). For the amplification of the 16S rDNA, the whole genomic DNA was isolated from the samples. The gene was amplified using AC907R,

AC341F and GC-clamp as primers. These primers were selected because they contained G and C bases at each end which brought about the stability to the DNA. These primers are also small and specific to 16S rDNA. These features made the primers to bind tightly and thus amplified the gene of interest with high affinity (Graves and Haystead, 2002).

There was high abundance of microorganism taking part in the transformation of nutrients in the rhizofiltration system. The results obtained indicated that ammonifier, *nitrosomonas* and denitrifying organisms were present in large quantities, which was favourable for the nitrifying and denitrifying reactions in the system. High microbial population density was obtained in the planted section, where high aeration and nutrient removal was obtained. Waki *et al.* (2010) found that with increased salinity, ammonia also increased and phosphate decreased in wetlands. In addition, methanogenic bacterial populations decreased. These results suggest that changes in salinity may affect how nutrients were used by microorganisms in a rhizofiltration and thus potentially affecting bacterial community composition. Rhizosphere colonization of macrophyte roots was increased with nutrient loading. Wang and Li (2011) studied rhizobium species from a single wetland site and found that macrophytes in dry nutrient poor soil media had different organisms than macrophytes in wet nutrient rich soils. Alarco (2011) attributed this to the species' specific preference for particular nutrient-tolerant or nutrient-sensitive macrophytes types. These findings were similar to this study where the rhizomes of *K. nemoralis* had higher microbial diversity compared to *P. australis*. Vymazal (2009) investigated the rate at which a single mycorrhizal species colonized a single macrophyte species at various phosphorus levels. He found bacterial colonization to occur only at low nutrient levels and that macrophyte biomass did not vary, suggesting that at low nutrient concentrations mycorrhizae may have assisted in macrophyte nutrient uptake.

The effects obtained in the rhizofiltration represented the relatively common responses of microbial activity to nutrient loading. They do not encompass all possible responses. Given the complexity of microbial communities within the vast array of differing rhizofilter types and hydrologic regimes, the range of responses are large. Because of the central importance of microbes in decomposition and nutrient recycling, any change in microbial activities might have large impacts on rhizofiltration functioning (Yu and Mohn, 2001; Lie *et al*., 2007).

Most microorganisms identified from the rhizofilter are known for their ability to remove nitrogen and phosphorus in wastewater or to reduce nutrient levels in wastewater (Cassamatta and Wickstorm, 2000).

Ibekwe *et al*. (2007) associated high microbial abundance and diversity on the wetlands' surface to aerobic and anaerobic zones provided by macrophytes that allowed growth of compound microbial communities on these sections. *Methylovourus* in the planted section was responsible for oxidation of ammonia to nitrite (Joanne *et al.*, 2008). *Azorhizobium* was also isolated and Arroyo *et al*. (2010) and Leek (2008) have linked this organism to nitrogen fixation in soil. Gammaproteobacteria was available in equal proportion on soil surfaces of both the planted and reference sections. At about 110 mm depth in the bottom of the system in the planted section Gammaproteobacteria was the most abundant and diverse functional group, *Pseudomonas* being the dominant specie while in the reference section the dominant group was Mollicutes. *Pseudomonas aeruginosa* is mostly found in soil, water and in surfaces of plants where it is considered nuisance organisms (Fricker, 2003; Bialowiec *et al.*, 2012). This organism is able to degrade a variety of nutrients in soil, including nitrogen (Truu *et al*., 2009). More organisms from the soil samples, particularly in the planted section, were found to be responsible for nitrogen degradation through the oxidation of ammonia to nitrite and nitrate (Figure 4.12-4.14). Nitrate was subsequently taken up by macrophytes. Nitrite may also have escaped into the atmosphere in a form of nitrous oxide.

Phosphate accumulating organisms responsible for phosphate removal were identified in the system. Vasileva and Topalova (2009) observed *Candidatus Accumulibacter phosphatis* to accept an electron from phosphate or orthophosphate for phosphorus removal. The observations also concurred with findings by Joanne *et al*. (2008) and Flower *et al*. (2009), who reported Accumulibacter to have accumulated up to 48% of phosphorus that was introduced to it in the aquaponic system. Most abundant organisms identified in the planted section were Gram negative bacteria (Figures 4.12-4.14). Willey *et al*. (2008) suggested most Gram negative microbes to possess a phosphatase enzyme in their periplasm which enable them to take-up and utilize phosphate and incorporate them to their cell mass. Truu *et al.* (2009) also found Alphaproteobacteria to be the most group in a study of a constructed rhizofiltration with high phosphorous loading. *Azorhizobium* was also a more abundant organism on the surface of the planted section. This section of the system had high phosphate concentrations

removed. *Azorhizobium* transformed phosphorus into orthophosphate, which then became available for macrophyte uptake.

Microorganisms belonging to *Delta proteobacteria* were identified at 110 mm depth in the rhizosphere of the planted section only while Mollicutes, Clostridia, Deniococcales and Gleobacteria were identified in the reference section (Figure 4.4). *Delta proteobacteria* are known to utilize nitrate, sulphur and trichloroacetic under anaerobic conditions (Suresh and Ravishanker, 2004; Gravity *et al*., 2005). Gammaproteobacteria was distributed across the system and were available throughout the study period.

4.5 CONCLUSIONS

- The soil media contained more complex microbial communities compared to influent and the effluent wastewater. The soil media filtered and supported the growth and development of diverse microbial populations in wastewater.
- Organisms introduced into the rhizofilter with the influent did not survive for long periods in the system because rhizofiltration was not their 'natural' environment. Thus, less microbial diversity was obtained in the influent compared to the effluent.
- Complex diversity as well as high abundance of microorganisms was obtained in the section planted with macrophytes. This explains high nitrogen and phosphorus reduction in the planted compared to the reference section.
- Nutrient reduction obtained in the rhizofiltration system was a direct result of microbial communities and their activities in the system which differed in planted and reference sections.

CHAPTER 5

EFFECTS OF MACROPHYTES ON ORGANIC AND INORGANIC NUTRIENT REMOVAL IN A CONSTRUCTED RHIZOFILTRATION SYSTEM

5.1 INTRODUCTION

The ability of macrophytes to accumulate nitrogen and phosphorus makes them useful for exploitation for use in wastewater treatment. Macrophytes have been reported to assist in maintaining the water quality in water bodies at the acceptable standards in natural reservoir (Kivaisi, 2001; Greenway, 2007; Vymazal and Kopfelova, 2009). Their effectiveness may be attributed to their interactions with other components found in the system (Kadlec *et al*., 2000; Show *et al.*, 2001; Scholz, 2006). While rhizofiltration systems may be a natural alternative to technical methods of wastewater treatment, understanding of the complex processes caused by macrophytes as well as their interaction with other components in the treatment efficiency is still incomplete. Apart from nutrient accumulation, macrophytes have been reported to release oxygen into the rhizosphere through their roots (Yoon *et al*., 2001; Brosnan and De Frank, 2008). Oxygen in wastewater is required by aerobic microorganism to degrade contaminants in the rhizosphere (Bedford *et al.*, 1991; Cooney, 1996; Sohsolam *et al.*, 2008). The amount of oxygen released in the sub-tropical region is higher and decreases with distance from the roots apex (Gumbricht, 1993; Brix, 1997). Another important role of roots has been the absorption of carbon dioxide from the rhizofiltration which serve as rich source of dissolved carbon (Sandurandivel and Vigneswaran, 2001; Vymazal, 2007; Wang *et al*., 2008). This phenomenon could lead to decreased carbon dioxide production, which also serves as a source of greenhouse gas.

Nitrogen and phosphorus are the two main nutrients found in wastewater. Nitrogen exists in the form of ammonium or nitrate while phosphorus may exist as phosphate and orthophosphates. These nutrients should be reduced to acceptable levels before wastewater can be released into the environment. The removal of nitrogen by macrophytes is achieved mainly through macrophyte interaction with microorganism in the root zone, and physical precipitation (Kadlec and Knight, 1996; Mitsch and Gosselink, 2000). The central pathway for nitrogen removal is nitrification followed by denitrification. The available nitrogen is converted into ammonium and nitrate. Macrophytes take up nitrogen in a form of ammonium and nitrate, which is in turn stored in an organic form in macrophytes biomass (Mitsch and Jorgensen,

2004). Nitrogen transformation and subsequent removal processes may be dependent on the concentration of oxygen. The available oxygen may be dependent on macrophyte diversity and population. The uptake of nitrogen is greater in the beginning of the growing season and decreases as the season progresses. The capacity of macrophytes to take up nitrogen may vary from 200 to 250 $kg.ha^{-1}year^{-1}$. Though macrophytes may remove nutrients, removal through plant uptake is usually smaller when compared to bacterial transformation (Sanduradivel and Vigneswaran, 2001).

The major removal route of phosphorus is mainly through uptake by macrophytes roots, adsorption and sedimentation. Absorption through roots and accumulation in the leaves and stems is usually very low and thus macrophytes account for most of removal at the beginning of the growing season (De Busk, 1999). Removal of phosphorus occurs in the rhizosphere through adsorption due to the reaction of phosphorus with iron, calcium and magnesium that is present in sediments. The absorption of calcium ions takes place under basic to neutral pH conditions. Thus, adsorption of phosphorus to its ions leads to phosphorus removal from wastewater. The reaction of calcium and phosphorus renders phosphorus in a chemical state suitable to be taken up by macrophytes. This process results in the storage of phosphorus in the organic matter which is eventually released. Growing macrophytes then take up the stored phosphorus as a source of nutrient and the uptake may vary between 30 and 150 $kgha^{-1}year^{-1}$ (Sanduradivel and Vigneswaran 2001).

Most of the world's constructed rhizofilters are based on plant monocultures consisting of only one type of macrophyte (Armstrong and Armstrong, 1988; Fisher *et al*., 2009, Mthembu *et al*., 2013). However, studies suggested that higher macrophytes diversity (mixed culture) in rhizofiltration system may results in higher removal of nitrogen and phosphorus (Barko *et al.*, 1991; Sorrell and Armstrong, 1994; Colemon and Meney, 2004). In this study, the synergistic role of *Phragmites australis* and *Kyllinga nemoralis* in a rhizofiltration system was investigated. The combination of these macrophytes has never been investigated and thus if successful, could provide effective means of nitrogen and phosphorus reduction in a rhizofiltration technology.

5.1.1 Aim

The aim was to investigate the combined role of *Phragmites australis* and *Kyllinga nemoralis* in nutrient removal using a constructed rhizofilter unit and to assess the effect of macrophytes on nutrient removal.

5.1.2 Objectives

a) To determine the bioaccumulation of nitrogen and phosphorus from wastewater on *Phragmites australis* and *Kylinga nemoralis* using the Kjeldahl and dry ashing methods.
b) To determine the effects of macrophytes on dissolved oxygen concentrations in planted and reference sections of the system.
c) To determine if nutrient removal efficiency by the system is enhanced due to macrophytes activities.
d) To correlate nutrient accumulation in macrophytes with nutrient removal efficiency measured in the rhizofiltration system.

5.2 METHODS

5.2.1 Macrophytes Sampling and Preparation

The complete plants of *Phragmites australis* and *Kyllinga nemoralis* were collected for total nitrogen and phosphorus accumulation. They were collected in alternate months between January 2012 and June 2013. Both *Phragmites australis* and *Kyllinga nemoralis* were uprooted from the sand in the constructed rhizofiltration system. Macrophytes were washed with tap water which was then followed by washing with distilled water. Samples were cut and segregated into leaves, stems and roots. Samples were covered with black plastic bags and then placed into a box and transported to the laboratory for analysis. In the laboratory, samples were initially dried at room temperature in order to shorten the subsequent drying period in an oven since long exposure to higher temperature may have resulted in the escape of nitrogen and phosphorus which may have led to incorrect results being obtained (Spence, 1967; Jones *et al*., 1991). Samples were then dried using an oven at 55^0C for 24 hours. After drying, the samples were chopped into small pieces using a pair of scissors. They were then ground into powder form using a one millimetre grading mill (Polychem), and the powder collected and stored in the sterile 10 ml brown bottle before analysis.

5.2.2 Methods Used to Study Nutrient Accumulation by Macrophytes

5.2.2.1 Estimation of total nitrogen

The Kjeldahl method was used to study the accumulation of nitrogen in macrophytes following the method by Julius (1912) and as modified by Plank (1992). The Kjeldahl method has a high precision and is a universal method for the determination of nitrogen. The problem associated with it was that it is labour intensive and time consuming. In the analysis, the following procedure for nitrogen quantification was followed:

Five hundred milligrams of plant sample was weighed into each Kjeldahl digestion tube. One hundred and ten milligrams of catalyst mixture (200 g potassium sulphate, 20 g curpric sulphate pentahydrate and 2 g of selenium) (Merck) was added into each Kjeldahl digestion tube. A digestion blank containing only reagents with sample was prepared by adding 101 mg of the catalyst mixture into a Kjeldahl digestion tube. Three milliliters of concentrated sulphuric acid was added to both Kjeldahl digestion tubes. The Kjeldahl digestion tubes were slowly heated at 200^0C using an oven until the frothing subsided in both Kjeldahl digestion tubes. Temperature was then adjusted to between 350-375^0C until the digestion was complete. The digester was allowed 20 minutes for cooling and 20 ml of de-ionized water was added and the mixture was vortexed. Twenty milliliters of 10 M NaOH was added and immediately transferred to Kjeldahl distillation apparatus and the distillate was collected. The distillate was titrated into boric acid solution using standard 0.01 M hydrochloric acid until the solution turned pink in colour. The samples were then read at 620 nm using a Spectroquant Pharo 300 spectrophotometer (Merck).

5.2.2.2 Estimation of total phosphorus

The dry ashing method allows the quantitative determination of both nitrogen and phosphorus concentrations in plants (Bureau of Nutritional Sciences, 1993). This method requires no added reagent and no blank subtraction. It can handle large number of samples at once and requires little attention. The use of this method may usually retain minerals in the solution with little or no loss from volatilization (Schulte *et al.*, 1987). This method was only used for phosphorus quantification. The procedure followed was as follows:

Plant biomass samples (10 g) were placed in 50 mm porcelain crucibles and ashed by heating for 48 hours in an oven at 250^0C. Ash residue was cooled and one gram of the sample was

added into a 100 ml volumetric flask. This was followed by addition of 5 ml 20% hydrochloric acid digester. The sample was agitated in an orbital shaker for four hours at 65 rpm. It was later filtered through a hydrochloric acid washed filter paper into 50 ml volumetric flask. The solution was then diluted up to volume in the volumetric flask with de-ionized water and then mixed well. The samples were read at 620 nm using a Spectroquant Pharo 300 spectrophotometer.

5.2.3 Result Analysis

The amount of total nitrogen and phosphorus accumulation in macrophytes using both Kjeldahl and dry ashing methods was expressed in percentage form using the formula:

$$\% N = \frac{(T-B)\times N\times 1.401}{g\ sample}\text{X}100$$

Where:
T = ml of sample titrated
B = ml of blank titrated
N = Acid normality
g = weight of sample = 0.5

The effect of physical and chemical parameters on nutrient accumulation in macrophytes was measured using linear and non-linear regression models. The choice of the model used in each case (where either linear or non-linear model used) was based on the physical observation and judgement on the plots/coordinates.

5.3 RESULTS

Analysis was conducted to determine the contribution of macrophytes in the removal of nutrients during wastewater treatment using the constructed rhizofiltration systems. Harvesting of *P. australis* was started in January 2012 while harvesting of *K. nemoralis* was started in May 2012. The difference in starting dates for sampling the two macrophytes was because *P. australis* was first planted in the system (June 2011), while *K. nemoralis* was planted later (October 2011). In 2013 harvesting for both macrophytes was between February and June. Nutrients measured in the macrophytes could be seen in Appendix 4.

5.3.1 Nitrogen Accumulation in Macrophytes

Phragmites australis had the highest percentage increase of total nitrogen uptake (91%) and *K. nemoralis* had 93% uptake when results were compared to the baseline results taken on macrophytes before were planted on the rhizofilter. The baseline results were *P. australis*: leaves = 2.44 mg/g, stems = 0.65 mg/g, roots = 0.94 mg/g; *K. nemoralis*: leaves = 0.25 mg/g, stems = 0.1 mg/g, roots = 0.08 mg/g. The baseline results were taken in June 2011 and October 2011 for *P. australis* and *K. nemoralis* respectively. In 2013 there was an overall increase in the accumulation of nitrogen and phosphorus by macrophytes when the results were compared to 2012. In 2012 the study involved using only pretreated pre-chlorinated wastewater for running the rhizofilter, and had less nutrient concentration which resulted in little nutrient availability for uptake by macrophytes. The wastewater used in 2013 in the system was richer in nutrients than pretreated water used in year 2012.

Nitrogen accumulation by *P. australis* was found to be higher in the leaves. While in general, leaves and stems showed an increase in nitrogen content in warm seasons and decrease in cold seasons, a trend decrease of nitrogen between May and September 2012 was observed. The results for nitrogen accumulation in macrophytes are presented in Figure 5.1. In 2013, a substantial increase in nitrogen uptake by different plant parts was observed compared to 2012. *Phragmites australis* had the highest percentage increase of total nitrogen accumulation (18%) on stem when compared to its baseline results and was observed in May 2012.

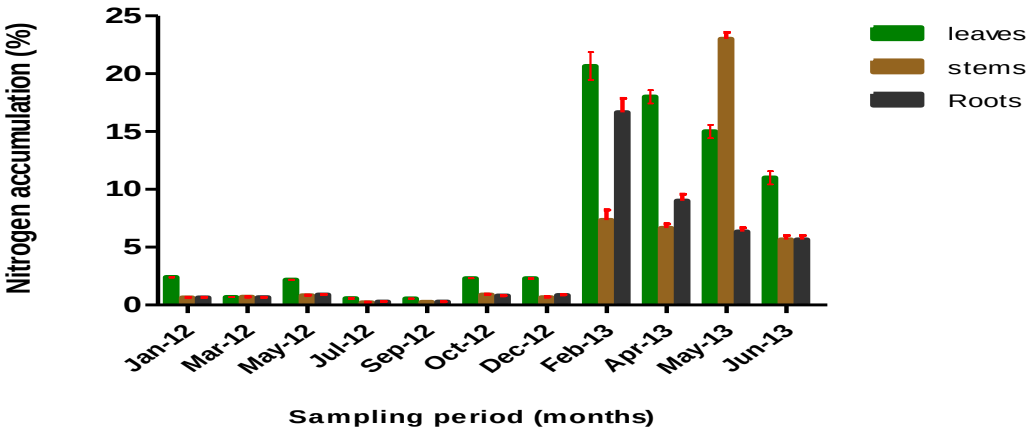

Figure 5.1: Nitrogen uptake by *Phragmites australis* in a constructed rhizofiltration system from January 2012 to June 2013. Mean values of nitrogen accumulation in *P. australis* shown, with whiskers representing standard errors of means.

Nitrogen uptake by *K. nemoralis* was found to be high compared to the uptake by *P. australis.* Like in *P. australis*, *K. nemoralis* accumulation of nitrogen was very high in 2013 compared to 2012. More nutrients were provided together with wastewater which ended up being taken up by macrophytes and thus leading nitrogen accumulation in plant biomass. While the uptake in leaves has shown similar variation in 2012 and 2013; there was a decrease in the accumulation of nitrogen in the stem between February and June 2013 while an increase in the uptake by the roots was observed (Figure 5.2). *Kyllinga nemoralis* had the highest percentage increase of total nitrogen of up to 93% cumulative uptake in 2013 when results were compared to baseline results taken before macrophytes were planted in 2012.

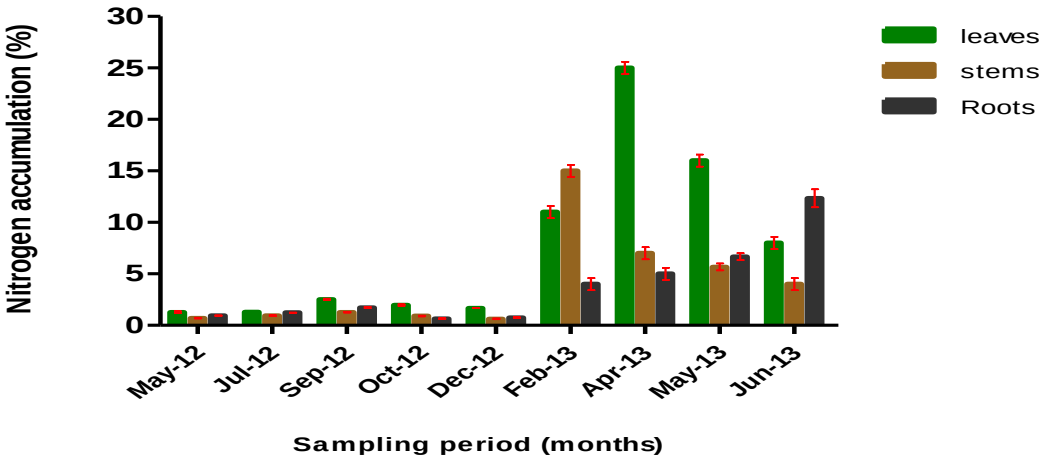

Figure 5.2: Nitrogen uptake by *Kyllinga nemoralis* in a constructed rhizofiltration system May 2012 to June 2013. Mean values of nitrogen accumulation in *K. nemoralis* are shown, with whiskers representing standard errors of means.

5.3.2 Phosphorus Accumulation in Macrophytes

Phosphorus accumulation was found to increase in *P. australis* from January and September 2012, with highest concentrations obtained in the leaves followed by the stems. The results for phosphorus accumulation in *P. australis* in 2012 and 2013 are presented in Figure 5.3. Phosphorus decreased in December 2012 may have been caused by system saturation. *Phragmites australis* had the highest percentage increase of total phosphorus uptake which was up to 89% increase (cumulative uptake) when the results were compared to the baseline results.

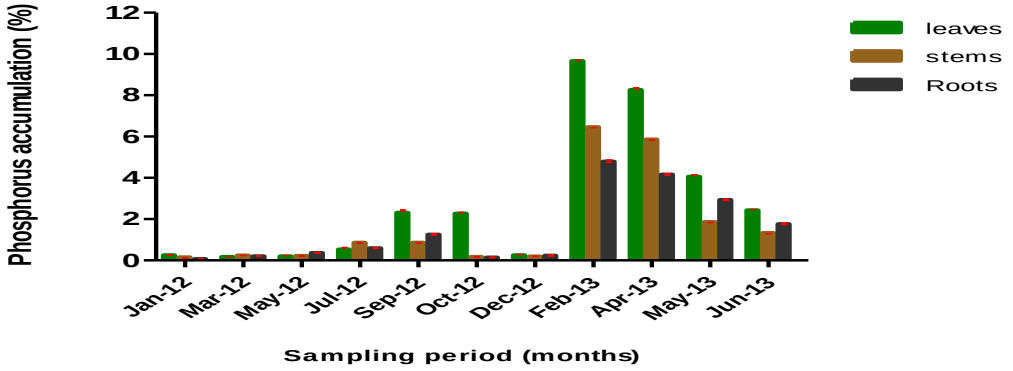

Figure 5.3: Phosphorus uptake by *Phragmites australis* in a constructed rhizofiltration system January 2012 to June 2013. Mean values of phosphorus accumulation in *P. australis* are shown, with whiskers representing standard errors of means.

Phosphorus concentration measured in *K. nemoralis* is presented in Figure 5.4. While phosphorus concentrations in *K. nemoralis* increased between May and December 2012 in the leaves, an increase between May and September as well as decrease between October and December 2012 was observed in both stems and roots. *Kyllinga nemoralis* showed 22% increase of total phosphorus accumulation when compared to the baseline results. In 2013 high phosphorus accumulation in *K. nemoralis* was recorded in the leaves followed by the stems while the roots had the least concentrations. There was however an exception in May 2013, where highest accumulation were observed in the stem, followed by the roots and then leaves. During this period the leaves had the highest uptake of phosphorus in April (13.1%). *Kyllinga nemoralis* had up to 98% increases (cumulative uptake) of total phosphorus accumulation when the results were compared to baseline results.

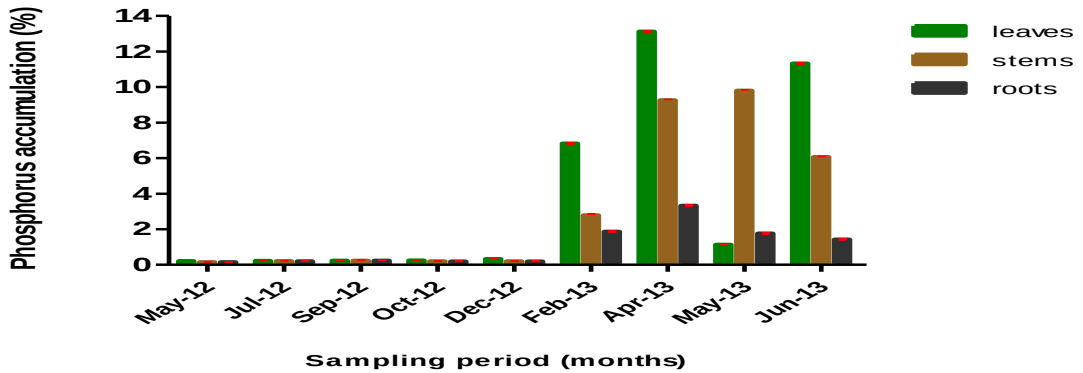

Figure 5.4: Phosphorus accumulation by *K. nemoralis* in a constructed rhizofiltration system from May 2012 to June 2013. Means values of phosphorus accumulation in *K. nemoralis* are shown, with whiskers representing standard errors of means.

5.3.3 Factors Affecting the Accumulation of Nitrogen and Phosphorus in Macrophytes

5.3.3.1 pH

High pH above 8 was found to decrease the assimilation of nutrients into the macrophytes. A very weak positive correlation was obtained between pH and total nitrogen in *K. nemoralis*, with 9.1% nitrogen accumulation per unit of pH increase. A very poor negative correlation was obtained between the pH and nitrogen removal in *P. australis* (r = -0.07), while a very weak positive correlation was obtained with *K. nemoralis* (r = 0.01) (Figure 5.5). Both macrophytes showed a weak negative correlation (r = -0.1 for *K nemoralis;* and -0.2 for *P. australis*) between phosphorus accumulation and pH (Figure 5.6). This study found assimilation of nutrients, particularly nitrogen into the macrophytes to be high at pH between 5.5 and 8.0.

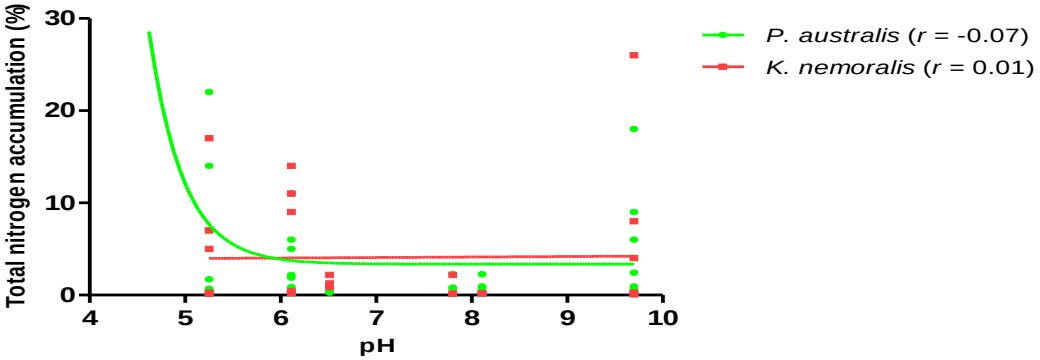

Figure 5.5: Effect of pH on total nitrogen accumulation in *P. australis* and *K. nemoralis* a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between nitrogen accumulation in macrophytes and pH.

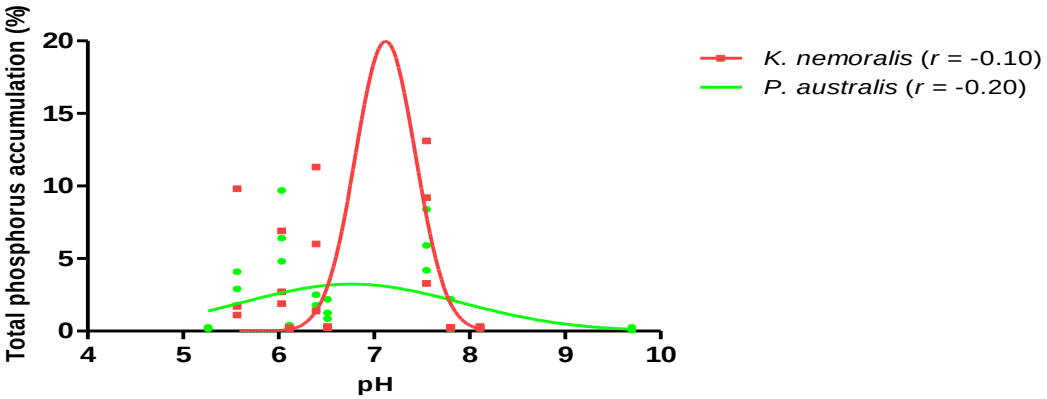

Figure 5.6: Effect of pH on total phosphorus accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between phosphorus accumulation in macrophytes and pH.

5.3.3.2 Temperature

Poach *et al*. (2004) reported temperature to be one of the factors that influenced the assimilation of nitrogen and phosphorus into the macrophytes. Temperature positively correlated weakly with both nitrogen (r = 0.19 for *K nemoralis*; and 0.25 for *P. australis*) (Figure 5.7) and phosphorus (r = 0.27 for *K nemoralis*; and 0.16 for *P. australis*) (Figure 5.8) accumulation in macrophytes. The accumulation of nitrogen per unit temperature increase per unit of degrees Celsius was 10.9% in *P. australis* and 3% in *K. nemoralis*, while for phosphorus it was 11% in *P. australis* and 4.2% in *K. nemoralis*. Nitrogen and phosphorus assimilated into the macrophytes was high in warm seasons. This was due to high microbial diversity and activities in warm season. Cheng *et al*. (2009) reported the diffusion of oxygen into the rhizosphere for the activity of microorganism, which transformed the nutrients, which subsequently accumulated into the macrophytes was favorable at temperatures between 20-25°C.

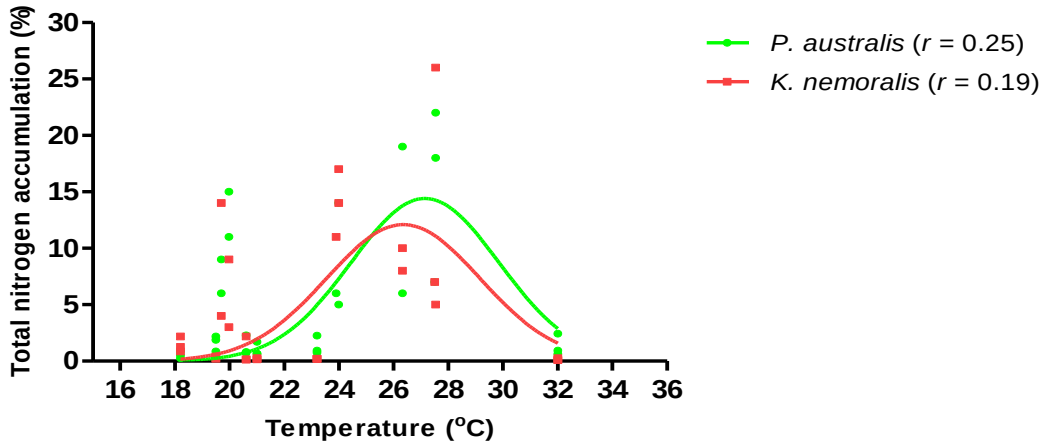

Figure 5.7: Effect of temperature on total nitrogen accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between temperature and nitrogen accumulation.

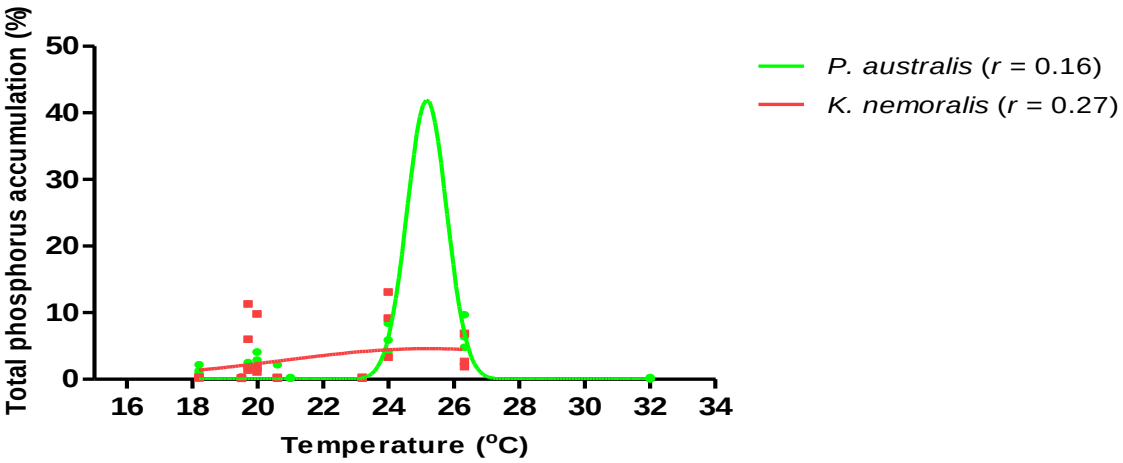

Figure 5.8: Effect of temperature on total phosphorus accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between temperature and phosphorus accumulation in macrophytes.

5.3.3.3 Dissolved oxygen and chemical oxygen demand

A moderate negative correlation between the DO and the total nitrogen (r = -0.69 for *K nemoralis*; and -0.56 for *P. australis*) (Figure 5.9) as well as DO and phosphorus (r = -0.57 for *K nemoralis*; and -0.12 for *P. australis*) (Figure 5.10) in both *P. australis* and *K. nemoralis* was obtained. The results were also supported by Greenway (2003) who reported nutrient accumulation in macrophytes to decrease with decreased dissolved oxygen concentration. The increase in the dissolved oxygen concentration resulted in the availability of microorganisms that degraded and transformed nutrients in the system, thus making the nutrients available for uptake by the plants. Oxygen was required by nitrifying and denitrifying bacteria in the rhizosphere for the removal of nitrogen. While nitrogen and phosphorus assimilation correlated negatively (very weak correlation) in *P. australis* with COD, a poor positive correlation was obtained in *K. nemoralis* (Figures 5.11 and 5.12), with 7.2% and 4.76% accumulation with increase in COD.

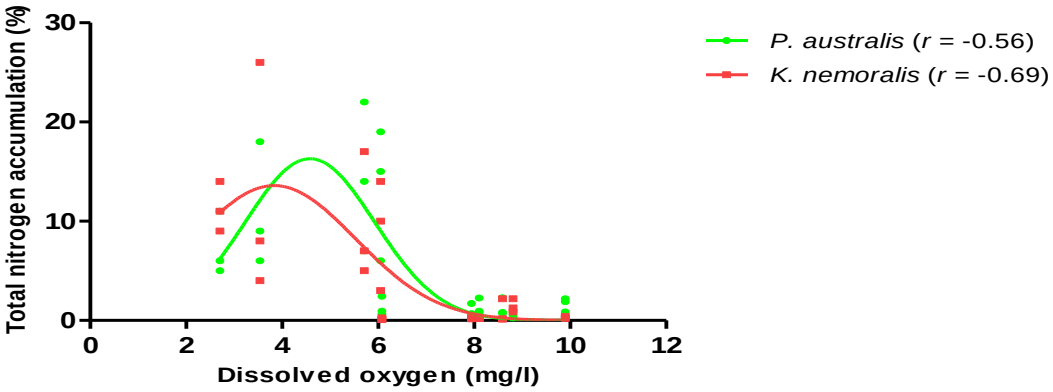

Figure 5.9: Effect of DO on total nitrogen accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the DO and nitrogen accumulation in macrophytes.

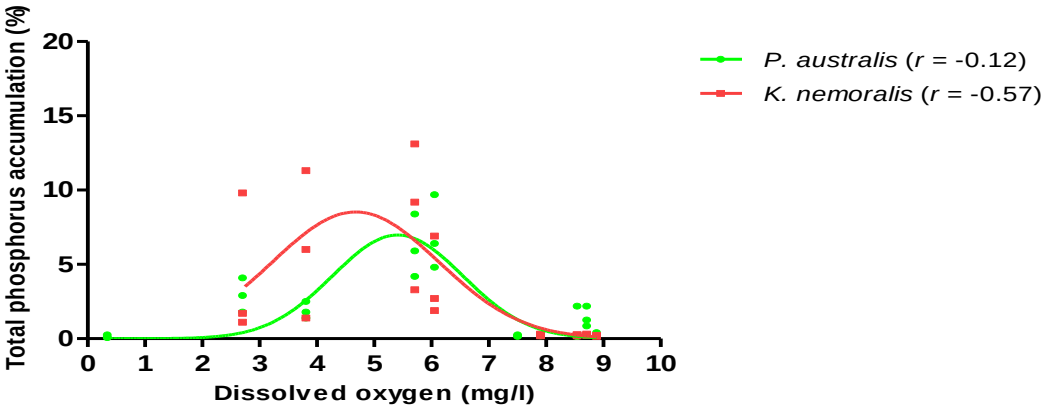

Figure 5.10: Effect of DO on total phosphorus accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the DO and phosphorus accumulation in macrophytes.

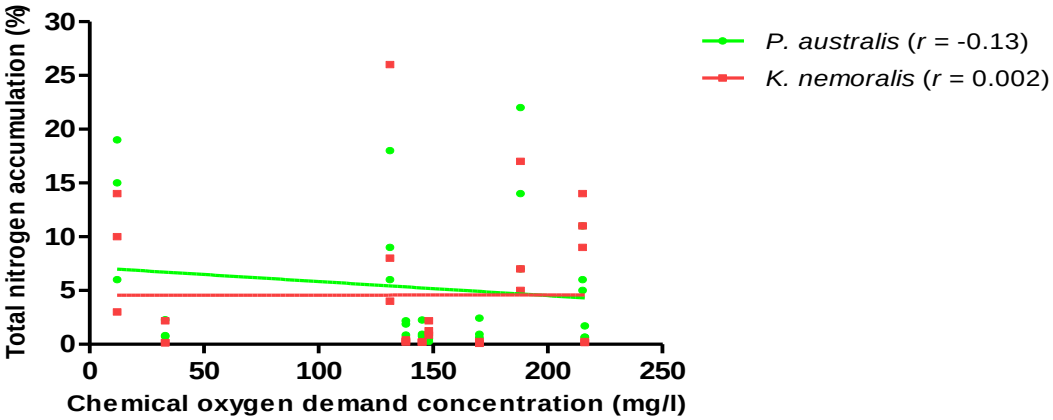

Figure 5.11: Effect of COD on total nitrogen accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between DO and nitrogen accumulation.

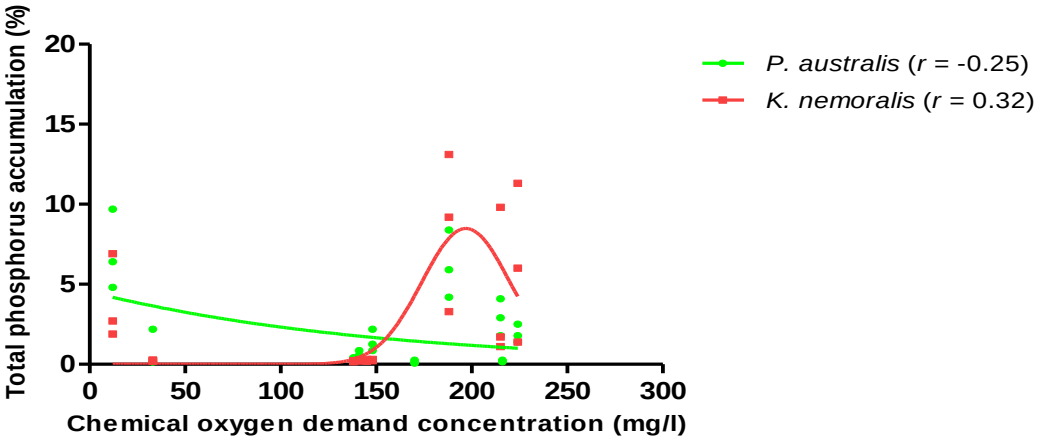

Figure 5.12: Effect of COD on total phosphorus accumulation in *P. australis* and *K. nemoralis* in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the DO and phosphorus accumulation.

5.3.3.4 Effects of nutrient loading on biomass accumulation in the system

Nutrient removal efficiency increased with an increase in total nitrogen and phosphorus accumulation in both *P. australis* and *K. nemoralis*, meaning the increased concentration of nitrogen and phosphorus in macrophytes decreased nutrient concentration in the system (Figures 5.14-5.17). The exception was with total nitrogen removal from the system, which showed a moderate to high positive correlation between ammonia removal and total nitrogen accumulation in both macrophytes (Figure 5.13), as well as well as in *P. australis* between orthophosphate removal total phosphate accumulations (Figure 5.17). A moderate negative correlation was also obtained in both macrophytes between nitrate removal and total nitrogen

accumulation (Figure 5.14). The accumulation rate of nitrogen in *P. australis* and *K. nemoralis* with increase in ammonia was 10.6% and 8.1% respectively. Nitrogen was transformed by nitrogen reducing and oxidizing bacteria isolated in the system into forms that were used by macrophytes as nutrients (Chen *et al.*, 2011). The abundance of these organisms were highest in the planted section, thus nitrogen transformation was high in the planted section. The accumulation efficiency of phosphorus in *P. australis* and *K. nemoralis* with increased orthophosphate concentration in the system was 10.6% and 8.1% respectively (Figure 5.17). Nutrient removal in the system increased with increased nutrient accumulation in macrophytes. Reddy and De Busk, (1987); Greenway (2003) and Cheng *et al.* (2009) reported macrophytes with faster growth rate and larger plant biomass to have more ability to take up large amount of nutrients than macrophytes with low biomass. The growth rate of *P. australis* and *K. nemoralis* was fast. The biomass for *P. australis* was also large. The use of these two macrophytes together ensured high removal of nutrients from the system.

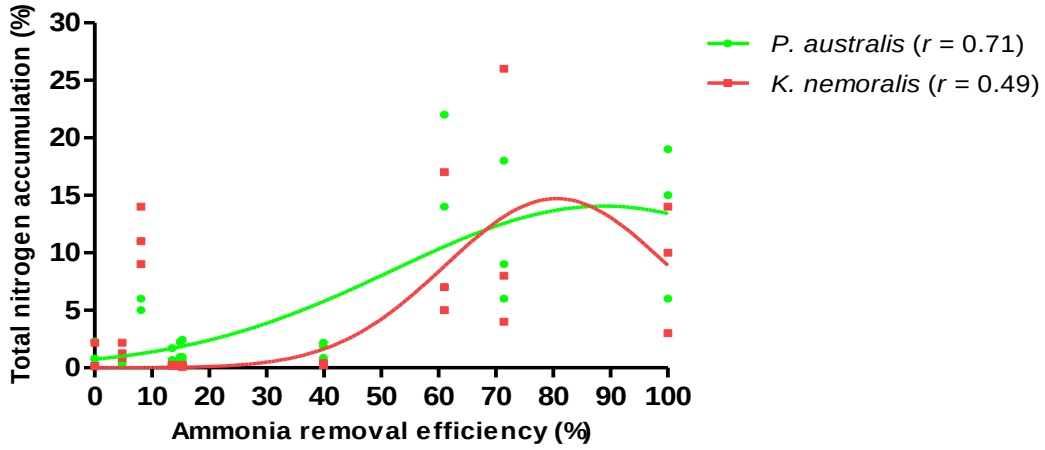

Figure 5.13: Effect of total nitrogen accumulation in *P. australis* and *K. nemoralis* on ammonia removal efficiency in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between ammonia removal efficiency in the system and nitrogen accumulation in macrophytes.

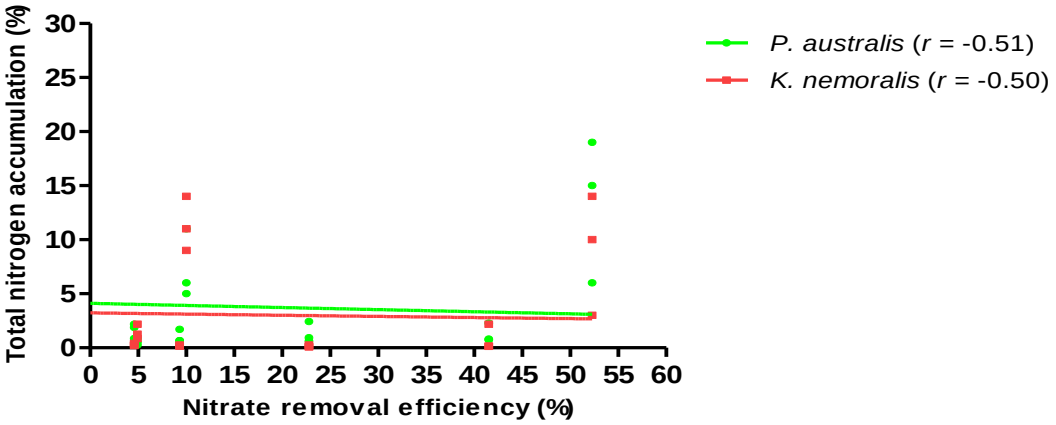

Figure 5.14: Effect total nitrogen accumulation in *P. australis* and *K. nemoralis* on nitrate removal efficiency in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between nitrate removal efficiency in the rhizofilter and nitrogen accumulation in macrophytes.

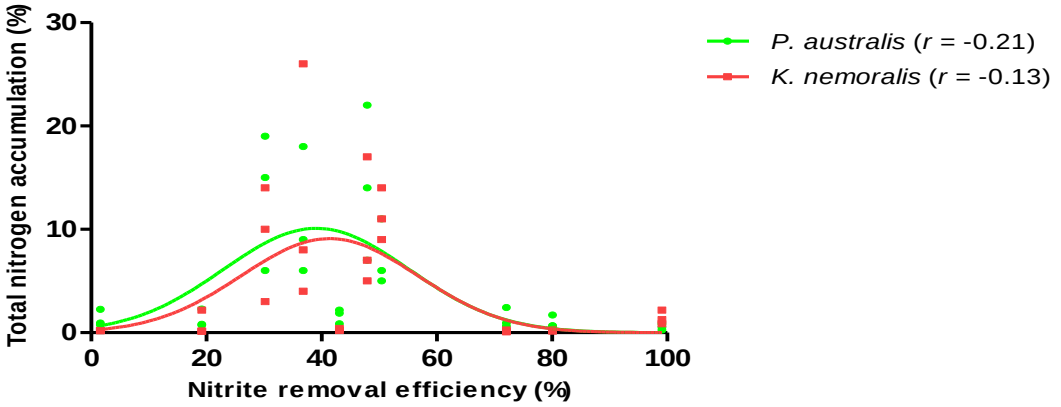

Figure 5.15: Effect of total phosphorus accumulation in *P. australis* and *K. nemoralis* on nitrite removal efficiency in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between nitrite removal efficiency and nitrogen accumulation in macrophytes.

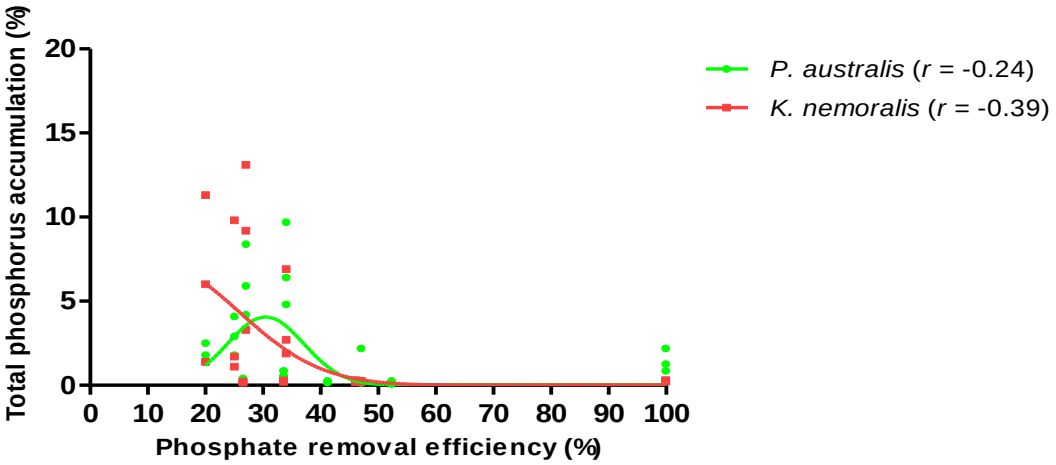

Figure 5.16: Effect of total phosphorus accumulation in *P. australis* and *K. nemoralis* on phosphate removal efficiency in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between phosphate removal efficiency in the system and phosphorus accumulation in macrophytes.

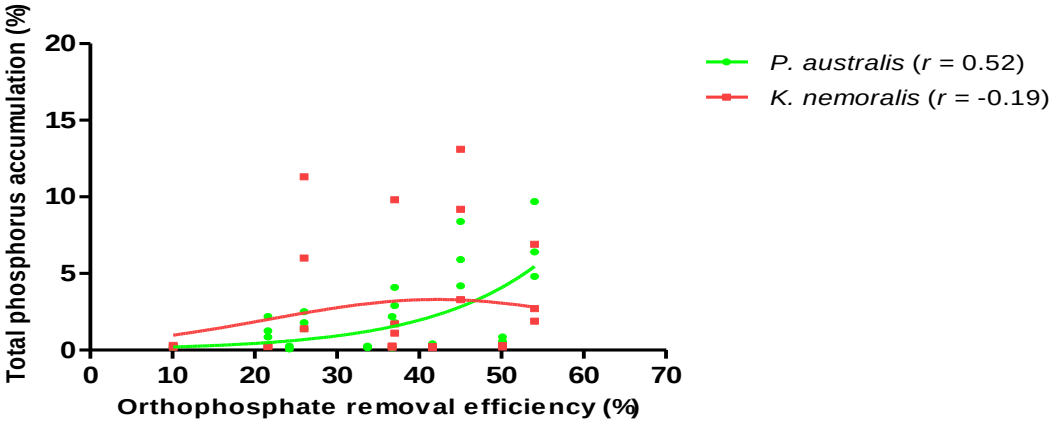

Figure 5.17: Effect of total phosphorus accumulation in *P. australis* and *K. nemoralis* on orthophosphate removal efficiency in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between orthophosphate removal efficiency in the system and phosphorus accumulation in macrophytes.

5.4 DISCUSSION

There are sufficient studies to indicate some roles being played by macrophytes in nitrogen removal though the significance of macrophytes uptake versus nitrification/denitrification is still questionable (Hua, 2015). Nitrogen can exist in various forms, namely Ammoniacal nitrogen (NH_3 and NH_4^+), organic nitrogen and oxidised nitrogen (NO_2^- and NO_3^-). The removal of nitrogen in the rhizofiltration system was achieved through

nitrification/denitrification, volatilisation of ammonia storage in detritus and sediment, and uptake and storage by macrophytes (Brix, 1997), with a majority removal occurred through either plant uptake or denitrification. *Phragmites australis* and *K. nemoralis* were the two macrophytes used in the study due to their nutrient absorption ability as well as their abundance and availability near and around the rhizofiltration site. Findings in this study have shown high accumulation of both nitrogen and phosphorus by macrophytes, especially in 2013 (Figures 5.1 – 5.4), and were also seasonal. In the autumn macrophytes in the system grew slowly. As a result, the assimilation of ammonia and the oxygen supply decreases markedly. This resulted in low reduction of nutrients during these periods.

Macrophytes have the ability to take up large quantities of nutrients and assimilate these nutrients through their roots system and store them in their leaves for building their tissue (Crank and Fennessy, 2001). Apart from reducing nutrients through incorporation into their cell tissues it is believed that macrophytes transported oxygen down their stems into their roots where it diffused into the sediment. Diffused oxygen into the soil media created a suitable environment for aerobic microbial activities in the rhizofilter. Macrophyte roots turnover may also have created macrospores in the constructed rhizofiltration system which provided space for the movement of oxygen in the rhizosphere (De Busk, 1999). Tanner *et al.* (2001) also found oxygen released by macrophytes to contribute to the formation of biofilm around macrophytes roots through proving aerobic microbial environment. The results were later supported by Stanley and Lazazzera (2004). The degradation of organic matter became initiated by microorganism and nutrients then absorbed macrophytes (Brix, 1997). These assumptions have been made due to high dissolved oxygen obtained in the planted section of the rhizofiltration unit as well as due to high abundance of aerobic organisms in that section, which all resulted to decreased nutrients in the planted section compared to the reference section.

Macrophytes in constructed rhizofiltration systems require nitrogen and phosphorus for growth and reproduction. Nitrogen was accumulated in macrophytes in a form of nitrate and ammonia (Figures 5.1 and 5.2). Phosphorus was accumulated in a form of orthophosphate (Figures 5.3 and 5.4). Macrophytes have the capacity of nutrient accumulation through direct uptake by macrophyte rhizomes and store the nutrients into cell mass, thus increasing in their biomass (Brix, 1997; Tanner, 2011). This study has shown the highest accumulation of nutrients in the leaves of both *P. australis* and *K. nemoralis* (Figures 5.1 – 5.4).

The highest accumulation of nutrient by macrophytes was observed in growing seasons (Figures 5.1 – 5.4), which coincided with high nitrogen and phosphorus removal in the system. Nutrients were also found to accumulate more in young plants. However no correlation was established between young plant growth and nutrient removal from wastewater. Greenway (2003) found high nutrient accumulation in younger plants to be due to their higher nutrient requirements for growth than was required by older plants.

The response of macrophytes to nitrogen and phosphorus loading was variable and dependent not only on the type and amount of nutrients, but also on the natural background conditions found in the rhizofiltration system. High nutrient loading resulted in increased macrophyte production and biomass formation, competition between the two macrophytes used, and shifts in community structure and composition (Phipps and Crumpton, 1994; Ciudad *et al*., 2005). The ways in which macrophytes responded to variation in nutrient concentrations and the ways in which these species exploited the nutrients, dictated the plant community density and structure of organisms that were present in the system. Nutrient enrichment in wastewater is essentially a change to the natural environment – an alteration of the conditions that macrophytes and other organisms have adapted to. Changes to the nutrient status of the rhizofiltration system resulted in the establishment of macrophytes that took advantage of increased nutrient availability. When nutrient concentration increased, large and fast growing *K. nemoralis* flourished and out-competed *P. australis.* This had an overall effect to nutrient removal efficiency by macrophytes, with *K. nemoralis* contributing to more removal of nutrients. Ponnamperuma (1972) and Strous *et al.* (1997) all reported macrophytes accumulated, sequestrated, and storage of the nutrients to vary and to depend on macrophyte physiology as well as background environmental conditions and the nature of nutrient inputs.

The presence of low effluent nitrogen and phosphorus concentrations in the planted section than in the reference section (Figures 3.8 – 3.12) suggested that macrophytes significantly increased the removal of these nutrients in the system. While all the nutrients were reduced, in the growing seasons of the plants, nitrite and nitrate were removed up to 99%. This circumstance suggests that all nitrate and nitrite formed through nitrification were removed either by denitrification in anoxic zones or were taken up by macrophytes (Bialowiec *et al*., 2012). Ammonia concentration also showed high variability over the period of the study (Figure 3.8). High variations in ammonia removal efficiencies (12–85%) for planted horizontal surface flow wetlands due to a higher density and activity of nitrifying organisms in the planted

systems were observed by Bialowiec *et al.* (2011). Analysis of microbial abundance has indicated more abundance in the oxygenated planted section. Organisms isolated were capable of transforming, and removing nitrogen and phosphorus with higher efficiencies in the planted section. The two macrophytes also released oxygen through diffusion into the rhizosphere. The released oxygen promoted the development of nitrifiers that aided in nitrification. Armstrong and Armstrong (1991) even suggested the existence of an aerobic microzone close to the root surfaces that enhances aerobic microbial mechanisms that contributed to organic and ammonia oxidation. The existence of this microcosm was a significant contribution to the overall removal of nutrients in the rhizofilter.

At the root-soil interface, atmospheric oxygen diffused into the rhizosphere through the leaves, stems, rhizomes and roots of the macrophytes and created an aerobic layer similar to those that existed in the media-water. Nitrogen transformation took place in the oxidised and reduced layers of media, the root-media interface and the below ground portion of the macrophytes. Ammonification occurred where organic N was mineralised to NH_4+-N in both oxidised and reduced layers. The oxidised layer and the submerged portions of macrophytes were were important sites for nitrification in which ammonia was converted to nitrite by the *Nitrosomonas* bacteria and eventually to nitrate by the *Nitrobacter* bacteria which was then taken up by the plants.

5.5 CONCLUSIONS

- The system planted with macrophytes removed more contaminants in a rhizofiltration unit than the reference system. It can therefore be concluded that macrophytes had a significant positive contribution to the reduction of contaminants in the rhizofiltration system.
- Apart from taking up nitrogen and phosphorus, macrophytes contributed to oxygen supply through diffusion, which in turn, supported the growth of aerobic organisms in the planted section. These organisms degraded and transformed contaminants in the planted section, which led to the subsequent reduction. The reference section lacked this phenomenon due to the lack of macrophytes, and thus had low nutrient reduction efficiencies.
- Phosphorus reduction decreased as the system aged. The decrease was due to system saturation, suggesting that its removal was mainly through physical entrapment.

- Mostly, in the parameters investigated, weak positive and negative correlations between nitrogen and phosphorus removal was obtained with varying parameters in the system. The exception was a strong positive correlation obtained between ammonia removal efficiency and total nitrogen accumulation in macrophytes.
- Nutrient reduction was high in warm seasons, and these were the macrophytes' growing seasons. This suggested that macrophyes absorbed the nutrients which accumulated in their cell mass.

CHAPTER 6

DETERMINATION OF THE CARBON FOOTPRINT OF THE RHIZOFILTRATION SYSTEM

6.1 INTRODUCTION

Climate change is a continuing global challenge due to increased production of greenhouse gases. Thus, every means to reduce the greenhouse gases (GHGs) in the environment should be considered. The main source of GHGs has always been considered to be the burning of fossil fuels. However, wastewater treatment systems have also proved to be a source of GHGs emission (Wallace and Nicholas, 1969; Hutsch, 2001). During wastewater treatment, nutrients are transformed by different microbial reactions and carbon dioxide, methane and nitrous oxide are released to the atmosphere (Martinko *et al*., 1997). Emission of GHGs negatively influences the quality of the air and increases GHGs effects. Increased production of carbon dioxide, methane and nitrous oxide have a direct influence on the environment; causing extreme weather changes, a global temperature increase, the deterioration of the ecosystem and potential health hazards to people (Dong and Sun, 2007). Climate change has been shown to affect the environment through increased frequency of heat waves, floods and droughts and may also results in mortalities (Haines *et al*., 2006).

Recently, fatalities have been witnessed globally due to climate change and the main cause remains an increase in GHGs emissions. Extreme environmental temperatures as well as roads and houses collapse due to heavy rains that leaves people with no shelter, food and transport witnessed all over the world in the past and may all be associated with abnormal events occurring in the environment due to climate change (Listowski *et al*., 2011; Guisasol *et al*., 2008). The abnormalities of these events mainly lie in the timing of the year at which they occur as well as their severity. The 2013 World Health Organization report (World Health Ogrnization, 2013), tied about 1000 000 annual deaths worldwide to climate change. These related deaths are expected to double by the year 2050 if no major steps or alternatives to human activities are put in place to replace activities deteriorative to the environment (Ugetti *et al*., 2012a; Liikanen *et al*., 2006). Introducing new alternatives in wastewater treatment that emit less GHGs may be a step towards saving the environment from the continued deterioration. Constructed rhizofiltration technology has been studied for this purpose.

Gas emission in wastewater treatment occurs through nitrification, denitrification and subsequent volatilization. Carbon dioxide, methane and nitrous oxide are the predominant gases produced during microbial degradation of nutrients in wastewater, contributing to the treatment efficiency of water. High loads of nitrogen and organic matter may increase the production of GHGs from the carbon cycle (Yan *et al.*, 2012). Carbon dioxide is produced indirectly as a result of fossil fuel combustion to generate the energy required for the operation of the wastewater treatment plant or may be produced directly during the respiration of organic matter. Nitrous oxide is emitted during biological nitrogen removal from wastewater through nitrification and denitrification processes. Nitrous oxide has a 300-fold stronger effect than carbon dioxide in the environment. Therefore, even minute quantities of N_2O emission into the environment may have undesirable detrimental effects. Methane is produced when the organic matter contained in wastewater is biodegraded by the methanogenic bacteria and either incorporated into new biomass or converted into carbon dioxide or methane under anaerobic conditions (Liikanen *et al.*, 2006). Thus, the degradable carbon content in the form of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic carbon (TOC) represent the amounts of methane and carbon dioxide producing potential of wastewater during treatment.

Conventional wastewater treatment systems contribute about 3% of GHGs to the atmosphere (Fuchs *et al.*, 2001). This percentage is enough to cause major destruction and health hazards considering daily production as well as the number and distribution of these wastewater treatment plants worldwide. It is thus important to consider alternative strategies for wastewater treatment to reduce GHGs emission for the purpose of environmental protection while avoiding carbon taxes and reducing energy costs.

During the past few decades the world has adopted the concept of 'carbon footprint' to refer to the total sets of GHG emissions caused by an organization, event, product or a person (Picek *et al.*, 2007; Lee, 2011). This study focuses on carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) as the three main GHGs emitted to the atmosphere and result in climate change. These gases can have adverse effects in the atmosphere since they act as GHG and cause global warming. Increased production of these gases into the atmosphere during wastewater treatment converts water pollution into an atmospheric pollution problem (Liikanen *at el.*, 2006). Waste and wastewater contribute 3% of GHGs to the atmosphere, whereas

forestry, agriculture, energy supply, buildings and industry contribute 17%, 14%, 26%, 8% and 19% respectively (IPCC, 2007).

Constructed rhizofiltration systems utilize natural methods for wastewater treatment. However, carbon dioxide, methane and nitrous oxide are produced. These systems are believed to produce less of these gases compared to other wastewater treatment technologies. If this could be proven, constructed rhizofiltration systems could be used as a useful tool in replacement of conventional methods of wastewater treatment because of their environmental friendliness in wastewater treatment. Studies comparing conventional wastewater treatment plants (activated sludge technology) to constructed wetlands have shown that the latter causes less of an environmental impact and emits less GHGs than conventional treatment (Fuchs *et al*., 2001; Daelman *et al.*, 2012). The main aim of this study was to investigate and seek an alternative low cost, environmentally friendly wastewater treatment technology with minimum contribution to the GHG production into the environment.

6.1.1 Aim

The aim of this chapter was to quantify the GHGs (CO_2, CH_4 and N_2O) emitted from the constructed rhizofiltration system.

6.1.2 Objectives

a) To estimate carbon dioxide and methane emission in the rhizofiltration system through quantification of COD and TOC in the influent, planted and reference section of the rhizofiltration unit.
b) To calculate and estimate nitrous oxide emission in a rhizofiltration system through the quantification of ammonia and nitrate in the influent and effluent of the rhizofilter unit.
c) To correlate the GHGs production with physicochemical parameters as well as nitrogen measured in the rhizofiltration system.

6.2 METHODS

6.2.1 Sample Collection

Wastewater samples were collected once a month from January 2012 to June 2013 and analyzed for temperature, pH, dissolved oxygen, total dissolved solids, salinity and electrical conductivity on-site using a portable Inolab multi-meter (Merk). Influent and effluent of both

planted and reference sections of the system were collected. Wastewater influent and effluents were collected from all sections of both planted (A, B, D and E) and the reference (F, G, I and J) sections. Samples were collected using autoclaved one-litre laboratory glass bottles and then transported to the laboratory for immediate further analysis. Chemical oxygen demand, total organic carbon, ammonia, nitrite, nitrate, phosphate and orthophosphate were analyzed in the laboratory using spectrophotometric methods as explained in Chapter 3, section 3.2.

6.2.2 Water Sample Analysis

Temperature, pH, dissolved oxygen, total dissolved solids, salinity and electrical conductivity were measured on-site using a calibrated Inolab multi-meter probe and results were recorded. Each sample was measured in triplicates. In the laboratory chemical oxygen demand, total organic carbon, ammonia, nitrite, nitrate, phosphate and orthophosphate were measured using spectrophotometric methods using a Spectroquant Pharo 300 spectrophotometer. The spectrophotometer was used with cell and reagent test kits obtained from Merck following the instruction manual. All the tests were conducted in triplicate. Biological oxygen demand is considered to be about eighty per cent of the COD value (Kadlec and Knight, 1996), and for its measurement, firstly, the pH of the samples was maintained within the range 5.5-7.5. Using the overflow measurement flask samples were filtered into 500 ml BOD bottles and reagents were added followed by the magnetic stirring rods and the bottles were sealed with gaskets. Sealed bottles were carefully placed onto the bottle rack equipped with the measuring device. They were then incubated for five days at 20˚C in the dark within the Aqualytic BOD-System AL606 (Polychem). Biological oxygen demand was read after five days. Chemical oxygen demand, TOC, ammonia and nitrate were measured as outlined in sections 3.2.2, 3.2.3, 3.2.4 and 3.2.5 respectively. After all the necessary parameters were measured, the following formulae were employed in the estimation of gases emitted in rhizofiltration systems (EPA, 2009):

Carbon dioxide estimation:

$$CO_2 = 10^{-6} \text{x} Q_{ww} \text{x} OD \text{ x } Eff_{OD} \text{ x } CF_{CO2} \text{ x } [\lambda \text{ (1-}MCF_s \text{ x } BG_{CH4})]$$

Methane estimation:

$$CH_4 = 10^{-6} \text{ x } Q_{ww} \text{ x } OD \text{ x } Eff_{OD} \text{ x } CF_{CH_4} \text{ x } [\lambda \text{ (1-}MCF_s \text{ x } BG_{CH_4})]$$

Nitrous oxide estimation:

$$N_2O_{WWTP} = Q_i \text{ x } TKN_i \text{ x } EF_{N_2O} \text{ x } \frac{44}{28} \text{ x } 10^{-6}$$

Where:

- CO_2: Emissions of carbon dioxide measured per hour
- CH_4: Emissions of CH_4 measured per hour
- Q_{ww}: Wastewater influent flow rate measured in m^3/hr.
- *OD*: Oxygen demand of influent wastewater which is determined as either biological oxygen demand (BOD_5) or chemical oxygen demand (COD) measured in mg/l
- *TOC*: Total organic carbon
- Eff_{OD}: Oxygen demand efficiency of the biological treatment unit.
- Eff_{TOC}: TOC removal efficiency of the biological treatment unit.
- CF_{CO2}: Conversion factor for maximum CO_2 generated
  = 1.375 g CO_2/g oxygen demand
  = 3.667 g CO_2/g TOC
- CF_{CH4}: Conversion factor for maximum CH_4 generation per unit of oxygen demand
  =0.5 g oxygen demand.
  =1.33 g TOC
- MCF_{WW}: Methane correction factor for wastewater treatment unit indicating fraction of the influent oxygen demand that is converted both aerobically and anaerobically
  Anaerobic = 0.8 and aerobic = 0
- BG_{CH4}: Fraction of carbon as CH_4 in generated biogas
  =0.65 g
- ƛ: Biomass yield (g C converted to biomass/g C consumed)
- Anaerobic=0.1g and aerobic= 0.65 g
- N_2O_{WWTP}: nitrous oxide emissions from the wastewater treatment plant (mg N_2O/hr)
- Q_i : wastewater flow rate measured in m^3/hr
- TKN_i: Amount of TKN in the influent (mg/l)
- EF_{N2O}: N_2O emission factor, 0.0050 g
- $\frac{44}{28}$: Molecular weight conversion, gN_2O per gN
- 10^{-6}: Units conversion factor (mg/g)

Aerobic wastewater treatment systems produce primarily CO_2, whereas anaerobic systems produce a mixture of CH_4 and CO_2. Thus, the equations used provide a general means of estimating the CO_2 and CH_4 emissions directly from any type of wastewater treatment process, assuming that organic carbon removed from the wastewater is converted to either CO_2 and CH_4 (RTI International, 2010).

6.3 RESULTS

Nutrient results from which carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emission were calculated, are presented in Chapter 3. The influent nitrogen, from which the effluent of the planted and reference sections were from, was the same. This only allowed/permitted nitrous oxide emission to be estimated or calculated from the whole rhizofilter unit instead of being differentiated into planted and reference sections.

6.3.1 Carbon Dioxide Emission

A trend of CO_2 emission had shown CO_2 to increase between January and April 2012, with a decrease obtained in May and in June 2012 (Figure 6.1). Decrease in gas formation was associated with a decrease in nutrient removal from which CO_2 was derived from. Between October 2012 and June 2013, an increase in the emission trend of CO_2 was recorded. Carbon dioxide was found to be seasonal with low emissions obtained in cold seasons (mean = 0.027 mg/day). This phenomenon was associated with reduced microbial functioning in cold temperatures. Emission of CO_2 was higher in the planted section, as well as in 2013 than in 2012 (Figure 6.1). Emission ranged between 0.005-0.453 mg/day in the planted section, while they were between 0.03-0.21 mg/day in the reference section. When the planted section was compared to reference section, a statistically significant difference was obtained in warm seasons in 2012 (January-April and between September and December) ($p < 0.05$), while in 2013, statistically significant difference was obtained between February and June.

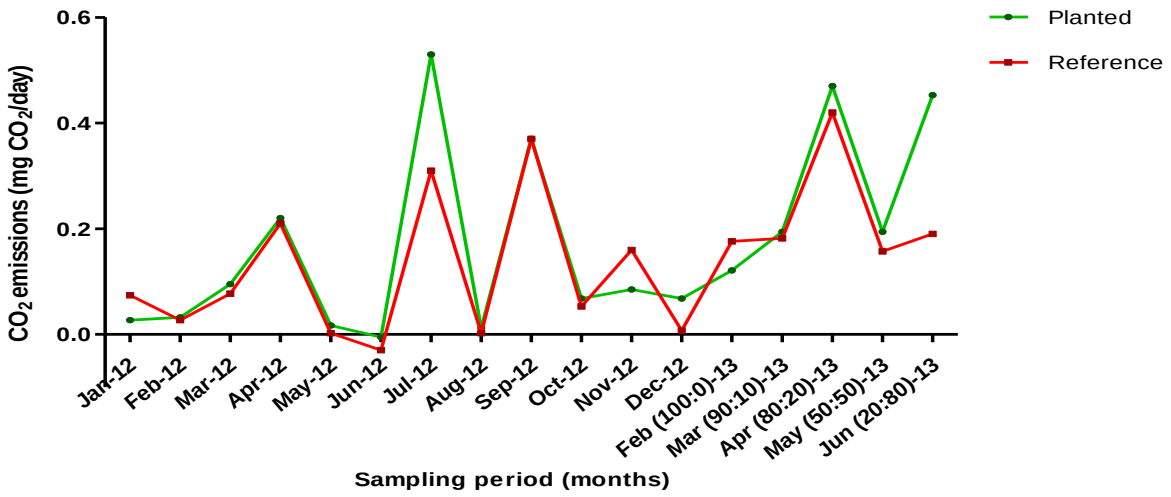

Figure 6.1: Means of carbon dioxide emissions obtained from January 2012 to June 2013 in the rhizofiltration system.

6.3.2 Methane Emission

It could be seen in Figure 6.2 that emission trends of methane were similar to CO_2 emissions, with the trend difference between the two gases observed in January 2012, where methane production was higher in the planted section compared to the reference section. Increased methane emission was also obtained in wastewater containing higher nutrient loading. In 2012, the planted section produced more methane. The ranges were between -0.002-0.171 mg/day, while in the reference section, they were -0.02-0.152 mg/d ($p < 0.05$). These results indicated

that methane was absorbed in the system during these periods. In 2013 a statistically significant difference was obtained between February and April (warm season), with the planted section producing more methane, the observations may be attributed to high methanogenic activities in summer.

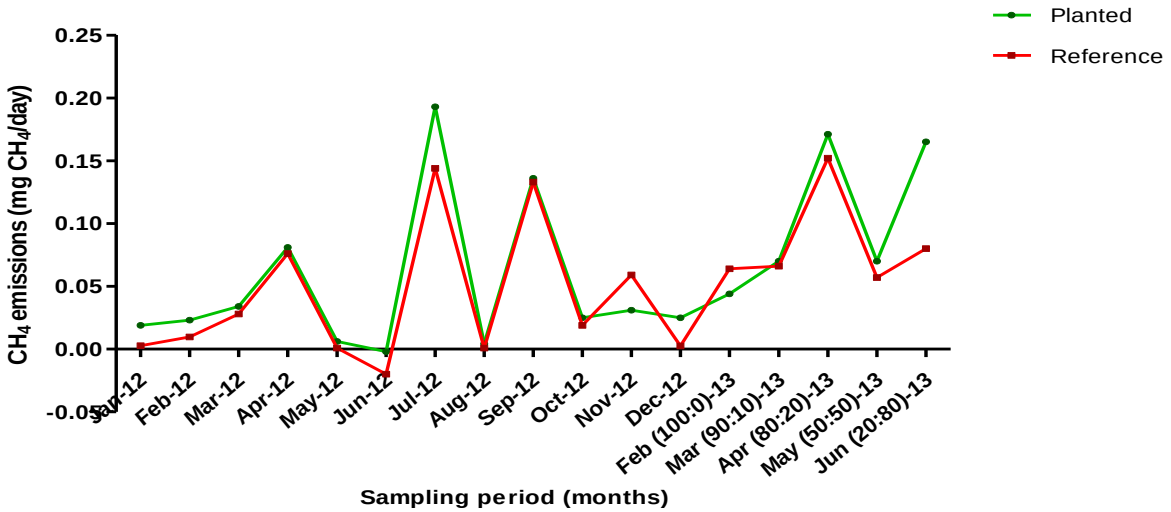

Figure 6.2: Means of methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013.

6.3.3 Nitrous Oxide emission

A trend of nitrous oxide emission in the rhizofilter showed an increase between January 2012 and June 2013 (Figure 6.3). While variable concentrations were obtained between July and December 2012, no seasonal variation could be established with the emission patterns obtained in the system. This is of particular interest since the nitrous oxide formation is strictly a microbially driven process. In 2013, a higher concentration of nitrous oxide was emitted by the system than in 2012 and this may be explained by high ammonia and nitrite concentrations obtained in the system (Figures 3.8 and 3.10 respectively).

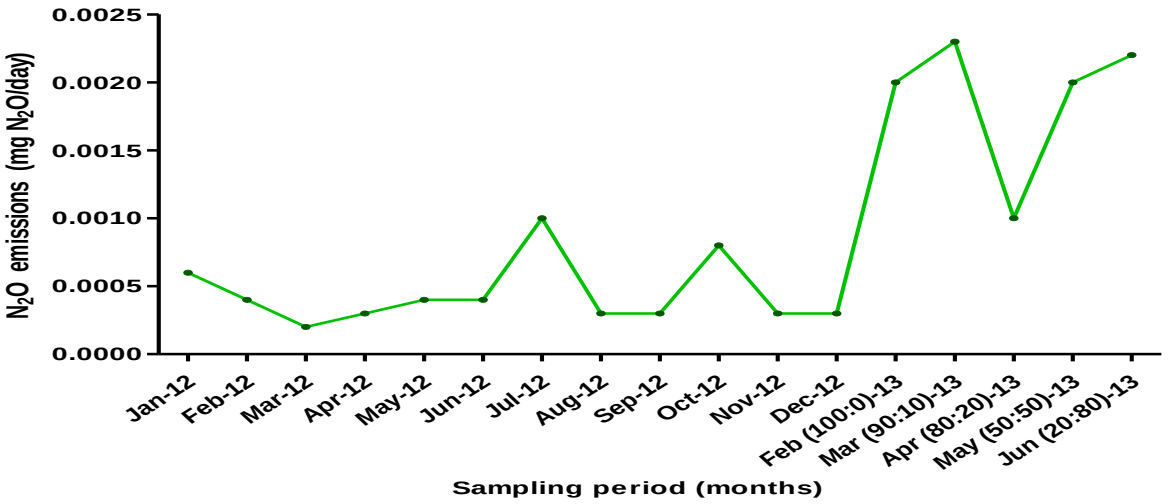

Figure 6.3: Means of nitrous oxide emission in the rhizofiltration system from January 2012 to June 2013.

6.3.4 Factors Affected the Emission of GHGs in the Rhizofilter System

There are several factors found to influence the production of the GHGs from the constructed rhizofiltration system. Physical parameters were monitored from the system and those found to have an impact in gas production are discussed below:

6.3.4.1 pH

Van der Star *et al*. (2007) pointed out losses of ammonium through volatilization from flooded soils and sediments to be insignificant at pH below 7.5 and very often the losses were found not to be significant when the pH was below 8.0. According to pH, high losses of ammonium through vitalization are not to be expected in constructed rhizofiltration systems. Most methanogenic bacteria function at pH between 6.7 and 7.4, but optimally around pH 7.0 and 7.2 (Wang *et al*., 2011). Gerard (2006) noted the rate of CH_4 formation in wastewater treatment plant decreased if the pH was lower than 6.3 or higher than 7.8. Results obtained in this study concurred with these reports since a decrease in quantities of methane emitted where the pH was higher than 7.8 or lower than 6.3 (Figure 6.5). High emissions were obtained at pH between 6.51 and 7.7. When the 2013 results were compared among themselves, May had lowest methane emissions, considering the high nutrient loading obtained in the samples (Figure 6.2). During these periods the pH was either lower than 6.3 or higher than 7.8. Highest emissions were obtained at pH between 7.4 and 7.68. A very poor negative correlation was obtained between carbon dioxide production and pH in 2012, while a moderate positive correlation was obtained in 2013. The trend of methane emission, as influenced by pH, was also observed with

carbon dioxide production from the rhizofiltration system. Production of methane, carbon dioxide and nitrous oxide are all microbial driven processes. Organism's activities are decreased by high pH values and thus unfavorable for their survival and functioning. While an increase in pH above neutral showed a decreased in carbon dioxide and methane in 2012, moderate positive correlation was obtained in 2013 when nutrient levels was high (Figure 6.5). Nitrous oxide emission decreased with increase in pH (Figure 6.6), with a rate of 0.2% decrease per unit pH increase.

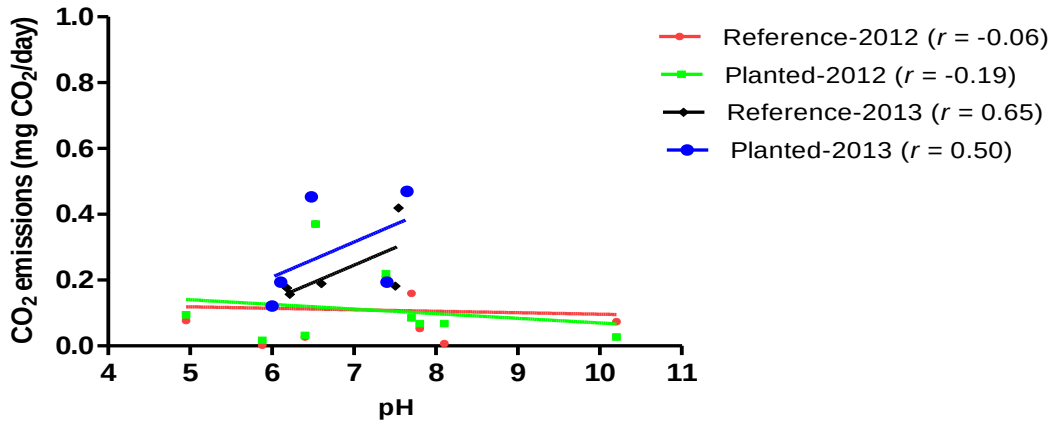

Figure 6.4: Effect of pH on carbon dioxide emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A linear curve fit was used to demonstrate the relationship between the pH and CO_2 emission in the rhizofiltration system.

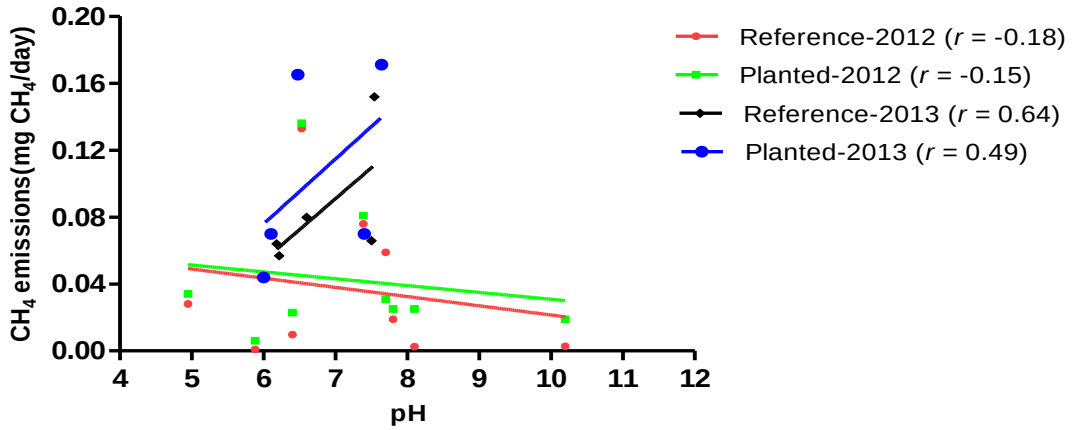

Figure 6.5: Effect of pH on methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A linear curve fit was used to demonstrate the relationship between the pH and CH_4 emission in the rhizofiltration system.

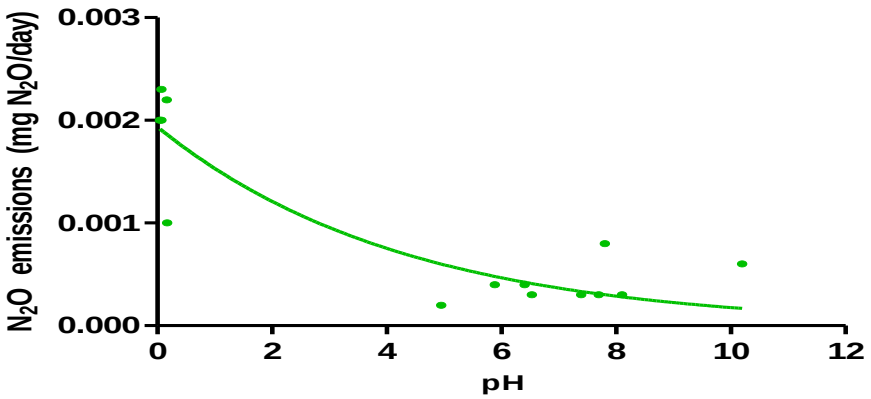

Figure 6.6: Effect of pH on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the pH and N_2O emission in the rhizofiltration system.

6.3.4.2 Temperature

A very weak negative correlation between temperature; methane (Figures 6.7) and carbon dioxide (Figures 6.8) emissions was observed in the system (positive correlations were obtained in 2013 in the reference section), while a weak positive correlation between temperature and nitrous oxide was obtained (Figure 6.9), with 0.1% nitrous oxide increase emission in the system per temperature increase. The rate of methane increase with an increase in temperature was 0.1% in the reference section in 2013, while that of carbon dioxide was 1.5%. Temperatures between 18 and 22^{o}C were optimal for carbon dioxide and methane production. Methane emissions decreased with an increase in wastewater temperatures. Gupta and Singh (2012) reported high temperatures favors methanogenesis which produced methane. The inhibition of methanogenesis at low temperatures has been reported in previous studies (Johansson *et al.*, 2004; Stradmark and Leonardson, 2005). Temperature supported organisms responsible for nutrient breakdown in the rhizofiltration system, and in the process, produced gases in the system.

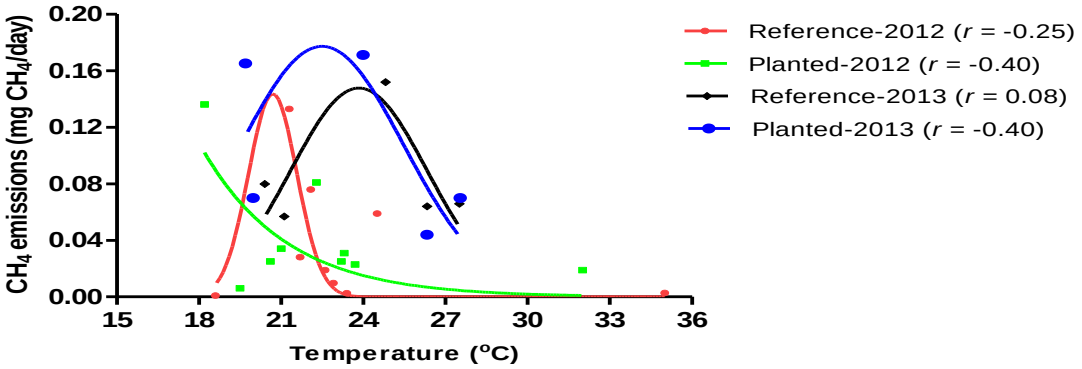

Figure 6.7: Effect of temperature on methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the temperature and CH_4 emission in the rhizofiltration system.

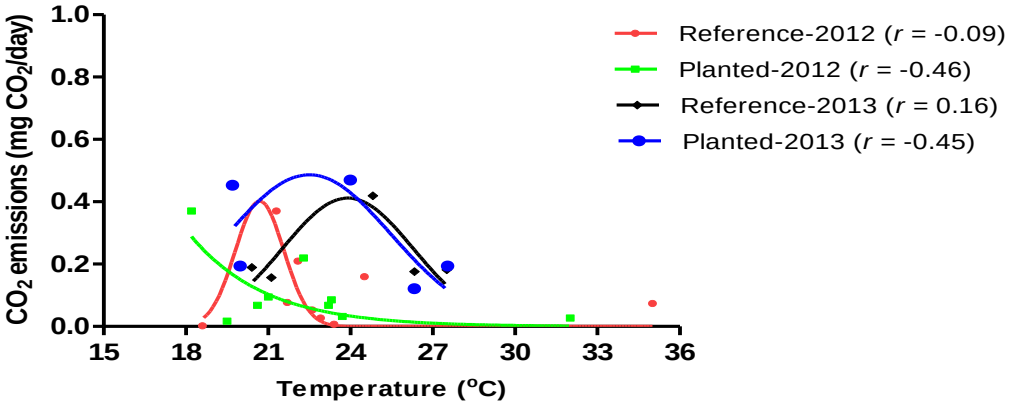

Figure 6.8: Effect of temperature on carbon dioxide emissions from January 2012 to June 2013 in a rhizofiltration system. A non-linear curve fitting was used to demonstrate the relationship between the temperature and CO_2 emission in the rhizofiltration system.

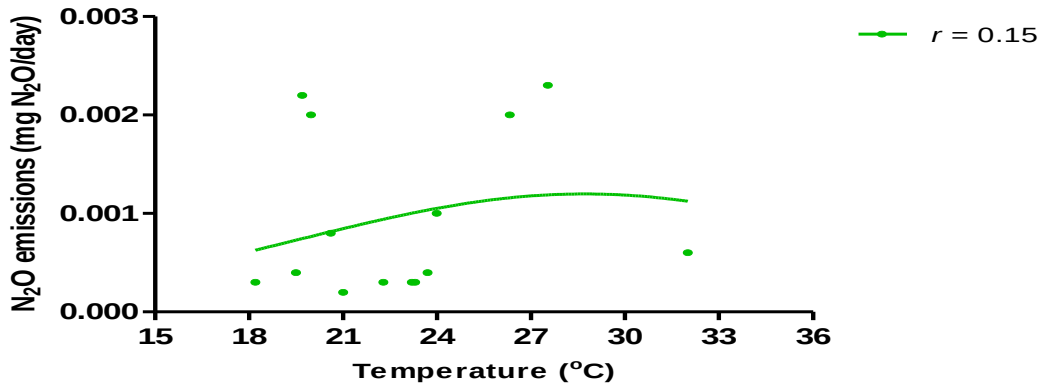

Figure 6.9: Effect of temperature on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the temperature and N_2O emission in the rhizofiltration system.

6.3.4.3 Salinity and conductivity

In the study by Waki *et al.* (2010) increased concentrations of salinity decreased the pH, which ultimately lowered the gases produced in the treatment of wastewater. In the present study it has been mentioned earlier that high concentrations of pH lowered the gases produced in the system. The pH of water changes with changes in type and concentration in a solution. Salinity readings obtained throughout the study were low. There was a poor correlation between carbon dioxide and methane with salinity (negative in 2012 while in 2013, it was negative reference section and positive in the planted section), with a positive correlation obtained in the planted section in 2013 (Figures 6.10 and 6.11 respectively). Carbon dioxide and methane are products of microbial metabolism and the results obtained in 2013 confirmed earlier suggestions that dissolved organic nutrients acted as an energy source and oxygen stimulated the growth of the halotolerant microorganisms, thus increasing the amount of produced carbon dioxide and methane. There was also a very poor negative correlation between salinity and nitrous oxide (Figure 6.12), where as a poor negative correlation between nitrous oxide and electrical conductivity was obtained (r = -0.45) (Figure 6.15). Nitrous oxide production is a microbially dependent process. These organisms have optimum pH ranges, below and above which the activities are decreased. While methane and carbon dioxide emission decreased with conductivity, a positive correlation was obtained between carbon dioxide (Figure 6.13) and methane (Figure 6.14), except in the reference section in 2013. .

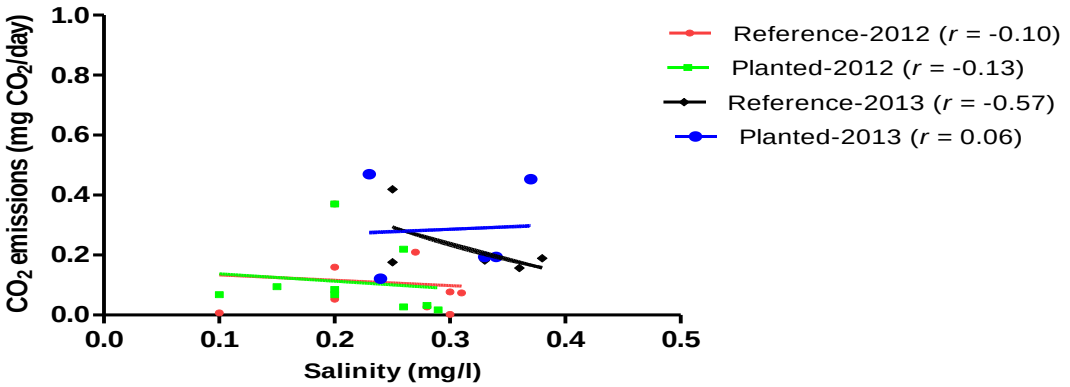

Figure 6.10: Effect of salinity on carbon dioxide emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A linear curve fit was used to demonstrate the relationship between the salinity and CO_2 emission in the rhizofiltration system.

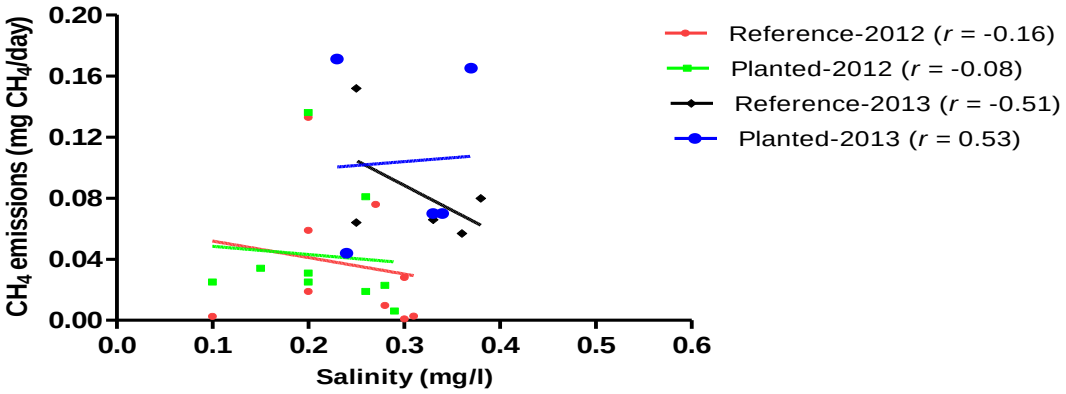

Figure 6.11: Effect of salinity on methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A linear curve fit was used to demonstrate the relationship between the salinity and CH_4 emission in the system.

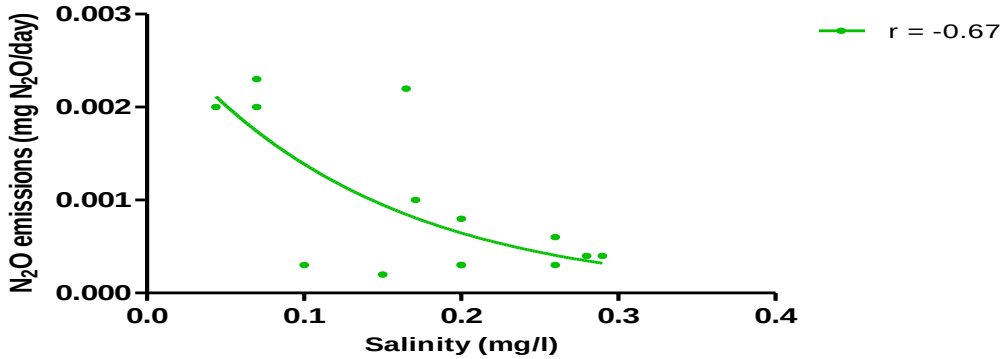

Figure 6.12: Effect of salinity on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the salinity and N_2O emission in the rhizofiltration system.

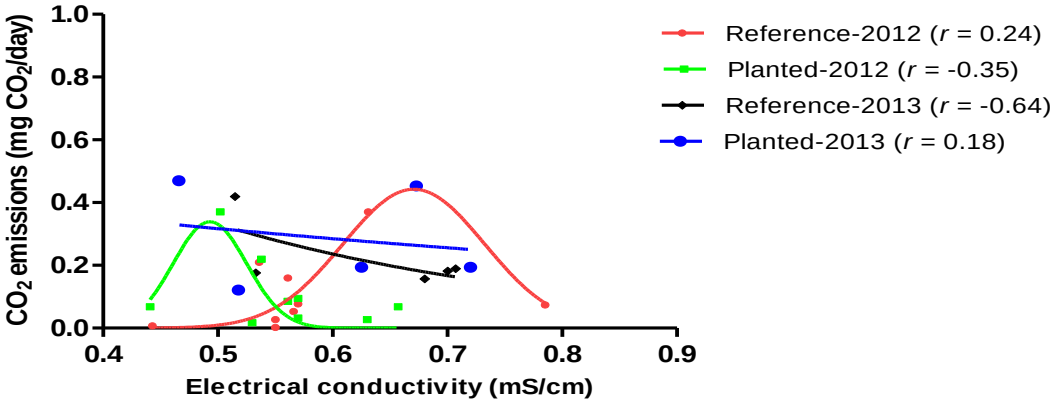

Figure 6.13: Effect of electrical conductivity on carbon dioxide emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A non-linear curve fit was used to demonstrate the relationship between conductivity and CO_2 emission in the rhizofiltration system.

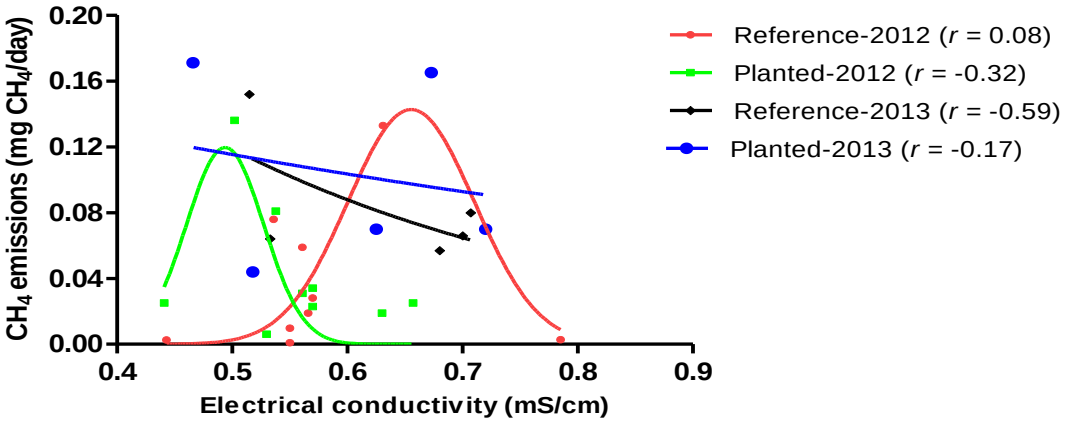

Figure 6.14: Effect of electrical conductivity on methane emissions in the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between conductivity and CH_4 emission in the rhizofiltration system.

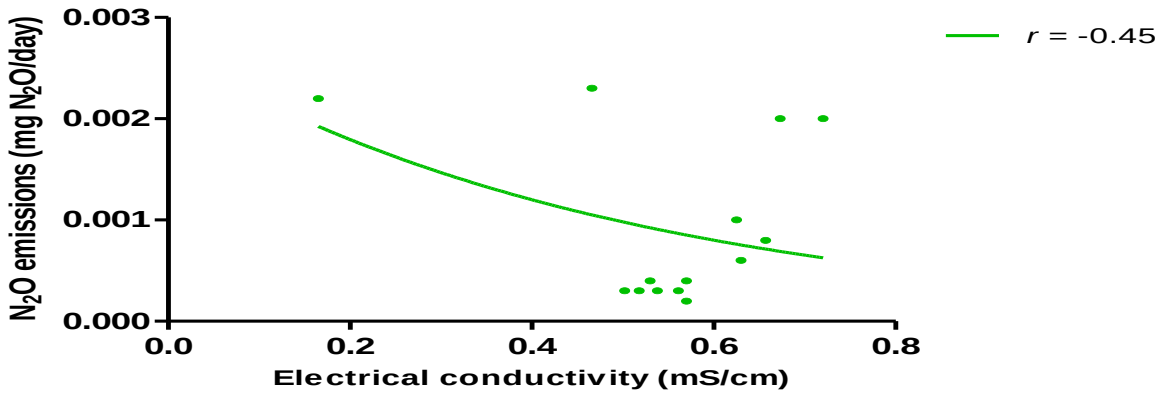

Figure 6.15: Effect of electrical conductivity on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the conductivity and N_2O emission in the rhizofiltration system.

6.3.4.4 Total organic carbon concentration on carbon dioxide and methane

Total organic carbon in wastewater positively correlated with carbon dioxide (Figure 6.16) and methane (6.17) emission, except in the reference section in 2012. While this correlation was very weak in other samples, it was moderate in the planted section in 2013. The rate of carbon dioxide increase per TOC increase in 2012 was 0.2% and 0.17% in the reference and planted section respectively; while in 2013 it was 6.3% in the planted section. Total organic carbon involved oxidation by combustion at higher temperatures and resulted to the formation of carbon dioxide and methane. Though there was a decrease in threshold value, the rate of methane increase per TOC increase in 2012 was 1.1% and 1.9% in the reference and planted section respectively; while in 2013 it was 0.7% and 6.3 respectively.

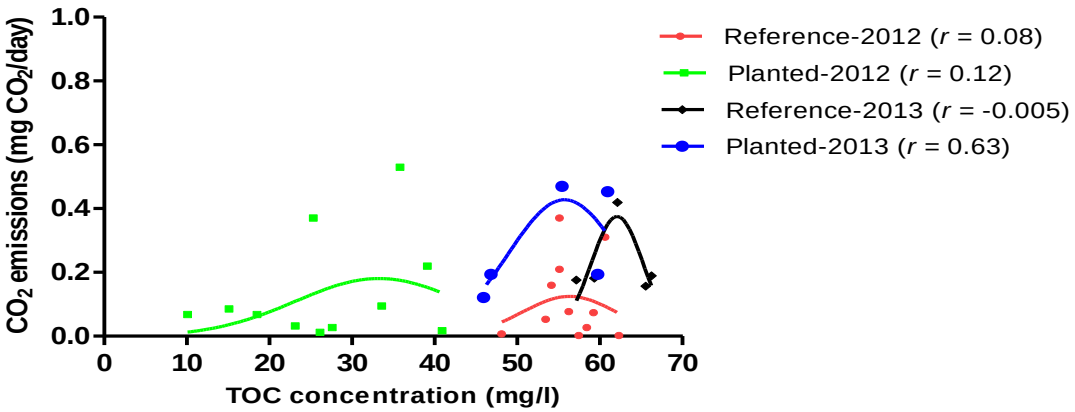

Figure 6.16: Effect of TOC on carbon dioxide emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A non-linear curve fit was used to demonstrate the relationship between TOC and CO_2 emission in the rhizofiltration system.

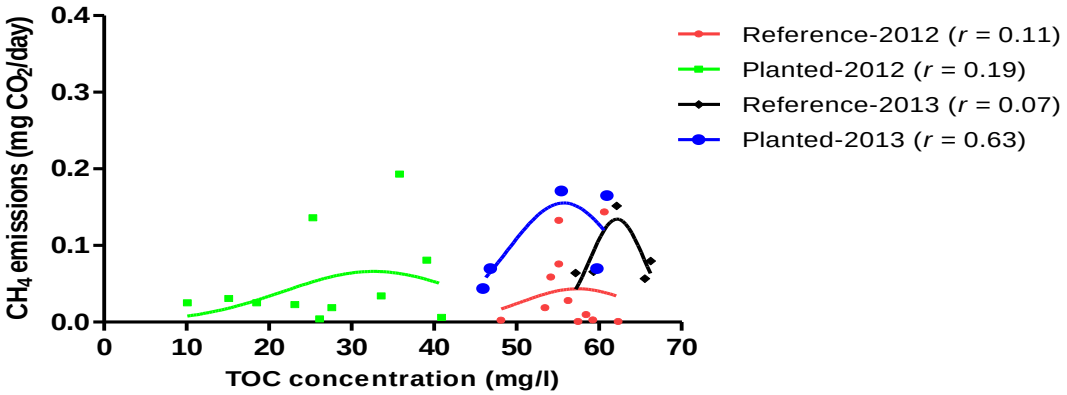

Figure 6.17: Effect of TOC on methane emissions against from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between TOC and CH_4 emission in the rhizofiltration system.

6.3.4.5 Dissolved oxygen

While the increased concentrations of the dissolved oxygen promoted high carbon dioxide (Figure 6.18) and methane emission (very poor positive correlation)(Figures 6.19), the emission of nitrous oxide inversely correlated weakly to dissolved oxygen (Figures 6.20). The rate of carbon dioxide increase per DO increase in 2012 was 1% in both the reference and planted section; while in 2013 it was 1.2% and 1.9% respectively. The methane emission rate was 1% and 0.2% in the reference and planted section respectively in 2012, while in 2013 it was 4% and 3% respectively. The correlation of methane and carbon dioxide with the DO was because in the aerobic layer of the rhizofilter system, the DOC was converted to bicarbonate and latter to carbon dioxide and methane. This process was faster in the planted section, with increased abundance of microorganisms (Figures 4.4; 4.7 and 4.8). In February, November and

December 2012, the DO was low. The absence of oxygen restricted nitrification and low supply of nitrite limited denitrification, hence lowered the efficiency of nitrite removal (Crites, 2008).

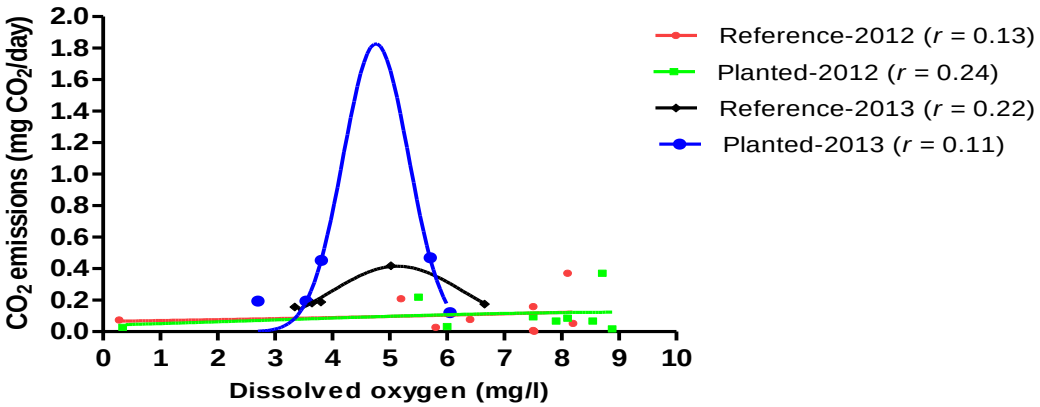

Figure 6.18: Effect of DO on CO_2 emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A linear (2012) and non-linear (2013) curve fit was used to demonstrate the relationship between the DO and the CO_2 emission in the rhizofiltration system.

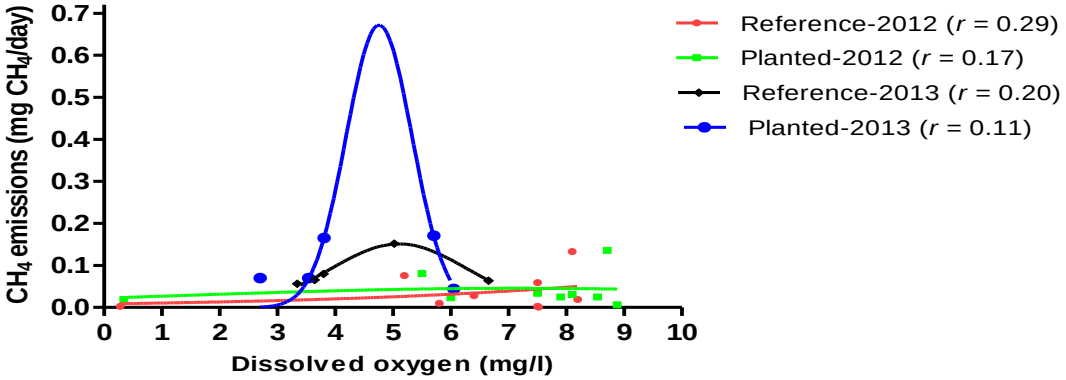

Figure 6.19: Effect of DO on methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A linear (2012) and non-linear (2013) curve fit was used to demonstrate the relationship between the DO and the CH_4 emission in the rhizofiltration system.

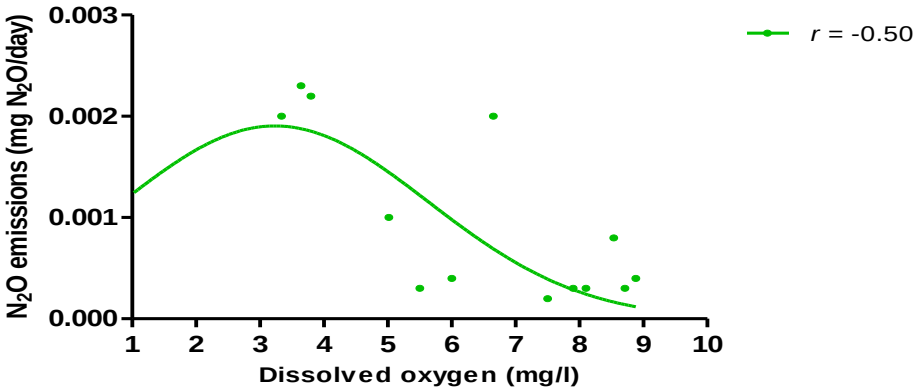

Figure 6.20: Effect of DO on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A linear curve fit was used to demonstrate the relationship between the DO and N_2O emission in the rhizofiltration system.

6.3.4.6 Chemical oxygen demand

Chemical oxygen demand concentrations varied seasonally in wastewater which led to the variation in carbon dioxide and methane emission. Chemical oxygen demand removal positively correlated (strong correlation in the planted section) to CO_2 and methane emissions (Figures 6.21 and 6.22 respectively), except in the reference section in 2012. Methane emission rate was 0.2% and 1% in the reference and planted section respectively in 2012, while in 2013 it was 0.1% and 3% respectively. The decrease rate of carbon dioxide in the reference section in 2012 was 0.1%, while an increase emission rate was 4% in the planted section. In 2013, the rate of increase of carbon dioxide emission with increased COD was 2 and 8% in the reference and planted sections respectively. A negative correlation obtained when COD was compared to nitrous oxide emission in the system was very poor (Figure 6.23), with 2% nitrous oxide decrease rate as COD concentration increase. Concentrations of chemical oxygen demand in the system and subsequent gas formation was influenced by the presence of macrophytes. Macrophytes supported the diverse microbial population that aided in breakdown of organic matter and COD into gases. Macrophytes in the planted section provided a large surface area for the attachment of microbial biofilms which offered diverse microbial processes (Wang *et al*., 2008).

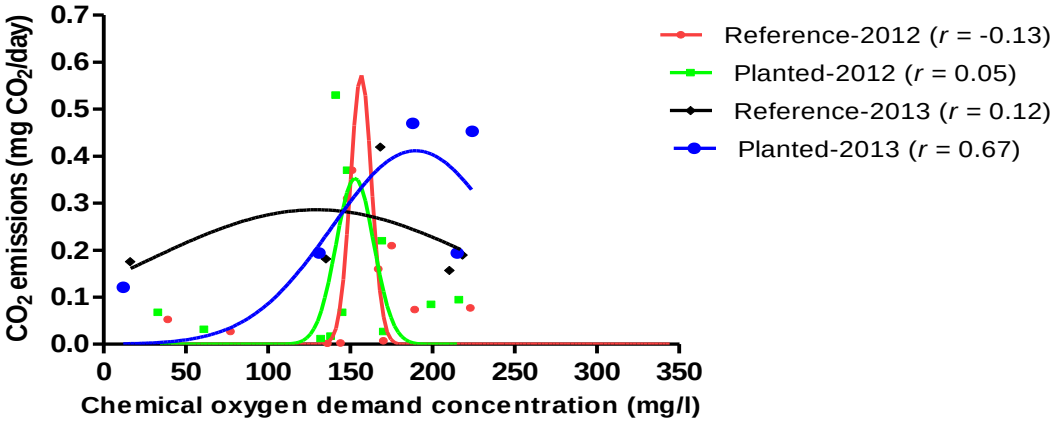

Figure 6.21: Effect of COD on carbon dioxide emissions obtained from January 2012 to June 2013 in a rhizofiltration system. A non-linear curve fit was used to demonstrate the relationship between the COD and CO_2 emission in the rhizofiltration system.

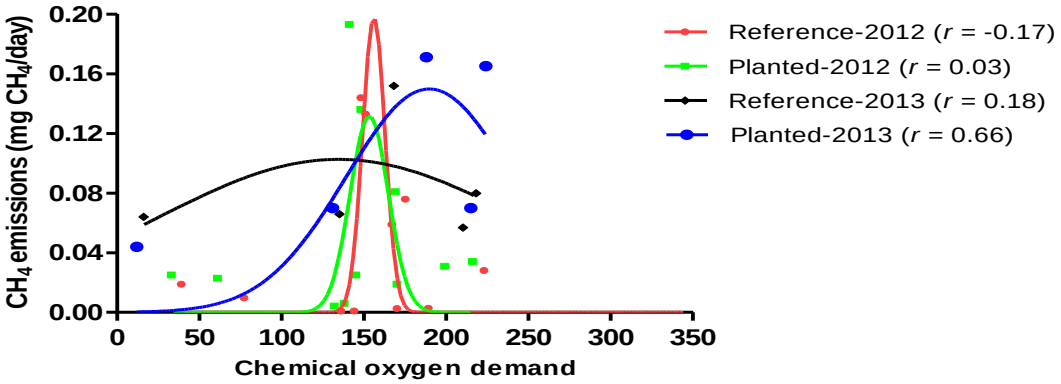

Figure 6.22: Effect of COD on methane emissions from the planted and reference sections of the rhizofiltration system from January 2012 to June 2013. A non-linear curve fit was used to demonstrate the relationship between the COD and CH_4 emission in the system.

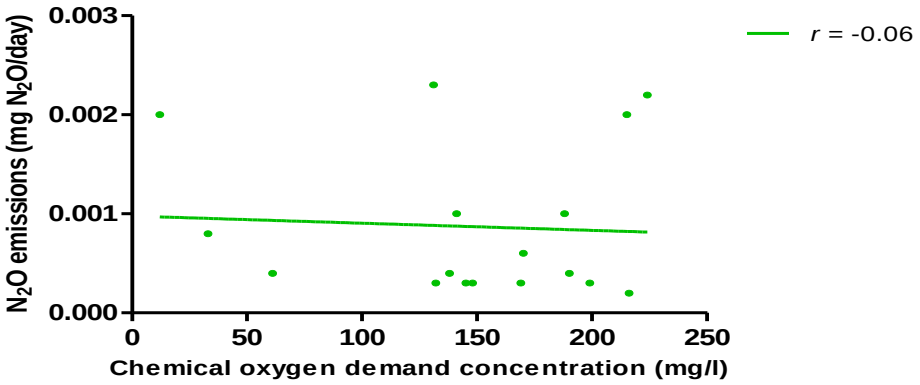

Figure 6.23: Effect of COD concentrations on nitrous oxide emission in a rhizofiltration system from January 2012 to June 2013. A linear curve fit was used to demonstrate the relationship between the COD and N_2O emission in the rhizofiltration system.

6.3.4.7 Effect of nitrate and nitrite removal efficiencies on GHGs emissions

Nitrous oxide production negatively correlation weakly to nitrate (Figure 6.24) and also had a very poor positive correlation to nitrite reduction efficiency as well (Figure 6.25). Studies are also available that reported high nitrate concentrations to effectively inhibit methane production in wastewater treatment process (Mohanakrishnan *et al.*, 2008; Banihani *et al.*, 2009). The rate of nitrous oxide formation as nitrate removal efficiency increase was 0.1%, while with nitrite removal efficiency; the emission rate was 3%. Nitrate also increased nitrous oxide formation during both nitrification and denitrification processes (Cornel and Krause, 2006).

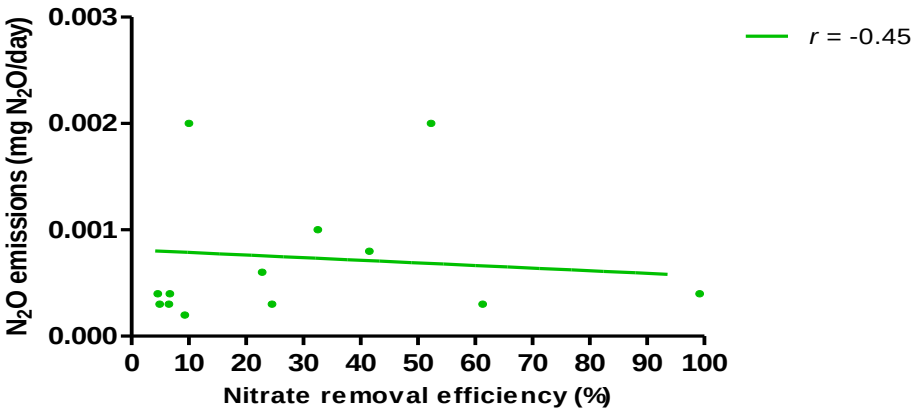

Figure 6.24: Effect of nitrate removal efficiency on nitrous oxide emission in wastewater treatment using rhizofiltration technology. Means of nitrate removal efficiency in the planted and reference sections were used against their corresponding means of nitrous oxide from January 2012 to July 2013 in the rhizofilter system. A linear curve fit was used to demonstrate the relationship between the nitrate and N_2O emission in the rhizofiltration system.

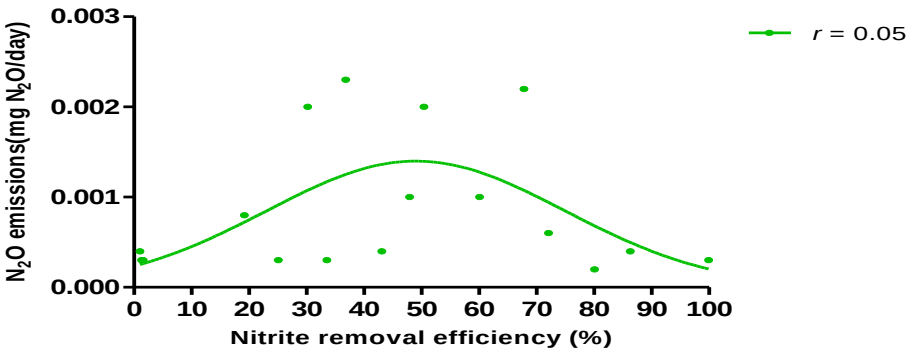

Figure 6.25: Effect of nitrite removal efficiency on nitrous oxide emission in wastewater treatment using rhizofiltration technology. Means of nitrite removal efficiency in the planted and reference sections were used against their corresponding means of nitrous oxide from January 2012 to July 2013 in the rhizofilter system. A non-linear curve fit was used to demonstrate the relationship between the nitrite and N_2O emission in the rhizofiltration system

6.3.4.8 Effect of ammonia removal efficiency on nitrous oxide emission

Different levels of nutrient concentration in wastewater resulted in different emissions of GHGs in the rhizofilter. When ammonia removal efficiency was high, gas formation also increased (a moderate positive correlation) (Figure 6.26), with 0.1% rate of nitrous oxide emission with an increase in ammonia. In 2012 pre-treated wastewater was analyzed. These samples were found to contain low concentration of nutrients. However in 2013 pre-treated wastewater was diluted with raw sewage, therefore constantly increasing the concentrations of nutrient loading in wastewater in the system. The result was a higher emission of gases in year

2013 compared to 2012. The increased in gas formation and production from the constructed rhizofiltration system was caused by the increased addition of raw sewage (diluents) in wastewater. A constant addition of raw sewage through wastewater dilution increased the organic matter to be degraded by microorganisms, thereby producing the gases.

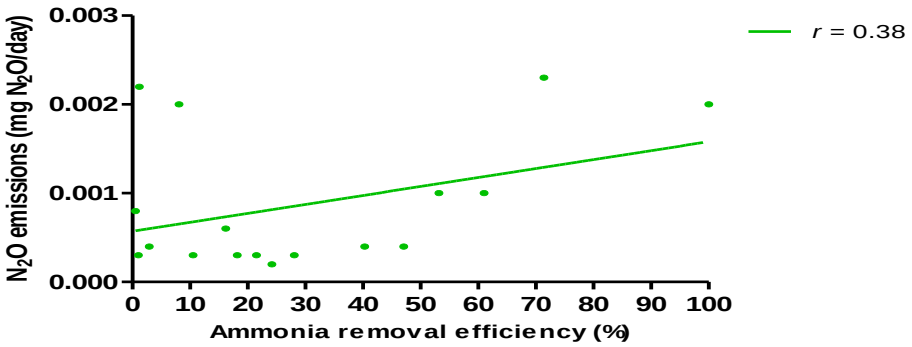

Figure 6.26: Effect of ammonia removal efficiency on nitrous oxide emission in wastewater treatment using rhizofiltration technology. Means of ammonia removal efficiency in the planted and reference sections were used against their corresponding means of nitrous oxide from January 2012 to July 2013 in the rhizofilter system. A linear curve fit was used to demonstrate the relationship between the ammonia and N_2O emission in the rhizofiltration system.

6.4 DISCUSSION

Carbon dioxide and methane emissions were quantified directly from the COD of wastewater. While the emitted carbon dioxide may have been produced from microbial degradation of TOC and macrophyte expiration, methane emissions resulted from anaerobic decomposition of organic matter present in wastewater. Methane was produced from the activities of methanogens and the rate of methanogenesis was primarily dependent on the concentrations of the wastewater COD (Figure 6.22). Nitrous oxide is associated with breakdown of nitrogen components commonly found in wastewater. Its production in the rhizofiltration system was a result of the two-phase process that involved *Nitrosomonas* spp. These nitrifying bacteria oxidized ammonia to form nitrate while denitrifying bacteria reduced nitrate into nitrogen gas, which was then released into the atmosphere. Nitrous oxide may have been released in both ammonia oxidization and nitrate reduction processes; though it is mainly associated with denitrification (Guisasol *et al*., 2008; Listowski *et al*., 2011).

The presence of macrophytes and microorganisms were the two main factors that controlled organic carbon availability and degradation during wastewater treatment in a rhizofiltration

system. Macrophytes may decrease (Wang *et al*., 2008) or increase (Zhang *et al*., 2011; Wild *et al*., 2001) the GHGs emissions. In the rhizofilter, macrophytes activity increased GHGs emissions. They stimulated the production of carbon dioxide. These observations were found in the planted section where the total carbon dioxide and methane emissions were high in the planted section compared to the reference section (Figures 6.1 and 6.2). Differences obtained in the planted and reference sections were mostly due to the difference in oxygen levels in these two sections. The presence of macrophytes in the planted section increased the level of oxygen. Methanogens are obligate anaerobes, thus high oxygen concentrations in the system inhibited the emissions of methane (Wang *et al*., 2011). While macrophytes in this rhizofiltration system contributed to GHGs production through creation of a suitable environment, microorganisms produced the gases through nutrient degradation, and subsequent removal from wastewater. Apart from supplying oxygen, macrophytes offered a large surface area for microbial attachment in the rhizosphere. *Nitrobacter* and *Nitrosomonas* spp. degraded ammonia and produced nitrate and nitrite, and nitrate being converted to nitrous oxide in the process (Figure 6.26).

Greenhouse gases production in the rhizofiltration system was seasonal; with more gas produced in warm climatic conditions than in the winter season, though higher than normal temperatures did not support gas formation (Figures 6.7 – 6.9). Gas formation and production also coincided with the growing season of the macrophytes, with more gases produced during macrophyte growing seasons in the planted section than during non-growing seasons. This further signified the importance of macrophytes in gas production from the system. High microbial diversity was further observed during the active season of the macrophytes, and these contributed to gas formation due to their nutrient degradation and transformation abilities. High levels of contaminated wastewater produced more gases (Figure 6.26). These observations were made in 2013 where increased nutrient loading was constantly introduced into the system through increased raw sewage in the dilutions. The results were an increased in gas produced per day in the system compared to the year 2012 results where undiluted pre-treated wastewater was used (Appendix 5).

Constructed rhizofiltration systems are complex bioreactors in which the removal of pollutants occurs by means of physical, chemical and biochemical processes (Yan *et al*., 2012; Wang *et al*., 2008). Methane and nitrous oxide emissions (based on mass units) corresponded to 0.25 and 2.98 CO_2 equivalents respectively, with time horizon of 100 years (Uggetti *et al*., 2012a,

Uggetti *et al*., 2012b). Taking into account the mean emissions values in reference and planted section, they correspond to 0.09 and 0.25 kg CO_2eq/m^2d, respectively. These values are among the lowest emissions in wastewater treatment considering that in 2012 the samples were collected throughout the year. Fuchs *et al.* (2001) demonstrated that CH_4 and N_2O emission from constructed wetlands was significantly higher during summer than in winter in his study based on seasonal variation of GHGs production in wetlands. However, longer measurement period in this study provided a more precise GHG emissions inventory.

Gas emissions in the rhizofilter were far lower when compared to emission from sludge treatment reed beds and other conventional systems of wastewater treatments (Fuchs *et al*., 2001; Correa *et al*., 2007). In a reed bed system, methane emissions was reported to range between 1000 and 3 700 mg CH_4/ m^2d^{-1} while nitrous oxide may range between 200 and 750 mg N_2O/ m^2d^{-1} (Kampschreur *et al*., 2009; Uggetti *et al*., 2012a). Uggetti *et al*. (2012b) reported methane emission to range between 10 and 5 400 mg CH_4/m^2d^{-1} while nitrous oxide emission ranged between 20 and 950 mg N_2O/m^2d^{-1} from sludge treatment wetlands. Activated sludge and anaerobic digestions have been reported to average between 1200 CH_4/m^2d^{-1} and 4500 CH_4/m^2d^{-1} while nitrous oxide averaged between 500 $m^2d^{-1}$and 1300 m^2d^{-1}. It could therefore be concluded that constructed rhizofiltration systems have little or no impact on greenhouse production when compared to other convectional wastewater treatment systems.

6.5 CONCLUSIONS

- The constructed rhizofiltration system was not a significant source of GHGs production. The system produced minute quantities of methane, carbon dioxide and nitrous oxide compared to other conventional systems.
- The presence of macrophytes in the planted section of the rhizofilter increased the GHGs emissions. More gases formed in the planted section were due to high microbial abundance, which utilized organic nutrients and transformed them into gases.
- Gases formation was affected by physical and chemical parameters, as well as by the presence of macrophytes in the system. Macrophytes and different levels of contaminants in wastewater differentially influenced the conditions by controlling the rate and production of GHGs, with more GHGs produced in the system containing high nutrient levels and macrophytes.
- Gas formation was seasonal, with warm temperatures supporting more gas formation.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 INTRODUCTION

Wastewater treatment is a growing concern worldwide, with issues spanning environmental, economic, and fiscal sectors. Of specific concern is the contamination of groundwater with heavy metals as a side effect of societal and industrial pollution. While moderate quantities of certain contaminants are acceptable for consumption, larger quantities may lead to acute poisoning (Dushenkov *et al.*, 1995), especially in countries where water resources are scarce and/or treatment and purification techniques are primitive. New technologies are a necessity to prevent these serious health crises.

Considering alternatives, rhizofiltration technology was cost effective and treated wastewater to the required effluent discharge standards, with low labour requirements. Large and established facilities may reduce costs by moving to this natural water treatment technique as an alternative to current methods while small populations devoid of such resources can apply rhizofiltration on a local and manageable scale. In the case of small populations, no new infrastructure would be necessary as macrophytes rhizofiltration may be applied directly to groundwater sources. The current state of the art in wastewater treatment systems generally involves significant investments in terms of financial strains and infrastructure where before any treatment occurs, water must be moved from its native location, often requiring an initial investment in kilometers of pipeline. Once at the treatment plant, a myriad of techniques ranging from aeration, digestion, flocculation, sedimentation, filtration, sludge treatment and disinfection are applied before reaching a final product with each step requiring additional technology and manpower to operate. While the complete process is effective, the resources needed to carry out this process are too great for certain populations. For example, populations in third world countries lack the fiscal acuity to invest in the infrastructure required for such extensive treatments. While current estimates for the construction of a fully operation filtration system range into the hundreds of millions of rands depending on the volume of water to be treated and the level of treatment needed, this was in stark contrast to the tens of rands per thousands of gallons of water treated via rhizofiltration system. This cost effective advantage was the result of the ease with which this plant-based filtration system was implemented. There was no heavy industry required to build this system, potentially permitting effective system

construction and use anywhere. The system was self-sustaining, as long as sufficient nutrients and sunlight for macrophytes and biofilms were available.

7.2 NUTRIENT REMOVAL PROCESS

Up to 60% nitrogen and 80% phosphorus were removed from the rhizofiltration system through the combination of efforts by microbial biofilm and macrophytes. Using the total Kjeldahl nitrogen measurement method, it was found that of the 40.3-61.3% of the total nitrogen removed from the system, 9% was removed by macrophytes and the remaining, primarily by nitrification/denitrification and sedimentation (Bigambo and Mayo, 2005; Lee *et al*., 2009). Bigambo and Mayo (2005) reported 48.9% total nitrogen removal in a constructed wetland with 29.9% removed by denitrification, 10.2% by macrophyte uptake and 8.2% by net sedimentation. Lee *et al*. (2009) reported that the denitrification processes may remove 60-70% of total nitrogen and 20-30% may be removed by macrophyte uptake in surface flow constructed wetlands. In the current study the retention time may have limited the denitrification process. During the establishment of the system, it was discovered that as wastewater was run over the system, it ended up creating defined path-ways within the system which allowed wastewater to pass directly through the system to form the effluent. Short-circuiting enabled a direct measure of effluent nutrients from the influent. Other researchers have reported short-circuiting and ineffective spreading of the influent water as it passes through the wetland system (Drizo *et al*., 2002; Mitsh and Jorgensen, 2004). In some instances, water will remain in backwater locations for more time than the theoretical retention time, and at other times it will flow faster through the wetland than the hydraulic retention time (Mitsh and Jorgensen, 2004). Total phosphorus was removed primarily by adsorption and precipitation reactions (Richardson, 1985; Drizo *et al*., 2002). Phosphorus effluent values varied widely due to pH shifts in the rhizofiltration system, especially during and after rain events. Both nitrogen and phosphorus were positively and negatively influenced by different physicochemical parameters. These either increased or decreased the removal in water.

7.3 EFFECT OF PHYSICAL AND CHEMICAL PARAMETERS ON NUTRIENT REMOVAL

This study investigated the effect of temperature, pH, salinity, electrical conductivity, dissolved oxygen, total dissolved solids and chemical oxygen demand on the removal of total nitrogen and phosphorus from the constructed rhizofiltration system. The rhizofiltration system was

located in a subtropical coastal region with high humidity and rainfalls. During summer seasons, high treatment efficiency was obtained, with nitrogen and phosphorus showing high reduction from the rhizofiltration system. High treatment values obtained was helped by high microbial population and diversity obtained during those periods. During warm seasons the rhizofiltration system created suitable conditions for microbial nutrient biodegradation, transformation and removal from the system. Thus microbial biofilms in the system improved the reduction efficiencies of nutrients. Increased nutrient loading led to decreased nutrient removal in the rhizofilter. Highly contaminated water contained increased concentration of electrical conductivity and salinity and other contaminants which did not allow the maximum and optimum performance of the biofilms in the system. Decreased microbial diversity led to low nutrient transformation, which affected their removal in the system. Nutrient removal obtained in the system showed that constructed rhizofiltration system technology was effective and could be used in wastewater treatment for nutrient reduction in wastewater containing low concentrations of nutrients. The system performed well when it was loaded with influent containing low concentrations of nutrients. However, it required maintenance as the decay roots and leaves resulted to an increase in nitrogen removal.

7.4 THE ROLE OF MICROBIAL BIOFILMS IN NUTRIENT REMOVAL

Microbial communities in the constructed rhizofiltration system were dominated by organisms that transformed nitrogen and phosphorus in the rhizofilter. Phosphate accumulating bacteria and nitrobacter were the main functional groups found in the system. The abundance of these organisms was high in the planted than in the reference section. The presence of these organisms in high numbers resulted in high removal of nitrogen in the planted section. Organisms responsible for phosphate removal were found in both planted and reference section. This could be due the fact that these organisms may also use nitrate as a source of electrons because of their ability to function under various condition such as aerobic and anaerobic conditions. Microbial activities (in this case measured by nutrient transformation) were found to be affected by pH, temperature, dissolved oxygen and nutrient concentrations in wastewater. Removal of phosphate and nitrogen in a form of ammonia was supported by high temperatures while nitrate removal was promoted at low temperature conditions.

7.5 THE ROLE OF MACROPHYTES IN NUTRIENT REMOVAL

Macrophytes used in rhizofiltration system had the ability to remove up to 60% of their dry weight as contaminants (Schnoor, 1997). The study has shown that there was a significant variation in the removal of nutrients between the planted and reference sections, with more nutrients removed in the planted section, which was caused by the effect of macrophytes in this section. *Phragmites australis* and *K. nemoralis* in the planted section improved wastewater treatment through nutrient uptake, oxygen supply as well as though microbial biofilm development with large surface areas. Macrophytes incorporated nutrients after being transformed by microorganisms in their own cell mass. This led to the growth of macrophytes. This process was high/fast during warm seasons, which corresponded to the fast growing seasons of the macrophytes, which also corresponded to the high treatment levels of wastewater. *Phragmites australis* showed greater efficiency toward the uptake of nitrogen while *K. nemoralis* showed greater efficiency towards phosphorus uptake throughout the study. Nutrient removal was positively influenced by dissolved oxygen concentration. The concentration of DO was higher in the planted than in the reference section of the rhizofiltration system. This DO was introduced by macrophytes, and also affected microbial diversity obtained in the two sections of the rhizofilter unit. Continuous harvesting of dead macrophytes component must be considered in order to reduce nutrient cycle which resulted to high bioaccumulation of residual nutrients in the system.

7.6 GREENHOUSE GAS PRODUCTION IN THE RHIZOFILTRATION SYSTEM

Both the planted and reference sections of the rhizofiltration system produced GHGs, with higher productions obtained in the planted section. The higher amounts of GHGs produced in the planted section was accompanied by higher treatment efficiency of wastewater when compared to the reference section. Gas production was seasonal with higher production obtained during warm seasons. High concentrations of nutrient loading in wastewater promoted more gas emission. When comparing conventional systems of wastewater treatment to constructed rhizofiltration, the rhizofiltration system produced very low quantities of GHGs, even when wastewater containing high nutrient load was used. Thus rhizofiltration system could be used as a greener technology for wastewater treatment with very little to no impact on gas production in the environment. The use of such systems may contribute to the prevention of global climate change and thus, significantly lower the effects caused by GHGs.

7.7 CONCLUSIONS

The use of constructed rhizofiltration technology in the treatment of wastewater is relatively new. However, the impressive results achieved thus far have prompted great expectations about this technology and about what it can still achieve. The rhizofilter can be used in a sustainable manner, by defining a clear design objective for the system to achieve its ultimate goal and close monitoring to assess the performance of the system and to ensure high treatment efficiency. The rhizofiltration system configuration, media, the macrophytes used, microbial biofilms formed, system layout and influent concentrations all played a role in determining the success of the system. Physical and chemical parameters differentially affected the removal of nutrients in the system. High success in the removal of nitrogen and phosphorus using a combination of the synergistic effect of macrophytes and microbial processes was achieved in the planted section of the system. This technology acted as a form of phytoremediation and used plant roots and associated microorganisms to remove target contaminants from water. Through absorption and eventual sequestering of specified contaminants by microorganisms and macrophytes, nutrients were efficiently removed from wastewater making water suitable for safe disposal. The system was also environmentally friendly due to its low GHGs production, making the system suitable to be used as a green technology in wastewater treatment.

7.8 RECOMMENDATIONS

It is recommended that since this technology is relatively new in terms of practical applications in industrial and municipal environments, there is a need for continuous research and development for sustainability and viability under various conditions, including applicability to different types of wastewaters, effectiveness under different climatic conditions and the use of different materials and macrophytes. The performance of existing constructed systems should be carefully monitored and additional research is required to optimize design and minimize construction costs. Local governments as well as international organizations involved in water and wastewater sector should promote this technology by building local capacity, and scaling up for more applications. The technology should be given a chance to prove its worthiness to local communities that may need it the most. This should be accompanied by extensive research to assess its performance.

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APPENDICES

Appendix 1: Publications Emanating from this Study

academicJournals

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African Journal of Biotechnology

Review

Constructed wetlands: A future alternative wastewater treatment technology

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Wastewater treatment will always pose problems if there are no new alternative technologies in place to replace the currently available technologies. More recently, it has been estimated that developing countries will run out of water by 2050. This is a course for concern not only to the communities but also a challenge to the scientist to find new ways of wastewater recycling. Water losses can be avoided through implementation of easy and inexpensive technologies for wastewater treatment. Environmental concerns over insufficiently performing septic systems and high expenses in the construction of sewer systems as well as their operations with centralized water purification systems have spurred investigation into the appropriateness of the use of wetland technology for wastewater treatment. Constructed wetland efficiency and potential application in wastewater treatment has been reported decades ago. However, the logistics and research for their commercial applications in wastewater treatment has not been documented in details. Research has shown that wetland systems can achieve high treatment efficiencies with regards to both organic and inorganic nutrients as well as pathogen removal if properly managed and efficiently utilized. This can have a profound effect in the management and conservation of our scarce and yet depleting water resources.

Key words: Constructed wetlands, rhizofiltration, microbial biofilms, wastewater treatment, treatment mechanism.

INTRODUCTION

South Africa is made up of approximately 850 municipal wastewater treatment plants, yet according to research by the South African Department of Water Affairs, less than 50% of the 449 wastewater treatment systems which have been assessed meet the regulatory national and international water quality standards for wastewater treatment. These findings are proof that South Africa's wastewater treatment systems are inadequate to meet the effluent required standards. This has resulted in the urgent need for the development and implementation of innovative systems to resolve the wastewater treatment constraints (Kalbar et al., 2012a). It is for this reason that interest has been sparked into the investigation of alternative wastewater treatment technologies for the treatment of wastewater. Constructed wetland systems are a good example of such alternative technologies which have the potential to meet the required influent treatment standards as compared to conventional methods. They are an old technology dating from wetland technology which was dated back in 1952 (Siedel, 1973) and has been in full scale operation from 1974 (Kickuth, 1977). The technology was developed through the

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Synergistic nutrient removal by *Phragmites* and *Kyllinga* species from a constructed rhizofilter system in Durban, South Africa

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Abstract

The role played by macrophytes need to be understood if constructed rhizofilter (CR) systems are to be considered an alternative technology in wastewater treatment. Influent and effluent nutrient concentrations of a CR were measured to determine the removal efficiency of the system. The unpaired *t*-test was used to assess the differences between phosphate removal in the planted and reference sections and it was found that the planted section containing two plants removed more phosphate 36.1±0.6% than when the section was planted with one plant as well as the reference section 12.6±0.4%. This was a statistically significant difference of between 22-73.5% (P=0.01 in both cases). Nitrogen removal rate was also more in the planted 46%±0.9 than in the reference section 40±0.2% (P=0.01). Findings suggest that when rhizofilter systems are planted with more than one high nutrient accumulator macrophytes can be efficiently used as a low cost alternative for the tertiary treatment of wastewater.

Keywords: constructed rhizofiltration; wastewater treatment; nutrient removal; microbial biofilms; macrophytes; rhizofiltration

1. Introduction

Environmental concerns over insufficiently performing septic systems and high expenses in the construction, operation and management of currently used conventional wastewater treatment systems have led investigations to finding alternative technologies for wastewater treatment. The appropriateness of constructed rhizofiltration systems for wastewater treatment had been reported from all over the world [1,2]. Aquatic plants and their root zone rhizofiltration medium are at the main helm into the processes occurring in the constructed rhizofiltration systems. *Phragmites australis* had been reported to remove nutrients from constructed wetlands [3,4,5]. However its role and treatment ability when used in combination with other macrophytes is not well and clearly documented. Rhizofiltration system uses natural processes like biological, chemical and physical mechanisms and mostly relies on plants in the removal of contaminants from wastewater [6,7]. Technical operation and the rate at which processes occur in the systems may vary due to variation in retention time as well as variations in the interactions between wastewater, soil matrix and macrophytes. In our work, a rhizofiltration system was constructed in Durban, South Africa, and was tested for its ability to remove nutrients from municipal wastewater through macrophytes activities. The main aim was to investigate the combination of the role of both *P. australis* and *Kyllinga nemoralis* in nutrients removal by comparing the results of inflow (influent) and outflow (effluent) phosphate, orthophosphate, ammonia, nitrate and nitrite from the system.

2. Materials and methods

A rhizofiltration system was designed and constructed in Durban, South Africa. It was first planted with *P. australis* and after three months of operation *K. nemoralis* was added. Influent and effluent were collected bimonthly for twelve (12) months from January 2012 to December 2012 and were analysed according to the Standard Methods for the Examination of Water and Wastewater [8]. Wastewater samples were collected

Appendix 2: Physio-chemical parameters measured in the rhizofiltration system

pH

Sampling Period	Influent			Reference section			Planted section		
Jan-12	9.73	7.12	7.3	7.12	6.51	7.12	9.7	7.12	8.11
Feb-12	6.6	6.11	6.44	6.11	7.8	6.11	6.5	6.11	6.7
Mar-12	4.78	7.75	6.44	7.75	7.7	7.75	5.26	7.75	6.03
Apr-12	7.4	7	6.44	7	8.11	7	7.12	7	7.37
May-12	5.54	6.5	6.44	6.5	6.7	6.5	6.11	6.5	7.548
Jun-12	7.76	6.51	6.44	6.51	6.03	6.51	7.75	6.51	5.565
Jul-12	6.95	7.8	6.44	7.8	7.37	7.8	7	7.8	6.393
Aug-12	7.3	7.7	6.44	7.7	7.548	7.7	6.5	7.7	7.302
Sep-12	6.44	8.11	6.44	8.11	5.565	8.11	6.51	8.11	6.293
Oct-12	6.44	6.7	6.44	6.7	6.393	6.7	7.8	6.7	6.599
Nov-12	6.44	6.03	6.44	6.03	7.302	6.03	7.7	6.03	6.18
Dec-12	6.44	7.37	6.44	7.37	6.293	7.37	8.11	7.37	7.5
Jan-13	6.44	7.548	6.44	7.548	6.599	7.548	6.7	7.548	7.541
Feb (100:0)-13	6.44	5.565	6.01	6.21	6.18	6.16	6.03	6	5.93
Mar (90:10)-13	6.44	6.393	7.4	7.5	7.5	7.52	7.37	7.4	7.39
Apr (80:20)-13	6.44	7.302	7.559	7.51	7.541	7.572	7.548	7.644	7.506
May (50:50)-13	6.44	6.293	6.336	6.188	6.216	6.224	5.565	6.104	6.086
Jun (20:80)-13	6.44	6.599	6.598	6.65	6.598	6.597	6.393	6.479	6.484

Temperature (°C)

Sampling period	Influent			Reference section			Planted section		
Jan-12	33.29	32.94	33.29	32	35	32	32.94	32	33.94
Feb-12	19.5	24	19.5	23.7	22.9	23.7	24	23.7	24
Mar-12	18.08	22	18.08	21	21.69	21	22	21	22
Apr-12	16.07	22.29	16.07	22.29	22.08	22.29	22.29	22.29	22.29
May-12	20.63	20.86	20.63	19.5	18.6	19.5	20.86	19.5	20.86
Jun-12	18.2	20.08	18.2	18.08	18.08	18.08	20.08	18.08	20.08
Jul-12	20.6	17.5	20.6	16.07	20.03	16.07	17.5	16.07	17.5
Aug-12	23.3	20.03	23.3	20.63	22.03	20.63	20.03	20.63	20.03
Sep-12	23.2	19.3	23.2	18.2	21.3	18.2	19.3	18.2	19.3
Oct-12	24.3	22.2	24.3	20.6	22.6	20.6	22.2	20.6	22.2
Nov-12	26.26	24.6	26.26	23.3	24.5	23.3	24.6	23.3	24.6
Dec-12	27.8	27.8	27.8	23.2	23.4	23.2	27.8	23.2	27.8
Jan-13	23.1	25.3	23.1	24.3	25.9	24.3	25.3	24.3	25.3
Feb (100:0)-13	26.42	26.41	26.42	26.26	26.53	26.26	26.37	26.26	26.32
Mar (90:10)-13	28.6	29	28.6	28.68	27.27	27.5	27.19	27.8	27.54
Apr (80:20)-13	23.85	23.71	23.85	24.07	24.4	24.81	24.56	23.1	23.99
May (50:50)-13	22.67	23.2	22.67	22.69	21.13	21.11	21.15	20.89	19.98
Jun (20:80)-13	22.26	22.37	22.26	22.11	20.24	20.4	20.64	19.24	19.7

Salinity (mg/l)

Sampling period	Influent			Reference section			Planted section		
Jan-12	0.21	0.35	0.4	0.38	0.31	0.38	0.24	0.26	0.3
Feb-12	0.24	0.28	0.24	0.15	0.28	0.2	0.22	0.28	0.28
Mar-12	0.22	0.3	0.22	0.26	0.3	0.19	0.2	0.15	0.4
Apr-12	0.2	0.28	0.2	0.29	0.27	0.24	0.3	0.26	0.22
May-12	0.3	0.3	0.3	0.28	0.3	0.33	0.2	0.29	0.22
Jun-12	0.2	0.3	0.2	0.4	0.28	0.27	0.21	0.28	0.21
Jul-12	0.21	0.4	0.21	0.2	0.4	0.37	0.24	0.4	0.2
Aug-12	0.24	0.24	0.24	0.2	0.22	0.2	0.35	0.2	0.3
Sep-12	0.35	0.22	0.35	0.19	0.22	0.3	0.28	0.2	0.2
Oct-12	0.28	0.2	0.28	0.2	0.21	0.2	0.38	0.19	0.21
Nov-12	0.38	0.3	0.38	0.1	0.2	0.21	0.2	0.2	0.24
Dec-12	0.2	0.2	0.2	0.17	0.1	0.24	0.19	0.1	0.35
Jan-13	0.19	0.21	0.19	0.24	0.19	0.35	0.24	0.17	0.17
Feb (100:0)-13	0.24	0.24	0.24	0.33	0.25	0.25	0.25	0.24	0.24
Mar (90:10)-13	0.33	0.35	0.33	0.21	0.33	0.33	0.33	0.33	0.33
Apr (80:20)-13	0.27	0.28	0.27	0.27	0.25	0.25	0.25	0.21	0.23
May (50:50)-13	0.37	0.38	0.37	0.37	0.36	0.36	0.36	0.35	0.34
Jun (20:80)-13	0.4	0.4	0.4	0.4	0.38	0.38	0.38	0.37	0.37

Conductivity (mS/cm)

Sampling period	Influent			Reference section			Planted section		
Jan-12	0.538	0.622	0.538	0.631	0.785	0.809	0.7	0.63	0.55
Feb-12	0.53	0.56	0.53	0.566	0.55	0.508	0.64	0.57	0.54
Mar-12	0.5	0.3	0.5	0.561	0.57	0.64	0.632	0.57	0.69
Apr-12	0.67	0.27	0.67	0.443	0.536	0.518	0.65	0.538	0.65
May-12	0.65	0.56	0.65	0.65	0.55	0.74	0.809	0.53	0.631
Jun-12	0.502	0.58	0.502	0.531	0.54	0.566	0.508	0.5	0.566
Jul-12	0.657	0.7	0.657	0.7	0.69	0.754	0.64	0.67	0.561
Aug-12	0.561	0.64	0.561	0.526	0.65	0.784	0.518	0.65	0.443
Sep-12	0.441	0.632	0.441	0.675	0.631	0.526	0.74	0.502	0.65
Oct-12	0.55	0.65	0.55	0.71	0.566	0.675	0.566	0.657	0.531
Nov-12	0.514	0.809	0.514	0.71	0.561	0.71	0.754	0.561	0.7
Dec-12	0.72	0.508	0.72	0.82	0.443	0.71	0.784	0.441	0.526
Jan-13	0.426	0.64	0.426	0.51	0.65	0.82	0.71	0.55	0.675
Feb (100:0)-13	0.56	0.518	0.56	0.518	0.531	0.533	0.356	0.514	0.518
Mar (90:10)-13	0.74	0.74	0.74	0.74	0.7	0.7	0.703	0.72	0.72
Apr (80:20)-13	0.545	0.566	0.545	0.547	0.526	0.515	0.509	0.426	0.466
May (50:50)-13	0.721	0.754	0.721	0.719	0.675	0.68	0.674	0.652	0.625
Jun (20:80)-13	0.772	0.784	0.772	0.769	0.71	0.707	0.71	0.673	0.673

Total dissolved solids (mg/l)

Sampling period	Influent			Reference section			Planted section		
Jan-12	321	328	3327	328	326	328	331	317	319
Feb-12	344	478	445	345	445	444	442	444	441
Mar-12	323	377	341	363	286	309	304	321	326
Apr-12	293	341	436	335	693	302	302	295	299
May-12	365	435	327	488	335	261	3.2	451	362
Jun-12	433	323	318	491	344	428	427	455	440
Jul-12	449	307	456	489	325	447	449	453	451
Aug-12	493	322	449	529	316	487	486	494	493
Sep-12	230	448	463	400	456	366	300	263	220
Oct-12	210	461	499	500	447	356	321	268	190
Nov-12	342	338	334	213	464	342	342	234	342
Dec-12	438	441	438	774	501	44	442	2439	439
Jan-13	336	336	334	902	341	34	334	2338	337
Feb (100:0)-13	327	321	316	187	333	329	326	225	327
Mar (90:10)-13	369	455	317	73	467	464	365	466	463
Apr (80:20)-13	344	437	439	135	447	451	445	447	345
May (50:50)-13	467	477	473	669	481	48	477	347	347
Jun (20:80)-13	498	504	503	559	513	506	503	501	503

Dissolved oxygen (mg/l)

	Influent			Reference section			Planted section		
Jan-12	3.8	6.95	7.9	4.34	5.47	6.3	6.08	8.54	8.88
Feb-12	5.8	3.45	7.61	6	2.3	5.3	6.5	8.1	4.6
Mar-12	6.4	5.54	3.74	7.5	3.5	4.6	7.94	7.9	4.1
Apr-12	5.2	2.69	5.52	5.5	2.22	5.47	6.2	7.2	3
May-12	7.52	3.62	4.19	8.88	2.81	2.3	9.9	6.51	5.6
Jun-12	3.9	7.5	4.85	4.6	5.65	3.5	5.3	4.22	8.54
Jul-12	3.5	7.5	6.95	4.1	2.19	2.22	5	6.15	8.1
Aug-12	2.7	6.5	3.45	3	2.94	2.81	5.1	5.76	7.9
Sep-12	8.1	5.47	5.54	5.6	2.57	5.65	8.81	4.88	7.2
Oct-12	8.2	2.3	2.69	8.54	6.3	2.19	8.59	7.61	6.51
Nov-12	7.5	3.5	3.62	8.1	3.5	2.94	8.39	3.74	4.22
Dec-12	7.5	2.22	3.2	7.9	6.3	2.57	8.1	5.52	6.15
Jan-13	6.5	2.81	3.1	7.2	5.3	3.2	7.9	4.19	5.76
Feb (100:0)-13	5.47	5.65	5.8	6.51	6.65	6.95	7.61	6.05	5.48
Mar (90:10)-13	2.3	2.19	2.23	4.22	3.64	3.45	3.74	3.54	3.29
Apr (80:20)-13	3.5	2.94	3.4	6.15	5.02	5.54	5.52	5.71	5.22
May (50:50)-13	2.22	2.57	1.41	5.76	3.34	2.69	4.19	2.7	2.1
Jun (20:80)-13	0.81	1.85	1.25	4.88	3.8	3.62	4.85	3.81	3.57

COD (mg/l)

	Influent			Reference section			Planted section		
Jan-12	192	216	344	189	223	141	170	90	216
Feb-12	88	169	148	77	175	132	61	70	169
Mar-12	255	138	136	223	144	148	216	150	138
Apr-12	261	190	151	175	344	33	169	145	190
May-12	145	141	39	144	148	199	138	120	141
Jun-12	157	132	167	344		145	190	165	132
Jul-12	148	148	170	148	136 151	145	141	130	148
Aug-12	137	33	175	136	139	12	132	85	33
Sep-12	302	199	16	151	167	134	148	120	199
Oct-12	61	145	135	39	170	186	33	90	145
Nov-12	234	145	160	167	175	122	199	150	145
Dec-12	173	12	215	170	116	90	145	55	60
Jan-13	180	134	210	175	135	80	145	59	60
Feb (100:0)-13	80	186	80	16	160	115	12	12	12
Mar (90:10)-13	205	222	206	135	135	135	134	131	129
Apr (80:20)-13	280	238	270	160	168	167	186	188	188
May (50:50)-13	285	285	284	215	210	209	222	215	212
Jun (20:80)-13	290	296	296	210	218	217	238	224	230

Appendix 3: Nutrients measured in the rhizofiltration system

Ammonia (mg/l)

	Influent			Reference section			Planted section		
Jan-12	0.31	0.21	0.41	0.11	0.35	0.21	0.11	0.21	0.1
Feb-12	3.8	3.9	2.7	3.7	2.7	3.9	3.7	1.9	0.9
Mar-12	3.2	2.2	3.2	3.2	1.8	2.2	2.8	1.2	1.2
Apr-12	3.9	3.5	3.5	3.5	1.2	3.5	3.2	1.5	2.5
May-12	4.5	3.7	2.7	2.7	2.4	3.7	2.4	2.7	3.7
Jun-12	4.6	3.6	2.6	2.6	2.8	3.6	2.8	3.6	3.6
Jul-12	33.5	29.8	27.8	25.8	15.8	29.8	15.8	20.8	14.8
Aug-12	6	7.7	6.7	5.7	4.3	7.7	4.3	4.7	3.7
Sep-12	2.9	3.9	3.9	2.9	2.9	3.9	2.9	2.9	1.9
Oct-12	0	0.9	0	0	0	0.9	0	0.2	0.9
Nov-12	3.8	3.2	3.2	3.2	3	3.2	3	2.2	0.2
Dec-12	2.3	2.1	2.1	2.1	2.2	2.1	2.2	2.1	0.1
Jan-13									
Feb (100:0)-13	7.4	10.3	10.6	0	0	0	2.6	2.6	2.6
Mar (90:10)-13	76.2	76.1	76	21.7	21.7	21.7	21.1	21.1	21.1
Apr (80:20)-13	61.2	61.2	61.2	11.6	11.7	11.7	29.1	29.2	29.2
May (50:50)-13	42.8	43.1	43.1	39.6	39.6	39.6	46.8	47	47.2
Jun (20:80)-13	73.8	73.7	73.5	75	75	75	78	78	78

Nitrate (mg/l)

	Influent			Reference section			Planted section		
Jan-12	17.3	12.4	16.3	16.9	15.9	13.9	13.4	11.4	13.4
Feb-12	7.7	6.8	6.7	7.6	6.6	7.6	7.2	6.2	6.5
Mar-12	3.2	7.1	3.2	3	13	3	2.9	2.8	2.9
Apr-12	3.2	9.3	3.2	3.1	4.1	3.1	3	2.3	1.3
May-12	4.4	3.5	4.4	4.3	4.3	4.3	4.2	3.2	1..2
Jun-12	6.5	11.2	6.5	0.06	0.6	0.3	0.5	0.6	0.7
Jul-12	0.9	8.1	0.9	0.76	1.76	0.76	0.61	0.81	0.71
Aug-12	4.3	5.2	4.3	4.3	4.9	4.3	4.1	3.1	4.1
Sep-12	22.1	21.4	21.1	14.4	13.4	14.4	13	13.6	11.9
Oct-12	5.9	1.5	5.9	5.8	5.9	5.8	2.3	2.9	2.3
Nov-12	6.7	5.6	6.7	7.1	7.1	8.1	6.9	6.6	6.9
Dec-12	6.1	5.9	6.1	9.3	9.3	8.3	5.5	6.1	5.5
Jan-13									
Feb (100:0)-13	11	7.9	7.9	11.2	11.3	11.3	12.7	13	15.3
Mar (90:10)-13	1.4	1.5	1.5	8.1	8.2	8.3	3.5	3.6	3.5
Apr (80:20)-13	1.1	1.1	1.2	5.2	5.3	5.4	6	5.9	5.9
May (50:50)-13	1.1	1.1	1.2	1.4	1.4	1.4	0.9	0.9	0.9
Jun (20:80)-13	0.8	0.9	1	1.5	1	0.9	0.8	0.8	0.74

Nitrite (mg/l)

	Influent			Reference section			Planted section		
Jan-12	0.42	0.32	0.62	0.3	0.3	0.39	0.12	0.02	0.22
Feb-12	0.07	0.17	0.27	0.07	0.07	0.27	0.07	0.7	0.17
Mar-12	0.1	0.11	0.12	0.05	0.05	0.25	0.008	0.08	0.018
Apr-12	0.06	0.16	0.66	0.05	0.05	0.25	0.04	0.4	0.14
May-12	0.07	0.27	0.17	0.07	0.07	0.17	0.06	0.6	0.16
Jun-12	0.5	0.35	0.56	0.06	0.06	0.16	0.07	0.7	0.17
Jul-12	0.22	0.42	0.32	0.11	0.11	0.31	0.09	0.9	0.19
Aug-12	0.03	0.33	0.43	0.01	0.01	0.31	0	0.6	0.1
Sep-12	2.3	2.93	2.13	2.1	2.1	2.41	1.86	1.6	1.16
Oct-12	0	2.1	0.1	0	0	1.2	0	2	2.1
Nov-12	0.05	0.35	0.15	0.07	0.07	0.17	0.05	0.5	0.15
Dec-12	0.13	0.33	0.23	0.12	0.12	0.32	0.11	0.21	0.11
Jan-13									
Feb (100:0)-13	0.01	0.06	0.31	0.112	0.115	0.111	0.04	0.08	0.04
Mar (90:10)-13	0.054	0.093	0.392	0.131	0.131	0.129	0.147	0.147	0.149
Apr (80:20)-13	0.06	0.059	0.459	0.158	0.156	0.156	0.155	0.155	0.155
May (50:50)-13	0.54	0.55	0.42	1.123	1.321	1.324	0.94	0.95	0.893
Jun (20:80)-13	0.694	0.632	0.71	1.98	2.04	2.121	1.32	1.32	1.423

Phosphate (mg/l)

	Influent			Reference section			Planted section		
Jan-12	1.6	1.3	1.6	1.4	1.9	1.3	1	1.1	2.1
Feb-12	3.5	3.9	2.5	1.6	1.3	1.3	1.2	1	1.2
Mar-12	6.3	6.13	5.3	5	1.5	2.5	4.1	3.1	2.1
Apr-12	2.59	2.39	2.89	1.98	1.78	1.98	0.36	0.26	1.2
May-12	1.62	1.52	1.32	1.24	1.34	1.94	0.3	0.2	1.3
Jun-12	1.2	1.9	1.3	0.94	0.34	0.74	0.67	0.57	0.63
Jul-12	5.9	5.29	5.5	5.2	3.2	3.2	3.6	2.6	3.3
Aug-12	3.5	3.9	3.6	3.2	4.2	3.2	2.99	2.59	2.92
Sep-12	0.4	0.8	0.2	0.3	1.3	0.5	0.3	0.1	0.2
Oct-12	5.9	5.7	5.9	4.9	3.9	4.1	3.9	1.9	3.4
Nov-12	0.5	1.5	0.5	0.4	1.4	1.4	0.4	0.2	0.4
Dec-12	0.8	1.8	0.8	0.81	0.11	0.51	0.79	0.19	0.69
Jan-13									
Feb (100:0)-13	3	3	3	2.1	2.1	2.1	2.7	2.7	2.7
Mar (90:10)-13	6	6	6	4.1	4.1	4.1	4.3	4.3	4.3
Apr (80:20)-13	6.3	6.3	6.3	4.7	4.7	4.7	4.8	4.8	4.8
May (50:50)-13	5.6	5.6	5.6	4	4	4	4.4	4.4	4.4
Jun (20:80)-13	6.25	5.99	5.97	5.1	5.02	4.9	4.5	4.9	4.687

Orthophosphate (mg/l)

	Influent			Reference section			Planted section		
Jan-12	4.2	3.2	4.3	4	4.9	4.2	3.2	1.2	2.2
Feb-12	0.7	2.7	0.9	0.7	0.76	0.6	0.6	0.2	1.6
Mar-12	0.6	1.6	0.8	0.45	0.55	0.45	0.4	0.3	1.4
Apr-12	0.7	1.7	0.9	0.62	0.52	0.32	0.51	0.71	0.01
May-12	0.29	2.29	0.7	0.23	0.33	0.33	0.17	0.27	0.7
Jun-12	0.65	1.65	0.7	0.56	0.46	0.76	0.53	0.33	0.53
Jul-12	1.76	2.76	1.26	1.24	1.34	1.94	0.89	0.59	0.89
Aug-12	3.81	3.31	3.91	3.43	3.33	3.93	2.99	2.39	2.19
Sep-12	3.56	3.96	3.86	3.21	3.11	3.61	2.25	2.35	2.15
Oct-12	4.9	5.9	4.29	4.5	4.9	4.3	4.3	4.6	4.13
Nov-12	3.1	3.9	3.3	3.1	2.1	3.1	2.8	2.1	2.8
Dec-12	2	2.1	2.4	1.1	0.1	1.3	1	1.1	0.99
Jan-13									
Feb (100:0)-13	2.4	2.4	2.4	1.6	1.6	1.6	2.4	2.4	2.4
Mar (90:10)-13	2.8	2.8	2.8	1.9	1.9	1.9	2.6	2.64	2.5
Apr (80:20)-13	7.547	2.8	2.8	2.53	2.9	2.9	6.42	2.9	3
May (50:50)-13	5.2	5.2	5.2	4	4.43	4.32	4.9	4.9	4.9
Jun (20:80)-13	5.31	5.42	5.64	4.812	4.411	4.211	5.011	5.146	5.261

Appendix 4: Nutrient accumulation in macrophytes from the rhizofiltration system

Nutrient accumulation in *P. uastralis* in 2012

Sampling period	Nitrogen (%)			Phosphorus (%)		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Jan-12	2.44	0.65	0.94	0.25	0.1	0.08
Mar-12	0.67	0.66	1.72	0.16	0.25	0.19
May-12	2.19	0.85	1.89	0.21	0.26	0.4
Jul-12	0.5	0.26	1.5	0.5	0.85	0.56
Sep-12	0.5	0.26	0.85	2.19	0.85	1.25
Oct-12	2.29	0.81	0.74	2.19	0.17	0.14
Dec-12	2.27	0.75	0.94	0.22	0.19	0.2

Nutrient accumulation *in K. nemoralis* in 2012

	Nitrogen (%)			Phosphorus (%)		
Months	Leaves	Stems	Roots	Leaves	Stems	Roots
May	1.12	0.76	0.99	0.21	0.16	0.23
July	1.28	0.98	1.22	0.22	0.2	0.29
September	2.55	1.23	1.66	0.24	0.23	0.3
October	1.95	0.97	0.6	0.26	0.19	0.25
December	1.68	0.67	0.68	0.31	0.19	0.24

Nitrogen accumulation in macrophytes in 2013

	P. australis (%)			*K. nemoralis* (mg/g)		
Months	leaves	stems	roots	leaves	stems	roots
	9.7	6.4	4.8	6.9	2.7	1.9
	9.7	6.5	4.7	6.8	2.8	1.9
February	9.6	6.5	4.9	6.8	2.9	1.8
	8.4	5.9	4.2	13.1	9.2	3.3
	8.3	5.9	4.1	13.2	9.3	3.4
April	8.1	5.8	4.2	13.1	9.3	3.3
	4.1	1.8	2.9	1.1	9.8	1.7
	4.2	1.9	3	1.1	9.8	1.8
May	3.9	1.9	2.9	1.2	9.7	1.8
	2.5	1.4	1.8	11.3	6	1.4
	2.4	1.3	1.8	11.3	6.1	1.5
June	2.4	1.3	1.7	11.4	6.1	1.4

Nitrogen accumulation in macrophyes in 2013

	Phragmites australis (%)			*Kylinga nemoralis* (%)		
Months	leaves	stems	roots	leaves	stems	roots
February	19	6	15	10	14	3
April	18	6	9	26	8	4
May	14	22	7	17	5	7
June	11	5	6	9	4	11

Appendix 5: Gas emission measured from the rhizofiltration system

Sampling periods	mg CO_2/day)		(mg CH_4/day)		mgN_2O/day)
	Planted	Reference	Planted	Reference	
Jan-12	0.027	0.074	0.019	0.0027	0.0006
Feb-12	0.032	0.027	0.023	0.0097	0.0004
Mar-12	0.095	0.077	0.034	0.028	0.0002
Apr-12	0.22	0.21	0.081	0.076	0.0003
May-12	0.017	0.0025	0.0061	0.0009	0.0004
Jun-12	-0.005	-0.03	-0.002	-0.02	0.0004
Jul-12	0.53	0.31	0.193	0.144	0.001
Aug-12	0.012	0.0023	0.0043	0.00085	0.0003
Sep-12	0.37	0.37	0.136	0.133	0.0003
Oct-12	0.068	0.053	0.025	0.019	0.0008
Nov-12	0.085	0.16	0.031	0.059	0.0003
Dec-12	0.068	0.007	0.025	0.0026	0.0003
Jan-13	0.155	0.165	0.15	0.16	0.0006
Feb (100:0)-13	0.169	0.179	0.044	0.06	0.001
Mar (90:10)-13	0.365	0.466	0.07	0.065	0.0002
Apr (80:20)-13	0.165	0.21	0.171	0.169	0.001
May (50:50)-13	0.194	0.18	0.07	0.076	0.1
Jun (20:80)-13	0.98	0.21	0.165	0.064	0.2