



**Assessment of heavy metals and pathogens removal from  
municipal wastewater using a constructed rhizofiltration  
system**

**Thesis submitted in fulfilment of the requirements for the degree of Doctor of  
Philosophy in Biotechnology in the Faculty of Applied Sciences at Durban University of  
Technology**

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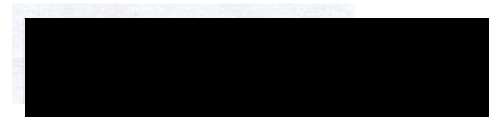
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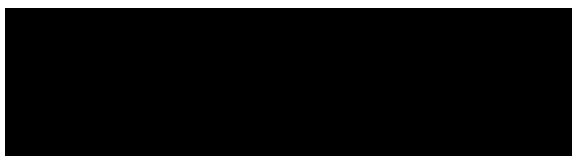
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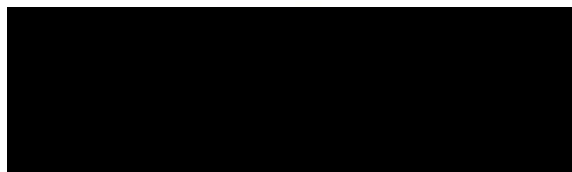


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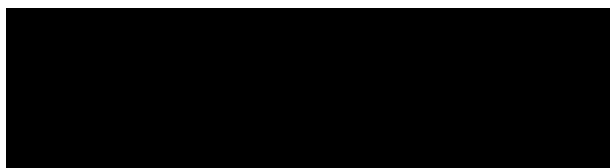


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**DEDICATION**

To my mother and my children

## ABSTRACT

Wastewater discharged from municipal treatment plants contain a mixture of organic contaminants, trace metals, enteric pathogens, viruses, and inorganic materials. The presence of such pollutants in wastewater poses a huge challenge to the choice and applications of the preferred treatment method. Conventional treatment methods are inefficient in the removal of some environmentally toxic pollutants and pathogens. This study evaluated the effectiveness of a constructed rhizofiltration system in the removal of heavy metals and enteric pathogens from municipal wastewater. The study was conducted at an eThekweni municipal wastewater treatment plant in Kingsburgh - Durban in the province of KwaZulu-Natal. The pilot-scale rhizofiltration unit included three different layers of substrates consisting of medium stones, coarse gravel and fine sand. The system had one section planted with *Phragmites australis* and *Kyllinga nemoralis* while the other section was unplanted and acted as the reference section.

Influent and effluent, plant tissue and sediment from the rhizofilter were sampled bi-monthly for a period of two years and assessed for the presence and removal of selected enteric pathogens, trace heavy metals and changes in physicochemical and biological parameters using standard methods. Antibacterial potential of the two experimental plants was determined by the agar-well diffusion method using plant root exudates exposed to selected pathogenic bacteria. Observation of details of plant morphology, distribution and assessment of the metals attachment onto the various plant tissues was determined using images from scanning electron microscopy (SEM). The Langmuir model was used to assess the heavy metal adsorption of the plants.

There was an increase in pH from 6.95 pH units to 7.55 pH units in the planted and 6.72 to 7.23 pH units in the reference sections. There was an average reduction in biochemical oxygen demand (BOD) by 79% and chemical oxygen demand (COD) by 75%. Suspended solids were reduced by 86% in the planted section and 59.8% in the reference section, Electrical conductivity was reduced by 7.7% in the planted section and 0.83% in the reference section, Total Dissolved Solids was reduced by 11.5% in the planted section and 3.5% in the reference section, temperature was

reduced by 11.9% in the planted section and 1.2% in the reference section, while dissolved oxygen was raised by 10% in the planted section and 5% in the reference section. Turbidity was reduced by 9.7 NTU in the planted section and 9.1 NTU in the reference section, while alkalinity was reduced by 46.3% in planted and 45.5% on reference sections of the rhizofilter. There was a significant reduction in organic loading in the system which was statistically significant (phosphorous,  $p = 0.029$ ; ammonia,  $p = 0.03$ ). These average reductions and increases were observed after the system was fully established. The results indicate a comparatively better removal efficiency in the planted than the reference sections of the system.

Considering the entire rhizofilter, heavy metals were accumulated at varying percentages of 96.69% on planted and 48.98% in reference sections for cadmium. Chromium was 81% and 24%, Copper was 23.4% and 1.1%, Nickel was 72% and 46.5, Lead was 63% and 31%, while Zinc was 76% and 84% in the planted and reference section of the rhizofilter respectively. The planted section had a much higher removal efficiency as compared to the reference section of the rhizofilter. The macrophytes were found to display some metals binding potential according to observations from SEM and EDX analysis. Significant amounts of Cu deposits were recorded on the roots of *K. nemoralis* at 0.31wt% with a peak at 0.6cps/eV than on *P. australis* which was at 0.31wt% with a peak at 0.6cps/eV. Further, higher deposits of Ni at 0.01 wt% with peak at 0.5 cps/eV and 0.0 wt% with peak at 0.2 cps/eV, Pb at 0.22 wt% with peak at 0.2 cps/eV and 0.21wt% with peak at 0.2 cps/eV were recorded on the roots of *K. nemoralis* and *P. australis* respectively. *Kyllinga nemoralis* was found to have greater metals adsorptive capabilities than *P. australis*.

The planted and reference sections had varied removal capacities of between 45% and 98% for the various pathogens detected in the influent wastewater. For example, the concentration of coliphage was reduced by 94.6% in the planted section and 93.6% in the reference section, *Candida* spp. removal was 64.7% in the planted section and 62.5% in the reference section. *Escherichia coli* was reduced by 65%-85% while *Salmonella* spp. was removed by 94% in the planted section compared to 78% in the reference section.

*Ascaris lumbricoides* was reduced by 77% in the planted section and 53% in the reference section. Accordingly, higher pathogens reduction was achieved in the planted section as compared to the reference section of the rhizofilter. Root exudates from *Kyllinga nemoralis* were found to display a wider zone of growth inhibition at  $9.97 \pm 0.19$  mm compared to *P. australis* which had a zone of  $8.63 \pm 0.22$  mm when exposed to cultured colonies of *Escherichia coli*.

**Keywords:** wastewater treatment, *Phragmites australis*; *Kyllinga nemoralis*; heavy metals; pathogens; rhizofiltration.

## PREFACE

### Publications

Mthembu MS<sup>1\*</sup>, **Odinga CA**<sup>2</sup>, Bux F<sup>2</sup> and Swalaha FM<sup>2</sup>. 2017. Constructed treatment wetlands: An emerging phytotechnology for degradation and detoxification of industrial wastewaters. The book entitled: Bioremediation of Industrial Wastes for Environmental Safety. Springer Nature, Singapore.

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**Conference presentations**

**Odinga, C. A., Mthembu, M. S., Swalaha, F. M., Otieno, F. A. O., & Bux, F. 2015.** Heavy metals removal from municipal wastewater using Rhizofiltration Technology. Conference presentation. International Conference on Sustainable Water Management 2015, at Murdoch University, Murdoch – Australia.

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**Odinga, C. A., Mthembu, M. S., Swalaha, F. M., Otieno, F. A. O., & Bux, F. 2012.** Oral presentation of a paper titled “Evolving technologies in heavy metals and pathogens removal from wastewater using a constructed wetland” at an International conference in Moi University- Kenya August 2012.



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**LIST OF ABBREVIATIONS AND ACRONYMS**

APHA	American Public Health Association
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EPA	Environmental Protection Agency
TENORM	Technologically enhanced naturally occurring radioactive materials
TDS	Total dissolved solids
EPA	U.S. Environmental Protection Agency
MCL	Maximum contaminant level
EC	Conductivity
DO	Dissolved oxygen
HACH DR/2000	Direct reading spectrophotometer by HACH Company
The IUPAC	International Union of Pure and Applied Chemistry
DNA	Deoxyribonucleic acid
WHO	World Health Organization
L.	Linnaeus (father of modern taxonomy)

## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### 1.1 INTRODUCTION

The world is currently faced with challenges related to management of wastewater and the related ecosystems (Hong *et al.*, 2017). This is due to an increase in the amount of wastewater arising mainly from mechanised farming, heavy industrialisation processes and population growth (Akpor and Muchie, 2010). Reuse of wastewater could easily mitigate the demand for potable water and environmental degradation especially in the developing world where quantity rather than quality is preferred (Sheng *et al.*, 2010; Jhansi and Mishra, 2013).

Wastewater from industries and municipalities is composed primarily of organic and inorganic constituents including metals, bacteria and viruses. Some of these constituents occurring beyond allowable limits in treated wastewater are likely to cause adverse effects on man and aquatic life (Dunn *et al.*, 2014). Wastewater is normally discharged into small and large water bodies. The wastewater then interacts with the biotic and abiotic factors leading to various forms of environmental changes (Basker *et al.*, 2009). In order to sustainably use water for purposes such as industrial and domestic supply, transport, crop irrigation, recreation, sport and commercial fisheries waste disposal and skilful management of water resources is inevitable (Akpor and Muchie, 2010; Baysal *et al.*, 2013). The main objective of water quality is thus, the holistic protection of all available water resources as any dangers posed to water bodies and aquatic life is likely to affect human life through the food chain (Arias *et al.*, 2003; Mamun *et al.*, 2012).

Quality of water in these receiving bodies depends largely on the wastewater treatment management and processes that have been applied (Dunn *et al.*, 2014). Domestic wastewater is treated using stabilization ponds, trickling filters, activated sludge and conventional systems for wastewater treatment while the industrial sector mainly uses physical, biological and chemical methods of wastewater pre-

treatment. Methods used for pre-treatment of industrial waste include grit removal, screening, removal of oil and grease and pre-settling as may be preferred from industry to industry (Anjum *et al.*, 2016). Anaerobic digestion is the preferred final pre-treatment process before the water is released into the receiving water body. In other instances, aerated stabilisation ponds are used as primary treatment and incorporate constructed wetlands as tertiary systems before final disposal into a river (Sheng *et al.*, 2010). Poor designs and management of conventional and stabilization ponds contribute to the inefficient removal of pollutants such as pathogenic microorganisms and heavy metals (Espinoza *et al.*, 2012; Varela and Manaia, 2013).

The use of natural wetlands for wastewater treatment faces challenges of management due to conflict of interest such as conservation efforts by the government authorities and local communities who may wish to utilize the resource for farming, grazing land, fishing and indiscriminately harvesting of some wetland products such as fuel wood (Khan *et al.*, 2009). The main reasons for wetlands construction are (i) improvement of water quality (ii) low maintenance cost (iii) provide mitigation means for floods, and (iv) food production according to Mwitari *et al.*, (2013).

Conventional treatment systems have been used to treat municipal wastewater over the years though some of these systems are not designed to remove pollutants such as heavy metals, nitrogenous compounds and pathogens (Abira, 2008: <http://www.vateknik.lth.se/vvan01/Arkiv/>). They are also expensive to manage, labour intensive, prone to system failure and are likely to increase pollution by the generation of sludge as a by-product (Baysal *et al.*, 2013; Hamadeh *et al.*, 2014). Conventional systems tend to retain large amounts of suspended solids and environmentally toxic agents such as some algae, a situation which eventually lowers their overall treatment efficiency (Hamadeh *et al.*, 2014).

Efficient disposal of wastewater, reuse and management of treatment systems remains one of the major challenges facing South Africa today (Dungeni and Momba, 2010). In the 2014 budget, it was mentioned that about 10% of the

wastewater treatment facilities in the country were not functional and some facilities required renovations. The need for new and alternative strategies to cost-effectively treat wastewater thus becomes a priority in view of the above-mentioned challenges. Reports from South African Department of Water Affairs indicate that only about 200 wastewater treatment facilities were found to be compliant with the national and international water quality regulatory standards (Oneale, 2014).

South Africa is one of the highly developed countries in Africa south of the Sahara, with a rapidly growing population density of about 52, 982 000 according to Africa, (2013). The country experiences water scarcity and depends on 14% of recycled water as additional supply from return flows, 9% from groundwater and 77% from surface water (Mosteo *et al.*, 2013).

In Durban (where the study site was located), the average volume of wastewater treated (averages approximately 450 million litres per day), prompted an investigation into the possibilities of recycling treated water in order to supplement and mitigate the domestic demand (Varela and Manaia, 2013).

The country boasts of several manufacturing industries, and a well developed agricultural and tourism sector. This has led to increased water demand and also the need to treat wastewater for reuse (Dungeni and Momba, 2010). Municipalities and the rapidly expanding manufacturing industry produce the bulk of wastewater which is discharged mainly into freshwater resources within the country and the oceans. Previous studies indicate that some of the effluent discharged into the receiving water bodies do not meet the expected regulatory requirements by the South African Department of Water Affairs (Mthembu *et al.*, 2013). The wastewater varies in composition and discharge mode which are through a point or non-point sources. Due to water scarcity, South Africa partially relies on return flows which form about 14% of the total freshwater supply. Freshwater sources are often polluted directly by poorly or partially treated wastewater through point and non-point sources (Dungeni and Momba, 2010). Thus, these water resources often require quality monitoring before applications in domestic and industrial utilities (Varela and Manaia, 2013).

Various species of macrophytes have been used for wastewater remediation in wetlands and for research. There is scarce information on the use of *Kyllinga nemoralis* as a plant used for wastewater treatment. This study will potentially add knowledge to the use of *Kyllinga nemoralis* as a wetland plant by providing data on its performance and potential to remove heavy metals and pathogens from wastewater. The aim of this study was therefore to assess the efficiency of a constructed rhizofiltration system and the effect of macrophytes in the removal of heavy metals and water-borne pathogens from municipal wastewater.

## 1.2 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to investigate the removal of heavy metals and pathogens from wastewater using a constructed rhizofiltration system in order to improve effluent water quality.

### 1.2.1 Objectives

- Establish steady-state operation in a constructed rhizofiltration system for nutrients and heavy metal removal.
- Investigate heavy metals the metal removal efficiency from planted and reference sections and assess metals uptake and distribution within the tissues of macrophytes in the rhizofilter.
- Assess metals bioaccumulation mechanisms and distribution within the tissues of the macrophytes by subjecting the plant roots to individual and mixed metals solutions and construct adsorption profiles.
- Determine the uptake and distribution of heavy metals within the tissue of the macrophytes by electron microscopy.
- Determine the efficiency of removal of pathogens within the rhizofiltration system.
- Determine the antimicrobial potential of root extracts of *P. australis* and *K. nemoralis* and the effect on selected pathogens. (\*)

**(\*) The objective which was not originally proposed, but was found to add value to the study.**

### **1.3 LITERATURE REVIEW**

#### **1.3.1 Introduction**

South Africa experiences varied climates unlike other states within the region. The coldest days (winter) lie between June-August, but Durban remains a little warmer during the winter. Average annual rainfall (about 450 mm) is higher in the East of the country and falls mainly during the summer months. Durban experiences a subtropical climate which has hot and humid summers while the winters (June – August) are warm and dry. In summer, the average temperatures are between 20°C-28°C and begin around November to mid-April when winter conditions start (Ziervogel *et al.*, 2014). Wastewater treatment and reuse can be achieved through various inexpensive treatment technologies such as rhizofilters (Sousa *et al.*, 2011). The inefficient performance in pollutant removal, management costs and environmental concerns of systems such as septic tanks have encouraged investigations into the applicability of wetland technology in wastewater treatment and reuse (Song and Li, 2014).

Conventional systems have been found to be labour intensive (Mthembu *et al.*, 2013), prone to system failure and may add residual pollutants such as sludge to the environment (Srivastava *et al.*, 2008; Barbagallo *et al.*, 2011). Conventional systems are also inefficient because they tend to retain large amounts of suspended solids and environmentally toxic agents like algae, a situation which eventually lowers their overall treatment efficiency (Gauss, 2008, Kim *et al.*, 2010, Rivas *et al.*, 2011).

Unsustainable use of freshwater resources, unplanned settlements in urban localities, industrialization, modern agricultural practices and forest destruction have led to increased water demand and pollution of surface and groundwater

reserves (Sayadi *et al.*, 2012; Mosteo *et al.*, 2013). Therefore, there is need to conserve the available water resources and recycle wastewater in order to mitigate water stress in areas that are experiencing water scarcity. But economic instability has placed developing countries with the challenges of wastewater treatment and recycling (Vera *et al.*, 2011; Abdel-Raouf *et al.*, 2012).

Many developing countries rely on the application of conventional wastewater treatment methods such as activated sludge processes and trickling filters (Werker *et al.*, 2002; Sundaravadivel and Vigneswaran, 2010). Sludge processes and trickling filters are cost-effective in chemical (Abira, 2008, Odinga *et al.*, 2013) and energy supply and have potential safety hazards associated with chemical handling, delivery, operation and by-products from disinfection (Sayadi *et al.*, 2012). Such systems, however, require additional treatment by adding disinfectants such as chlorine, ozone and ultraviolet radiation in order to achieve acceptable discharge limits of pathogens. (Werker *et al.*, 2002, Sundaravadivel and Vigneswaran, 2010). Stabilization pond systems which have been in use by many developing countries, cannot eliminate pollutants like heavy metals and pathogens which is the ultimate goal to efficient wastewater treatment (Mahmood *et al.*, 2013).

The potential of rhizofiltration treatment systems in pollutant removal from wastewater has been investigated in the past (Vymazal, 2009; Vivien *et al.*, 2012). However, detailed mechanisms that are involved in pollutants removal and systems functionality of rhizofiltration treatment systems are remote (Momba *et al.*, 2006). The systems are reported to achieve high pollutant removal efficiencies of pathogens, organic and inorganic pollutants (Leiviska *et al.*, 2008; Sousa *et al.*, 2011). Management challenges associated with rhizofiltration systems include the temperature of the wastewater inflow which is likely to give inconsistent results, the flow rate of influent, seasonal weather changes and infiltration from other adjacent polluted ponds according to Sundaravadivel and Vigneswaran, (2010). Clogging of the filtration matrix or sediment on which the plants grow may be experienced due to organic load and some metal deposits. This situation tends to

compromise removal efficiency of the systems (Vymazal, 2010; Pedescoll *et al.*, 2011; Thayaparan *et al.*, 2013).

### 1.3.2 Constructed Wetland Rhizofiltration

Wetlands are areas which are naturally inundated with water (fresh, brackish or salty) throughout or for the most part of the year (Vymazal, 2010). Constructed wetland systems are designed to use various species of plants with potential to accumulate pollutants (Mustafa, 2013; Qureshi *et al.*, 2013). Some of the plant species that have been used in the past include- *Phragmites australis*, *Typha latifolia* and *Eichhornia cracipes* (Garcia *et al.*, 2010). The potential of *Kyllinga nemoralis* as a wastewater treatment plant species has not been investigated, however many studies have investigated its use as a medicinal plant (Qureshi *et al.*, 2013).

Rhizofiltration involves the mutual interaction of plant roots and microorganisms to remove contaminants from wastewater (Prasad, 2011). More specifically, it involves the filtration, adsorption, concentration and assimilation of pollutants such as heavy metal ions and pathogens from the wastewater into the root systems (Rawat *et al.*, 2012; Arroyo *et al.*, 2010).

A constructed rhizofilter was built at Kingsburgh municipal wastewater treatment works in eThekweni, KwaZulu - Natal to assess its efficiency in removing heavy metals and pathogens from wastewater. In this study, removal of selected metals that are normally associated with human and environmental toxicity in wastewater, and enteric pathogens of the genus *Ascaris*, *Salmonella*, *Shigella*, *Candida*, and enteric viruses was evaluated. Presence and removal of heavy metals such as lead, zinc, cadmium, chromium, nickel and copper which are associated with industrial effluents and stormwater according to Zhang *et al.*, (2011) was assessed.



### 1.3.3 Heavy Metals

According to Mukesh and Thakur, (2013), heavy metals are those that have densities of about  $5 \text{ g/cm}^3$  or more, and atomic numbers above 20. These are generally metals in group IIA, IVB, VB, IIIB, and VIB in the periodic table. Heavy metals are also naturally occurring elements that are potentially hazardous to both terrestrial and aquatic environments when present above permissible limits (Rawat *et al.*, 2012). Main sources of metals pollution in the environment arise from battery factories, mining activities, e-waste smelting activities, automobile emissions and paints according to Mukesh and Thakur, (2013), atmospheric deposition and fossil fuel combustion (Thayaparan *et al.*, 2013; Lum *et al.*, 2014; Song and Li, 2014). Traces of heavy metals usually occur in water where some get immobilized in the sediment and form complexes with oxides and hydroxides of Iron (Fe) and other particulate matter in the sediment. It is, therefore, necessary to effectively treat wastewater suspected of having metal concentration beyond acceptable limits. Efficient wastewater treatment in conformity with the set standards (Table 1.1), will eliminate potential toxicological effects on humans, animals and aquatic environment (Baysal *et al.*, 2013).

Table 1.1. Maximum contaminant limits (EPA) for domestic supplies and human health indications for selected heavy metals. Adapted from Barakat, (2011).

Heavy metal	Toxicities	MCL (mg/l) EPA	MCL for South Africa (mg/l)	
			Recommended maximum limit (µg/l)	Maximum allowable limit (µg/l)
Cadmium	Renal disorder, kidney damage, carcinogenic	0.005	10	20
Mercury	Diseases of kidney, nervous system and circulatory system, rheumatoid arthritis	0.002	5	10
Copper	Wilson's disease, liver damage, insomnia	1.3	500	1000
Nickel	Nausea, dermatitis, chronic asthma, carcinogenic, coughing	0.0002	-	-
Chromium	Nausea, carcinogenic, diarrhoea, headache	0.1		
Lead	Kidney disease, circulatory system, damage to foetal brain, nervous system damage	0.015	50	100
Arsenic	Visceral cancer, vascular disease, skin ailments	0	100	300
Zinc	Lethargy, neurological signs (such as tingling in feet or hands), depression, increased thirst	5	1	5

#### 1.3.4 Wastewater Treatment Methods for Removal of Heavy Metals

The most common methods of metals treatment from wastewater include removal of metal ions by chemical precipitation, ion exchange, coagulation, solvent extraction, hydroxide precipitation, electrodialysis and reverse osmosis according to Thayaparan *et al.*, (2013). Some of these methods have been tested and proved to have limitations including the formation of residual metallic compounds that require extra treatment of the effluents (Pei *et al.*, 2014). New methods such as the application of rhizofiltration systems which are based on the application of aquatic plants (normally roots) to remove metals through adsorption and absorption processes have been adapted (Franco *et al.*, 2012).

Hydroxide precipitation is one of the treatment methods that is considered to be simple as compared to conventional types (Baysal *et al.*, 2013). The technique is based on metals precipitation from wastewater using various hydroxides such as calcium hydroxide. For example, ions of Cu, As, Zn, Pb and Cd may be co-precipitated with pyrite. This is likely to form insoluble sulphides which are not easily available to biota (Franco *et al.*, 2012).

#### 1.3.5 Metals in Wastewater

Metal deposits occurring in excess of allowable limits, in the environment potentially pollutes the environment and causes adverse effects to humans and plants (Table 1.1 and 1.2). For example, copper results from the release of Cu by fibre – producing, mining and metal plating industries as well as copper pipe corrosion (Mukesh and Thakur, 2013). Some fertilizers and fungicidal sprays applied in agriculture also contain copper deposits. When excess quantities exceeding 1.5 mg/l, are exposed to the human body, Wilson’s disease of the brain, liver and skin may occur. The toxicity of copper, according to Espinoza *et al.*, (2012), which causes “Wilson’s” disease, arises from its accumulation in the lenticular nucleus found in the brain.

As the 23<sup>rd</sup> most abundantly occurring element, the concentration of zinc in the environment is constantly rising due to human activities (Deng *et al.*, 2009). Zinc occurs as a group IIB element in the periodic table. Its addition and accumulation in the environment are through activities such as steel processing, mining and batteries manufacture, fertilizers and electroplating industries. Wastewater from the above - mentioned activities contributes to excess zinc in the aquatic environment. Excessive amounts up to 5 mg/l of zinc in the environment may cause leaf fall and retarded plant growth, leaf necrosis, chlorosis and leaf discolouration thereby hampering photosynthetic activities Kandziora-Ciupa *et al.*, (2013). Humans exposed to high levels of zinc through inhalation may develop pulmonary dysfunction expressed through a cough and throat irritation and chest pain. Elevated levels of zinc are reported in urine and plasma. Two soldiers exposed to zinc chloride smoke while on some military training evolved respiratory distress syndrome and succumbed after 32 days (Elzbieta *et al.*, 2015).

Cadmium compounds are mainly found in sedimentary rocks. About 15 mg cadmium/kg is accumulated in marine phosphates. Most of the cadmium compounds are water soluble which makes Cd readily bio-available and easily accumulated in tissues of aquatic organisms (Tchounwou *et al.*, 2014). Due to its solubility characteristics, it could be an environmental pollutant capable of causing kidney damage when left to accumulate beyond acceptable limits in tissues of animals (Kihampa, 2013). Cadmium poisoning resulting from exposure to levels exceeding 0.005 mg/l, may result in the manifestation of a disease called Itai-Itai (diagnosed in Japan in the 1950's) whose symptoms include multiple fractures due to osteomalacia. Much research on the toxicity of Cd since the discovery of this disease has been conducted and has yielded tangible information about the sources of Cd in the environment, other toxicity related illnesses, removal from aquatic environments and mitigation measures (Han *et al.*, 2009; Zhang *et al.*, 2012; Kabir *et al.*, 2014). Kidney stones in humans (especially women) are caused due to heavy accumulation of cadmium in the body according to a study by Ferraro *et al.*, (2011). Nickel is an essential element to plants and animals but can be very toxic (Table 1.2) when in either greater or less than the amount required for plant or animal

uptake (Cempel and Nikel, 2006). For example, nickel levels in excess of 0.0002 mg/l are considered toxic to human beings when inhaled or through oral exposure. It's natural occurrence is in various isotopic forms: 58(67.8%), 60(26.2%), 61(1.2%), 62(3.7%), and 64(1.2%). Nickel is a group VIII B element and is resistant to corrosion. Nickel is used in the production of nickel alloys and stainless steel as an alloy. Nickel is mostly present in effluents from electroplating industries, silver refineries, and battery industries and thus its presence in wastewater (Kabir *et al.*, 2014). In drinking water, nickel occurs from metals used in the supply systems. In groundwater, nickel occurs from the dissolution of rocks that bear nickel ore. Generally, a huge proportion of the population has been found to be exposed to Ni poisoning through inhalation, water and occupational exposure (Cempel and Nikel, 2006). Elevated levels of nickel (Ni) in humans is known to cause cancer of the bone and lungs. Symptoms of nickel poisoning in humans (reported cases are rare) are shortness of breath, nausea, vomiting, cyanosis and tightness in the chest. The absorption of Ni into human tissue is dependent upon its physicochemical form. Certain skin ailments such as allergic dermatitis are also associated with Nickel poisoning (Paulo *et al.*, 2017).

Table 1. 2. Effect of excess levels of heavy metals on plant growth and tissues. Adapted from Akpor and Muchie, (2010).

Metal	Maximum acceptable levels (mg/kg/l) (WHO, 1996; EPA, 2010 )	Effects
Mercury (Hg)	0.005	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline
Cadmium (Cd)	0.02	Decreases seed germination, lipid content, and plant growth; induces phytochelatins production
Copper (Cu)	10	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area
Chromium (Cr)	1.30	Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root damage
Zinc (Zn)	0.60	Reduces Ni toxicity and seed germination; increases plant growth and ATP chlorophyll ratio
Lead (Pb)	2	Reduces chlorophyll production and plant growth; increases superoxide dismutase
Nickel (Ni)	10	Reduces seed germination, dry mass accumulation, protein production, chlorophylls and enzymes; increases free amino acids

Lead occurs mainly as particulate matter with fairly low bioavailability (Campbell and Ogden, 1999). Lead particularly does not increase the food chain and is not one of the essential microelements required by plants and animals. However, the available lead in the food chain may be transferred to humans (Tchounwou *et al.*, 2014). In aquatic systems, Pb binds strongly to sediment. It is one of the metals that occur in industrial effluents of most developing countries where law enforcement on effluent standards is not strong. These are regions in the world where 97% of the four million children under threat from Pb poisoning live (Thayaparan *et al.*, 2013). Human exposure to levels above  $20 \mu\text{g}/\text{m}^3$  may cause lead poisoning. Some Pb compounds inhibit enzymes that catalyze reactions of haemoglobin biosynthesis, cause damage to the central nervous system, kidney damage and unclear reports on its carcinogenicity are available (Baysal *et al.*, 2013).

Chromium is an element that occurs naturally in the Earth's crust. It is an element that is more stable in its trivalent compounds. It enters the environment through various anthropogenic activities such as welding of stainless steel, production of chromate processes, processing of metal and tannery industries (Tchounwou *et al.*, 2014). From these activities, chromium is released into the environment, mainly in its hexavalent state which may be very toxic if found in the ordinary environment (levels above  $20 \mu\text{g}/\text{m}^3$  are considered very toxic). Human exposure to chromium occurs through inhalation or ingestion of foods or water containing chromium (Baysal *et al.*, 2013). Chromium poisoning may occur upon exposure of skin to chromium contaminants whose toxicities are dependent on the prevailing oxidation state. Chromium (VI) also has a potential of triggering damaging conditions on chromosomes within the DNA. However, chromium is also a micronutrient which is required in various amounts for growth in plants. Chromium supplements may be used to improve glucose intolerance, corticosteroid-induced diabetes and gestational diabetes (Chekade *et al.*, 2009).

### 1.3.6 Pathogens occurring in wastewater

The fate of enteric pathogens (bacteria, yeast, nematodes) once released from their hosts and into wastewater systems and aquatic or natural environments, has been investigated by a number of scholars (Chaidez *et al.*, 2014; Wu *et al.*, 2014). Their presence and quantity of wastewater form a major determinant of the quality of wastewater discharged into various ecosystems (Varela and Manaia, 2013). This is because some of the pathogenic microorganism survive the treatment system, whereas others have adaptations that compromise their removal depending on the type of wastewater treatment applied (Al-Jaboobi *et al.*, 2013). Some pathogens are very resilient and change their forms (form cysts e.g. *Ascaris lumbricoides*) in order to survive harsh environmental conditions. (Abbadi *et al.*, 2012; Odinga *et al.*, 2013; Rani *et al.*, 2014). Some wastewater microorganisms are also resistant to common conventional disinfection methods such as chlorination. These could possibly be removed by alternate biological treatment methods such as rhizofiltration systems (Abreu-Acosta and Vera, 2011). It is thus necessary to know the rate of survival of the pathogens, characterize the pathogens and evaluate the effectiveness of the treatment facility employed to ensure efficient wastewater treatment.

Viruses cause many human illnesses such as meningitis, paralysis myocarditis and hepatitis (Charles *et al.*, 2014). For example, some waterborne viral epidemics were reported in Italy where about 344 people got infected with norovirus (NoV) and 460 people also got infected with enterovirus in Belarus during an outbreak (Jurzik *et al.*, 2010). These previous reports contributed to the conclusion by some researchers that the determination of viral load in wastewater should form part of quality reference parameters in wastewater (Hot *et al.*, 2003).

*Salmonella* spp. normally occur in foods such as meat, egg products and unpasteurized milk (Rani *et al.*, 2014). A majority of the population in many third world countries are at a high risk of contracting illnesses caused by *Salmonella* spp. (Chaidez *et al.*, 2014). These include countries where many cultural activities are practised *in situ* along the water courses where the water is normally used without

any form of treatment at all. Such activities include recreation, religious rituals, bathing and drinking (Chaidez *et al.*, 2014). The population is thus at a high risk of contracting illnesses such as paratyphoid fever and typhoid fever (Bazaka *et al.*, 2012). The illnesses associated with *Salmonella* spp. have caused high mortality rates and economic burden to many countries in the developing world (Chaidez *et al.*, 2014). These illnesses are caused by the ingestion of food that is contaminated with as little as between 15-20 cells of typhoidal serovars such as Typhi and Paratyphi (Zaki *et al.*, 2009). There are three types (*Salmonella enterica*, *Salmonella bongori* and *Salmonella subterranean* (lower classification in the animal kingdom) that are normally isolated from wastewater according to Al-Jaboobi *et al.*, (2013). Report from a previous study by Henriette *et al.*, (2012), indicate that *Salmonella* spp. (which causes high mortality rate during epidemics) can survive for long periods (up to 1 year) in sediment and soil. According to Abel, (2002) and Charles *et al.*, 2014, some pathogens have the potential to remain viable in the wastewater for long periods (Up to one year in the case of ova of *A. lumbricoides*).

*Shigella* is a gram-negative non-spore-forming bacterium associated with human apes and birds. *Shigella* spp. is classified as one of the major bacterial causes of diarrhoea globally with estimated cases of about 90 million each year especially in the developing world (Ghosh *et al.*, 2009). Less than 100 cells are capable of causing shigellosis when ingested. The strains mostly associated with pathogenicity are *S. flexneri* and *S. dysenteriae*. *Shigella* has more than 40 strains, however *S. flexneri* and *S. sonnei* are the strains normally isolated in wastewater (Zaki *et al.*, 2009). *Shigella* spp., commonly associated with many ailments in children is known to cause acute gastrointestinal illnesses (Garcia *et al.*, 2010). The disease known as Shigellosis is caused by either of the four serogroups of the genus *Shigella* viz:- *Shigella dysenteriae* belonging to group 'A', *Shigella flexneri* group 'B', *Shigella boydii* group 'C' and *Shigella sonnei* of group 'D' (Rani *et al.*, 2014). *Shigella* species is reported to have some survival strategies which make them very difficult to eliminate from wastewater. Some of these survival strategies are dependent on the nature of pollutants in wastewater, temperature and pH. When

they invade the intestinal walls, they cause dysentery which is accompanied by fever, diarrhoea and abdominal pain according to a review by Cabral, (2010). Their transmission is primarily through person-to-person contact (handshake from unwashed hands after visiting toilet), the practice of gay sex and occasionally when outbreaks occur and by consuming contaminated foods (Baer and Claassen, 1999; APHA, 2005).

Most fungal infections are caused by yeasts. *Candida* spp. belong to this category and are reported as one of the leading contributors to fungal diseases in the world (Garcia *et al.*, 2010). *Candida* spp. cause many diseases especially in humans who are immune-suppressed. *Candida albicans* causes candidiasis in humans. *Candida* spp. has been isolated from municipal wastewater giving an indication that they originate from the human alimentary canal (Biedunkiewicz and Ozimek, 2009). Infections by *C. albicans* cause denture stomatitis, cystic fibrosis and mucocutaneous candidiasis in man. Other genera of fungi found in wastewater are *Penicillium*, *Aspergillus* and *Rhizopus*. Their presence in wastewater suggests that they originate from the human body (Lee *et al.*, 2014).

The rod-shaped facultative bacteria *Escherichia coli* (*E. coli*) is a species normally found in the gut of warm-blooded animals including man. Most types are said to be harmless or may cause mild diarrhoea to humans (Liu, 2009). However, some serotypes of *E. coli* such as EDL933 of the 0157:H7 group, can cause food poisoning with symptoms of chronic diarrhoea, stomach cramps and severe vomiting. Poisoning normally occurs due to exposure to contaminated water or food (Garcia *et al.*, 2010). Investigation and isolation of the pathogens in water, wastewater and other foods is usually done under varied conditions with each type having specific treatments and growth media type (Abbadi *et al.*, 2012).

*Ascaris lumbricoides* forms one of the most important groups of nematodes because of their parasitic nature in humans. The eggs are mainly found in wastewater and in the soil and have the ability to remain viable for a very long time (up to a period of



10 years or more) under unfavourable conditions. It grows up to a length of 35 cm at adult stage (Molleda *et al.*, 2008). It causes the condition ascariasis in man through the ingestion of mature ova. Its manifestation is associated with poverty and poor hygiene in a population (Arroyo *et al.*, 2010). The major route of removal from wastewater is filtration through the matrix, length of the wetland, hydraulic loading and retention period of the wetland system (Garcia *et al.*, 2010). Helminths, for example, pose serious health implications and illnesses such as pneumonitis (Loeffler's syndrome) and intestinal obstruction in children (Gerardi and Zimmerman, 2004). *Ascaris lumbricoides* is considered as one of the very common parasitic infections in the world today, though mortality rates associated with it remains relatively unclear (Scott, 2008; Buckley *et al.*, 2008).

### 1.3.7 Rhizofiltration and Constructed Wetlands Systems

Conventional treatment systems such as trickling filters, up-flow anaerobic sludge blanket, waste-stabilization ponds, land treatment are cost-effective in terms of land area, machine maintenance, labour and basically remove pathogens by reduction of turbidity and filtration (Arroyo *et al.*, 2010; Shelef *et al.*, 2013). Infiltration systems such as rhizofilters can efficiently remove or reduce the concentration of pathogens in wastewater by adsorption and straining through the matrix (Stevik *et al.*, 2004; Periasamy and Sundaram, 2013).

Wetlands are known to achieve better treatment of wastewater including the removal of heavy metals, suspended solids and wastewater pathogens, unlike the conventional treatment systems which partially remove the above pollutants (Boutilier *et al.*, 2011; Jia *et al.*, 2014). Purification of wastewater by wetland systems is notably achieved through processes such as rhizofiltration, phytoextraction, phytovolatilization and phytoaccumulation (Kamarudzaman *et al.*, 2011).

Wetlands have been defined as:-

“Wetlands are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh,

brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six metres” (Ramsar, 1971).

Wetlands also occur in floodplains, hillsides, glacial valleys and along shorelines (Hey and Philippi, 1999). Wetlands act as biofilters due to their ability (macrophytes) to withstand harsh effluent conditions, to remove sediments and pollutants such as heavy metals and pathogens from the wastewater (Jia *et al.*, 2014; Nedunuri *et al.*, 2014).

A constructed wetland (CWs) is an artificial marsh or swamp (Sayadi *et al.*, 2012) created for anthropogenic discharge such as wastewater, stormwater runoff or sewage treatment (Reed *et al.*, 1995; Mthembu *et al.* 2013). These systems are also able to remove pollutants through direct killing by plant toxins and other microorganisms, natural die off, temperature and ultraviolet radiation according to Abdel-Raouf *et al.*, (2012). Indicator micro-organisms such as *E. coli* can be greatly reduced to non-detectable levels by use of constructed wetland (McCarthy *et al.*, 2011; Odinga *et al.*, 2013). One of the factors influencing removal is hydraulic retention time (Kumar *et al.*, 2010). Pathogen removal by these systems may occur even at few hours retention time depending on the type of pathogen. However, a retention time of 20 days is sufficient to remove significant amounts of pathogens including *Salmonella* and *E. coli*, however, high rainfall may reduce retention time thereby increasing the time required for pathogen removal to more than 20 days in order to achieve higher removal efficiencies (McCarthy *et al.*, 2011).

Aquatic plants (through phytoremediation) in constructed wetlands play a major role in wastewater treatment efficiencies (Shelef, *et al.*, 2012; Ebrahimi *et al.*, 2013). This treatment ability is based on the particular plant species, metabolic activities within plant and pollution tolerance rates (Tuttolomondo *et al.*, 2014). Their root systems are resilient to various shocks from pollution loading, climatic variations and pollutants that have very high salinity contents with reported levels of up to 11,500 mg/l as total dissolved solids (Reddy and Delaune, 2009). Others include total phosphorus and electrical conductivity levels of 14 mg/l and 16  $\mu$ S/cm

respectively (Calheiros *et al.*, 2010; Vymazal, 2011). Pollutant removal by CW is also based on water depth, hydraulic loading, hydraulic efficiency and influent concentration (Haarstad *et al.*, 2012).

#### 1.3.7.1 Types of constructed wetlands

Due to variations in pollutant composition and disposal requirements, wetlands may have different design characteristics in order to achieve maximum pollutant reduction (Vymazal, 2011). For example subsurface flow constructed wetlands (SSF CWs) are extensive systems widely used for the treatment of wastewater generated in small communities (Odinga *et al.*, 2013). Low energy requirements and non-specialized manpower for plant management are among the most important advantages of SSF CWs in comparison to conventional alternatives such as the activated sludge processes (Wallace and Knight, 2006; Jia *et al.*, 2014).

It is widely accepted that clogging is the worst operational problem of the treatment wetlands technology (Wallace and Knight, 2006; Knowles *et al.*, 2011). This is because clogging limits the lifespan of the systems, thus impacting negatively on wastewater treatment efficiency (Caselles-Osorio *et al.*, 2006; Gunes *et al.*, 2012).

#### 1.3.7.2 Free water surface constructed wetland rhizofilters (FWS CWs)

Free water surface constructed wetland rhizofilters consist of a sealed basin or a sequence of basins, which contain rooting soil (20-30 cm) and usually have a water depth of 20-40 cm (Figure 1A). The subsurface is normally fitted with an impervious geotechnical membrane to prevent seepage of water into the ground (Vymazal, 2010). At the surface, there is dense emergent vegetation covering almost 50% of the total wetland area (Noor *et al.*, 2010). Free water surface CWs are commonly used for municipal wastewater treatment and storm water runoff purification. In FWS CWs, the water flows above ground in a manner that mimics natural wetlands. Treatment is achieved when the water flows slowly through the wetland while being regulated by the plant stalks and litter resulting in settlement of particulate matter (Noor *et al.*, 2010; Nilsson *et al.*, 2012).

### 1.3.7.3 Subsurface flow Constructed Wetlands (SSF CWs)

These are extensive systems with shallow basins underlain by an impermeable material with substrate media to support the growth of emergent macrophytes as shown in Figure 1B (Vymazal, 2010; Shelef, *et al.*, 2013). There are two types of subsurface CWs namely Vertical Flow Subsurface (VFSS) and Horizontal Flow Subsurface (HFSS) as illustrated in Figure 1.1, A and B, with various pollutant removal kinetics and efficiencies (Vymazal, 2007; Zhang *et al.*, 2011). A study by García *et al.* (2010) reported that vertical sub-surface constructed wetlands have higher removal efficiencies in comparison to the other designs like horizontal sub-surface constructed wetlands. The vertical flow sub-surface (VFSS CW) provides suitable environmental (aerobic) conditions for removal of suspended solids, organics and nitrification processes within the wetland while the HFCW removes pollutants by providing anaerobic/anoxic conditions for denitrification based on Siedel's concept (Kalipci, 2011; Nilsson *et al.*, 2012). In these systems, pre-treated wastewater is fed slowly through the filter medium either vertically or horizontally (Jia *et al.*, 2014). As the water passes through the reed bed and the filter medium, it is exposed to a network of either anoxic/anaerobic or aerobic zones where pollutants are removed (Arroyo *et al.*, 2010). The subsurface treatment systems are best suited for treating primary wastewater since the design does not allow for direct contact between the atmosphere and the water column (Kropfelova *et al.*, 2009). These designs are recommended because of restricted breeding of disease-causing parasites like vermin (Jia *et al.*, 2014). However, system blockage caused by sludge occurring around the inlet zone is a common problem associated with horizontal flow systems (Vymazal 2011). The blockage normally occurs due to either poor hydraulic design, inappropriate choice of filtration media or poorly regulated flow distribution within the inlet zone (Zhang *et al.*, 2010). This situation slows down the performance of the system resulting in poorly treated or substandard effluents (Osorio *et al.*, 2007; Zhang *et al.*, 2010).

#### 1.3.7.4 Hybrid wetlands

Hybrid wetlands consist of a series of other constructed wetlands that are used in combination in order to achieve higher treatment efficiencies especially of pathogens and total nitrogen removal (Vymazal and Kropfelova 2005; Baskar *et al.*, 2009; Sayadi *et al.*, 2012). The optimum combination of the different types of CWs depends largely on the target pollutants (Vymazal, 2009). For example, HF CWs are normally characterized by low levels of dissolved oxygen leading to slow nitrification processes, while VF systems are designed to transport oxygen at a greater capacity, which provides a better environment for nitrification (Kalipci, 2011). In order to enhance the removal of ammonia, HF CWs may be used in combination with VF CWs in well-engineered systems (Vymazal, 2009; Vymazal, 2011). Another technology that embodies the combination of FWS CWs and sub-surface flow CWs and FWS CWs with sub-surface flow CWs (Borkar and Mahatme, 2010). This combination is designed with a focus on efficient removal of organic material and suspended matter (Vymazal, 2011).

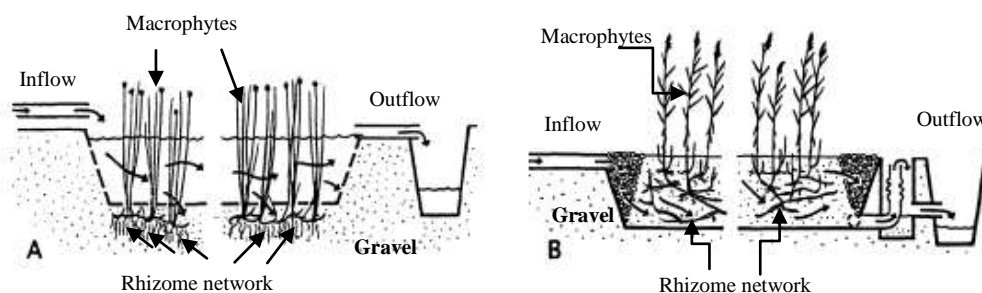


Figure 1.1. A & B. Typical arrangement of a constructed wetland with (A) free water surface flow and (B) subsurface horizontal flow. Adapted from Jenssen *et al.* (1993).

#### 1.3.7.5 Rhizofilters

Constructed wetlands rhizofilters are based on mechanisms that employ processes of filtration by use of plants (phytofiltration). The pollutant filtration works through processes of phytostabilization and phytoextraction (Bouasria *et al.*, 2012). Reports from previous studies indicate that plants used in rhizofilters are effective in the

removal of environmentally toxic heavy metals such as Cu, Cd, Cr, Ni, Pb and Zn (Akpor and Muchie, 2010; Elias *et al.*, 2014). The design of CWS (Figure 1.1) is based on the presence/absence of water (subsurface flow or surface), the presence of macrophytes (submerged, free-floating, and emergent) and flow regimes (horizontal or vertical) as shown in Figure 1.2.



Figure 1.2. The constructed Rhizofiltration unit in Kingsburgh,-Durban, South Africa showing the established macrophytes and influent channel to the planted and reference vertical flow channels.

#### 1.3.8 Wastewater Treatment

Treatment mechanisms involved in a constructed wetland system are the hydraulic retention time (HRT) and volumetric flow rate (VFR) of water (Mitsch and Gosselink 2000). Increased HRT coupled with reduced VFR is believed to provide maximum exposure of water to the root surface of the plant providing sufficient time for the uptake of the nutrient ions and other chemical changes (Srivastava *et al.*, 2008).

Pollutant removal in CWs is due to plant uptake through the rhizosphere; however, the main contribution has long been attributed to microbial activity and filtration through the rhizofiltration matrix (Hechmi *et al.*, 2014). Another process by which plants influence microbial communities within CWs is through the release of

oxygen to the rhizosphere (the root surface and environment directly influenced by the root) (Borkar and Mahatme, 2010). This enhances the activities of specific microbial functional groups and thus promote organic carbon degradation (Faulwetter *et al.*, 2009). Some studies have reported an increase in microbial activity and density within the rhizosphere (Haarstad *et al.*, 2012). Other studies have incorporated molecular techniques to determine the effect of plants on the microbial dynamics within CW systems but most of what we know is still largely based on inference (Faulwetter *et al.*, 2009).

Constructed wetlands are self-repairing systems which give them an extended shelf life over the conventional systems (Steudle, 2000). In this regard, CWs have become a comparatively viable option for further filtration and efficient treatment of wastewater either in an integrated manner or as single phase treatment (Sohsalam *et al.*, 2008).

The major CWs treatment mechanisms involve processes of phytoremediation where dynamics encompass the different actions of plants and their associated rhizospheric bacteria through rhizofiltration, phytodegradation, rhizodegradation and phytovolatilization (Vymazal, 2011; Barbagallo *et al.*, 2011). The rhizosphere forms a very dynamic region within the wetland as it is the actual interaction point of the pollutants and the plant roots (Kalipci, 2011; Xu, 2014). Haarstad *et al.*, (2012), in his study of CWs planted with *Phragmites australis* reported pollutant reductions after increasing retention periods (Table 1.3).

Table 1. 3. Successive reduction of pollutants (%) after an increase in retention period from a period of three to five days. Adapted from Haarstad *et al.*, (2012).

Parameter	% Pollutant reduction with time		
	After 3.96 days	After 4.56 days	After 5.4 days
Biochemical Oxygen Demand	64.5	65.1	71.2
Chemical Oxygen Demand	68.0	70.8	75.1
Total Phosphorus	21.0	22.7	24.5
Total Nitrogen	20.7	21.9	23.5
Suspended Solids	79.7	81.8	87.3

In a study of wetlands conducted by Hamadeh *et al.*, (2014), there were significant removals of TSS by 98%, BOD 96%, COD 88%, Phosphate 92% and Ammonium 98%. These results show evidence of improved efficiency with longer retention periods. Pollutant removal by CWs also depends on the water depth, hydraulic loading, hydraulic efficiency and influent concentration (Haarstad *et al.*, 2012, Kalipci, 2011). Other pollutant removal mechanisms in CWs are based on sedimentation, filtration, chemical precipitation, microbial interactions, and adsorption on the root, soil/s, surface and absorption into the tissues of aquatic macrophytes (Vymazal, 2011).

There are some pitfalls for wastewater treatment wetlands according to a review by Haarstad *et al.*, (2012). Constructed wetlands form habitats and breeding grounds for mosquitoes, which are disease-carrying agents and therefore, a health risk to the human population around CWs facilities (Popko *et al.*, 2006). Constructed wetlands are also prone to system clogging caused by suspended matter and dead plant material. Considering the climatic conditions at the site, macrophytes may take a long time for root zone growth and the subsequent efficient wastewater treatment to be achieved (Sundaravadivel and Vigneswaran, 2010; Knowles *et al.*, 2011; Odinga *et al.*, 2013). Another limitation on these systems is the challenges faced by the site locations, for example, areas with the high water table and steep slopes may not be favourable for their construction as such sites will hinder their process functionality (Kalipci, 2011).

#### 1.3.9 Classification and Characteristics of some Wetlands Macrophytes

In studies by Srivastava *et al.*, (2008) and Vymazal, (2010), macrophytes are classified according to their growth forms as emergent macrophytes (e.g. *Phragmites australis*, *Typha latifolia*), free-floating macrophytes (e.g. *Eichhornia crassipes*, *Lemna minor*), submerged macrophytes (e.g. *Myriophyllum spicatum*, *Potamogeton*, *Elodea*) and floating-leaved macrophytes (e.g. *Nuphar luteum*). Floating aquatic macrophytes (FAMs) are mainly found in tropical wetlands



whereas the emergent macrophytes dominate the temperate wetlands (Borkar and Mahatme, 2010). Floating aquatic macrophytes, such *Potamogeton* sp. add value to wastewater treatment systems because they serve as the carbon source to decomposers that play a major role in the treatment process (Karathanasis *et al.*, 2003). Submerged aquatic macrophytes (SAMs) are found in the regions between land and open water and are associated mainly with nutrient cycling (Kamarudzaman *et al.*, 2011). These plants possess some buffering mechanisms like nutrient uptake and bicarbonate utilization that help maintain a clear water state in the wastewater treatment process (Vymazal, 2010; Vymazal, 2011). The SAMs are known to remove nutrients and heavy metals using processes like denitrification and nitrification, which are normally limited by ammonium ( $\text{NH}_4^+$ ) as used by e.g. *Potamogeton* spp. and *Elodea* spp. (Sayadi *et al.*, 2012).

Aquatic macrophytes possess adaptive features that enable them to survive the harsh environmental conditions and be able to act as treatment sinks to wastewater remediation (Srivastava *et al.*, 2008; Lum *et al.*, 2014). One of the common aquatic macrophytes, *Phragmites australis* has many adaptive features that make it suitable as the macrophyte of choice in a constructed wetland for wastewater remediation (Kalipci, 2011). Additional beneficial features of *Phragmites australis* include the ability to produce large amounts of seeds which spread vegetatively using rhizomes and stolons (Srivastava *et al.*, 2008). Rhizome segments containing at least one axillary bud are also an effective propagule for plant establishment, a characteristic that makes *Phragmites australis* a quick colonizer of any region where it is first introduced (EPA, 2010). Table 1.4 shows information on the distribution and desirable limits for some of the major environmental factors that influence the choice of macrophytes in CWs (DWAF, 1998).

Table 1.4. Emergent aquatic plants for wastewater treatment and their environmental requirements.

Organism name / Common	Growth Temperature (°C) Desirable Limits	Seed Germination (days)	Maximum Salinity tolerance (ppm*)	Effective pH range	References
<i>Typha</i> spp. / Cattail	10-30	12-24	30	4-10	EPA, (1998)
<i>Phragmites communis</i>	12-23	10-30	45	2-8	EPA, (1998)
<i>Phragmites australis</i>	20-30	10-20	20	3.7-8.0	(EPA, 1998; Swearingen and Saltonstall, 2010; Marks <i>et al.</i> , 2012)
<i>Juncus</i> spp. / Rush	6-26	30	20	5-7.5	EPA, (1998)
<i>Scirpus</i> spp. / Bulrush	18-27	30	20	4-9	EPA, (1998)
<i>Carex</i> spp. / Sedge	14-32	30	20	5-7.5	EPA, (1998)

\*ppm = parts per million (DWAF, 1998).

Macrophytes play an important role in the wastewater treatment process by providing an appropriate environment for microbial growth and significantly improving the transfer of oxygen (Vymazal, 2011) into the root zone (Table 1.5). Macrophytes, in association with aquatic microorganisms and periphytons, enhance the uptake of nutrients from wastewater (Vymazal, 2010). Periphytons potentially remove metal cations and nutrient anions such as  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$  by direct absorption from the water column (Scinto and Reddy, 2003; Srivastarva *et al.*, 2008). Some microalgae such as *Chlorella pyrenoidosa* could also be potentially responsible for the death of bacteria due to competition for food in treatment rhizofilters (Singh *et al.*, 2014). Apart from oxygen transfer, macrophytes also transport approximately 90% of the oxygen available in the rhizosphere creating conditions that are suitable for the sedimentation and settling of suspended solids (Srivastarva *et al.*, 2008; Vymazal, 2011). This stimulates both aerobic decompositions of organic matter and promotes the growth of nitrifying bacteria and periphytons (Zhang *et al.*, 2010). Some of the plants that are used in sub-surface wetland treatment systems include cattails (*Typha* sp.) and the common reed (*Phragmites australis*) systems (Borkar and Mahatme, 2010).

Table 1. 5. Summary of major roles that macrophytes components play in constructed wetlands. (modified from Vymazal, 2011).

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

\*Roles important in HF CWs in *italics*

Macrophytes are assumed to be the main biological component of a constructed wetland (Haarstad *et al.*, 2012), with *Phragmites australis*, *Vetiveria zizanioides*, *Scirpus spp.*, *Typha spp.*, *Glyceria spp.*, *Lemna spp.*, being the common species of choice for wastewater treatment due to their root structure formations (Kadlec and Reddy, 2001; Srivastava *et al.*, 2008).

Aquatic plants play an important role in the treatment process by providing an appropriate environment for microbial growth (Table 2.1). Plants facilitate the growth of bacteria colonies and other microorganisms which form a biofilm attached to the surface of roots and substrate particles (Adefemi *et al.*, 2013; Mthembu *et al.*, 2013). In the case of the reed, there is a massive network of roots and rhizomes which maintain a high biological activity in the constructed wetland (Sayadi *et al.*, 2012; Singh *et al.*, 2014). The aquatic plants normally accumulate

ions of metals from their locality (Cooper *et al.*, 2005). This has led to the investigation of their possible role in metals removal from municipal wastewater using a constructed wetlands facility (Singh *et al.*, 2012).

The growth, function and distribution of plant roots play the important remediation process in treatment wetlands (Arroyo *et al.*, 2010). These properties include high root porosity, higher radial oxygen loss, photosynthetic rate, root activity, above - and below-ground growth rates, and nutrient removal rates, which can be comparable to the fibrous-root plants (Zang *et al.*, 2010).

### 1.3.10. Adaptive Strategies of Macrophytes to Various Growth Processes

#### 1.3.10.1 *Phragmites australis*

The ‘common weed’ *Phragmites australis*, is a tall (1.5-4.0 m) coarse perennial grass found primarily in brackish and freshwater wetlands (Lee and Scholz, 2007, Odinga *et al.*, 2013). It is distributed worldwide but is endemic to North America (Lee and Scholz, 2007). In wetlands, *Phragmites australis* is sometimes considered a nuisance species because it colonises a region and once established, acts like a climax species (Hauber *et al.* 1991; Swearingen and Saltonstall, 2010; Dolinar and Gaberscik, 2010).

*Phragmites australis* has many adaptive features that make it suitable as the macrophyte of choice in a constructed wetland for wastewater treatment (Vymazal and Kropfelova, 2005). These range from, reproduction, apical dominance, mechanical adaptations and gaseous exchange (Hara *et al.*, 1993; Marks *et al.*, 1994; Zehra and Khan, 2007; Dolinar and Gaberscik, 2010).

The rapid growth of up to 10 metres under optimum conditions is possible because the horizontal stems of *Phragmites* exhibit strong apical dominance (Verboven *et al.*, 2011; Sayadi *et al.*, 2012). This is a feature that promotes the use of *P. australis* in wastewater treatment because the uptake of pollutants will be enhanced by their rapid growth (Cowie *et al.*, 1992; Kropfelova *et al.*, 2009; Howard, 2010).

*Phragmites* species possess abundant aerenchyma cells which are responsible for internal gas exchange. This increases the suitability of this plant in enhancing oxygenation of the rhizosphere in the treatment system (Shimamura *et al.*, 2010; Malik *et al.*, 2010; Shiono *et al.*, 2010; Hazelton *et al.*, 2014). Light attenuation by the elevated *Phragmites australis* leaf canopy, imparts increasing stress to shorter understory wetland plant species. Therefore *P. australis* remains the dominant species as the wastewater treatment is achieved (Vymazal and Kropfelova, 2005; Kalipci, 2011; Lum *et al.*, 2014). These stems tend to persist but when they break, they generally do so at the first or second node above the soil surface (Stanton, 2005). As a consequence, *Phragmites* colonies tend to form a dense thatch that is elevated above the soil (Howard, 2010; Gill *et al.*, 2014). The dense thatch that is created by *P. australis* colonies creates self-mulch which assists in moisture conservation. When this occurs, the *phragmites* population is protected from competition from other plants and therefore improves its performance in pollutants removal (Howard, 2010, Luca *et al.*, 2011; Kalipci, 2011).

#### 1.3.10.2 *Kyllinga nemoralis*

Available literature on the potential of *Kyllinga nemoralis* to remove heavy metals from wastewater is scanty. A sedge of the family Cyperaceae, which forms a mat and grows up to 50 cm high, *Kyllinga nemoralis* is considered a common weed (Majumder, 2013; Odinga *et al.*, 2013). The perennial weed spreads by means of creeping rhizomes which contain allopathic oils (Kawabata *et al.*, 1994). This plant mainly propagates through rhizome cuttings and seeds (Majumder, 2013). *Kyllinga nemoralis* possesses an achene fruit which measures about 1.2-1.5 mm long by 0.5-0.7 mm wide. The leaves contain the active chemical such as essential oils ( $\beta$ -selinene, terpenes  $\alpha$ -cyperone, and  $\alpha$ -humulene), while the rhizomes also possess glycosides, triterpenoids and flavonoids (Majumder, 2013). These compositions render the roots and leaves of *Kyllinga nemoralis* highly medicinal (Das *et al.*, 2010). Some of the ailments that can be treated using the leaves and roots include skin rash, reduction of malarial chills, diarrhoea, and diabetic conditions. Greenhouse studies indicate that *Kyllinga nemoralis* cannot withstand drought conditions, a

characteristic that potentially increases its use as a wetland macrophyte (Vacca *et al.*, 2005; Nedunuri *et al.*, 2014). In a study by Sindhu *et al.* (2014), *Kyllinga nemoralis* root extract was successfully used to inhibit the growth of pathogenic bacteria, *Escherichia coli*. Hence it is a wetland plant that may potentially be used in phytoremediation processes to remove pollutants such as bacteria from wastewater. Ray and George, (2009) studied metals deposition and accumulation on Cyperaceae family along the roadside. They discovered that the *Kyllinga* species accumulated metals in its tissues. This discovery confirmed that *Kyllinga nemoralis* has the potential to remediate heavy metals from wastewater. This study was also a maiden investigation of the macrophyte *Kyllinga nemoralis* and successfully proved its ability to remove pollutants from wastewater.

The potency of root exudates against various pathogens has been investigated by several researchers especially in the field of medicine, but little research has been focused on the potential effects of wetland plants in the removal of pathogens from wastewater (Gruyer *et al.*, 2013). For example, Rojas *et al.*, (2006) investigated the antibacterial potential of *Cecropia peltata* L., *Bidens pilosa* L., *Cinchona officinalis* L. against *Bacillus cereus*, *Escherichia coli* and *Candida albicans*. Abeysinghe, (2010) studied the potential of mangroves such as *Exoecaria agallocha* L., *Rhizophora apiculata* L., *Bruguiera sexanula* L., against *Proteus* spp. and *Staphylococcus aureus* while Mwitari *et al.*, (2013) investigated *Warbugia ugandensis* L., *Plectranthus barbatus* L., *Withania somnifera* L. and *Prunus africana* L. against *Escherichia coli* and *Staphylococcus aureus*. They all concluded that the extracts from their investigated plants portrayed high antimicrobial potentials against the target microorganisms. Garg *et al.*, (2013) conducted a study on *C. spectabilis* and concluded that this plant poses some antimicrobial compounds that can be combined with other antibiotics to enhance its treatment efficiency.

### 1.3.11 Heavy Metals in Wastewater

Due to their harmful and toxic effects, environmental pollution caused by heavy metals has become one of the major research interests all over the world (Mami *et al.*, 2011). There exist about 90 naturally occurring elements, 53 of which are heavy metals (Smith, 2009; Luca *et al.*, 2011; Tangahu *et al.*, 2011; Espinoza *et al.*, 2012; Elias *et al.*, 2014).

Heavy metals are categorized as those metals with a specific gravity that is greater than 5 g/cm<sup>3</sup> (Rai, 2008). Heavy metals in wastewater originate from large manufacturing enterprises, fertilizers, municipal wastes, pesticides, sewage, mining and smelting of metalliferous ores and burning of fossil fuels (Sorme and Lagerkvist, 2001). The presence of heavy metals in wastewater treatment systems and final discharged effluents from treatment systems into receiving waterways may pose serious health threats to both aquatic and human life (Rai, 2008; Zhang *et al.*, 2014b). Some of the common heavy metals found in wastewater include copper (Cu), mercury (Hg), cadmium (Cd), lead (Pb), zinc selenium (Se), nickel (Ni) and chromium (Cr). Heavy metals are toxic to the environment and may cause ecological damage when left to accumulate to high levels (Cheng *et al.*, 2002; Xilong *et al.*, 2005; Vymazal, 2010; Tangahu *et al.*, 2011; Espinoza *et al.* 2012; Elias *et al.* 2014). Mercury, arsenic, lead and cadmium are ranked as toxic substances by US Agency for Toxic Substances and Disease Registry (ATSDR) (Rai, 2008). Arsenic, cadmium, lead, mercury and silver can be harmful to both aquatic plants and animals even at very low concentrations. Additionally, Copper, Cadmium and Zinc ions are extensively applied in the industrial sector according to Katsou *et al.*, (2011) and Mukesh and Thakur, (2013) in wood processing, pigment manufacturing, petroleum refining and photographic operations. These are of great concern in environmental health (Cheng *et al.*, 2002; Tangahu *et al.*, 2011; Banflavi, 2011).

Arsenic causes disorders of the nervous system, cadmium, mercury and chromium are known to cause damage to the kidneys and Lead causes mental retardation and

anaemia to humans (Vasudevan *et al.*, 2011). It has also been reported that copper levels above 1.0 mg/l are detrimental to duck weed species (*Lemna gibba*) (EPA, 2010) and may also cause Wilson's disease in humans (Mukesh and Thakur, 2013). A study by Veroli *et al.*, (2014) reported that heavy metal pollution (chromium, zinc, copper, cadmium) causes mouthpart deformities (by about 56%) in certain aquatic macroinvertebrates (*Chironomus riparius*). This kind of deformity hinders or reduces the normal metabolic activities of the macroinvertebrate thereby reducing their potential as an indicator of water quality in freshwater ecosystems (Odinga *et al.*, 2011).

Heavy metals pollution also alters soil microbial diversity and tends to mediate gas evolution by greenhouse biogenic processes (Zhou *et al.*, 2014). However, some heavy metals are essential to both humans and plants. According to Manivannan and Biju, (2011) and Tangahu *et al.*, (2011), plants require trace amounts of barium, boron, chromium, cobalt, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, selenium, sulfur, and zinc for their growth. Constructed wetlands wastewater treatment systems are commonly used for the removal of pollutants such as organics, suspended solids, microbial population and nutrients. Information on heavy metals removal by CWs is currently insufficient to make any conclusive CWs treatment statements (Vymazal, 2010; Islam *et al.*, 2015).

Various methods and techniques have been used to remove heavy metals from wastewater. Ion exchange, lime precipitation and electrolytic techniques have been used in the recent past to reduce heavy metals concentration in wastewater (Madhavi *et al.*, 2013). The conversion of metals from one form to another such as Cr(VI) to the less toxic Cr(III) using ferrous sulphate contributes to particle size increase by coalescing together and causing sedimentation and eventual removal from wastewater (Wang and Fu, 2011). These methods, however, contribute to environmental pollution and have also been reported to exhibit some limitations towards the total elimination of metals pollution from wastewater (Graillet *et al.*, 2013).



Heavy metal accumulation in plant tissues is not confined to a particular part of the plant but is distributed based on the type of plant, type of metal and the CWs hydraulics (Barakat, 2011). The increase of environmental diversity by wetland plants in the rhizosphere, act as a catalytic phenomenon to various biochemical and chemical reactions that promote purification of the wastewater (Ahmad *et al.*, 2014). According to Tangahu *et al.*, (2011), the highest arsenic (As) accumulation was observed in *Pteris vittata* L. species, which recorded more than 0.7 mg As/g dry weight of the plant. Within the plant root system, the highest Arsenic accumulation was observed in *Populus nigra* as  $> 0.2$  mg As/g of the plants dry weight (Sharma, 2012). Other plants that accumulated  $> 50$  mg/g of dry weight included species of *Brassica campestris* (L.), *Brassica carinata* A., *Brassica juncea* (L.) Czern (L.), and *Brassica nigra* (L.) (Wang and Fu, 2011; Tangahu *et al.*, 2011). The removal mechanism of heavy metals within the CWs is mainly through the wetland vegetation, wetland hydrology and the soil substrate. The actual removal kinetics involves accumulation in tissues (Figure 1.3), adsorption (Mukesh and Thakur, 2013) on the root zone of the emergent and free-floating macrophytes, and through the leaves in case of both floating and submerged leaves (Achyut *et al.*, 2010).

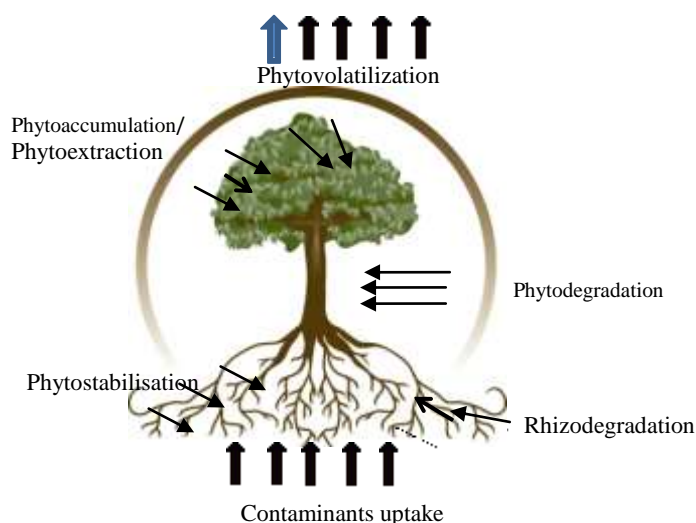


Figure 1.3. Mechanisms involved in the heavy metals uptake by the phytoremediation technology. Adopted form Tangahu *et al.* (2011).

Sorption methods are economical and are reported to efficiently remove traces of metals from wastewater with minimal adverse effects to the environment (Graillot *et al.*, 2013). Zinc, for example, is readily absorbed by plant tissues while Cu is best absorbed or attached to sediment material according to a study by Smith, (2009). This absorption and attachment tendency is pH dependent as high soil pH hinders the metals transfer into plant tissues (Smith, 2009). According to Wood and Shelley, (1999), metal biosorption is highly influenced by the organic carbon in binding metal and acid volatile sulphide (AVS).

Heavy metal removal processes through phytoremediation include (i) phytoextraction/phytoaccumulation, which involves the use of plants to remove heavy metals (Figure 1.3) from the soil matrix (Mwitari *et al.*, 2013). (ii) Phytovolatilization employs the use of plants that are genetically modified such that they are able to absorb the elemental forms of the metals from the soil and convert them biologically to gaseous species (He *et al.*, 2012). In this state, the elements can easily be released into the atmosphere. This process has met several controversies to the effect that metals such as mercury and selenium, when released to the atmosphere in gaseous forms, could be detrimental to the health of the environment (Gill *et al.*, 2014). (iii) Phytostabilization is a technique that hydraulically suppresses the metal's movement and restricts its exposure to the soil matrix only, a situation that ensures the contaminants neither migrate and contaminate groundwater resources nor find their way into the aboveground biomass (Achut *et al.*, 2010).

*Agrostis tenuis* Sibth of the Poaceae family which is a poor translocator of metal contaminants may be used for phytostabilization of Zn, Cu and Pb within the soil matrix (Prasad and Freitas, 2003; Kumpiene *et al.*, 2008). Other removal mechanisms, especially in anoxic conditions, occur by decomposition of organic matter through reduction reactions and precipitation of metal sulphides in an organic substrate (Lum *et al.*, 2014). This process eventually influences sulphate reduction. The decomposed organic matter then accumulates on the surface of the wetland sediment where heavy metals are potentially absorbed (Mukesh and

Thakur, 2013). The submerged rooted plants remove the heavy metals through extraction from sediments and water (Yadav *et al.*, 2011). Heavy metals removal by plants may also be achieved by passive water movement in the aqueous phase through the cracks found in the cuticle or through the stomata and cell wall (Tangahu *et al.*, 2011). Further removal may also be through the plasmalemma and the phytochelatin cells. These are the sites, which are involved in the detoxication of heavy metals in plant cells (Achyut *et al.*, 2010). According to Sheoran and Sheoran, (2006), excess heavy metals can bind to cell walls in the process of metathiolate formation, which involves mercaptide complexes.

Annual harvesting of the wetland plants also plays a significant role in the removal of heavy metals that are used up by the plants in the tissues and adsorbed onto the root surfaces (Achyut *et al.*, 2010). For better removal efficiency, the plant harvesting ought to be done in such a way as to pluck out the total plant including the root systems (Yadav *et al.*, 2011). Phytoplanktons are also known to remove heavy metals from wastewater. This observation was made by studying algae such as *Nitella* and *Chara*, which were used to reduce uranium in mining effluent according to Sheoran and Sheoran, (2006).

The uptake mechanism of heavy metals by different plant species is influenced by a number of factors. These include plant species, properties of the medium, the chelating agent added, uptake mechanisms, the bioavailability of the metal and chemical properties of the contaminant. Environmental conditions and the root zone composition of the study macrophyte also play a major role in heavy metal uptake (Tangahu *et al.*, 2011).

Cheng *et al.* (2002) and Tangahu *et al.*, (2011) conducted studies on the kinetics of heavy metal removal from a constructed wetland planted with *Cyperus alternifolius* and *Villarsia exaltata* with a vertical/reverse flow system design. In this study, two chambers were separated which shared a common drainage layer. The plants used were first cultivated under normal conditions in an open field. Upon attainment of the required growth level, the plants were uprooted and planted in the vertical flow (inflow) and the reverse-vertical flow (outflow) chambers of the CW. In order to

acclimatize the plants to wastewater conditions, the plants were watered daily using 40 L of water, which was enriched with mineral nutrients. The CW preparation/optimization period was achieved after three months. Artificial wastewater of varying concentrations of Al, Cd, Cu, Mn, Pb and Zn was applied to the wetland. Using various HRT regimes, water samples at inlet and effluent chambers were collected and analyzed for heavy metals using ICP-MS methods according to Cheng *et al.*, (2002) and Islam *et al.*, (2015). Consequently, the leaves, shoots, rhizomes, main roots and lateral roots of the *C. alternifolius* were prepared according to APHA, (2005) and analysed for heavy metals. Removal of heavy metals after a period of five months was almost 100%, except for manganese which was at 42.2%. Other possible removal rates by CWs as reported by Sheoran and Sheoran, (2006) are 75-99% for cadmium, 26% for lead, 75.9% for silver, and 66.7% for zinc. In the above study, the heavy metals were found to accumulate in the leaves, shoots and rhizomes with the main roots and lateral roots having the highest content, while the lowest concentrations were found within the shoots (Cheng *et al.*, 2002).

In a study by De Souza *et al.*, (1999), it was reported that certain bacteria are responsible for the accumulation of metals especially selenium and mercury in the roots of constructed wetland plants. This action by bacteria contributes to the removal effects of heavy metals from wastewater. The mechanisms by which bacteria influence heavy metal removal from wetlands are through interactions with plants tissues (Sayadi *et al.*, 2012). Their association is believed to trigger production of some plant root exudates that facilitate metal accumulation in plants. This is supported by findings of Schaller *et al.*, (2011) that bacteria in the biofilm accumulate large amounts of metals. The bacteria are also responsible for the health and pollution reduction through the biofilm (Kumar and Goel, 2010). Consequently, they are also known to transform certain elements into forms that are easily absorbed into plant roots. Rhizospheric bacteria stimulate the protein that transports sulphate and selenite within the rhizosphere. This action enhances heavy metal removal by plants (De Souza *et al.*, 1999).

### 1.3.12 Pathogens in Wastewater

Wastewater from municipalities is known to carry various groups of pathogenic microorganisms like protozoa, viruses, bacteria and helminths (McCarthy *et al.*, 2011). These pathogens mainly emanate from the human gastrointestinal tract or are normally associated with human and animal gut (Colomer-Lluch *et al.*, 2014). The diversity and abundance of the pathogens depend largely on the socio-economic activities of the population releasing the wastewater (Nasser *et al.*, 2012). Waterborne pathogens are known to infect over 250 million people globally each year with most of the infections resulting in 10 to 20 million deaths especially in developing countries (Reinoso *et al.*, 2008; Odinga *et al.*, 2013). This situation is attributed to lack of awareness and low sanitation levels within the affected population (Vymazal, 2010). Coliform bacteria in wastewater and recycled water are indicators of contamination from human and animal waste while *Salmonella* and *Shigella* find their way into wastewater through faeces of infected individuals (McCarthy *et al.*, 2011). Enteric viruses, on the other hand, are shed into wastewater through faecal matter from infected individuals either by vaccination or ingestion of contaminated food (Nasser *et al.*, 2012).

Ova of other parasitic pathogens such as *Ascaris lumbricoides* enter the wastewater through faeces of healthy and infected persons. *Ascaris lumbricoides* cause human disease *Ascariasis*. *Salmonella*, *Clostridium* and *Shigella* are causative agents for diarrhoea in adults and children resulting in death if not treated (Sundaravadivel and Vigneswaran, 2010). The presence of pathogens in recycled wastewater remains a major challenge in many countries where water reuse may be the only viable solution to irrigation of food crops (Redder *et al.*, 2010). Indeed, some viruses were detected in a borehole 27.5 m below a field where crops were irrigated with reclaimed water (Kayyali and Jamrah, 1999).

### 1.3.12.1 Pathogens removal in constructed wetlands

Constructed wetlands have emerged as very efficient systems for the removal of pathogens especially in tropical countries due to their low installation and management costs (Arroyo *et al.*, 2010). The use of CWs rhizofiltration systems to eliminate ova and (oo) cysts of helminthes, protozoa bacteria, viruses from wastewater is one of the latest technologies that has emerged and is expected to solve the problem of efficient pathogen removal from wastewater (Iasur-Kruh *et al.*, 2010; Lilach *et al.*, 2010). According to Reinoso *et al.*, (2008), CWs systems are able to remove 97.6% of the protozoan pathogen like *Cryptosporidium parvum* and 94.8% of *Giardia lamblia*. Constructed wetlands are also able to reduce coliform bacteria from  $4.1 \times 10^6$  MPN/100ml to  $3.5 \times 10^4$  MPN/100ml (Arroyo *et al.*, 2010).

Microorganisms that occupy wetland systems play a major role in pathogen and pollution indicator organisms' reduction, with removal rates of up to 95% being achieved (Paluszak *et al.*, 2003; Sundaravadivel and Vigneswaran, 2010). Table 1.6 outlines a summary of *E. coli* removal rates by CWs systems. Other pathogen removal mechanisms are through the biofilms (Iasur-Kruh *et al.*, 2010) formed on the surfaces of the filter material, where the contaminant decomposition and transformation are believed to occur (Faulwetter *et al.*, 2009).

Table 1.6. Comparison of Total coliform removal from three surface flow wetlands and two subsurface flow wetlands in Queensland Australia (Greenway, 2005).

Location	Cains	Emu Park	Townsville	Blackall	Oxley	Logan
Wetland type	Surface	Subsurface	Surface	Surface	Subsurface	Melaleuca
Effluent	Secondary	Secondary	Secondary	Secondary	Secondary	Primary
HRT (days)	16	11	7-10	7	4-5	2
Influent (CFU/100 ml)	79500	79500	84000	36000	1600	$1 \times 10^7$
Effluent (CFU/100 ml)	1100	2900	300-700	110-195	300-900	<1000

HRT – Hydraulic Retention Time

HLR – Hydraulic Loading Rate

A study by Boutilier *et al.*, (2011) reported *E. coli* removal efficiencies of between 52% and 99.9% from HSSF CWs. Greenway, (2005) and Barbagallo *et al.*, (2011)

in their studies, reported a 95% pathogen removal by CWs systems (Table 1.6). Reinoso *et al.*, (2008) reported 99% removal of helminth eggs by constructed wetlands. Helminth eggs are, however, a more resilient group and most difficult to remove from wastewater since their eggs have high viability and can survive over long periods under unfavourable conditions (Kayyali and Jamrah, 1999; Nasser *et al.*, 2012). Thus, their removal by CWs is a major breakthrough in wastewater treatment. Werker, (2002) and Vacca *et al.*, (2005) classified previous studies on pathogen removal by CWs treatment systems only as “black box” field studies mainly aimed at comparing the influent and effluent levels and argue that, there is need for more research on the treatment efficiencies and applications of CWs (Bouki *et al.*, 2013).

The depth of the wetland plays a major role in microbial community growth, dynamics, composition and diversity (Truu *et al.*, 2009; Sundaravadivel and Vigneswaran, 2010). Lasur-Kruh *et al.*, (2010) and Lilach *et al.*, (2010) in their studies, found that the biofilm assembly and the kinetics within the biofilm and rhizospheric functions play a major role in pathogen and suspended solids reduction from wastewater. Other pathogen removal mechanisms include physical, chemical, and biological systems (Molleda *et al.*, 2008). The physical systems involve various forms of filtration, which have been reported to remove between 98% and 99.8% of pathogens in CWs while chemical processes involve oxidation and UV irradiation mechanisms (Jillson, (2000); Paluszak *et al.*, 2003).

Biological removal mechanisms occur through adsorption on the biofilm, predation (by protozoon and virus) and the release of toxic antibiotics (that are able to kill the pathogens) from the plants and other microbes (Sundaravadivel and Vigneswaran, 2010). Boutilier *et al.*, (2011) in their study, reported that temperature, solar radiation and media filtration are other efficient mechanisms by which totalcoliforms may be removed from wastewater in CWs systems. However, with time as the wetland vegetation attain longer heights, this becomes ineffective due to the shadow effects of the plants (Elias *et al.*, 2014).

### 1.3.12.2 Role of plants in pathogens removal from constructed wetlands

The combination of anaerobic reactor and HF CWs processes is envisaged to improve pathogen removal efficiencies (Gikas *et al.*, 2007). Planted wetland beds have higher microbial populations than unplanted beds and with increasing root development, there is likely to be an increase in community diversity and therefore greater pathogens removal within the rhizosphere (Zhang *et al.*, 2011). This creates an environment for much greater competition and a higher level of predation within the microbial community, a phenomenon that may be seen to increase the CW's treatment efficiency (Truu *et al.*, 2009). Oxygenation by macrophyte rhizomes, which occurs primarily at sub-apical regions of young roots, causes an increase in oxidation and allows aerobic degradation of pathogens as wetland vegetation matures over a period.

Packer *et al.*, (2017) conducted a study of reed beds, and concluded that *Phragmites australis* beds (due to their fibrous extensive root structure), exhibited higher pathogen reduction rates than *Typha* beds. These high removal rates may also be a result of the differences in oxygen release rates by the plants and possibly associated with temperature variations (Srivastava *et al.*, 2008; Achyut *et al.*, 2010). It is important to note that performance of CWs in pollutant removal is poor in the winter months. This situation is attributed to reduced dissolved oxygen concentration (Arroyo *et al.*, 2010) in the root zone (due to the die-off of hydrophytes above ground level) and low general metabolic activity in the root zone resulting in reduced levels of antibiosis and bacterial predation (Vymazal, 2010). Large retention time with sufficiently low pathogen loading rates has been reported to yield high reduction rates for all pathogen species and a high level of the CWs pollutant removal efficiency (Zhang *et al.*, 2010).

### 1.3.13 Constructed Wetlands Wastewater Treatment Efficiencies

The major treatment mechanisms involved in a constructed wetland system are the hydraulic retention time (HRT) and volumetric flow rate (VFR) of water (Vidales-Contreras *et al.*, 2010). A higher HRT and lower VFR provide maximum exposure



of water to the root surface of the plant. This provides sufficient time for the uptake of the nutrients and other chemical changes that increase biodegradation and sedimentation of organic substances (Cota *et al.*, 2011; Aguilar and Woods, 2014). Studies by Kantawanichkul, (2011) reported high pollutant removal efficiencies based on low hydraulic loading rate (HLR) (2 cm/d) and long HRT. In their study, the organic loading was 2, 8, 12 and 16 g/m<sup>2</sup> for COD and the removal efficiencies were 49.0, 46.1, 42.4 and 38.7% respectively. The settled effluent was fed into the wetland system where organic matter was processed by microbes and plants. This process prevented the accumulation of materials at the bottom of the wetland to avoid clogging problems normally associated with these treatment systems (Knowles *et al.*, 2011). Pollutants were thus removed as the water was allowed to flow through the gravel substrate in the wetland by physical (sedimentation and filtration), chemical (precipitation and adsorption), and biological processes (US EPA 1999; Boutilier *et al.*, 2011). Table 1.7 outlines some of the pollutant removal processes involved according to the various pollutants.

The pollutant reductions progressed effectively after 25 days of retention time. The loadings decreased with increasing number of days, for example, the BOD<sub>5</sub> reduced from 158.2 mg/l on the initial day to 42.7, 30.0, 19.8 and 18.0 mg/l with a retention time of 10, 15, 20 and 25 days respectively. The removal trend was seen across all the parameters tested. Moreover Schnabel and White, (2001) in their study, highlighted that mycorrhizal fungi also played an important role in rhizosphere degradation through their metabolic activities and growth kinetics.

Table 1. 7. Summary of pollutant removal processes in wetlands systems. Adapted from Sundaravadivel & Vigneswaran, (2010).

Pollutants	Processes
Organic material (measured as BOD)	Biological degradation, sedimentation, microbial uptake
Organic contaminants such as pesticides	Adsorption, volatilization, photolysis and biotic/abiotic degradation
Suspended solids	Sedimentation, filtration
Nitrogen	Sedimentation, nitrification/denitrification, microbial uptake, plant uptake, volatilization
Phosphorus	Sedimentation, filtration, adsorption, plant & microbial uptake
Pathogens	Natural die-off, sedimentation, filtration, predation, UV degradation, adsorption
Heavy metals	Sedimentation, adsorption, plant uptake

#### 1.3.14 Applications of Constructed wetlands

A study by Odinga *et al.*, (2011), assessed the effectiveness of an HF CW planted with *Echinocloa pyramidalis* and *Cyperus papyrus* in removing nutrients from sugar factory effluent. The wetland system was designed to accommodate eight wetland ponds with an HF system and measuring between 1: 20 ml × 3mW× 1mD and 21 ml × 3mW× 1 mD. The eight CWs were planted with two different macrophytes (*E. pyramidalis* L. and *C. papyrus* L.) in an alternate sequence. All wetland cells (ponds) were waterproofed at the base with high-density polyethylene and lined with fine sand (Cooper *et al.*, 2005). The effluent was introduced into the wetland at different velocities with a retention time of seven days. Findings from the study indicated that microorganisms such as bacteria and fungi, binding on the surface of the plants' rhizomes and adsorption onto the soil matrix could have played the major role in nutrient removal from wastewater. Further studies on the soil matrix characterization were recommended from the study (Odinga *et al.*, 2011). Valigore *et al.*, (2012), in their study of the effects of hydraulic and solids retention times on productivity and settleability of microbial (microalgal–bacterial) biomass grown on primary treated wastewater as a biofuel feedstock concluded that pH levels increased when there was more light that increased the rate of photosynthesis.

The DO levels increased during the same period as the microalgae used more of the CO<sub>2</sub> and DO increase during the light periods when CO<sub>2</sub> was consumed. The study noted that shorter HRT yielded low DO levels and reduced bacterial growth and that the levels were increased by the microbes and atmospheric oxygen release. Longer retention time was found to inhibit microalgal growth because of the higher DO levels. Garc ia *et al.*, (2010) and Song *et al.*, (2014), assessed the effectiveness of CWs in the reduction of microbial threats every four weeks for a period of one year. The study aimed at assessing the wastewater microbial reduction using post-mechanically and post-biologically treated wastewater. Levels of D-group streptococci, *E. coli*, *Salmonella* spp. were assessed. Bacterial reduction in the wastewater that was mechanically treated was not significant as compared with the biologically treated wastewater. The study also reported that the reductions of the different bacterial groups were dependent on the various seasons of the year.

Sayadi *et al.*, (2012), determined the potential of hybrid CWs to treat landfill leachate, domestic, river polluted water, hospital, industrial, rainwater runoff and agricultural wastewater. The wetland systems were designed on a laboratory scale, pilot and a full scale with prescribed configurations. The hybrid wetland effectively removed COD, BOD<sub>5</sub> and SS while nutrient removal was dependent on the systems operational conditions and properties. The study concluded that hybrid systems are more stable and efficient in pollutant removal as compared with other treatment systems.

#### 1.3.15 Human Health Concerns

Constructed wetlands that receive primary effluent are known to produce noxious odours and should be areas restricted only to authorized personnel. Human and animal health concerns become significant especially when the water is to be reused or discharged into sensitive receiving rivers, ponds and lakes (Dong *et al.*, 2017). Moreover, whenever there is water shortage and need for irrigation using wastewater, hygiene concerns and infection from potential pollutants become major

concerns on the direct users of the treated water (Lilach *et al.*, 2010). Crops grown through irrigation by treated wastewater may contain pathogenic microorganisms based on the treatment method applied. Other health concerns regarding the reuse of treated wastewater include risks of infection by protozoan parasites like *Cryptosporidium*, *Giardia* spp. and *Ascaris lumbricoides*. However, CWs have demonstrated effective removal of protozoan parasites mainly with horizontal and vertical subsurface flow gravel-based systems (Redder *et al.*, 2010). Other risks include skin contact and potential transmission of viral and other air-borne diseases normally associated with wastewater. Mosquito populations also pose health effects in the environments around these systems especially in warmer regions where the encephalitis mosquito (*Culex tarsalis*) is dominant and breeds well by extension due to the wet conditions provided by the wetland. The mosquito population may be controlled by a two-tiered pond design, which concentrates the prey in smaller areas for consumption by predators such as the mosquito fish and also the introduction of macroinvertebrate predators into the wetland system as suggested in a study by US EPA, (1999) and Greenway, (2005).

#### 1.4 SUMMARY OF LITERATURE

Constructed wetlands rhizofiltration systems are engineered and managed to remove various types of pollutants from wastewater. They are cost-effective in operation, maintenance and low-energy requirements. Conventional biological systems usually transfer problems of pollutants with time and space, but CWs are free from some of these problems such as generation of sludge as a by-product of conventional systems.

Some CWs (FWS CWs) may form an ideal disposal system for sludge generated from conventional treatment systems to be used for purposes such as nutrient source. Constructed wetland treatment systems have proved to be reliable in the efficient treatment of wastewater from industries, agricultural runoff and municipal effluents. Research findings on CWs planted with aquatic macrophytes show that both submerged and free-floating designs are able to effectively improve

wastewater quality by removing and reducing heavy metals and pathogens (Gikas *et al.*, 2012).

Heavy metals removal is primarily through adsorption, absorption and the interaction with microorganisms and wetland plants/sediment while pathogens are removed by physical, chemical and biological mechanisms such as filtration, die-offs, chemical emissions from plant roots and adsorption by the biofilm, respectively. Heavy metals and pathogens in the environment pose serious health effects and their removal through CWs systems adds great value to the use of CW systems (Jacob *et al.*, 2013).

## CHAPTER 2

### PERFORMANCE OF A PILOT CONSTRUCTED RHIZOFILTRATION SYSTEM FOR WASTEWATER PURIFICATION

#### 2.1 INTRODUCTION

The performance and efficiency of rhizofiltration systems in pollutants removal is greatly influenced by physicochemical parameters. For example, nutrients are potentially removed through the action of microorganisms attached to the filter media, sedimentation and root attachment within the macrophytes (Vymazal, 2011). Other parameters that influence pollutant removal in wetland systems include pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved oxygen (DO), Salinity, Turbidity (Srivastava *et al.*, 2016). An investigation into the levels of pH in rhizofiltration systems is in a bid to assess and identify the effect on neutrophils, acidophilus and alkalophiles which are the bacteria acting on the degradation of organic matter to form other compounds (Al-Jaboobi *et al.*, 2013). Various levels of pH in rhizofiltration systems are likely to impact on the plasma membrane of microorganisms and render them incapable of nutrient uptake. This situation is likely to hamper the nutrient removal efficiency and eventual death of the microorganisms. Temperature variations in the rhizofiltration systems are likely to affect the removal of pollutants in various ways. For example, nitrifying bacteria function better in temperatures between 15°C–25°C. Consequently, nitrification in wetland systems is favoured by temperatures ranging between 16.5°C–32°C (Vymazal, 2010). Raised salinity levels in rhizofiltration systems are likely to inhibit the activities of microbial community and therefore reduced denitrification leading to a reduced supply of nutrients to the root system of the rhizofilter. High levels of electrical conductivity (EC) reduces denitrification resulting in raised salt content in the system, a situation that hampers nitrogen removal (Nilsson *et al.*, 2012). Turbidity in these treatment systems is caused mainly by suspended matter, while increased turbidity reduces the amount of light penetrating the rhizosphere

and therefore reduces macrophyte growth and pollutant removal efficiency according to Jin *et al.*, (2012). The rhizosphere acts as a filter media of pollutants through the root network of the macrophytes (Nilsson *et al.*, 2012). The rhizosphere is also well aerated and provides aerobic conditions which favour microbial activities thereby enhancing removal of pollutants such as nutrients through processes of denitrification, nitrification and precipitation (Vymazal, 2011). It is, therefore, necessary to study and understand the possible influence and limits of physicochemical parameters in pollutants removal in rhizofiltration systems. For example, *P. australis* and *K. nemoralis* grow better in temperature ranges between 12°C and 25°C beyond which, their nutrient absorption ability is decreased (Nilsson *et al.*, 2012). The choice of *K. nemoralis* as the study plant was based on the fact that the plant is categorized as a pioneer plant, it is mainly found within rock crevices, shaded meadows and by the roadside growing under very unfavourable environmental conditions (Sindhu *et al.*, 2014). They are also found growing on wet grasslands in association with *Bacopa monnieri* and *Eleocharis geniculata* (Ching, 2009; Shaw *et al.*, 2011). *Kyllinga nemoralis* grows well in full sunshine and that is also one reason why the plant was selected for this study based on climatic conditions in Durban and the design of the rhizofiltration system. Though Sindhu *et al.*, (2014) consider *K. nemoralis* a terrestrial plant, it is best to place it under transition plant since it grows very well in both environmental conditions in agreement with Vymazal, (2011). These characteristics support the choice of *Kyllinga nemoralis* as a study plant in this research. It was introduced in the rhizofiltration unit in order to compare and blend with *P. australis* (which had retarded growth during rhizofilter acclimatization period) which has been widely used in treatment wetland studies due to its morphological and ecophysiological characteristics, though *P. australis* is reported to take a much longer time to acclimatize (Rodríguez and Brisson, 2015).

This study investigated the establishment of a rhizofiltration system, its optimal pollutant removal stage and system efficiency in the reduction of pollutants.

### 2.1.2 Aim

To assess the potential of a constructed rhizofiltration system in removal of pollutants from wastewater.

### 2.1.3 Objectives

- To construct a rhizofiltration system and establish macrophyte growth as well as achieve steady-state operation.
- To assess and optimize the physicochemical parameters for efficient operation of the system.
- To compare the potential for metals removal by the planted and reference sections of the rhizofiltration system.
- To determine removal efficiency of selected pathogenic microorganisms by the constructed rhizofiltration system.

## 2.2. METHODS

### 2.2.1 Establishment of the Rhizofiltration Unit

A study was conducted at the Kingsburgh wastewater treatment works located in Amanzimtoti, South – East of Durban, in the province of KwaZulu-Natal (Figure 2.1). The Little Amanzimtoti River that receives the final effluent from the treatment works, empties into the Indian Ocean. A combined vertical and horizontal flow (VHFS) rhizofiltration system was constructed in 2011 with the aim of assessing its efficiency in pollutants.



### 2.2.2 Study Site

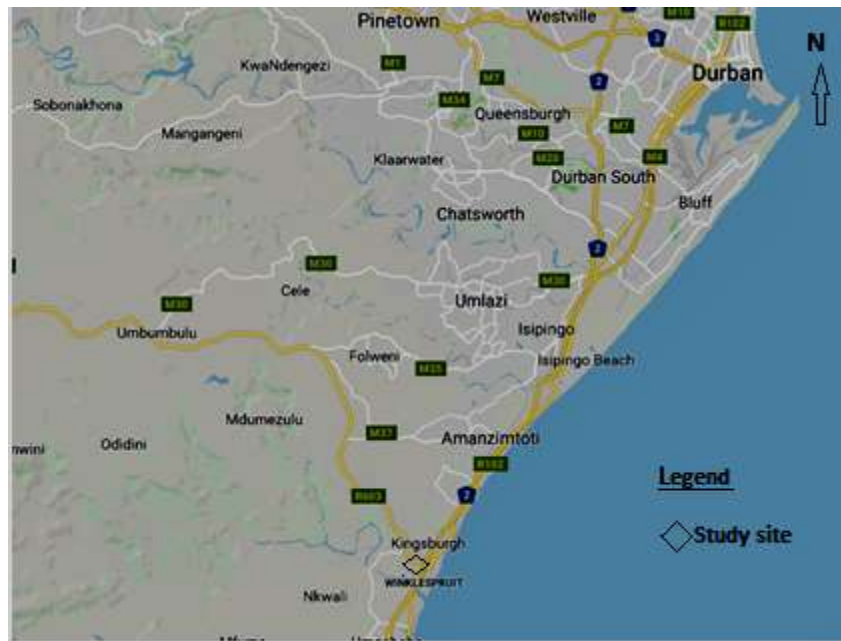


Figure 2.1. Map of KwaZulu–Natal province and study site located at Kingsburgh wastewater treatment plant 11.07.13.

<https://www.google.com/maps/place/Kingsburgh,>◇ denotes the exact study site.

The rhizofiltration system measured approximately 9 m (L) x 2 m (W) x 1 m (D) and was packed with filter material arranged in three zones/layer (coarse rock zone was 250 mm thick, small stone and crushed aggregate zone was 150 mm thick while topmost zone of small stones and river sand was 200 mm thick) according to the study design. The first zone was composed of coarse material of half broken bricks and blocks (100-120 mm Ø and 250 mm thick) while the second zone was composed of crushed stone of between 63–150 mm sizes and the third zone was a mixture of small tones (19-25 mm) gravel and coarse river sand (Figure 2.2). The sand layer at the top was overlaid with fine gravel to shield the sand from being blown away by the wind.

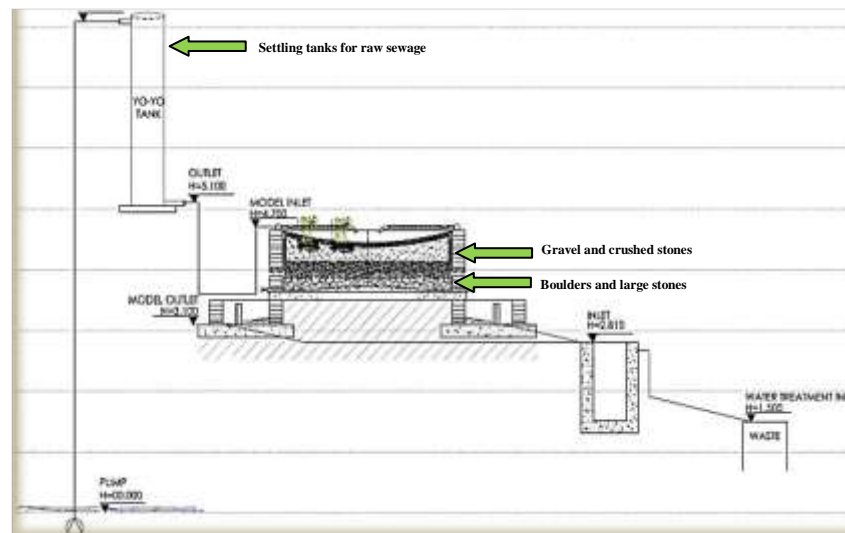


Figure 2.2. Schematic representation (not to scale) of the rhizofiltration system in Kingsburgh showing gradient flow of pre-settled wastewater as adapted from Wilsenach *et al.*, (2012).

The study adopted the vertical flow design for the experiments with 10 inlet zones that opened directly onto the sand media. A flow rate of between 25-50 m<sup>3</sup> /d from the settling tank to the rhizofilter was adopted according to the systems design. The designer outlets (top overflows and bottom outlets for low flows and effluent sampling – Appendix 3) were considered for channelling different flow regimes. The design favoured an inlet feed into the rhizofilter and drainage back to wastewater treatment works by gravity (Figure 2.2). This figure also shows the internal design of the rhizofiltration system highlighting the layout of the filter matrix composed of boulders and gravel. The combined vertical (VFCW) and horizontal flow (HFCW) rhizofiltration system had two zones to compare pollutant removal efficiencies (planted and reference). In the planted section of the rhizofilter 20 rhizome stalks each of two species of macrophytes (*Phragmites australis* and *Kyllinga nemoralis*) were initially planted (well-established rhizomes of about 25 cm in length were used) according to the method by Geller, (1997). *Phragmites australis* was the pioneer species (Figure 2.4A), which was planted in two rows, then *Kyllinga nemoralis* was added after a period of 2 months in order to increase the plant cover and also as a second experimental macrophyte to compare its efficiency against *P. australis*. Tap water was used to acclimatize plant growth for a

period of one week, after which pre-treated wastewater from the tanks was periodically applied for system establishment, that is the biofilm establishment which was to assist in pollutants removal at the steady-state operation. The mature rhizome stalks of the plants were obtained from a local field adjacent to the wastewater treatment plant and therefore were well adjusted to the environmental and climatic conditions of the study area according to the methodology adopted by Luca, (2011).

Freshwater was applied to the plants in the system for a period of one month in order to stimulate growth. This was followed by application of settled raw sewage from the municipal treatment plant inlet which was channelled through the Jojo tanks. This application was done in stages from low concentration to high concentration of the raw sewage using settled sewage from the primary settling tank. Mix ratio of 10%, 20%, 30%, 50%, 80% and finally 100% of the settled wastewater against treated water was applied for a period of three months. This was to establish the treatment process (microorganisms and biofilm establishment) based on a method by Cortes-Esquivel *et al.*, (2012). Adjacent to the rhizofiltration system were two settling tanks of 10,000 m<sup>3</sup> (large) and 5,000 m<sup>3</sup> (small) capacity. Raw sewage was pumped into the 5000 m<sup>3</sup> 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 channelled by gravity into the vertical feed system of the rhizofilter in comparison to the designs by Solano *et al.*, (2004) and Gikas *et al.*, (2007). Further, the settled sewage was channelled to flow into the vertical feed system of the rhizofilter by gravity. The second tank (large) was connected also connected to the post-secondary-treatment within 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.

According to Lim *et al.*, (2001) and Vymazal, (2011), a system is said to achieve a steady state of operation when there is a significant reduction in organic loading and suspended matter according to wastewater discharge standards (Vera *et al.*,

2011). The biomass is also at optimum and steady state when about 80% of the soil is covered by the plants (Figure 2.4B). The hydraulic loading of the rhizofiltration system was tested by performing flow rate test based on influent and effluent discharges and was adjusted using valves. The design of this system accommodated a flow rate of 25-50 m<sup>3</sup>/d from the pre-settling tank to the inlet of the rhizofilter. The system was fitted with 10 sampling points (A-E on planted and F-J on the reference) on the opposite sides (Appendix 3) where water samples were taken periodically for various tests to assess the efficiency of the system in pollutant removal (Figure 2.3). Raw wastewater from the conventional municipal treatment system was channelled to the 5,000 m<sup>3</sup> and allowed to settle before being released into the 10,000 m<sup>3</sup> tank which finally channelled the wastewater to the rhizofiltration unit by gravity flow. The system was designed to release about 25-50 m<sup>3</sup>/d which was regulated by the valves.

Investigations of the efficiency of the system in pollutant removal were based on the flow types and regime (mainly vertical batch flow), the design of two sections of planted and reference and well-controlled inflow and effluent flow as was desired during the various sampling procedures.

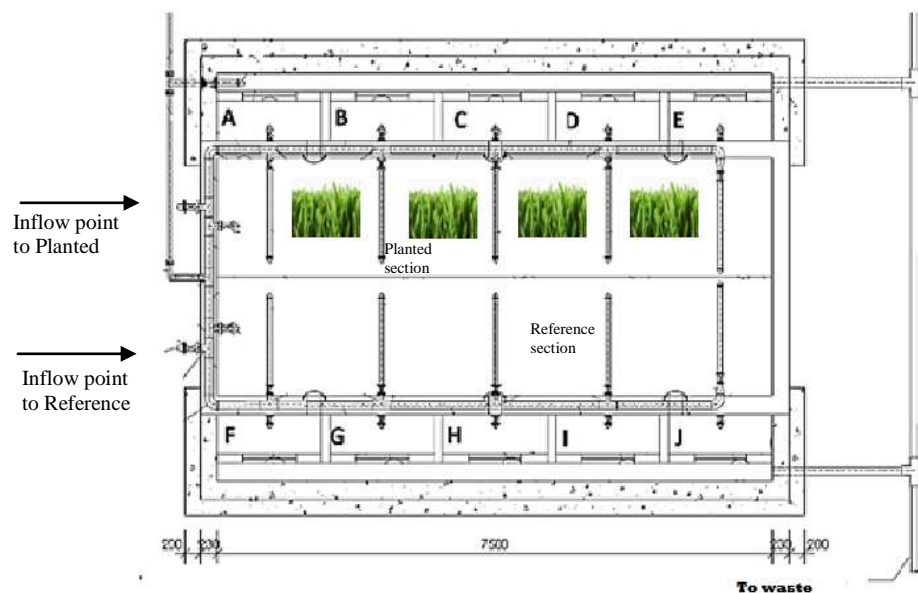


Figure 2. 3. Design of the rhizofilter showing outlet sampling points A-E (planted) and F-J (reference) sections. Adapted from Wilsenach *et al.*, (2012).

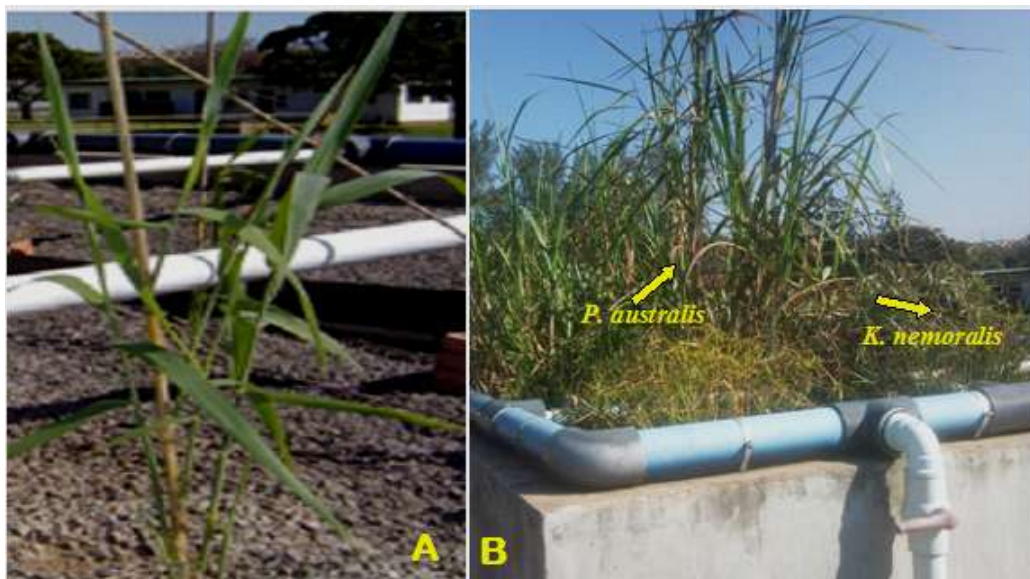


Figure 2. 4 Healthy growth of *Phragmites australis* at the initial stages of the rhizofilter establishment (A), and a section of optimum Macrophyte growth showing the mature plants, *P. australis* and *K. nemoralis* (B).

### 2.2.3 Measurement of Flow Rates in the Rhizofilter

The flow rate of raw wastewater from the municipal treatment inlet into the 5,000 m<sup>3</sup> tank was measured and time taken for the tank to fill up was noted and regulated from time to time. The water level in both tanks was checked using a transparent graduated tubing with 10 cm interval markings, which was fitted at the side of the tank. The flow of the water entering the rhizofilter was measured by observing the decrease and increase in the markings and regulated using valves as was required from time to time. Flow rate from the various outlet sampling points was measured using a graduated bucket which was allowed to fill up and time is taken to fill it up was also noted and used to calculate the flow rate from each sampling point against time.

Water and plant samples were taken monthly for analysis of the various parameters to establish system functionality and efficiency from June 2011 to December 2011, when the system reached a steady state of operation.

#### 2.2.4 Sampling Procedures for Wastewater and Plant Material

Water samples were collected from the inlet to the rhizofiltration unit every fortnight in 1 L sterile Schott bottles, placed in a polystyrene cooler box packed with ice and transported to the laboratory for the various analyses. Various physicochemical tests such as pH, turbidity (Turb), dissolved solids (TDS) and suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD) were carried out using conventional procedures according to APHA, (2005). Likewise, three cuttings (weighing approximately 200 g) of each specimen of *K. nemoralis* and *P. australis* were randomly collected from the rhizofiltration unit using a pair of secateurs according to APHA, (2005). The specimens were placed in transparent plastic bags, labelled and taken to the laboratory for analysis.

All glassware used in the various experiments were washed using detergent and rinsed with tap water. They were then immersed in 10% nitric acid (Analar grade) to ensure zero residual metal deposits. Finally, the glassware was rinsed with tap water and finally with deionised water.

#### 2.2.5 Measurement of Physico-chemical Parameters

A calibrated electronic meter (YSI 556 MPS-United Kingdom) was used in the field for the determination of electrical conductivity ( $\mu\text{S}/\text{cm}$ ), salinity ( $\text{mg}/\text{l}$ ), total dissolved solids ( $\text{mg}/\text{l}$ ), pH (pH units), dissolved oxygen ( $\text{mg}/\text{l}$ ) and temperature ( $^{\circ}\text{C}$ ). The electrode was rinsed each time between sample measurements using distilled water to avoid contamination from the preceding sample.

#### 2.2.6 Biochemical Oxygen Demand (BOD) Determination

Biochemical oxygen demand (BOD) is one of the major tests done on wastewater to determine the quality (Jouanneau *et al.*, 2011). In this study, BOD

was determined by the respirometric method using the OxiTop TS 606/2-i system according to methods by APHA, (2005). In this procedure, the oxidation of ammonia is inhibited and oxygen is consumed, resulting in the release of carbon dioxide. Carbon dioxide thus released, is absorbed making the pressure in the bottle and amount of gas to drop too. The drop in pressure results in the sensor electronics displaying the amount of oxygen consumed. This method is recommended because of its simplicity in terms of sample treatment (no dilutions required) and also oxygen consumption readings can be observed at any time or day before the five days and the readings after five days are also direct (no further calculations required).

The required volume of water for the BOD test was calculated based on the COD values according to the manufacturer's manual (WTW, Weilhem, Germany). In this study, the optimum amount ranged between 90-432 ml each time.

The volume of the sample for BOD determination was calculated each time (such as 42 ml, 32 ml), measured and transferred into clean, previously autoclaved 500ml OxiTop BOD bottles. The sample volume was different each time the influent COD concentration was varied. About 9 drops of 5 g/l N-allylthiourea (WTW No. NTH 600) solution was added to the sample to suppress nitrification. A small amount (2 pellets) of NaOH was also added to the top compartment of the special BOD bottle to absorb the CO<sub>2</sub> formed during the incubation period. A magnetic stirrer was carefully dropped into the sample bottle. The OxiTop sensor tops were fitted to the bottle and tightly capped. The bottle was then placed on the OxiTop meter stirrers and uniform stirring was commenced using magnetic stirrers placed in the bottles. The process was started using the OxiTop controller keyboard for a period of 5 days at 21°C. After five days, the BOD values were read directly from the OxiTop meter.

### 2.2.7 Chemical Oxygen Demand Determination

The collected water samples were preserved at 4°C at pH of 2 using sulphuric acid for further analysis. The closed reflux colorimetric method according to the design by APHA, (2005) was used for the analysis.

Five ml of sample was measured and transferred to a microwave Teflon tube. The Teflon tube was inserted into the microwave safety vessel. About three ml of digestion solution (0.01667M, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and seven ml of sulphuric acid reagent were carefully added to the sample. The sample was carefully mixed, tightly capped and digested at 150°C for 55 minutes using the microwave (Milestone START D). A reagent blank was also digested with the sample each time. After cooling, the solution was filtered and then transferred into Gallery cuvettes for colorimetric analysis using the Thermo Gallery photometric analyser (Germany). Results were recorded in mg/l.

### 2.2.8 Alkalinity Determination

About 100 ml of the water sample was measured and transferred to an Erlenmeyer flask. A few drops of indicator solution (mixed bromcresol green-methyl red indicator solution, mixed in proportions of 100 mg bromcresol green sodium salt and 20 mg methyl red sodium salt in 100 ml distilled water) was added and swirled to mix according to APHA, (2005). This was titrated against 0.02M H<sub>2</sub>SO<sub>4</sub> to the endpoint. Alkalinity was calculated using the formula:-

$$\text{Alkalinity mg/l} = \frac{\text{ml titrant} \times 1000}{\text{ml of sample}}$$

### 2.2.9 Suspended Solids Determination

Suspended solids were determined using the filtration method as outlined by APHA, (2005). Glass fibre filter disks (GF/A Cat No. 1820 047 with 47 mmØ) were prepared by washing and drying them at 103°C-105°C with a total of 60 ml



of distilled water using 20 ml each time. This procedure was done at three successive intervals and each time the filter paper was dried in an oven until a constant weight was achieved. About 100 ml of a well-mixed sample was filtered through the glass fibre filter disc. The filter disc plus residue retained on it was dried at 103°C -105°C using an Incoterm (Labotec) oven to a constant weight. After drying and cooling in the desiccator, the paper was removed and weighed. The difference between the weight of the empty filter disk and that of filter disk plus residue constituted the suspended solids. The total suspended solids were calculated using the formula:-

$$\text{Total Suspended Solids in mg/L} = \frac{(A-B) \times 1000}{C}$$

Where:-

A = weight of filter and dish + residue in mg, B = weight of filter and dish in mg, C = volume of sample filtered in ml.

## 2.2.10 Metals Detection

### 2.2.10.1 Preparation and Analysis of Water Samples

Triplicate samples were filtered through 0.45 µm Whatman No. 1 filter paper for total dissolved metals and acidified using 1:1 ratio of concentrated trace-metal grade nitric acid to pH of 2 (Hou *et al.*, 2006). A portion of the sample (45 ml) was carefully transferred into the microwave Teflon tube (Milestone START D). Each Teflon tube was covered with a safety shield. A 3 ml sample of reagent grade HNO<sub>3</sub> (65%) and 2 ml of HCl was carefully added to the reaction mixture in a fume cupboard. The solutions were carefully mixed to ensure homogeneity and digested for 30 min. using a microwave digester system (Milestone START D) equipped with a revolving carousel with a capacity of 12, 100 ml type quartz tubes to a maximum temperature of 165°C ±5°C. The reaction mixture was

evaporated on a hot plate and the residue diluted to 50 ml using double distilled water. The combination of the two strong acids is recommended since the wastewater sample is composed of readily oxidizable organic matter (Marin *et al.*, 2011). Blank samples were prepared with each batch and treated in the same manner as the experimental samples. Metal analysis was done on the clear supernatant using the inductively coupled plasma-optical emission spectroscopy (ICP-OES) according to a method by Luca *et al.*, (2011).

#### 2.2.10.2 Preparation of Plant Material

The specimens were washed with distilled water to remove attached soil particles and placed in a sieve to drain out excess water. The plant specimens were further immersed in a solution of HCl (0.01 M) in order to remove any exterior metal deposits. The material was then rinsed with double distilled water for about 1 minute. The material was then partitioned into leaves, stems and roots and then shredded using a pair of scissors and secateurs. The sample was air dried to remove excess water and then dried at 80°C for 24 hours. The material was blended to a fine powder using a Milestone start D Mellerware 50 g capacity blender.

The homogenized dried plant material (0.3 g) was weighed in triplicate and placed in a microwave Teflon tube. The tube was introduced into the Teflon safety shield and 8 ml of HNO<sub>3</sub> (65%) followed by 2 ml of H<sub>2</sub>O<sub>2</sub> (30%) were carefully added and mixed in a fume cupboard. The solution was gently swirled to ensure homogeneity. The Teflon tube was tightly closed and placed in the microwave (Milestone START D) cavity and digested for 35 minutes at 180°C. The residue was evaporated on a hot plate at 50°C and diluted using double distilled water to a volume of 50 ml. The metal content was determined using Inductively Coupled Plasma optical emission spectrometer (ICP-OES) as described by Rai, (2008).

### 2.2.11 Protozoa Detection using modified Bailenger method (MB)

An estimated amount of 1 litre of the sample was collected from the inlet and 5 L from the designated effluent sampling points in the planted and reference sections respectively. The water sample was vigorously shaken and 1.5 litres carefully transferred into the open-topped straight-sided container to settle overnight. Approximately 90 % of the supernatant was carefully syphoned out and discarded. The remaining sample was centrifuged at 1000 x g for 15 minutes. The supernatant was discarded and the pellet resuspended in an equal volume of aceto-acetic buffer (pH 4.5). Two volumes of Ethyl acetate was added and the sample was vortexed and centrifuged at 1000 g for 15 minutes. The volume of the pellet was noted and a fine needle was used to loosen the fatty layer above the solution. The solution with the fatty layer was carefully poured out in one smooth action leaving the pellet. The volume of the pellet was noted and the pellet re-suspended in five volumes of zinc sulphate (33%) solution. The volume of the final product was recorded ( $X$  ml) and the sample thoroughly mixed using a vortex mixer. An aliquot was quickly removed using a Pasteur pipette and transferred onto a McMaster slide that holds about 0.3 to 1.5 ml. After about 5 minutes, (to allow the eggs to float to the surface) the eggs were examined and enumerated under 100 magnification using a light microscope (Nikon 81- Japan). The total number ( $N$ ) of the eggs per litre sample was determined from the formula:-

$$N = \frac{AX}{1.5 * P}$$

Where:- ( $N$ ) number of eggs per litre of sample, ( $A$ ) number of eggs counted in the McMaster slide or the mean of counts from two or three slides, ( $X$ ) volume of the final product (ml), ( $P$ ) volume of the McMaster slide (0.3 ml or 1.5).

### 2.2.12 Coliform Bacteria Detection

Total coliforms and *Escherichia coli* (*E. coli*) were detected using the Colilert<sup>®</sup> - 18 methods (based on the MPN, USEPA protocol – 40CFR136) - IDEXX Defined Substrate technology as outlined by Borges *et al.*, (2008) and Calheiros *et al.*, (2010). Water Samples were taken from the inlet, planted and reference sections of the rhizofilter, placed in 500 ml sterile glass bottles and transported to the laboratory for analysis. Several dilutions were made and 100ml each of replicate samples was transferred into sterile Schott bottles (Jouanneau *et al.*, 2011). One snap package of the reagent having the Colilert<sup>®</sup>-18 media was added to the sample. The bottle was aseptically capped and thoroughly mixed till there was no foam. The bottle was placed in a shaking water bath at 35°C for 20 minutes after which the solution was carefully transferred into a Colilert<sup>®</sup>- quanti-tray (for 100 wells) avoiding contact with the foil tab and sealed using the IDEXX Quanti-Tray<sup>™</sup> sealer. The tray was incubated for 18 hours at 35°C  $\pm$ 0.5°C. Yellow wells (without fluorescence with a 6 watt, 365 nm, UV light) were evaluated and counted as total coliforms while the yellow fluorescent wells under UV were counted as positive for *E. coli*. The colourless wells were evaluated as negative for both total coliforms and *E. coli*. Sterile controls were also used in each batch of the sample. The results were reported as MPN/100 ml sample.

### 2.2.13 Nutrients Detection

#### 2.2.13.1 Nitrates

Water samples were collected as described in chapter 2.2.4. The sample was stored at pH of 2 until analysis. Approximately 4 ml of NO<sub>3</sub>-I reagent was measured, transferred into a 20 ml test tube and 0.5 ml of sample was carefully added according to a method outlined in the instructions manual for Merck Spectrophotometer 14556 (Merck). The tube was tightly closed and carefully

swirled repeatedly to mix. The tube was cooled for 10 minutes. The solution was transferred to the 10 mm spectrophotometer cuvette and concentration was established using a spectrophotometer (Spectroquant Pharo 300) at 620 nm.

#### 2.2.13.2 Phosphates

Water samples were taken as described in 2.2.4. Samples were kept at pH 2 using  $\text{H}_2\text{SO}_4$  till analysis according to the outlined procedure in the manual for Merck Spectrophotometer 14729, phosphate cell test. Into 5 ml sample in a reaction cell, 1 cap of P-1K was added. The mixture was swirled carefully to ensure proper mixing and placed for 30 minutes in a preheated thermoreactor to  $148^\circ\text{C}$  and removed. After cooling, 5 drops of P-2K was added and mixed until dissolved. After 5 minutes, the cell was placed into spectrophotometer cell compartment and absorbance was read at 620 nm using Spectroquant Pharo 300 spectrophotometer.

#### 2.2.13.3 Ammonia

Ammonia cell test 14559 by Merck was used to analyse ammonia content in the sample. About 500  $\mu\text{l}$  of  $\text{NH}_4\text{-I}$  reagent was measured and transferred into 10 ml tube. Approximately 0.20 ml of the water sample and one micro-spoon of  $\text{NH}_4\text{-2}$  were carefully added to the tube and mixed to dissolve. After 15 minutes, the solution was transferred into a 10 mm spectrophotometer cuvette and measured at 620 nm using Spectroquant Pharo 300 spectrophotometer (Merck).

### 2.3 Data Analysis

Data for the inferential tests were analysed using Microsoft Excel (version 2007) and GraphPad Prism software, version 5.01. (GraphPad Software Inc., San Diego, CA USA; 2005). A one-way analysis of variance (ANOVA) and Tukey HSD (honestly significant difference) was also obtained. The pollutant removal

rates were estimated on the basis of percentage removals and mass removals. Percent reductions and removals were calculated using methods by APHA, (2005) and also according to a method by Abdelhakeem *et al.*, (2016).

## 2.4 RESULTS

Average concentrations of the parameters of study at inflow, planted and reference sections in this study were averaged. The macrophytes achieved optimum growth after a period of one month, despite the plant cover on the rhizofilter being less than 100%. However, initial tests were carried out on influent and effluent from both sections of the rhizofilter. *Kyllinga nemoralis* grass species was added as an experimental macrophyte. After a period of three months, the rhizofilter had about 90% cover of the macrophytes and this was therefore perceived as optimum plant growth (Figure 2.5). This was followed by bi-monthly sampling from the influent and effluents for analysis of the various parameters as outlined in the objectives.



Figure 2.5. The rhizofiltration unit at Kingsburgh showing about 80% plant cover in the planted section, three months after the initial planting of the macrophytes.

### 2.4.1 Flow Rate Measurements

Figure 2.6 shows the measurements of flow rate experienced between the various sampling points at the establishment of the rhizofilter. The settling tank

which received the raw sewage first before the rhizofilter had a flow rate of 0.62 l/s. from this tank, the flow rate increased to 1.12 l/s. The flow rate was varied within the various sampling points of the system. The flow rate was optimized by ensuring a constant head above the filter. The lowest recorded flow rate was from sampling point “E” at 0.3942955 l/min in the planted section while highest was at sampling point “J” at 0.8710061 l/min in the reference section. The valves that released or channelled the flow into the rhizofilter further regulated the flow by reducing it to 0.02 l/s. The reduction was found to form some head of about 100 mm on the filter surface of the unit. This phenomenon may have caused pressure and eventual channelling of the flow through some specific areas within the rhizofilter. This may also be the reason for the difference in flow rates at the various sampling points of the system. Similar findings were reported in a study by Lai *et al.*, (2011). The other factor that may have positively influenced the soil hydraulics in the rhizofilter was the distribution of the different sizes of the grain in the matrix. The topmost layer which was a mixture of sand and fine gravel played one major role of contaminant removal. Similar observations were reported by Stottmeister *et al.*, (2003) during their study on the effects of plants and microorganisms in constructed wetlands for wastewater treatment.

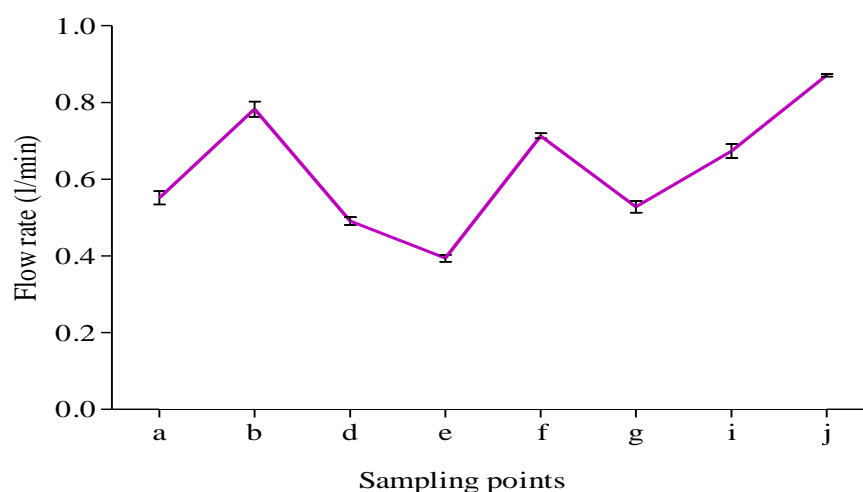


Figure 2.6. Variations in flow rates at the various sampling points. Whiskers represent standard deviations of 12 means.

### 2.4.2 Physicochemical Parameters

The physicochemical parameters that were studied gave variations according to the sampling points and season (Figure 2.7). The pH increased on average from 6.95 to 7.55 pH units, and 7.23 in the planted and reference sections respectively (Figure 2.8). High pH values were observed between the months of February and April while comparatively low values were recorded in May and July. However, the pH values were not statistically different between the planted ( $7.32 \pm 0.01$ ) and reference ( $7.29 \pm 0.12$ ) sections (three samples t-test,  $p = 0.9925$ ). The flow rate was varied within the various sampling points on the planted and reference sections of the system. The lowest flow rate was from sampling point 'E' at 0.3942955 l/min in the planted section while highest was from sampling point 'J' at 0.8710061 l/min in the reference section.

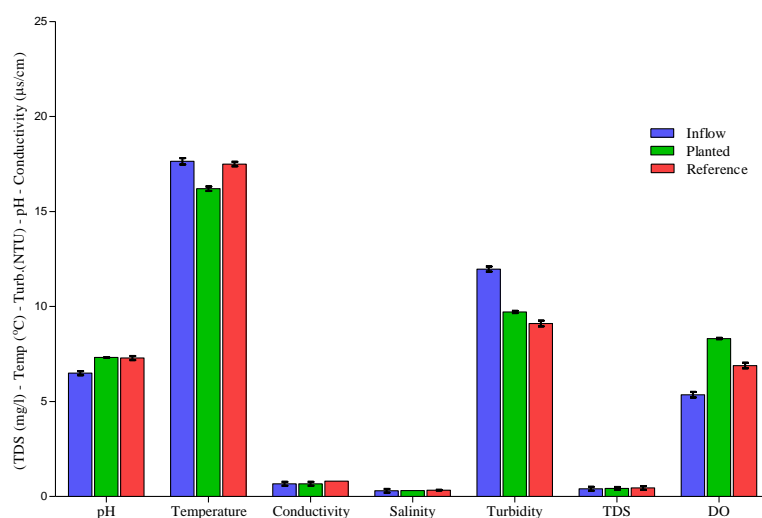


Figure 2.7. Average variations in physicochemical parameters recorded over 12 months in 2012. Whiskers represent standard deviations of 12 means.

The first three months of study recorded removals of 38% in the planted section and 16% on reference for BOD while COD was reduced by 16.5% and 9.6% for planted and the reference section (Figure 2.9) respectively. Table 2.1 displays result for various parameters including BOD<sub>5</sub> at 79% and COD at 75% based on optimum removal efficiency by the system after a period of 6 months. The low values of COD and BOD in the influent may have resulted from the effect of



influent pre-treatment by the conventional municipal treatment system and the potential microbiological treatment within the Jojo tanks. The means for the various tests did not differ significantly ( $p = 0.9925$ ). Also, there was no significant difference on the means from planted and reference sections of the rhizofilter ( $p = 0.9717$ ). Total suspended solids were reduced by 86% in the planted section while the reference section witnessed a 59.8% reduction. Electrical conductivity (EC) was reduced on average by 7.7% in the planted section while the reference section had an average reduction of 0.83%. The low reduction in conductivity levels during this initial system establishment could be attributed to an increase in nutrient and mineral levels occasioned by addition of compost manure which was required to enhance the growth of the macrophytes. Total dissolved solids (TDS) was reduced by 11.5% for the planted section and 3.5% in the reference section of the rhizofilter. Similar findings were also reported in a study by Rizwana *et al.*, (2014). The temperature was reduced by 11.9% in the planted section and 1.2% on reference section. The highest value of 35.3°C in the reference section was recorded in February while the lowest temperature reading of 17.1°C in the planted section was recorded during the month of June (Figure 2.10). Dissolved oxygen was raised by 10% in the planted section and 5% in the reference section (Figure 2.7).

Table 2.1. Summary of means ( $\pm$  standard error) of physicochemical parameters from influent and effluent from the planted and reference sections.

Parameter /unit	Influent (pre-chlorinated sewage) Mean $\pm$ S.D	Effluent (Planted) Mean $\pm$ S.D	Effluent (Reference) Mean $\pm$ S.D	Discharge limits (maximum) UNEP/WHO, (1996); HABITAT/WSSCC, (2004)
pH (pH units)	6.9 $\pm$ 0.08	7.5 $\pm$ 0.01	7.2 $\pm$ 0.07*	5.5-9.5
Temperature ( $^{\circ}$ C)	21.9 $\pm$ 0.05	20.0 $\pm$ 0.15	21.5 $\pm$ 0.10	30/ Not to alter ambient temperature
EC ( $\mu$ s/cm)	0.6 $\pm$ 0.05	0.5 $\pm$ 0.01	0.5 $\pm$ 0.05	70- 250
Salinity (mg/l)	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05*	1
Turbidity (NTU)	10.7 $\pm$ 0.10	9.9 $\pm$ 0.17	11.1 $\pm$ 0.29*	5
TDS (mg/l)	0.3 $\pm$ 0.05	0.4 $\pm$ 0.05	0.3 $\pm$ 0.05*	250
TSS (mg/l)	1.1 $\pm$ 0.05	0.8 $\pm$ 0.15	0.9 $\pm$ 0.05*	25-30
DO (mg/l)	3.8 $\pm$ 0.09	6.7 $\pm$ 0.08	5.7 $\pm$ 0.10*	Objectionable
COD (mg/l)	53.9 $\pm$ 1.71	30.9 $\pm$ 1.06	39.9 $\pm$ 1.43*	75
BOD (mg/l)	19.7 $\pm$ 0.83	7.3 $\pm$ 0.29	8.9 $\pm$ 0.36*	20

\* p <0.05 Significance was detected.

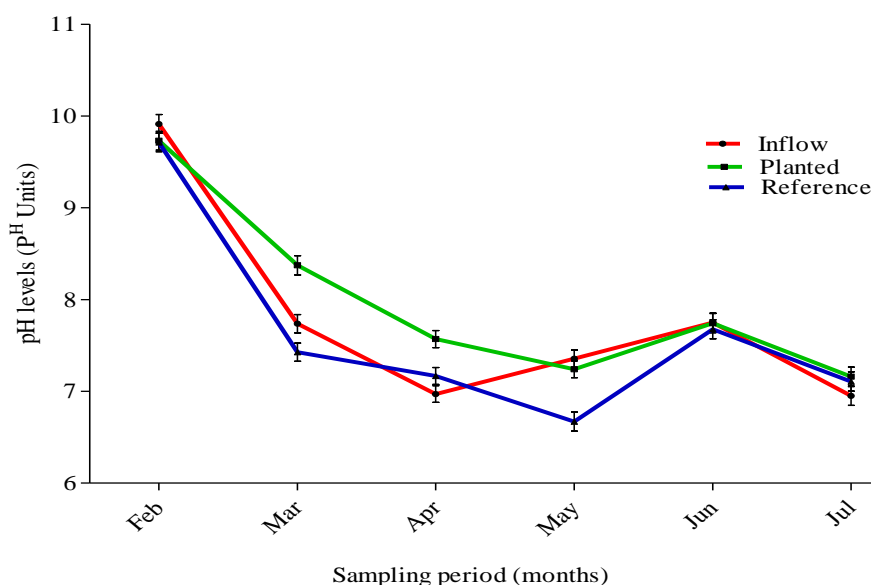


Figure 2.8. pH variations covering hot (February–April 2012) and cold seasons (May–July). Whiskers represent standard deviations of three means.

The average pH values obtained during the study were in the range of 9.97 and 6.67 pH units as shown in Figure 2.8. These results were observed on samples collected during the warm and cold seasons. The variations were not statistically significant between the planted and reference sections ( $p = 0.1048$ ). pH is a significant water quality parameter because of its influence on the biological and

chemical processes that occur in water and that influence the water quality. Acidic tendencies were observed in the months of February, May and June 2012. This reduction in pH value may have occurred due to either bacterial decay of organic matter (raised levels of CO<sub>2</sub>) or by respiration of the plants. Low pH is reported to enhance the level of nutrients for plants use in wastewater (Newcomb *et al.*, 2017). Low pH levels may have encouraged the removal of phosphorus, nitrogen and COD during the warm months of the year.

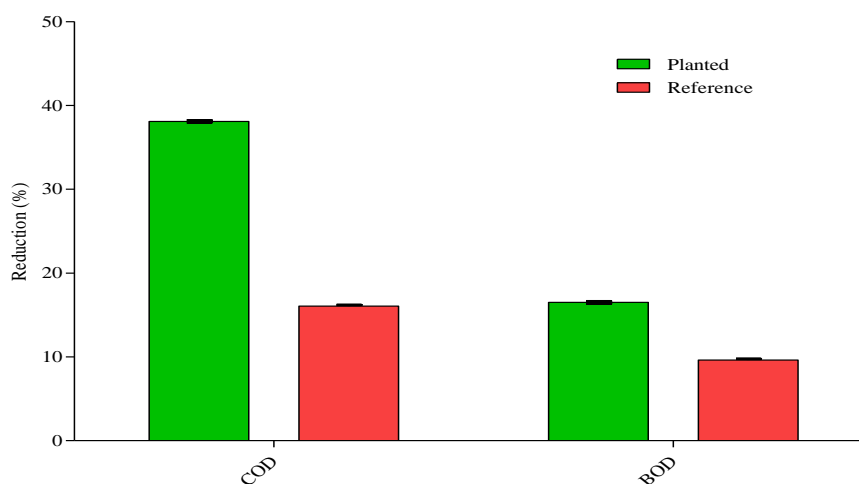


Figure 2.9. Reductions (%) in BOD and COD (mean  $\pm$  SEM) in the planted and reference sections of the rhizofilter between January - March 2012. Whiskers represent standard deviations of three means.

There were reductions in COD and BOD levels though the difference in the reductions was not particularly significant ( $p = 0.3681$ ) during the first three months of system establishment (Figure 2.9). The presence of macrophytes in the system may have played a key role in the reduction of organic matter and microbial activity, therefore, the reductions in COD and BOD. This was evidenced by the higher efficiency of reductions of COD and BOD from the planted section as compared to the reference section. On the contrary, a study by Vymazal, (2011) suggested that increased microbial activity is attributed to the participation of macrophytes which allow for more microbe attachment and greater microbial activity associated with the rhizomes. The macrophytes also provided a pedestal (stems, roots and leaves) which supported the growth of microorganisms responsible for organic molecules break down. These reductions (%) in COD may

have also been dependent on temperature (Figure 2.10) since organic matter removal and increased microbial activity tended to increase in summer months. The season between January and March have average temperatures of 28°C in Durban (Morris *et al.*, 2011; Mthembu *et al.*, 2013). The mass reduction rates for COD and BOD were similar to those reported by Abdelhakeem *et al.*, (2016).

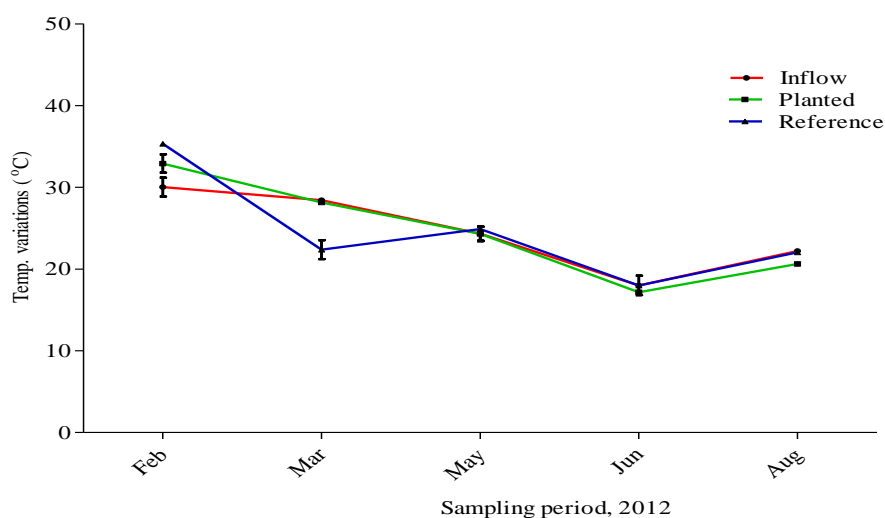


Figure 2.10. Variations in temperature at the various sampling points calculated from six means covering both warm (February – April) and cold (May-August) seasons. Whiskers represent standard deviations of 12 means.

Temperature showed a general decrease from February to August during this monitoring stage (Figure 2.10) within the influent, planted and reference sections, but the variations in the decrease were not significantly different ( $p = 0.9995$ ). Temperatures in all sections remained same between May and June but varied again in the month of August.

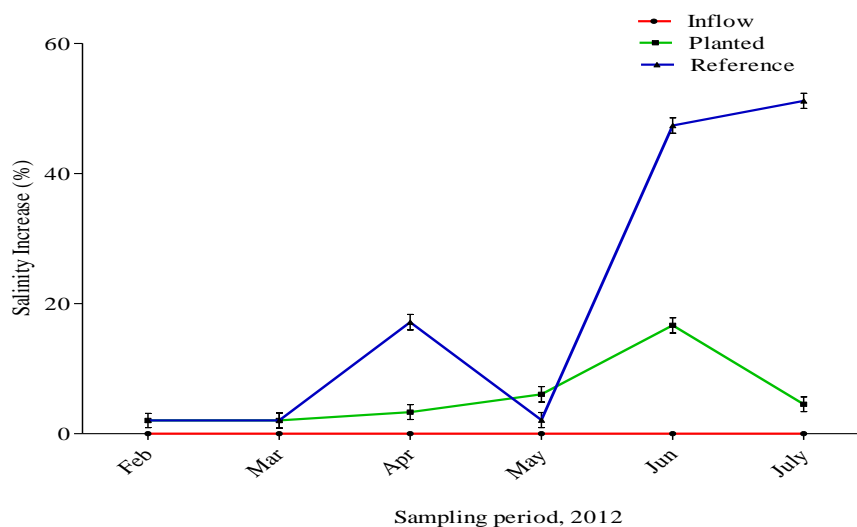


Figure 2.11. Variations (%) in salinity at the inflow, planted and reference sections covering both warm and cold seasons. Whiskers represent standard deviations of three means.

Salinity values remained constant between all the sampling points but were slightly higher in the reference section at 0.34 mg/l as compared to 0.31 mg/l in the planted during the study period. The variations were not significantly different ( $p = 0.0523$ ). Comparatively higher values were recorded in July while the lowest values were recorded in May (Figure 2.11). Alkalinity values were found to vary between the different sampling points of the rhizofilter as seen in figure 2.13. The means calculated between the various sampling points were significantly varied at  $p = 0.0004$ . Highest removal rates of 46.3% were observed in sampling point B, 46.3% (Sampling point F) and 45.5% (Sampling point I) were recorded in April. The lowest reduction rate of Alkalinity at 5.6% (Sampling point A) was recorded in the month of May (Figure 2.12). Turbidity was varied between 8 and 11 NTU at the Inflow and the various sampling points ( $p = 0.0270$ ). February and May recorded highest turbidity levels. The reductions were recorded at 11.97 NTU at the inflow to 9.7 NTU and 9.1 NTU on planted and reference sections respectively (Figure 2.13).

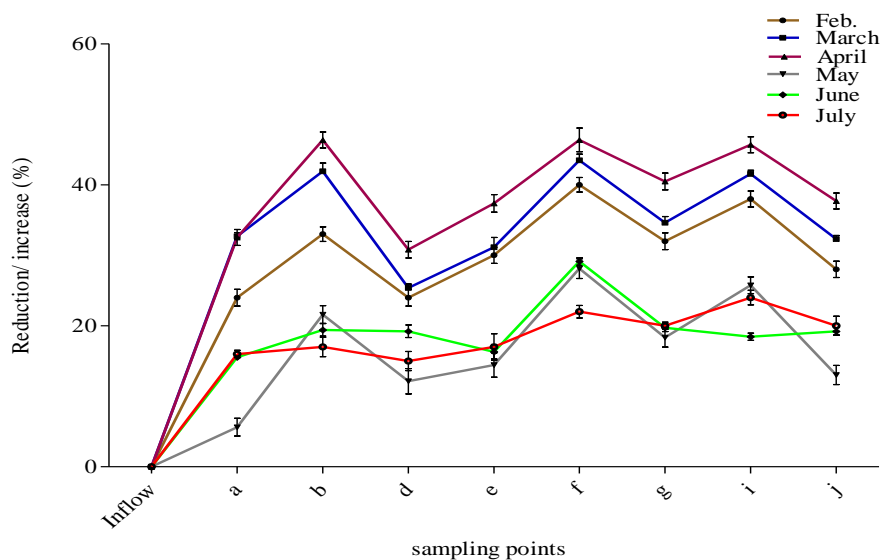


Figure 2.12. Variations (%) of Alkalinity at the various sampling points, A-J, between February and July 2012. Whiskers represent standard deviations of 12 means.

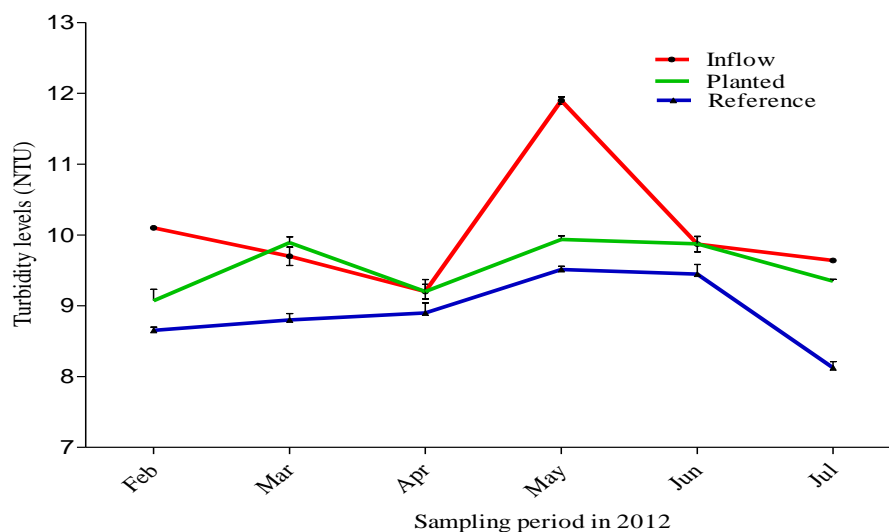


Figure 2.13. Mean variations in turbidity covering both warm and cold seasons. Whiskers represent standard deviations of 12 means.

The month of May recorded some of the highest turbidities (up to 11.9 NTU) at the inflow during the study. This was reduced to an average of 8.3 NTU mostly in the reference section of the rhizofilter. The presence of macrophytes in the unit contributed to the reduction of turbidity and suspended solids in varying percentages. Suspended solids may have been removed by the filtration

component and physical suspension offered by the extensive root network of the macrophytes. The process of pre-settling the raw sewage at the Jojo tanks before the gravity flow to the unit, also contributed to the reduction of both turbidity and suspended solids and prevented system clogging.

#### 2.4.3 Metals determination

There were statistically significant increases and reductions in the metals composition (ANOVA,  $p < 0.05$ ) within the components of the rhizofiltration unit after a period of three months from the initial system set up. Figure 2. 14 and 2. 15 show the initial concentrations of the metals in the plant parts, 1 month after system establishment. Results show that the planted section had a higher increase of metals than the reference section. Also noted is that the means for the different metals were varied in the planted and reference sections  $p=0.0145$ , though the variances were found to be equal within the triplicate samples analysed according to Bartlett's test. However, according to Tukey's multiple comparison tests, there were no significant variances to be declared between the means from the columns  $P < 0.05$ .

Table 2. 2. Mean concentration of metals in the inlet, effluent from planted and reference sections of the rhizofiltration system after three months of study (mg/l).

	<b>Inlet</b>	<b>Planted</b>	<b>Reference</b>
	Mean + SD	Mean + SD	Mean +SD
Cadmium	0.031±0.03	0.030±0.001****	0.031±0.11
Chromium	0.067±0.06	0.047±0. 011	0.051±0.01
Copper	0.061±0.06	0.068±0. 001	0.082±0.01
Nickel	0.048±0.05	0.046±0. 001****	0.041±0.04
Lead	0.540±0.14	0.588±0. 003	0.625±0.01
Zinc	0.471±0.17	0.430±0. 001	0.544±0.05

\*\*\*\* Means not significantly varied at  $p=0.9312$  (Bartlett's test).

Initial results from inlet and effluents from both planted and reference sections show that there were some reductions and increases in the concentrations of metals of some metal ions (Table 2.2). For example, initially Cd was at 0.031 mg/l and reduced to 0.03 mg/l on effluent from planted section and 0.031 mg/l on effluent from the reference section, Cr was 0.07 mg/l at inflow and reduced to

0.046 mg/l on planted and 0.047 mg/l on reference section. This could be attributed to uptake by the plants and the amount in the reference section may have resulted from the adsorption onto the sediment. Similar observations were reported by Mukesh and Thakur, (2013) during their study on heavy metal Cu, Ni and Zn: Toxicity, health hazards and their removal techniques by low-cost adsorbents. Copper was initially 0.061 mg/l and increased to 0.068 mg/l on planted section and 0.078 mg/l on reference section, Ni was 0.0481 mg/l and reduced to 0.046 mg/l on planted section and 0.041 mg/l in the reference section while Zn was 0.47 mg/l initially and reduced to 0.43 mg/l in the planted section but increased in the reference section to 0.54 mg/l. Zinc is one of the micronutrients required by plants for growth. This explains why there was a reduction in the planted section as more zinc may have been taken up by the plants for growth, while residual Cu from the plant tissues may have contributed to the increase witnessed in the planted section (Vymazal, 2011). The accumulation of metals on the stem and leaves of the wetland plants could be attributed to translocation. This means that the macrophytes were able to translocate the metal ions from the roots to stem and leaves. Similar observations were reported by Adefemi and Awokunmi, (2013) in their study on metals uptake by tomato (*Lycopersicum esculentus*).

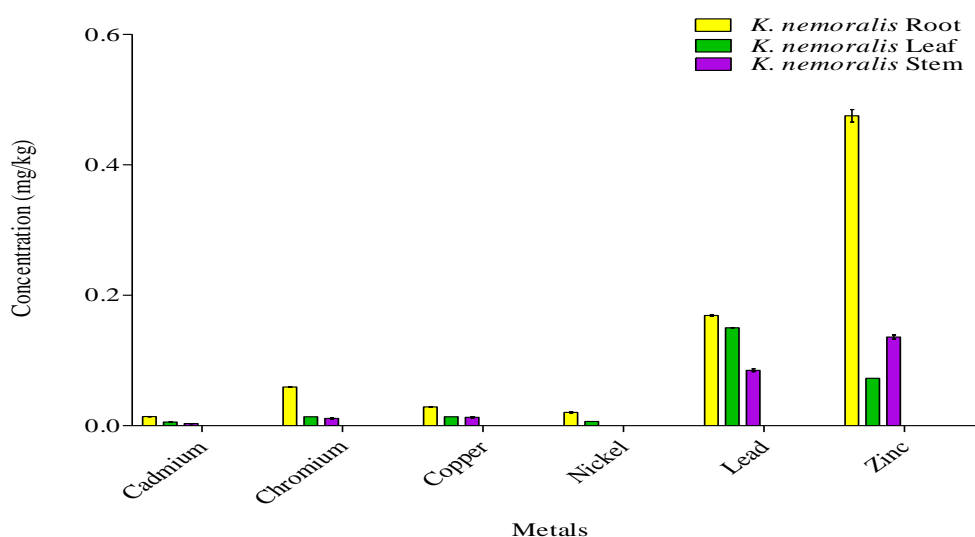


Figure 2.14 Metals (mean) concentration in tissues of *K. nemoralis* during the first month of study. Whiskers represent standard deviations of 12 means.



Zinc increased by 124% in the planted section but was found to be 44% in the reference section. Cadmium increased by 61% in the planted section against 50% in the reference section. The highest increase was seen in zinc at 124% while the least increase was on the lead at 40%. Figure 2. 16 shows the increase (%) and a decrease of the metals within the plant tissues of the rhizofiltration system. After 3 months of system establishment, there were notable increases in the concentration of cadmium (80%), copper (60%) and lead (50%) while chromium decreased by 40% in the root of *K. nemoralis*, and 70% in the root of *P. australis*. The means differed significantly ( $p= 0.0145$ ). Nickel decreased by 40% in the root of *P. australis* while zinc decreased by 20% on the leaf of *K. nemoralis*.

There was an increased deposit of zinc on the root of *K. nemoralis* and on the stem tissue of *P. australis*. A significant amount of nickel was deposited on the root of *K. nemoralis* and stem tissue of *P. australis*. Results of sediment analysis also show that more metal deposits were witnessed in the planted section as compared to the reference section of the rhizofilter. Notably, higher levels of zinc were deposited in the planted section (Figure 2. 17).

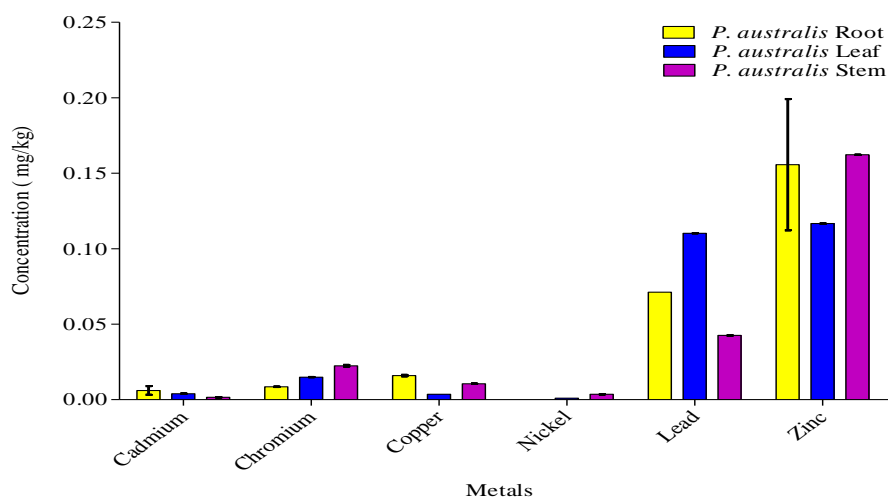


Figure 2.15 Concentration of metals (mean) in tissues of *P. australis* during the first month of study. Whiskers represent standard deviations of 12 means.

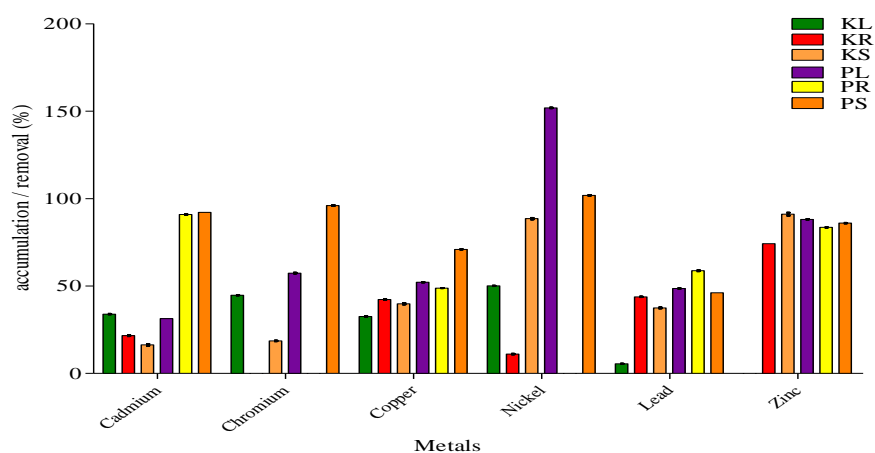


Figure 2.16 Accumulation and reduction (%) in metals on the plant tissues after 3 months of study. Whiskers represent standard deviations of 12 means.

KEY - KL (*K. nemoralis* leaf), KR (*K. nemoralis* root), KS (*K. nemoralis* stem), PL (*P. australis* leaf), PR (*P. australis* root) and PS (*P. australis* stem).

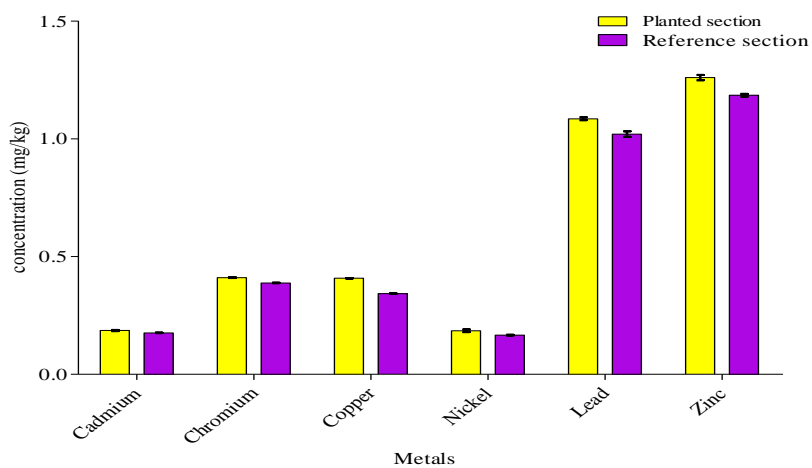


Figure 2.17 Metals concentration on the sediment of planted and reference sections of the rhizofilter after three months of system establishment. Whiskers represent standard deviations of six means.

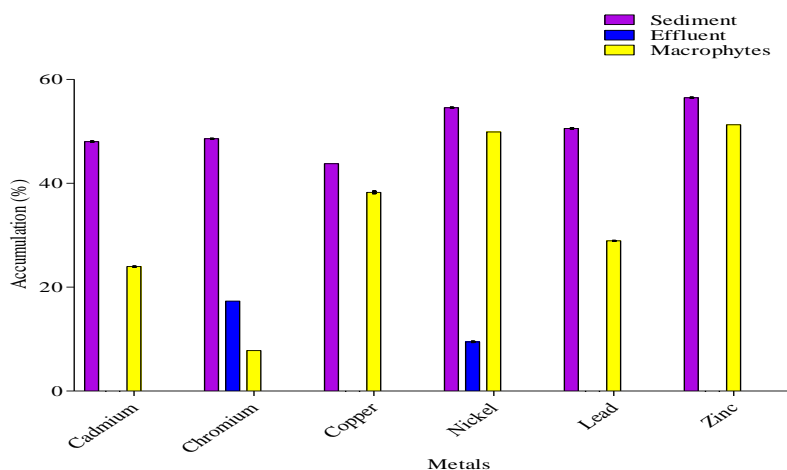


Figure 2.18. Mass balance of accumulation (%) of metals in the various components of the rhizofiltration system during the first year of study. Whiskers represent standard deviations of six means.

Considering the distribution of the metal ions in the components of the rhizofilter, a significant amount of metal ions was deposited on the sediment in comparison to the plant parts and the final effluent. The total amount of metal ions adsorbed by the sediment, absorbed by the plant tissues and concentration in wastewater were considered in order to create a mass balance. The highest accumulation was on sediment with zinc recording 56%. The highest concentration in the effluent was observed in chromium and Nickel at 17% and 9% respectively (Figure 2.18). Means of concentrations recorded were found to vary significantly at  $p < 0.0001$ . The highest accumulation on the macrophytes was witnessed on zinc at 51%. The raised amount of zinc could also be attributed to the fact that zinc is a micronutrient required by plants and that there may have been already some substantial amount of zinc deposits on the plant tissues before the exposing to the wastewater in agreement with Mukesh and Thakur, (2013) in their overview of heavy metal Cu, Ni and Zn: Toxicity, health hazards and their removal techniques by low cost adsorbents. However, some amount of metal ions could not be accounted for and was assumed to have been taken up by bacteria and fungi present in the rhizosphere according to Salam *et al.*, (2011), when they investigated the removal characteristics of heavy metals from wastewater by low-cost adsorbents.

#### 2.4.4 *Ascaris lumbricoides* and Coliform bacteria

Effluent from the planted section recorded a lower (10 ova/l) concentration of the ova of *A. lumbricoides* (Figure 2.19) than the reference section (16 ova/l) after 3 months of system establishment. The calculated means were found to be statistically significant ( $p < 0.0001$ ). These values were further reduced to 3.5 ova/l and 5.8 ova/l in the planted and reference sections respectively after 6 months of study. The removal rates were 82% in the planted section and 63.4% in the reference section of the rhizofilter.

Results for the coliform analysis indicate that reduction was higher in the planted section compared to the reference section (Figure 2.20). The calculated means were statistically significantly varied ( $p < 0.0001$ ). The highest inflow was recorded during the month of October (26.0 x 10<sup>-5</sup>MPN/100ml) and lowest in August (5 x 10<sup>-5</sup>MPN/100ml). The average reductions were in the range of 2.0 x 10<sup>-5</sup>MPN/100ml in planted and 3.5 x 10<sup>-5</sup>MPN/100ml in the reference section during the warm season (October and November) and between 3.2 x 10<sup>-5</sup>MPN/100ml and 5.2 x 10<sup>-5</sup>MPN/100ml in the planted reference sections respectively.

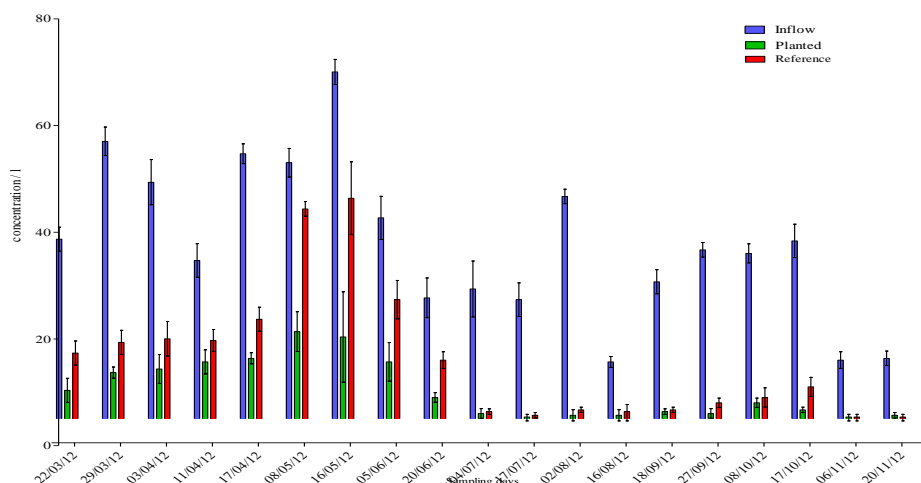


Figure 2.19 Concentration of and seasonal variations on ova of *Ascaris lumbricoides* at the inflow, planted and reference sections of the rhizofilter during the first year of study from March to November 2012. Whiskers represent standard deviations of 12 means.

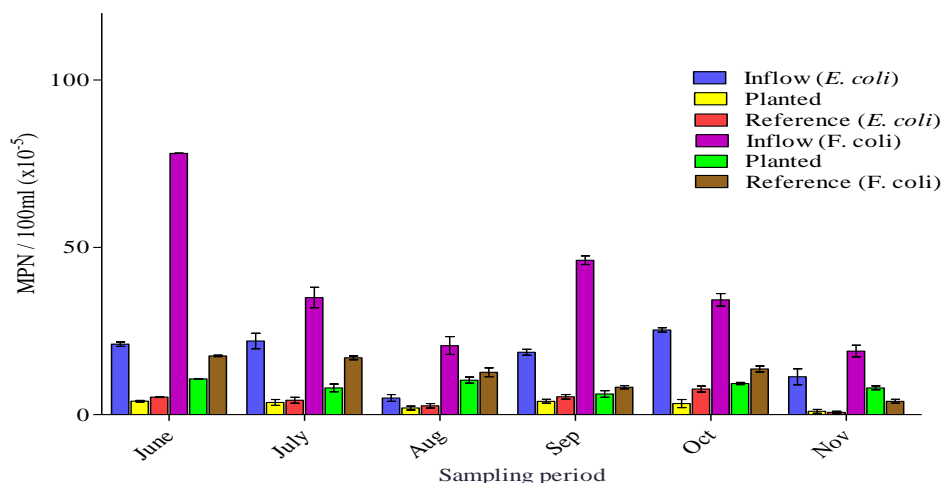


Figure 2.20 Concentration of *E. coli* and *F. coli* within the inflow, planted and reference sections over a six month period covering cold (June-Aug) and warm (Sept – Nov) seasons. Whiskers represent standard deviations of 12 means.

## 2.4.5 Nutrients

### 2.4.5.1 Nitrate removal

Nitrate is one of the nutrients required for plant growth. However, when nitrate occurs in excess of the acceptable limits, it may cause water quality deterioration including eutrophication with adverse effects on aquatic life (Odinga *et al.*, 2013). For example at levels above 10 mg/l, dissolved oxygen levels deplete.

Concentration of nitrate was found in the range of 0.6 mg/l - 24 mg/l in the influent, 0.6 mg/l – 18 mg/l and 0.5 mg/l – 15 mg/l in the reference and planted sections respectively. High value of nitrate in the influent could have been a result of its accumulation due to increased oxygen levels resulting from aeration in the municipal conventional treatment processes. The efficiency of the rhizofilter in nitrate removal was noted at 98% and 99% in the reference and planted sections respectively (Figure 2.21). There were statistically significant variations on the means from both planted and reference sections especially during the warm seasons ( $p < 0.05$ ), unpaired t-test. The highest removal of nitrate was observed in the month of June for both the planted and reference sections of the rhizofilter.

This could have been due to the low nitrate concentration in the influent during that season. However, there was an increase in nitrate removal during the month of June, which could be attributed to the rise in levels of dissolved oxygen which was recorded at between 6.3 mg/l and 6.8 mg/l in the reference and planted sections respectively. The available oxygen was probably used by the nitrifying bacteria to convert ammonia to nitrite and finally nitrate. This is in agreement with the findings of Gikas and Tsihrintzis, (2012), that ammonia removal is also highly influenced by nitrate removal under aerobic conditions. The removal of ammonia is interrelated to the concentration of nitrate. This was also reported by Vymazal, (2011), that ammonia removal was enhanced by nitrification and that aerating the soil within the rhizofilter led to an increase in nitrification.

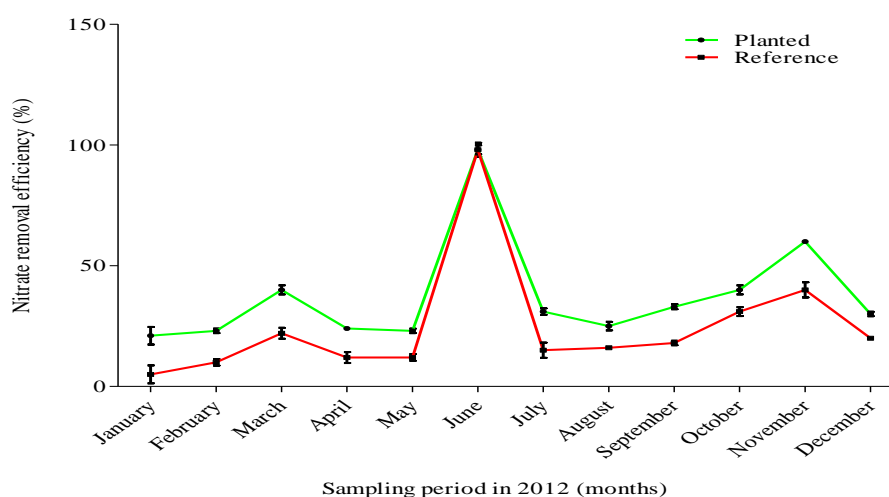


Figure 2.21 Removal efficiencies (%) for nitrate measured from influent and effluent of planted and reference sections of the rhizofilter during the study period in 2012. Whiskers represent standard deviations of 12 means.

#### 2.4.5.2 Phosphate removal

Removal of phosphate was varied between the various sampling months in the year 2012 (Figure 2.22). The concentrations in the planted and reference sections ranged between 0.1 – 6.8 mg/l at the influent, 0.1-5.8 mg/l and 0.1 – 5.1 in the reference and planted sections respectively. These results were found to be within the acceptable discharge limits of 5 mg/l according to EPA, (2009). The

concentrations were found to be lower in the cold seasons than the warm seasons with removal percentages ranging from 12% - 94% and 6%-65% in the planted and reference sections respectively. There was the statistically significant difference in the removal efficiencies ( $p = 0.032$ ). More phosphate was removed in the planted section as compared to the reference section of the rhizofilter and was observed to rise during the early stages of system development.

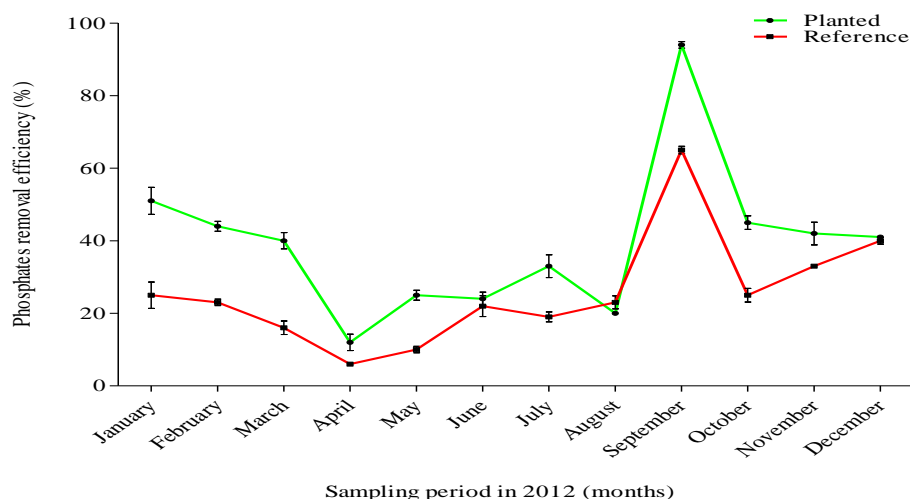


Figure 2.22 Removal efficiencies (%) for phosphate measured from influent and effluent of planted and reference sections of the rhizofilter during the study period in 2012. Whiskers represent standard deviations of 12 means.

#### 2.4.5.3 Ammonia removal

The level of ammonia ( $\text{NH}_3$ ) in surface water is considered low, however, in municipal wastewater, levels could rise up to about 30 mg/l depending on the treatment levels of the wastewater in municipal treatment plants (Jouanneau *et al.*, 2011). The concentration of ammonia in the effluent of the rhizofilter was found to be within acceptable limits of 16.0 mg/l in the planted section, 30.2 mg/l in the influent and 25.8 mg/l in the reference section. Removal efficiency was at the level of 75% and 89% for the reference and planted sections respectively (Figure 2.23). Removal of ammonia may have been dependent on the presence of

macrophytes for the planted section and the soil media for reference section of the rhizofilter based on a study by Odinga *et al.*, (2011) and Tuttolomondo *et al.*, (2014). Removal of ammonia was higher during the warm seasons with means occurring between 40% - 60% and 14% - 50% for the winter period in the reference and planted sections respectively. There were significant variations ( $p < 0.05$ , Tukey's test) in removal efficiency of ammonia between summer and winter seasons. The whiskers represent the means calculated from a total of six separate readings.

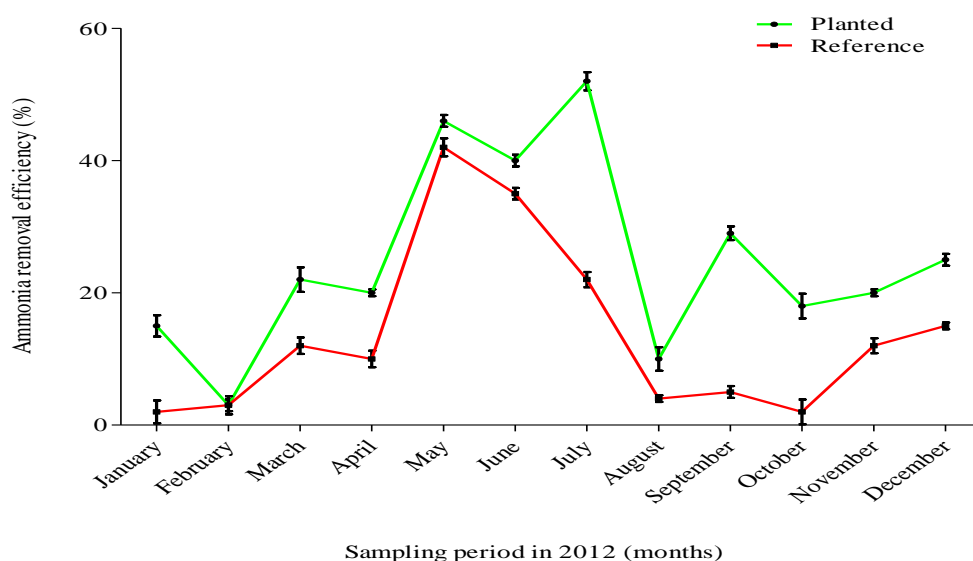


Figure 2.23 Removal efficiencies (%) for ammonia measured from influent and effluent of planted and reference sections of the rhizofilter during the study period in 2012. Whiskers represent standard deviations of 12 means.

## 2.5 DISCUSSION AND CONCLUSIONS

Results of the physicochemical parameters obtained from January to December 2012 are outlined in this section. Raw settled sewage and pre-chlorinated water samples were used during this preliminary study.

### 2.5.1 Physico-chemical Parameters

Wastewater content that was received by the rhizofilter and removal rates was based on the source (which was around Kingsburgh) and the flow rate into the



system (Figure 2.1). The wastewater was collected at the bottom of the collection chamber after grit removal and pumped into the system using a pump that was fitted at the bottom. Pre-settling of raw wastewater in treatment plants and wetlands as studied by Vymazal, (2011) reported that this aided in the removal of suspended solids. The wastewater was received into the rhizofilter by vertical flow system as was designed for the study (Figure 2.2). The vertical flow was preferred due to its efficient pollutant removal through direct down flow into the filter matrix. This process provides easy trapping of the pollutants such as suspended solids. Vertical flow is also not prone to system clogging as this may be prevented through the channelling of the flow, in comparison to the horizontal flow. Similar sentiments have been also reported by Vymazal, (2011). Initial flow measurements were taken in order to establish the optimum flow rates into the rhizofiltration unit. The flow rates were found to vary (Figure 2.6) according to the solid content and viscosity (Sundaravadivel and Vigneswaran, 2010). Removal of the various pollutants was dependent on the components of the rhizofilter such as macrophytes and sediment (Figure 2.3) which provided a complex network of the root system known to harbour microorganisms whose activities contribute to the reduction of pollutants such as organics. Initial macrophytes were planted (Figure 2.4) and initial tests performed on the efficiency of the system in agreement with a study by Chen, (2014). The system assumed optimum pollutant removal efficiency after it attained a plant cover of about 80% (Figure 2.5) according to Vymazal, 2011. The pollutant removal efficiency of the system was enhanced by the easy gradient flow that was effectively designed on the system (Figure 2.2). This enabled the free flow of the wastewater after the pre-settling and sedimentation which was facilitated by the Jojo tanks. Wastewater treatment systems employing pre-settlement have been investigated in the past by Vymazal, (2011) who investigated the performance of a wetlands system using pre-treated wastewater. This formed the basis of this study which was conducted at Kingsburgh. This system may be very suitable in communities where wastewater is pre-treated. The efficiency of the system was tested by ensuring that the effluent was sampled at various designated points (Figure 2.3) which allowed for comparison of the various parameters. The

rhizofilter was filled with filter media having various components with different sizes. This media was the platform on which the macrophytes grew and also was the filtration unit for solids removal. The roots of the plants preserved the permeability of the unit, while their horizontal continuous rhizomes assisted in applying oxygen to the rhizosphere. Similar sentiments were also shared by Chen, (2014).

Physic-chemical parameters were investigated in order to determine the conditions under which the various metals and pathogens were reduced or added to the rhizofiltration unit (Figure 2.7). The choice of physic-chemical parameters followed the regime that was adopted by Gikas and Tsihrintzis, (2012). For example, the choice to investigate temperature changes was based on the fact that above a certain threshold, macrophytes cannot grow and also below, nitrogen-converting bacteria would not efficiently work, thus there would be no food for the plants. The temperature was reduced by 11.9% in the planted section and 1.2% on reference section. The highest value of 35.3°C in the reference section was recorded in February while the lowest temperature reading of 17.1°C in the planted section was recorded during the month of June (Figure 2.10). Reductions in effluent temperature in the planted section were due to the plant cover and water retention, which created some macroclimate within the rhizosphere. The pH of the effluent increased on average from 6.95 to 7.55 pH units, and 7.23 pH units in the planted and reference sections respectively (Figure 2.8). Comparatively higher pH values were observed in the month of February and comparatively lower values were recorded in May and July (Shelef *et al.*, 2012). The pH values were not significantly different as the mean value for all sampling points was an average of 6.5-7.2 pH units. This suggests that the wastewater was majorly from the domestic source according to Gikas and Tsihrintzis, (2012). The flow rate was varied within the various sampling points of the system. The lowest recorded flow rate was from sampling point “E” at 0.3942955 l/min in the planted section while highest was at sampling point “J” at 0.8710061 l/min in the reference section. The variations in flow rate within the sampling points could be attributed to established channelling caused by

variations in flow rates as water flowed through the substrate in concurrence to a study by Vymazal, (2011). Other attributes to the difference in flow rates could have been the particle consistency of the wastewater and the location of the pump at the bottom area of the Jojo tank which may have hampered continuous flow.

The first three months of study recorded removals of 38% in the planted section and 16% on reference for BOD while COD was reduced by 16.5% and 9.6% for planted and reference respectively. Table 3 displays the significant reductions in the various parameters including BOD<sub>5</sub> at 79% and COD at 75% at the optimum removal efficiency by the system (Figure 2.9). The reductions of BOD and COD within the system is an indicator of heavy removal of organic pollutants by the rhizofilter, an observation which was also reported by Kropfelova *et al.*, (2009), Borkar and Mahatme, (2010) and Sayadi *et al.*, (2012) in their study of wastewater treatment with vertical flow constructed wetland using *Phragmites australis* where they reported reductions of up to 95% for BOD and 94% for COD. The organic matter could possibly have been reduced through aerobic reactions and adsorption of solid particles within the rhizosphere which contributed to the breakdown of organics in conjunction with the macrophytes which provided oxygen to the root system. Major mechanisms for BOD and COD reduction were probably filtration by the filter matrix, sedimentation and microbial metabolism within the rhizofilter. The BOD and COD removals were higher in the planted as compared to the reference section of the rhizofilter as seen in Figure 2.9. This was consistent with the findings of Sayadi *et al.*, 2012.

Total suspended solids were reduced by 86% in the planted section while the reference section witnessed a 59.8% reduction. The reduction of SS may have occurred due to sedimentation especially in the settling Jojo tanks and further onto the substrate of the rhizofilter (Mahmood *et al.*, 2013). Electrical conductivity (EC) was reduced on average by 7.7% in the planted section while the reference section had an average reduction of 0.83%. The reduction of EC by 7.7% on planted and 0.83% in the reference section may have been due to the

reduction of phosphorus and nitrogen salts by the microorganisms and plants interaction within the porous substrate of the rhizosphere according to Adhikari *et al.*, (2011) and Singh *et al.*, (2012). Evapotranspiration and temperature variations in summer have also been reported as one of the mechanisms involved in the reduction of EC in treatment wetlands (Adhikari *et al.*, 2011). This could be the reason for higher reductions of EC during the warm months. Total dissolved solids (TDS) was reduced by 11.5% on the planted section and 3.5% in the reference section of the rhizofilter (Ali *et al.*, 2013; Shelef *et al.*, 2013). Consequently, higher removal efficiency was observed in the planted section than the reference section.

Dissolved oxygen was raised by 10% in the planted section and 5% in the reference section. The recorded values were in the range of 3.2 mg/l at the inlet to an average of 6.5 mg/l and 6.0 mg/l in the planted and reference sections respectively according to Lee *et al.*, (2013). The increase in DO levels resulted from the physical and biological mechanisms of plant roots within the rhizosphere.

Turbidity values were significantly higher at the onset of the cold season in May and witnessed reductions from 11.97 NTU at the inlet to 9.7 NTU and 9.1 NTU on planted and reference sections respectively (Figure 2.13), an observation that concurred with findings from a study by Shelef *et al.*, (2013). Turbidity was reduced at a steady rate in the reference section while the planted section had inconsistent reductions which were attributed to the flow channelling caused by the root network of the macrophytes and the flow rate into the system according to Vymazal, 2011.

Salinity values remained constant between all the sampling points but were higher in the reference section at 0.34mg/l as compared to 0.31mg/l in the planted (Figure 2.11). Higher values were recorded in July and while the lowest values were recorded in May. Alkalinity was varied between the different sampling points of the rhizofilter (Figure 2.12). Highest Alkalinity removal rates

of 46.3% (Sampling point B), 46.3% (Sampling point F) and 45.5% (Sampling point I) were recorded in April. The lowest reduction rate at 5.6% (Sampling point A) was recorded in May. Alkalinity reductions may have been achieved through the variations in pH at the inflow and effluents. Low pH increased the Alkalinities while high pH which was experienced at the sampling taps contributed to the reduced Alkalinity at the effluents especially in the planted section in concurrence with the findings of Maine *et al.*, (2006). This advanced that the calcium carbonate precipitation in the rhizosphere represented a trace governed by the inflow pH.

### 2.5.2 Metals Removal

Results after three months of study from inception indicate that there was additional metal accumulation (Figure 2.16) on the tissues of the experimental macrophytes. For example, initial results show that cadmium on the leaves of *K. nemoralis* was 0.033667 mg/kg and increased by 33% to 0.05095 (Figure 2.14). On the root system of *K. nemoralis*, the initial concentration of cadmium was 0.041 mg/kg and increased by 21% to 0.054 mg/kg. The concentrations of were varied between the different plant parts (Figure 2.16) which may have been based on the different metal binding capacities of the plant tissues according to Hamadeh *et al.*, (2014). Zinc and lead were found to be most abundant (Figure 2.15) in the tissues of *P. australis* though the means for the variations were not significant ( $p=0.07969$ ). Zinc is one of the micronutrients found in plant tissues, and its elevated levels here may be explained by this phenomenon. The highest accumulation of metal ions was observed in the sediment (Figure 2.17) of the planted section of the rhizofiltration unit. it can be concluded that there was a significant difference ( $p<0.0001$ ) in metal accumulation on the various components of the rhizofilter (Figure 2.18). The highest accumulation as mentioned earlier was noted at 56% for zinc on the sediment which was followed by a 51% of the same for the macrophytes and zero for effluent while the highest percentage noted for the effluent was for chromium at 17%.

Metal concentrations at the influent were very low giving the rhizofilter high percentage removals which could have mostly (Cd, Cu, Cr, Ni) been achieved through filtration and adsorption onto the filter matrix and rarely through the macrophytes except for Pb and Zn.

### 2.5.3 *Ascaris lumbricoides* and Coliform Bacteria Removal

The removal of ova of *A. lumbricoides* by the system was mostly by filtration through the heavy root network of the plants and the rhizofiltration unit substrate. Similar reports were also given in a study by Gikas and Tsihrintzis, (2012). Total coliform bacteria may have been removed by the presence of macrophytes in the rhizofilter, since removals were higher in the planted section as compared to the reference section (Figure 2.19). The main mechanism of removal could be associated primarily with UV radiation and an element of higher temperatures on the sections of the rhizofilter that were exposed. The total coliforms reduction (Figure 2.20) could have been achieved through the action of the macrophytes which excreted some inhibiting metabolites through root exudates (Hamadeh *et al.*, 2014). Further, the presence of the macrophytes could have also stimulated the growth of some preying microorganisms within the rhizosphere according to Maine *et al.*, (2006) and Lee *et al.*, (2013).

### 2.5.4 Pollutant Removal by the Rhizofilter

The removal of pollutants by the rhizofilter was determined by the concentration at influent and effluent of the system. The specific values were derived based on points of sampling at every stage of the study (Figure 2.3). Cumulative removal values were also recorded at the end of the study period. The organic matter removal and eventual BOD reduction could be attributed to the attachment of large particles on the substrate and large root surface (for microbial augmentation) provided by the plants. Activities of anaerobic and aerobic bacteria in the rhizosphere could have played some significant role in organic

pollutant removal from the system according to Adhikari *et al.*, (2011) and Vymazal, (2011). The organic loading rates and pollutant removal was also established by the determination of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total suspended solids (TSS). The results showed some reductions in the organic load though the variations on consecutive samplings did not exceed 10% and were not significantly varied ( $p = 0.3280$ ) unpaired t-test. Organic loading may have been reduced through biodegradation by bacterial action within the rhizosphere and adsorption onto the extended root network of the macrophytes and on the sediment. Total suspended solids were removed by the pre-treatment through settling at the inlet Jojo tank. This was in agreement with the findings of Kayyali and Jamrah, (1999), Maine *et al.*, (2006) and Hamadeh *et al.*, (2014).

Effluent from the planted section recorded a slight increase on suspended solids. This may have been caused by movement of plant roots and increased evapotranspiration, a situation that was also observed and reported by Hench *et al.*, (2003). Due to penetration of plant roots into the filter matrix, the supply of dissolved oxygen is enhanced through the roots. This was confirmed by the raised DO levels in the planted section (10%) as compared to the reference (5%) section. The raised levels of DO in the system led to the oxidation of organic matter which may be the reason for the reduction of BOD levels in the planted section. It is also notable that the high levels of DO are recorded in the planted section against the reference section according to Rawat *et al.*, (2012).

The Rhizofiltration performance is affected by a number of factors including operating mode (batch or continuous, horizontal or vertical), loading rate and environmental conditions such as climate and season (Rawat *et al.*, 2012). A study by Wood, (1999) reported that overloading such systems may result in clogging, decreased treatment efficiency and odour emissions, as well as large oxygen demand upon roots and resultant death of the plants. This agrees with the findings of Faulwetter *et al.*, (2009) and Morris *et al.*, (2011) that higher organic loads result in hydraulic dysfunction due to internal clogging. The increase of suspended solids in the planted section of the rhizofilter may have been caused by movement of plant roots and increased evapotranspiration. The average

concentrations of the tested parameters were found to be within the allowable discharge limits according to WHO, UNEP and South African Department of Water Affairs (DWAF) 2004, discharge standards.

Metals may have been removed through various processes including precipitation and the ability of organics in the wastewater to bind to metal ions. This binding ability of metals onto organic matter may explain the reason for metals decrease in above-ground plant parts (Figure 2.18). Another metals removal process may have occurred through the accumulation of organic matter from shoots and leaves of the macrophytes in the rhizofilter. The metal ions present bound directly onto the organic matter which provided carbon and therefore energy for the microbial metabolism. Removal efficiencies during the first six months of study for the various metal ions showed significant differences ( $p < 0.0001$ ) considering the mass balance accumulation in plant tissues, effluent and sediment (Figure 2.16). It was on this basis of pollutant removal rate that the system was considered fully established for the continuation of the other investigations of the study. The planted section of the rhizofiltration unit was considered to have removed more pollutants than the reference section (Figure 2.17, 2.19 and 2.20). The efficiency of removal of nitrate (Figure 2.21), phosphate (Figure 2.22) and ammonia (Figure 2.23) was found to increase with the development of the system in both planted and reference sections. The drop that was witnessed in the month of August for the three parameters was attributed to changes in temperature and therefore changes in microbial activities as well. Similar sentiments were aired by Morris *et al.*, (2011). This study observed that the rhizofiltration system with macrophytes may be considered as efficient in reduction of pollutants from wastewater (Figure 2.16).



## 2.6 CONCLUSIONS

- This study demonstrated the potential of the rhizofilter in pollutant removal to some acceptable levels. This was initially achieved through constant monitoring of the functionality of the system in general pollutants removal.
- Monitoring for the effective functioning of the system is dependent on well-regulated flows and optimum macrophyte growth which was achieved at 80% cover within three months of system establishment.
- There was an increase in dissolved oxygen concentrations after 10 months of study.
- The physicochemical parameters were effectively reduced by the rhizofiltration system after three months of establishment. This means that the system had achieved optimum pollutant removal efficiency. For example, turbidity became stable after the third month with values reducing from 12 NTU to between 8-9 NTU (Figure 2.13).
- A vertical flow system such as the one used in this study has the potential of functioning efficiently without minimal clogging challenges because the raw sewage is settled first before being allowed to flow into the system.
- The planted section was found to remove more pathogens than the reference section (Figure 2.19 and 2.20).
- In as much as the planted section performed better in terms of pollutant removal, the reference section also played a key role in the pollutants removal. For example, the exposed surface of the reference section played a role in the elimination through filtration of some pathogens such as *E. coli* and *ova* of *A. lumbricoides*.
- After a period of six months of study, the pollutant removal efficiencies were found to be consistent for many parameters. Due to this, the system was assumed to be fully established for further research.

## CHAPTER 3

### ASSESSMENT OF HEAVY METALS REMOVAL FROM WASTEWATER USING CONSTRUCTED RHIZOFILTRATION SYSTEM

#### 3.1 INTRODUCTION

Heavy metals are introduced into the municipal wastewater as a result of runoff from different industrial processes, anthropogenic activities, traffic emissions, atmospheric deposition, prolonged contact with the zinc, copper or lead piping or tanks and from agricultural practices (Zhang *et al.*, 2014b). Other sources are through activities such as mining, manufacturing and irrigation using inefficiently treated wastewater (Dana and Mohammed, 2014). In sediment, metals are available in phases such as biologic and organic matter, sulphide, quartz, clay, carbonate and iron and manganese oxide (Walli, 2015).

Copper used as an additive in animal feeds occur in the animal excreta which ends up in the wastewater system with elevated levels of copper (Abel, 2002; Faulwetter *et al.*, 2009; Luca *et al.*, 2011; Elias *et al.*, 2014; Nazeera *et al.*, 2014).

Lead (Pb), chromium (Cr), nickel (Ni), cadmium, copper and zinc (Roy and McDonald, 2013) have been identified as the metals commonly investigated in wastewater research (Suruchi and Khanna, 2011; Espinoza *et al.*, 2012). This is because they are associated with high toxicities and have been documented to accumulate in the environment where they negatively affect the plant, animal and human health through the food chain (Suruchi & Khanna, 2011; Dana and Mohammed, 2014). The discharge of metals into the environment ultimately/subsequently ends up in low capacity or inefficiently treated wastewater and significant amounts of metals are likely to enter the receiving water bodies (Singh *et al.*, 2012; Wu *et al.*, 2014). If this occurs, the chances of assimilation into the human body through the food chain are very high (Chibuike and Obiora, 2014; Walraven *et al.*, 2015).

The occurrence of heavy metals in water sources can contribute to either beneficial or detrimental effect on the environment (Dana and Mohammed, 2014, Elias *et al.*,

2014). Some metals are required for growth and development in both plants and animals (zinc and copper) in trace amounts while some, even in trace concentrations are phytotoxic and cytotoxic (Akpore and Muchie, 2010, Singh *et al.*, 2012). Their toxic effects are attributed to bioaccumulation and the ability to form organo-metallic complexes with some of the rhizospheric components (Abel, 2002; Hooda, 2007; Zhang *et al.*, 2011). These effects are determined by the concentrations and sources of heavy metals in the water according to APHA, (2005). Heavy metals are known to accumulate in tissues of plants and animals through the food chain and potentially have various adverse effects in plants as shown in table 3.1 which was adopted from Khan *et al.*, (2009). Consequently, wetland sediment remains the major pollutant reservoir in wetlands according to Luca *et al.*, (2011). For this reason, the rhizofilter sediment was also investigated to assess the metals adsorption potential.

Table 3. 1 Effects of heavy metals on plant growth and tissues on exposure beyond acceptable limits. Adapted from (Akpore and Muchie, 2010).

Metal	Effects on plants
Copper (Cu)	Retards plant growth and reproduction by inhibiting photosynthesis. Decreases surface area of thylakoid thereby reducing light absorption.
Nickel (Ni)	Reduces production of protein, germination of seed, enhances accumulation of dry mass and also raises levels of free amino acids.
Cadmium (Cd)	Reduces germination of seed, lipid levels and plant growth and induces production of phytochelatins.
Chromium (Cr)	Causes damage to membranes reduces enzyme activity which retards plant growth, causes chlorosis and damage to roots.
Zinc (Zn)	Increases the growth of plant and ratios of ATP and chlorophyll. Also reduces the toxicity of nickel in the rhizosphere and plant tissues.
Lead (Pb)	Causes decrease in production of chlorophyll and growth of plant and damage to cells by increasing superoxide dismutase.

Plants absorb metals from water by extracellular or intracellular mechanisms, though it is documented that soil is the main source of metal absorption into plants (Akpore and Muchie, 2010). Metal absorption involves the exchange and replacement of cations and protons by metals ions in solution (Schneider *et al.*, 2001). Adsorption, on the other hand, is influenced by the electrostatic attraction between the cations in solution and the strength of the pollutant in an aqueous state.

Metals absorption into plant tissues is also influenced by functional groups within the biomass and the electrostatic attraction among charges in the species.

Metals adsorb onto plant roots with some degree of specificity based on various conditions. For example, the uptake by the roots depends on a number of factors such as soil pH, type of soil, the presence of organic matter, microbial activity and drainage status (Tangahu *et al.*, 2011). The uptake of metals by plants depends on the accessibility to plant roots which is highly heterogeneous in each plant species, the season of collection and the metallic form (Roy and McDonald, 2013). A decrease in pH improves the accessibility of cobalt (Co), manganese (Mn) and Ni and is the reason poorly drained soils release Co and Ni at a faster rate (Tangahu *et al.*, 2011). Some plants are known to accumulate specific metals from water and soil (Gerhardt *et al.*, 2009). For example, *Astragalus* spp. accumulate more selenium (Se) if present, while *Sebertia accuminata* is reported to accumulate more of Ni, Zn and Cd. The availability of these heavy metals to the roots of *Thlaspi caerulescens* is enhanced by a decrease in soil pH (Chibuike and Obiora, 2014). Apart from the roots, metals may enter plant tissue through the leaves (Singh *et al.*, 2012). High levels of Pb have been reported in plants growing near busy highways, a discovery that confirms potential accumulation of these metals by the leaves. Levels of up to  $44.30 \pm 18.384 \mu\text{g/g}$  of Pb were reported by Naveed *et al.*, (2010) after a study on roadside trees of Quetta. Metals that are able to dissolve in rainwater may enter the plant through the leaves (Tangahu *et al.*, 2011).

Many conventional treatment systems cannot efficiently remove heavy metals from wastewater water due to their design limitations (Luca *et al.*, 2011). Metal deposits are often lodged in the sludge which is normally a by-product of these treatment systems. Conventional systems have many management challenges because they are labour intensive, expensive to run, are prone to system failures and need constant monitoring (Adhikari *et al.*, 2011). Recent studies have coined some designs that incorporate biotechnology into wastewater treatment systems. These systems involve the use of phytoremediation with applications of constructed wetland rhizofiltration systems for wastewater remediation. This is the application of plant roots to accumulate, concentrate, absorb and precipitate pollutants in order

to achieve the desired phytoremediation efficiency (Khilji and Firdaus-E-Bareen, 2008). In a rhizofiltration system, plants, water, substrate, organic matter and microorganisms interact forming a complex system (biogeochemical filter) to biologically, chemically and physically remediate wastewater (Luca *et al.*, 2011).

Several studies have been carried out to seek biological solutions to wastewater treatment for small communities especially in the developing world (Zhang *et al.*, 2014a). Chibuike and Obiora, (2014) investigated the use of macrophytes in the remediation of soil polluted with heavy metals and reported that a combination of both plants and microorganisms ensures a more efficient removal of heavy metals. Naveed *et al.*, (2010) conducted a study on metals pollution of roadside plants and they detected significantly high levels of Pb in the leaves of the study plants. In a study by Xu, (2014), it was reported that efficient removal/reduction of parameters such as total phosphorus (TP) and total nitrogen (TN) is affected by temperature and that low reductions are experienced in cold seasons while biochemical oxygen demand (BOD), suspended solids (SS) and chemical oxygen demand (COD) remain unaffected by seasonal climatic changes. Kulbat *et al.*, (2003) reported heavy metals removals of 80% Cr, 76% Pb, 1% Ni and 50% Zn during their study of metals removal from wastewater using biological systems. Allende *et al.*, (2014) studied the removal of metals in constructed wetlands and reported that the main removal mechanisms were based on cation exchange and precipitation.

The rhizofiltration process is affected by a number of factors. These include operating mode (batch or continuous, horizontal or vertical), loading rate and environmental conditions such as climate and season of the year (Faulwetter *et al.*, 2009). Arienzo *et al.*, (2009), reported that overloading may result in clogging, decreased treatment efficiency and odour emissions, as well as large oxygen demand by the roots and resultant death of the plants.

The rhizosphere harbours a great number of bacteria which enhance the wastewater treatment through various metabolic and chemical complexes with metal ions and the organic components within the rhizosphere (Vymazal, 2011; Ali *et al.*, 2013).

The size of rhizosphere varies according to the morphological differences of the plant roots. This size is normally restricted to the 1 mm region surrounding the root. The diversity and population of bacteria in the rhizosphere are further activated by the large surface area provided by the root network and carbon source (for denitrifiers) through the root exudates (Odinga *et al.*, 2013). The plants used in rhizofiltration are adapted to mechanisms involving the rapid growth of roots and leaves (Yadav *et al.*, 2011). Rhizofiltration relies heavily on the chemical synthesis of plant roots by the plant exudates (Rawat *et al.*, 2012). The process is highly pH-dependent as this promotes the precipitation of metals onto the plant root surfaces. When plant root exudates such as phenolics are released by decaying cells, they change the metal status and speciation causing protons to be released (Shelef *et al.*, 2013). The release of protons causes acidification of the rhizosphere which promotes the transport of metal ions and their bioavailability (Vymazal, 2011). Rhizofilters are normally designed to use young plants (mainly shrubs and weeds), but recently, trees with deep roots have been discovered to be suitable for pollutant reduction in wetlands. Such trees have been used for water purification in a study by Karak and Bhattacharyya, (2010). Trees have proved to be even more cost-effective and with longer life spans than shrubs which require some maintenance such as harvesting for replacement, have a shorter lifespan and quite often lack the ability to tolerate high heavy metals toxicities (Yadav *et al.*, 2011).

The various rhizofiltration processes are constantly faced with certain limitations such as constant pH regulations which require continuous monitoring. Initial growth and establishment of the plant bio-accumulators prove to be time-consuming since they must mature before transplanting into the wetland (Khan *et al.*, 2009). The interaction of different plant species and metals speciation has to be optimized before the onset of rhizofiltration process and monitored periodically (Sood *et al.*, 2012). Various wetland and terrestrial plants have been used in several studies to assess their phytoremediation potentials (Tangahu *et al.*, 2011). *Phragmites australis* has been used in several studies in Australia, Europe, Canada and Africa for wastewater treatment (Vymazal, 2011). *Phragmites australis* occurs in marshy areas and brackish water where plant diversity is limited and therefore its

rapid colonization of such areas does not significantly affect other plant diversities (Meyerson *et al.*, 2000). Bragato *et al.*, (2006) and Shelef *et al.*, (2013), studied metals removal from wastewater using *Bolboschoenus maritimus* L. and *P. australis* and reported higher metals uptake on the shoot of *P. australis* than in *B. maritimus*. Shelef *et al.*, (2013) in their study of phytoremediation potential of *Bassia indica* concluded that the annual plant has a 10% salt content removal capability.

A study conducted by Fibbi *et al.*, (2012), reported low accumulation of Cr in above ground tissues and slightly higher accumulation in the roots of *P. australis*. Salinity tolerance of *P. australis* clones was studied by Achenbach *et al.*, (2013) and they concluded that the clones can tolerate higher salinities than the original plant. In addition, they reported that the osmotic control of *P. australis* is achievable by varying concentrations of similar solutes such as  $K^+$ . Other plants that have been used in wetlands for wastewater purification include *Typha* spp. such as *T. latifolia*, *T. angustifolia* and *T. domingensis*. Other emergent species have been used in wetlands remediation studies, for instance, *Scirpus* spp., *Phalaris arundinacea* and *Iris* spp. according to a report by Vymazal, (2011).

### 3.1.1 Aim

To evaluate the removal of heavy metals from municipal wastewater by using a constructed rhizofiltration facility planted with *Phragmites australis* and *Kyllinga nemoralis*,

### 3.1.2 Objectives

- To assess the removal efficiency in the rhizofilter by comparing metals concentrations at inflow and effluents from planted and reference sections.
- To assess the distribution of metals uptake by the leaves, roots and stem of the macrophytes.
- To assess the contribution of rhizofiltration sediment in adsorption of metals as part of the removal process.

## 3.2 METHODS

### 3.2.1 Site Description

This study was carried out in the eThekweni municipality, Durban South at Kingsburgh wastewater treatment works. Durban experiences about 320 days of sunshine annually. Temperatures in winter range from 16-25°C and in summer, a range of 23-33°C is experienced. The summer season occurs between September to April with the hottest month being January with records of temperatures of  $\pm 32^{\circ}\text{C}$  daily. The Mozambique current brings warm currents along the coast even during winter and this is one reason why Durban remains warm throughout the year (Ziervogel *et al.*, 2014). Design of the rhizofiltration system is described in chapter 2.2.1.

### 3.2.2 Preparation and Analysis of Wastewater Samples and plant material is described in chapter 2.2.10.1.

### 3.2.3 Collection, Preparation and Analysis of Sediment Samples

Sediment samples (approximately 500g) were collected in triplicate from the planted and reference sections of the rhizofilter using a PVC scoop (3cm diameter) from a depth of 10 cm and placed in glass bottles. Monthly samples were taken from three designated points adjacent to taps A, C, and E on the planted section and taps F, H, and J on the unplanted section of the rhizofilter.

The sediment material was kept in a cool box with ice and transported to the laboratory for metals analysis according to USEPA method 3050B. The sediment was dried at 80°C for 24 hours (Kruopiene, 2007; Zhang *et al.*, 2011) and sieved through 40 mm sieve. A portion of the sediment sample (1.0 g) was weighed and transferred into Teflon tubes. The prepared material in triplicate Teflon tubes was then introduced into the microwave tube safety shield and 6 ml of  $\text{HNO}_3$  (65%) followed by 4 ml of HF (40%) was carefully added to the sample as suggested by Damodhar and Reddy, (2012). The solution was digested using a microwave



digester Milestone START D for 30min at  $180\pm 2^{\circ}\text{C}$ . The residue was dried on a hot plate, cooled and diluted with 10 ml of distilled water in a 50 ml volumetric flask. The solution was then brought to the mark using double distilled water. Three replicates were prepared and analysed each time. The heavy metal content was determined using inductively coupled plasma optical emission spectrometry (ICP OES) on a Shimadzu ICPE, Model-ICPE- 9000 spectrometer according to the method by Bakar *et al.*, (2013).

### 3.2.4 Plant and Sediment Samples Preparation for Electron Microscopy

In order to assess the possibility of metals attachment or adsorption onto plants and sediment of the rhizofilter, Scanning Electron Microscopy (SEM) was performed on sediment collected from specific locations within the rhizofilter.

Electron microscopy on plant and sediment samples was done in the electron microscopy laboratory at the University of KwaZulu-Natal – South Africa.

The plant specimens were collected as described in 2.2.10.1, washed with distilled water and cut into small pieces of approximately 1 cm each. The shredded material was kept in wells flooded in liquid  $\text{N}_2$  to acquire a freezing point of  $-2^{\circ}\text{C}$ . The sample was freeze-dried for 48 hours under vacuum at  $-40^{\circ}\text{C}$ . The samples were removed and left to warm up to room temperature. The treated pieces were carefully lifted using a tiny pair of forceps and adhered on carbon sticky tape which was then mounted on 14 mm diameter aluminium stubs. The samples were gold sputter-coated using Polaron SC 500 Sputter coater for 5 minutes. Consequently 500 g of sediment samples were taken from depths of 10cm from the planted and reference sections using a plastic scoop, placed in polythene containers and sealed in order to minimize oxidation and stored at  $4^{\circ}\text{C}$  for analysis by SEM according to the USEPA, (2003) methods as described by Hammerschmidt and Burton, (2010) and Beh *et al.*, (2012). Sediment samples were examined using SEM in order to identify potential trace element carriers and metal ions mobility and binding phase in the rhizofilter. Samples were dried in the oven at  $60^{\circ}\text{C}$  for 72 h and mixed by gently crushing in a mortar. Each dry sample was adjusted to 1 g and stored in clean clear plastic containers for SEM analysis. A few grains were chosen using

forceps and transferred to sticky tape. The tape with grains was mounted onto aluminium stubs and gold sputter-coated for 5 minutes using a Polaron SC 500 Sputter Coater. The prepared sample was then observed under field emission scanning electron microscope (Zeiss - Ultra Plus FE-SEM). The gold-plating of plant and sediment material was preferred in order to show the required elemental availability and composition of the biomineral components.

### 3.3 DATA ANALYSIS

The data were statistically analysed using Microsoft Excel and GraphPad Prism software, version 5.01. (GraphPad Software Inc., San Diego, CA USA; 2005). The means in each factor and group of factors (metals concentrations and removal rates) were checked by one-way analysis of variance (ANOVA) according to methods by (Lee *et al.*, 2014). The observed variations were accepted and reported as significant at a probability of  $p < 0.05$ .

### 3.4 RESULTS

#### 3.4.1 Metals in Wastewater

Table 3.2 Mean concentration ( $\pm$ SD) of metals in wastewater obtained during the warm season, February to April 2012 (Summer temperatures in Durban range from 23°C- 33°C).

	Inflow	Planted	Reference	DWA 2010, limits
Cadmium (mg/l)	0.026 $\pm$ 0.033	0.026 $\pm$ 0.003*	0.0197 $\pm$ 0.0002	0.005
Chromium (mg/l)	0.036 $\pm$ 0.059	0.038 $\pm$ 0.002***	0.0256 $\pm$ 0.0002	0.11
Copper (mg/l)	0.075 $\pm$ 0.081	0.076 $\pm$ 0.002**	0.0557 $\pm$ 0.0002	0.01
Nickel (mg/l)	0.015 $\pm$ 0.069	0.017 $\pm$ 0.002***	-0.0008 $\pm$ 0.0002	No current limits
Lead (mg/l)	0.483 $\pm$ 0.636	0.510 $\pm$ 0.003*	0.4122 $\pm$ 0.0012	0.01
Zinc (mg/l)	0.449 $\pm$ 0.518	0.377 $\pm$ 0.001**	0.3046 $\pm$ 0.0167	0.1

\*Indicates means statistically Significant (using t-test at 95% confidence level at  $p < 0.05$ ; \*\* Significant at  $p < 0.005$ ; \*\*\* significant at  $p < 0.001$

The concentration of zinc was significantly high during both warm and cold seasons. Zinc was most abundant (Figure 3.1) during winter occurring at 0.97 mg/l in the planted section and 0.5 mg/l in the reference section as seen in figure 3.2.

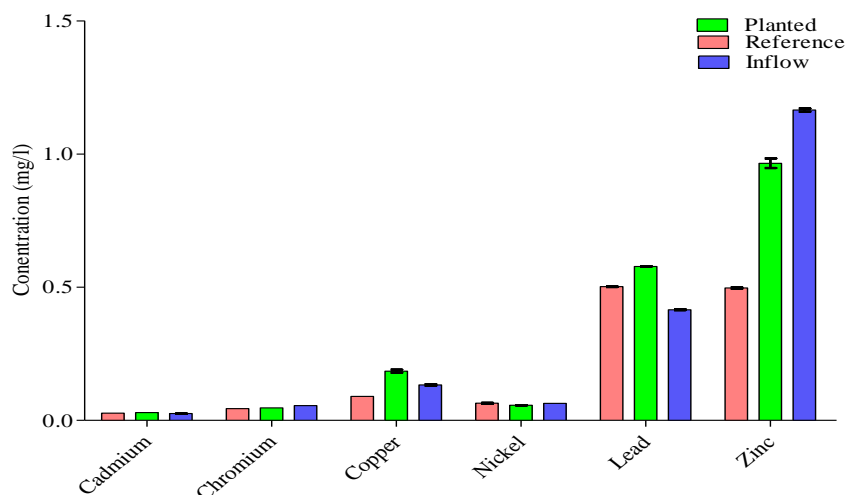


Figure 3.1 Average metals concentration in wastewater at the inflow, planted and reference sections of the rhizofilter covering six months of hot and cold seasons. Whiskers represent standard deviations of 12 means.

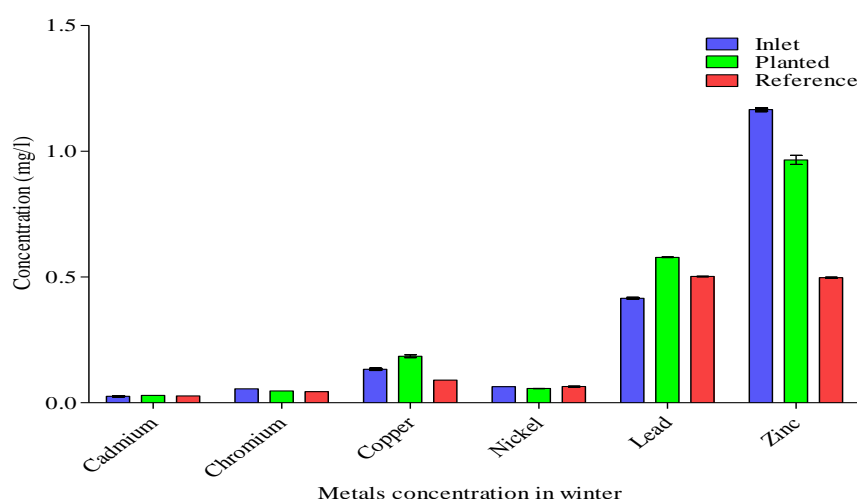


Figure 3.2 Metals concentration in wastewater at the inflow, effluent from planted and reference sections of the rhizofilter during the cold season. Whiskers represent standard deviations of 12 means.

The means were found to vary significantly ( $p = 0.0007$ ). The inlet had a value of 1.17 mg/l which was decreased in the reference section than the planted section of the rhizofiltration system. The lead was found to be the most abundant metal in the wastewater during the summer period (Figure 3.3) occurring at 0.5 mg/l in the planted section and 0.4 mg/l in the reference section. The occurrence of higher levels of zinc in the planted section (0.97 mg/l) against 0.5 mg/l in the reference

section could be attributed to the existing zinc ions as micronutrients in plant tissues (Lee *et al.*, 2014).

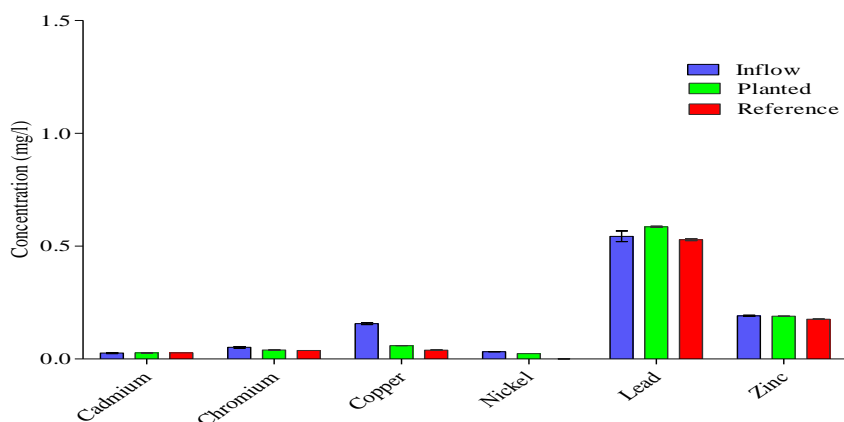


Figure 3.3 Metals concentration in wastewater at the inflow, effluent from planted and reference sections during warm (November- February 2012) season. Whiskers represent standard deviations of three means.

#### 3.4.2 Metals in sediment

The rhizofiltration system was designed to remove heavy metals from wastewater through plants and sediment by uptake and adsorption. But some metals such as zinc form part of the essential nutrients of plants, the concentration from the inlet may have raised the zinc levels within the rhizofilter as shown in Figure 3.4.

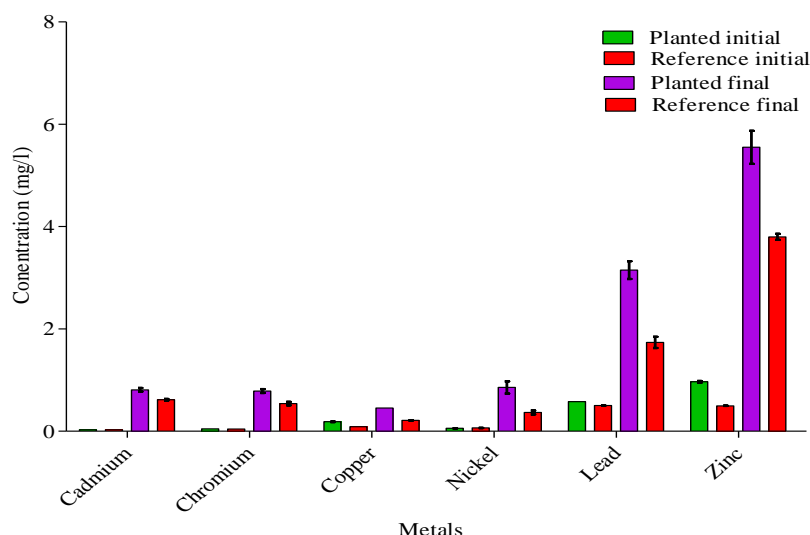


Figure 3.4 Concentration of metals in sediment from planted and reference sections during the first three months of study. Whiskers represent standard deviations of three means.

The concentration of Zinc increased from an initial average of 0.95 mg/l to 5.5 mg/l in the planted section and from an initial average concentration of 0.49 mg/l to 3.8 mg/l in the reference section. Lead, nickel, copper, chromium and cadmium all had increased from the initial concentrations to the ranges of 3.2 mg/l, 0.9 mg/l, 0.5 mg/l, 0.8 mg/l and 0.8 mg/l in the planted section respectively. Consequently, the reference section also had increases of 1.7 mg/l, 0.4 mg/l, 0.2 mg/l, 0.5 mg/l and 0.6 mg/l for lead, nickel, copper, chromium and cadmium respectively.

During the study, cadmium was found to have been deposited from the wastewater to the various components of the rhizofilter at the rate of 96.69% on the planted section and 48.98% on reference section. These percentages represented an average increase of 83.7% in the planted and 43.4% in the reference sections respectively. Chromium deposits were increased by 81% on the planted section and 24% in the reference section, while Cu increased by 23.4% in the planted and 1.1% in the reference section. Nickel deposits increased by 72% in the planted section and 46.5% in the reference section while Pb increased by 63% in the planted section and 31% in the reference section of the rhizofilter. Zinc was deposited at 76% in the planted section and decreased by 84% in the reference section. The metals

deposited in the planted section through the influent, followed the pattern of Cd>Cr>Zn>Ni>Pb>Cu while in the reference section, the order was Ni>Cd>Pb>Cr>Cu>Zn. These results concur with those of Marchand *et al.*, (2010) and Ali *et al.*, (2013). The overall result shows a higher metals deposit on the planted section than the reference section.

### 3.4.3 Metals in Plant Structures

Both *P. australis* and *K. nemoralis* had significant concentrations ( $p=0.01$ ) of Zn and Pb initially (Figure 3.5 and 3.6) but *K. nemoralis* was found to have eventually accumulated more of the metals than *P. australis*. In the leaf of *K. nemoralis*, Pb was found to be accumulated at  $0.22 \pm 0.01$  mg/kg while in the root it was  $0.22 \pm 0.06$  mg/kg. The stem had the least amount of accumulation of Pb at  $0.1 \pm 0.11$  mg/kg. This was followed by Cu at  $0.12 \pm 0.001$  mg/kg,  $0.12 \pm 0.05$  mg/kg and  $0.095 \pm 0.02$  mg/kg for leaf, root and stem respectively. This indicated that there was more Cu accumulated in the root than plant parts above the ground.

Chromium accumulated more in the roots than the above-ground tissues. Nickel was accumulated in the leaf at a higher concentration than the root of *K. nemoralis*. Zinc accumulated more on the above ground than the root tissues, while Cd was mainly accumulated in the roots and the stem region. There was no accumulation or deposition of Cd in the leaves. The availability of metals in the tissues of *K. nemoralis* followed the decreasing order of Pb> Cu >Ni> Zn> Cr> Cd in comparison with the findings of Karak and Bhattacharyya, (2010) and a report by Marchand *et al.*, (2010).

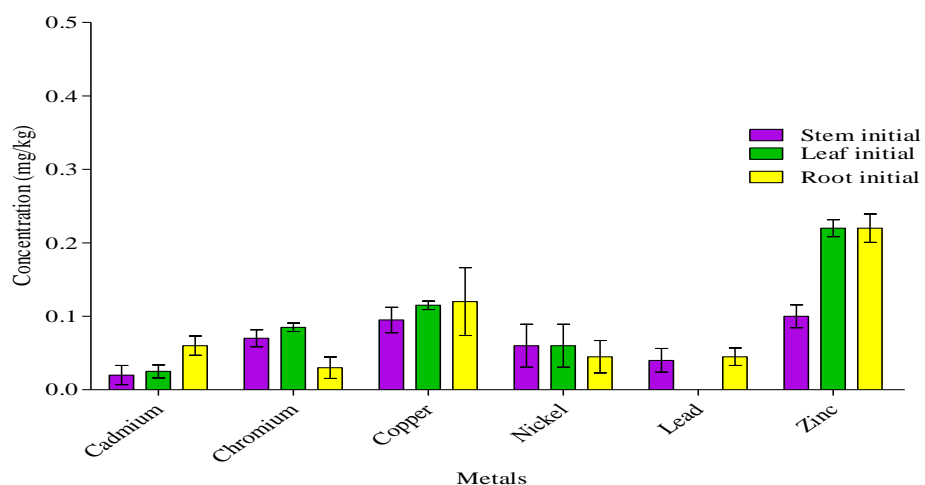


Figure 3.5 Initial concentration of metals in the leaf, stem and root tissues of *K. nemoralis* before the experiment. Whiskers represent standard deviations of 12 means.

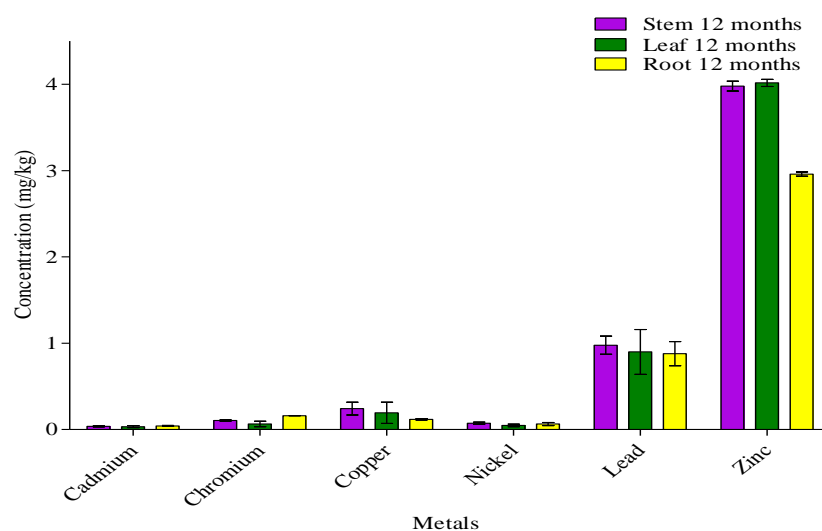


Figure 3.6 Concentration of metals in the leaf, stem and root tissues of *K. nemoralis* after 12 months of study. Whiskers represent standard deviations of 12 means.

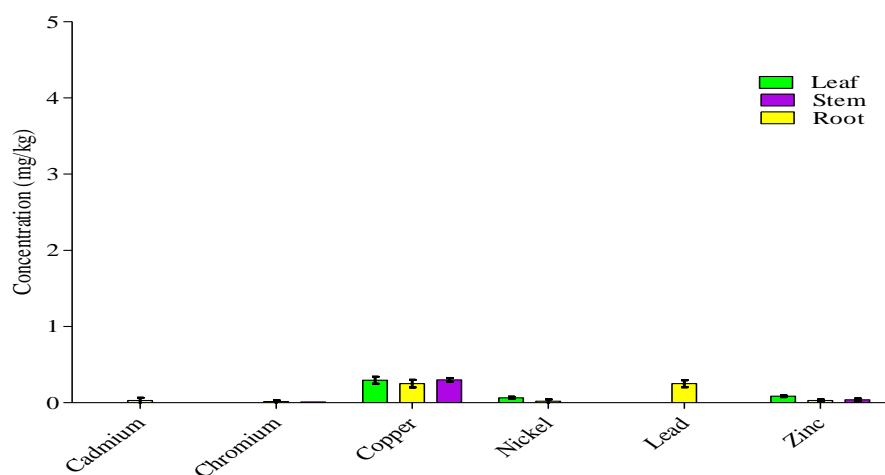


Figure 3.7 Concentration (initial) of metals in the leaf, stem and root tissues of *P. australis* before the experiments. Whiskers represent standard deviations of 12 means.

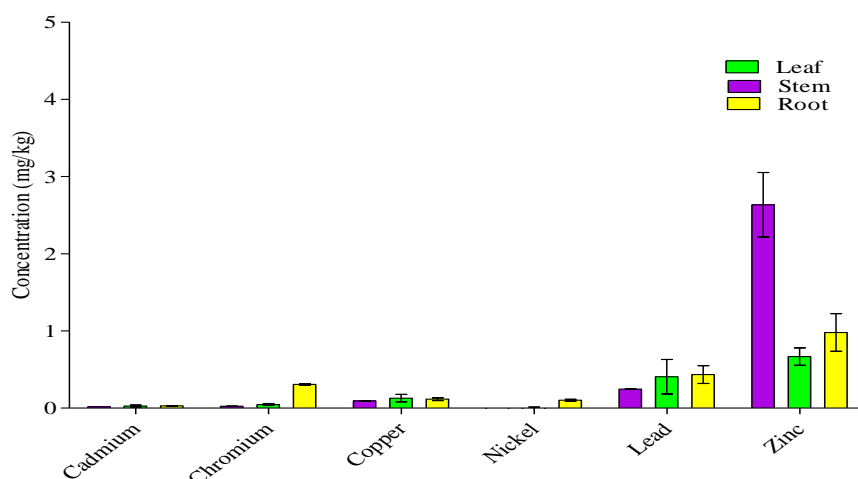


Figure 3.8 Average concentration of metals in leaf, stem and root tissues of *P. australis* after a period of 12 months of study. Whiskers represent standard deviations of 12 means.

The leaf of *P. australis* had a significant amount of Zn (Figure 3.7). Lead and Cu accumulated on the roots of *P. australis*. Nickel and Zn accumulated on the leaves and Cd and Cr were least available on the tissues of *P. australis* (Figure 3.8.) Statistical comparison with t-test revealed that the metals relocation followed the decreasing order of Cu, >Pb, >Zn, >Ni, > Cd >Cr.



### 3.4.4 Scanning electron microscopy (SEM) and Energy-dispersive X-ray (EDX) Analysis

The SEM micrographs display the metals attachment regions within the plant tissues and on sediment (Figure 3.9). Figure 3.9 C is the stem of *K. nemoralis* before exposure to metal ions and figures 3.14 shows the layered image of the stem with metal deposits exposing the highest Cu peaks at 0.45cps/eV (Figure 3.10C) on the EDX spectrum. These observations were found to be in agreement with reports from a study by Zhang *et al.*, (2011). Similarly, Figure 3.9F shows the stomatal openings of the stem of *P. australis* before metals exposure and the corresponding layered image (Figure 3.23) showing metals deposition and wide distribution on sections of stomatal openings. The corresponding EDX spectrum also indicated Cu peaks at 0.3 cps/eV (Figure 3.10F).

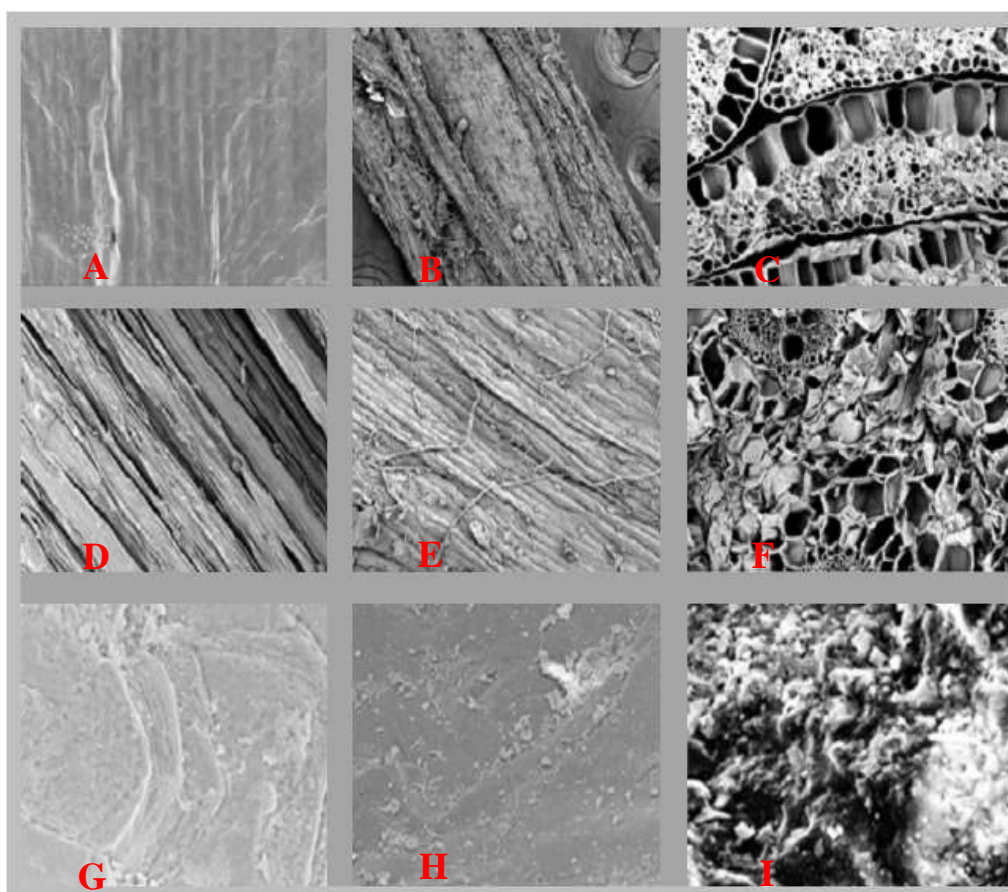


Figure 3.9 Scanning electron, micrographs of *K. nemoralis* A, B, C (leaf, root, stem), *P. australis* D, E, F (leaf, root, stem), and sediment G, H, I (Reference N,

Reference, Planted). Letters in figure 3.10 show the location of the corresponding EDX analysis.

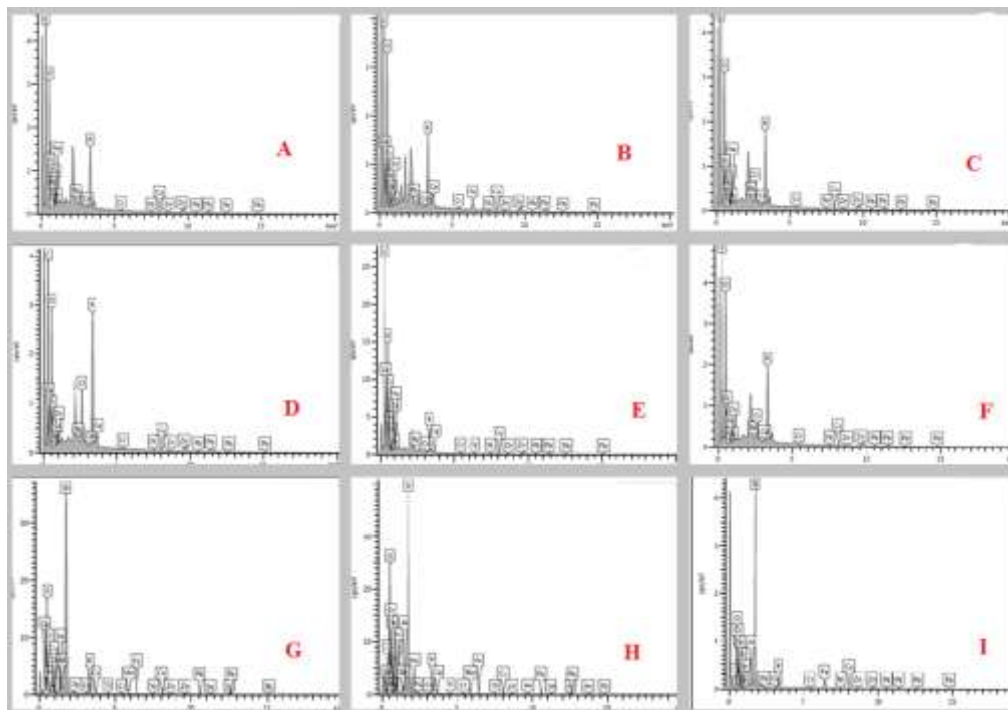


Figure 3.10. The electron probe X-ray microanalysis spectrum of *K. nemoralis* leaf, root and stem corresponding to Fig. 3.9 (A–C); *P. australis* leaf, root and stem corresponding to (D–F) and sediment spectra reference N, reference; and planted section (G–I).

EDX was to determine the composition and availability of the elements before and after adsorption by the metal ions. According to the methodology applied, the specimen was coated with gold to reduce charging. Figure 3.11 shows the image of the normal leaf of *K. nemoralis* showing well differentiated epidermal cells and the layered image of the same structure showing metal deposits (Figure 3.12). The lead was found to be most abundant at 0.74wt% on the scanned section with EDX peak at 1.6cps/eV (Figure 3.10).

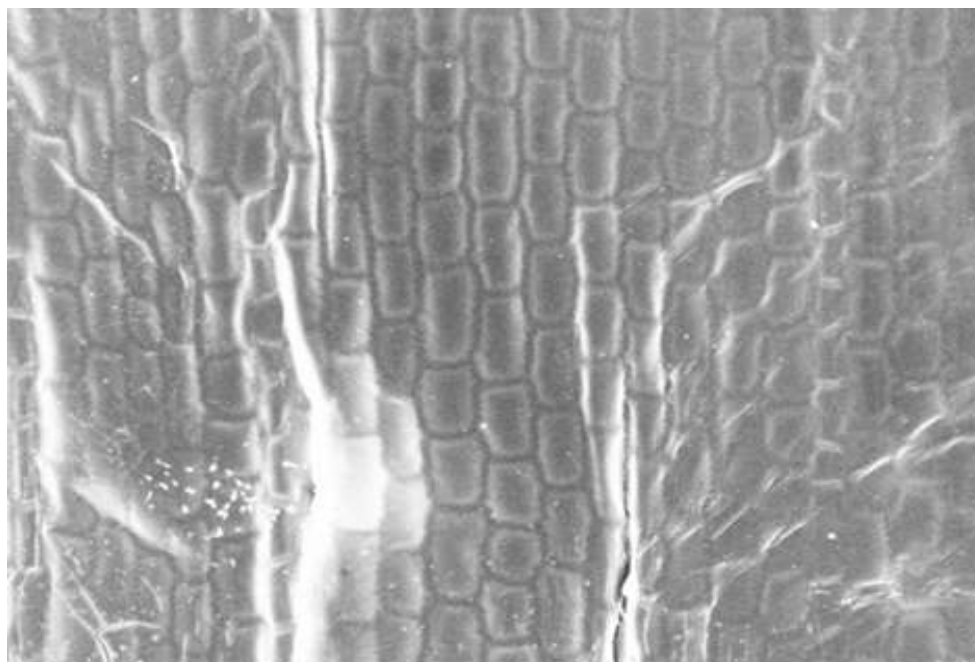


Figure 3.11 Scanning electron micrograph of the normal leaf of *K. nemoralis* showing internal cellular structure (142 x mag.).

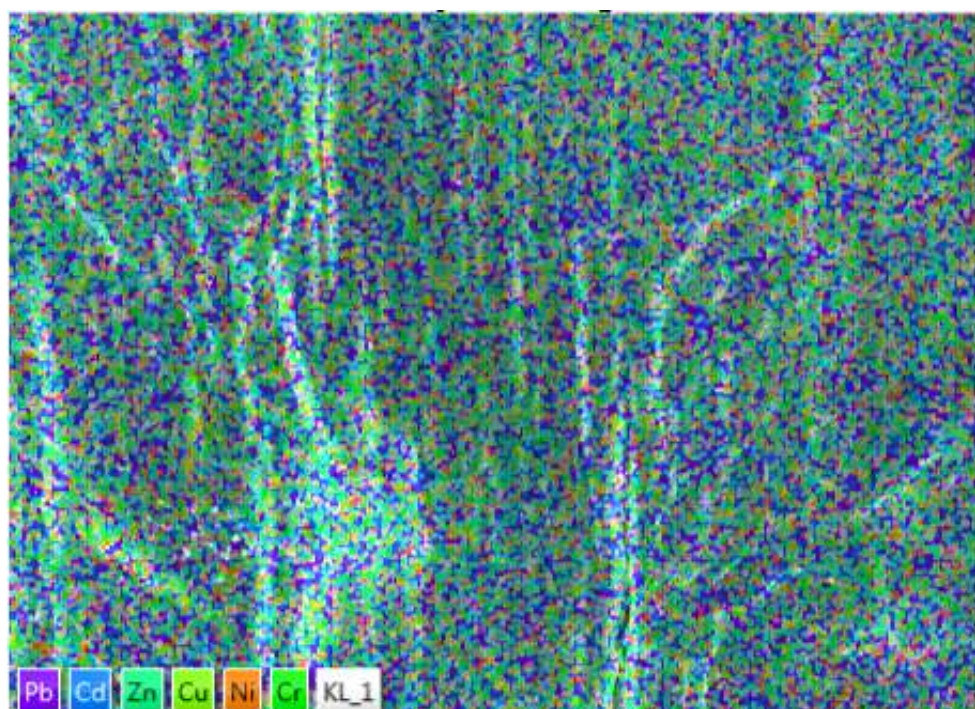


Figure 3.12 Layered image of *K. nemoralis* leaf showing metal deposits on the cellular structures. (142 x magnification).



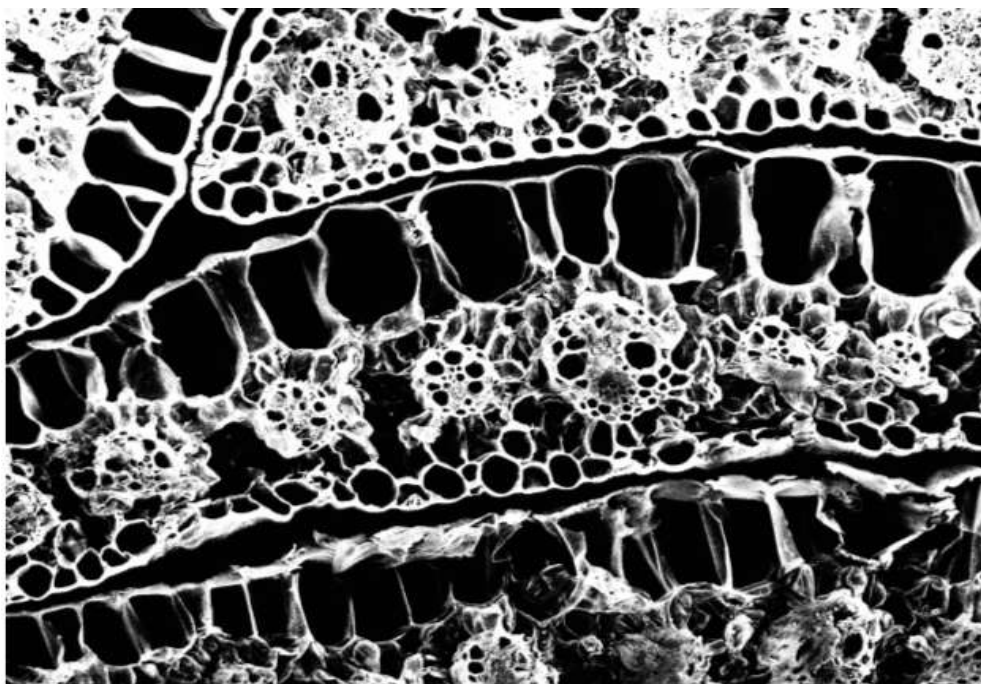


Figure 3.13 Scanning electron micrograph of normal *K. nemoralis* stem showing parenchyma cells (100 x magnification).

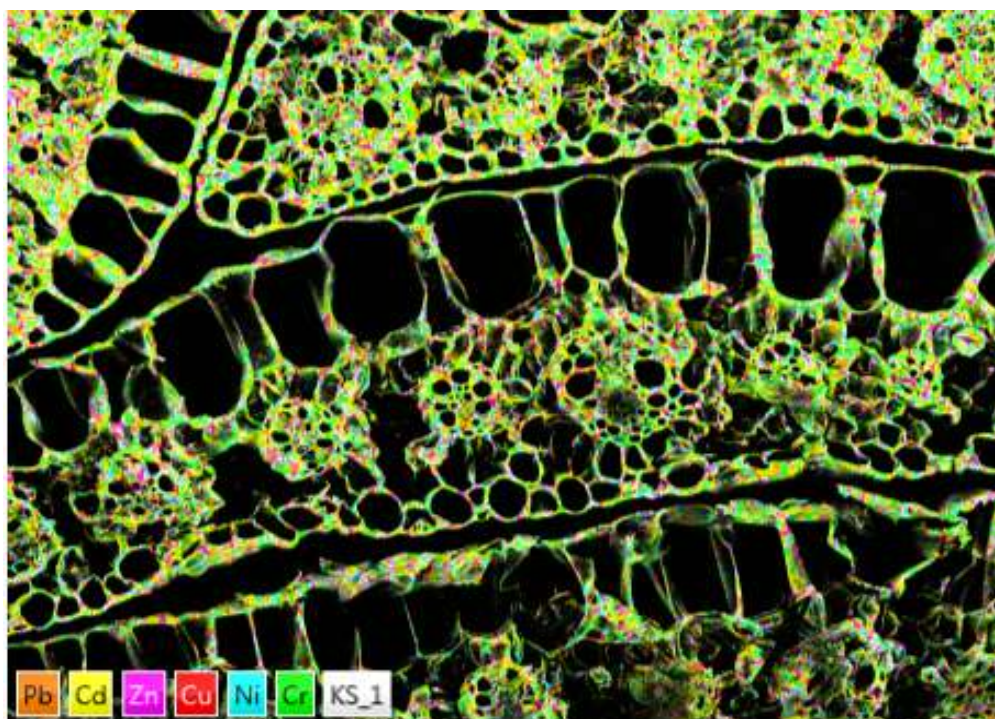


Figure 3.14 EDS Layered image of *K. nemoralis* stem showing metal deposits in the circular waxes. (100 x magnification).



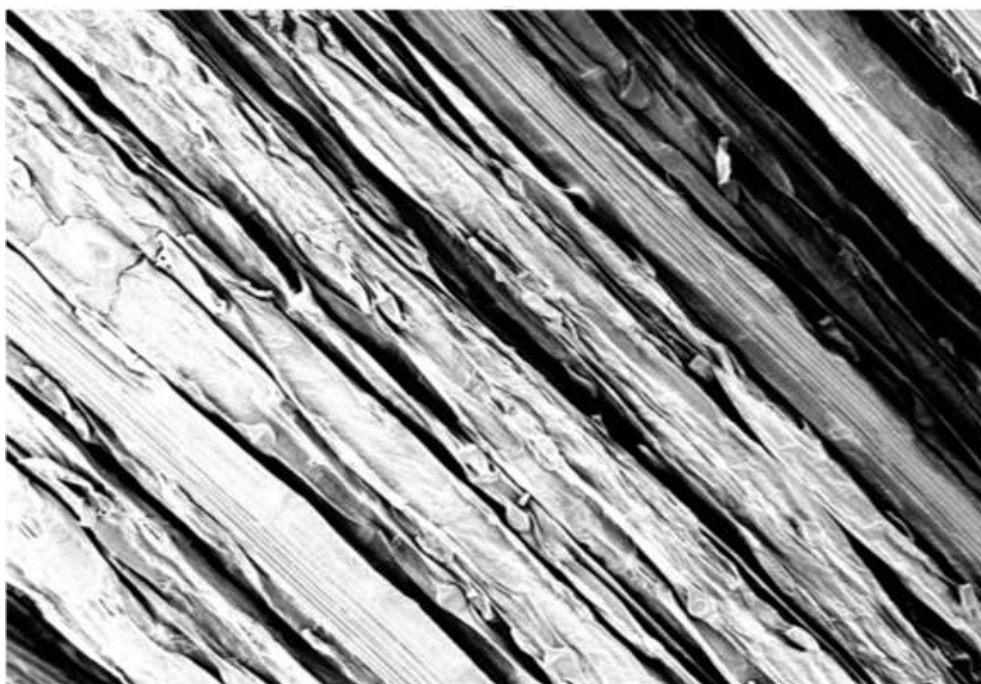


Figure 3.15 Scanning electron micrograph of normal leaf of *P. australis* without metal deposits. (142 x magnification).

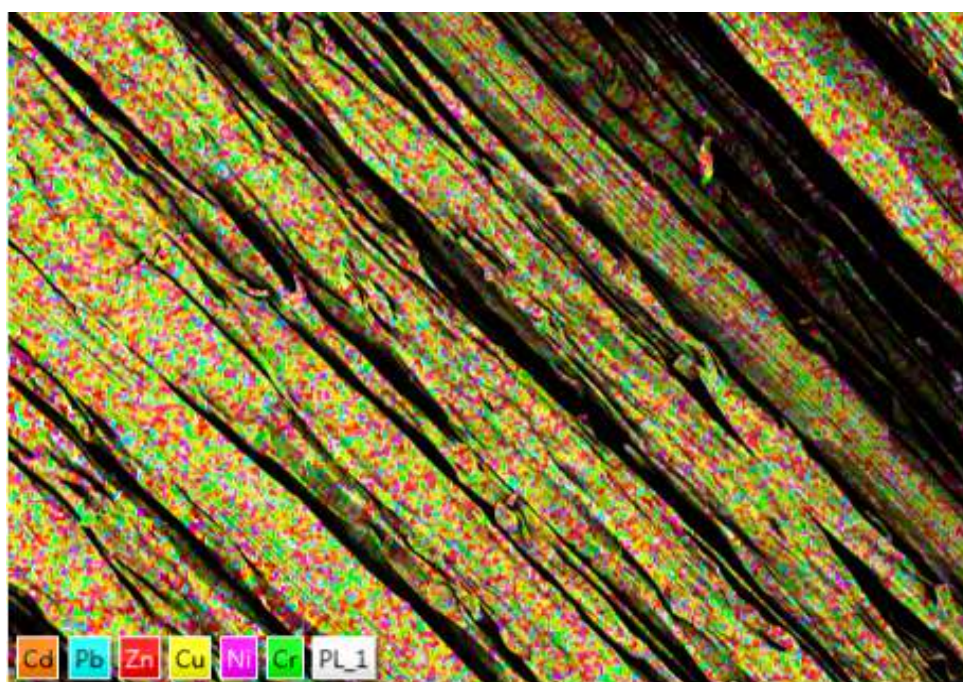


Figure 3.16 EDS layered image of *P. australis* leaf showing metal deposits denoted by the various colours. (142 x magnification).

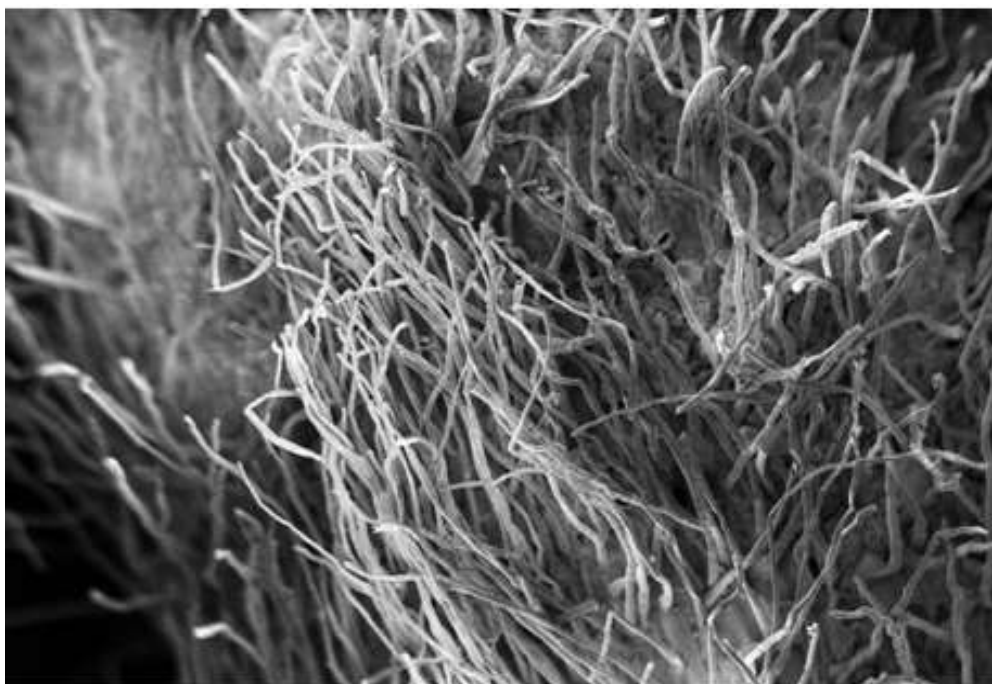


Figure 3.17 Normal root morphology of *K. nemoralis* showing the fibrous network that is significant in metal ions uptake. (96 x magnification).

Metal deposits were clearly seen on the EDS scanned images of the roots of *K. nemoralis* (Figure 3.18) and *P. australis*. *Kyllinga nemoralis* (Figure 3.20) had more Cu deposits on the roots at 0.31wt% with peak at 0.6cps/eV while *P. australis* (Figure 3.20) had 0.31wt% with EDX peak at 0.6cps/eV.

Figure 3.17 shows the fibrous root structure of *K. nemoralis*. The root structure supports the development of a network that encourages the formation of biofilm for attachment of microorganisms. The microorganisms play a key role in the removal of organic matter and heavy metals according to D'Urešová *et al.*, (2014). Differentiation of metal deposits between the original plant tissue and tissue after uptake was observed by the colour intensity of the layered images and height of the peaks observed on EDX analysis.



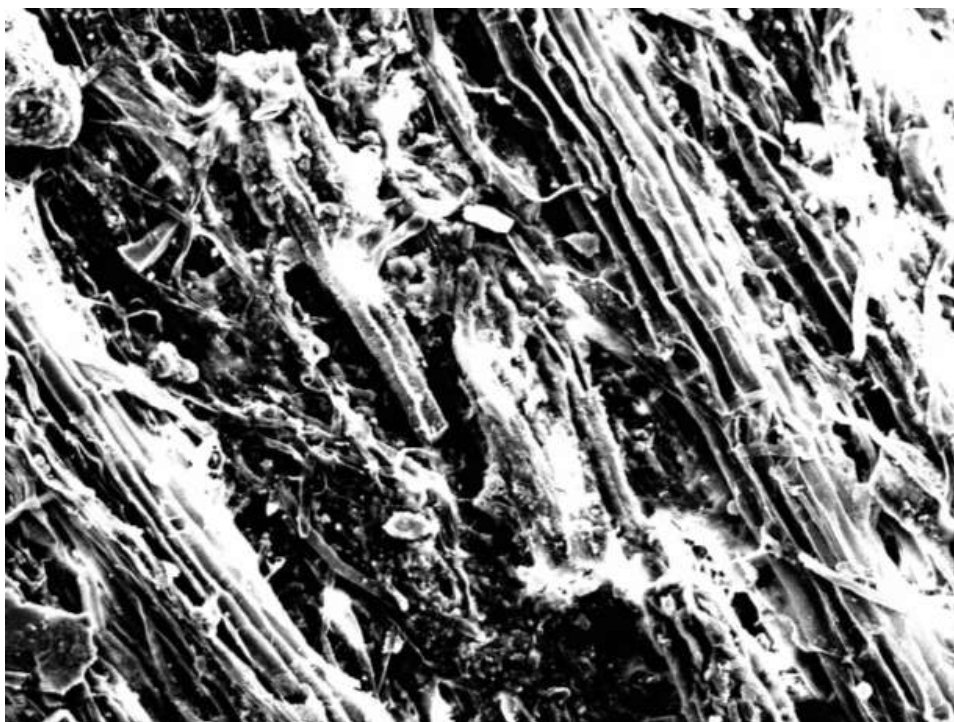


Figure 3.18. Scanning electron micrograph showing normal root of *K. nemoralis* without metals deposits. (100 x magnification).

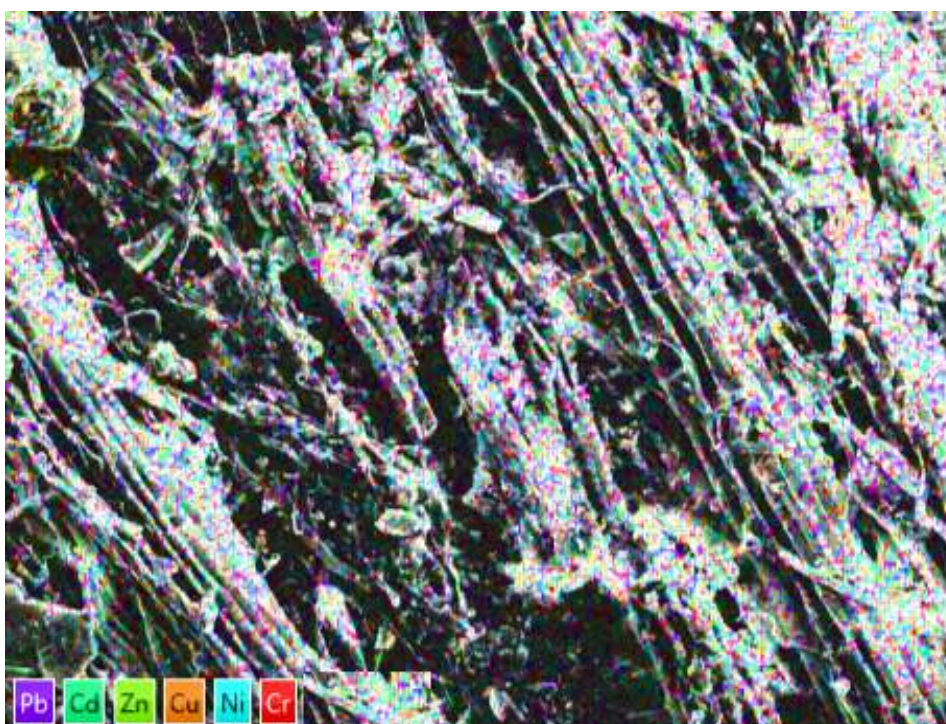


Figure 3.19 EDS layered image of metals deposition on the root of *Kyllinga nemoralis*. denoted by the various colours. (100 x magnification).



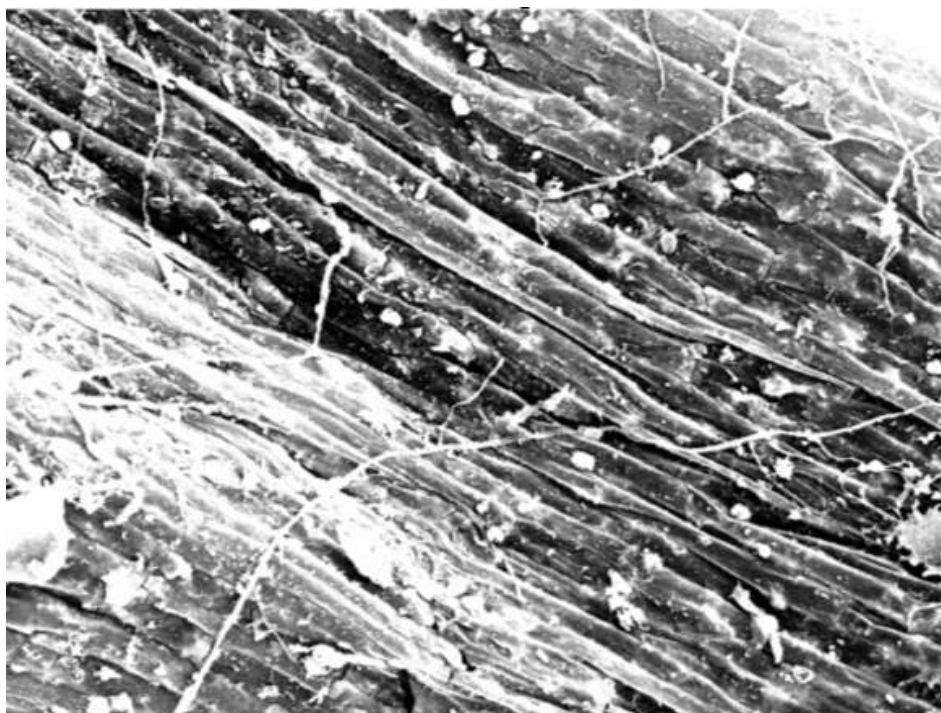


Figure 3.20 Scanning electron micrograph of normal of the root of *P. australis* showing metal deposits. (100 x magnification).

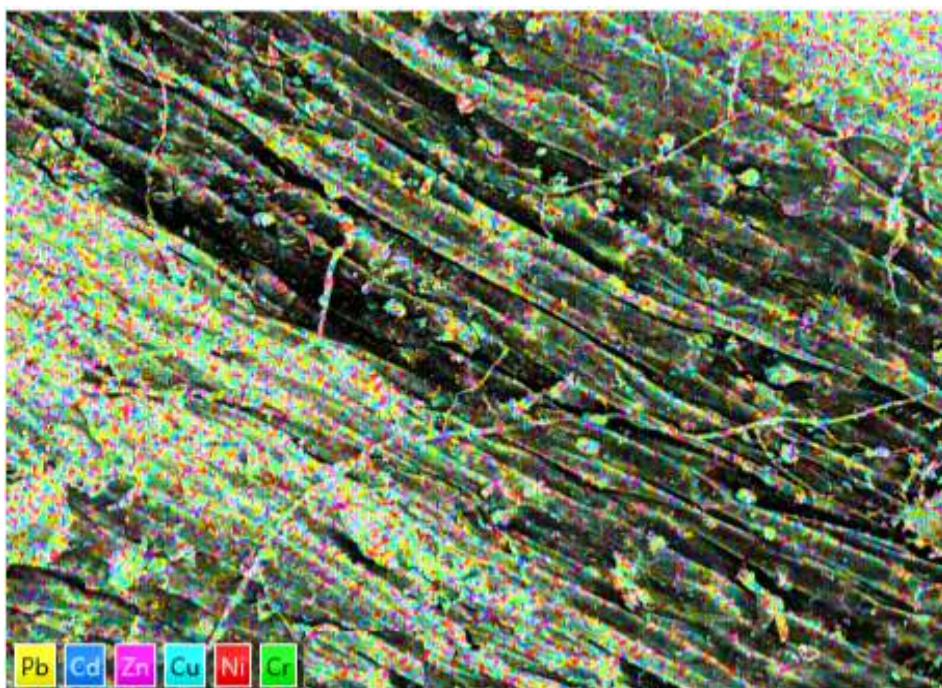


Figure 3.21 EDS layered image of the root of *P. australis* showing the deposits of metals denoted by the various colours. (100 x magnification).



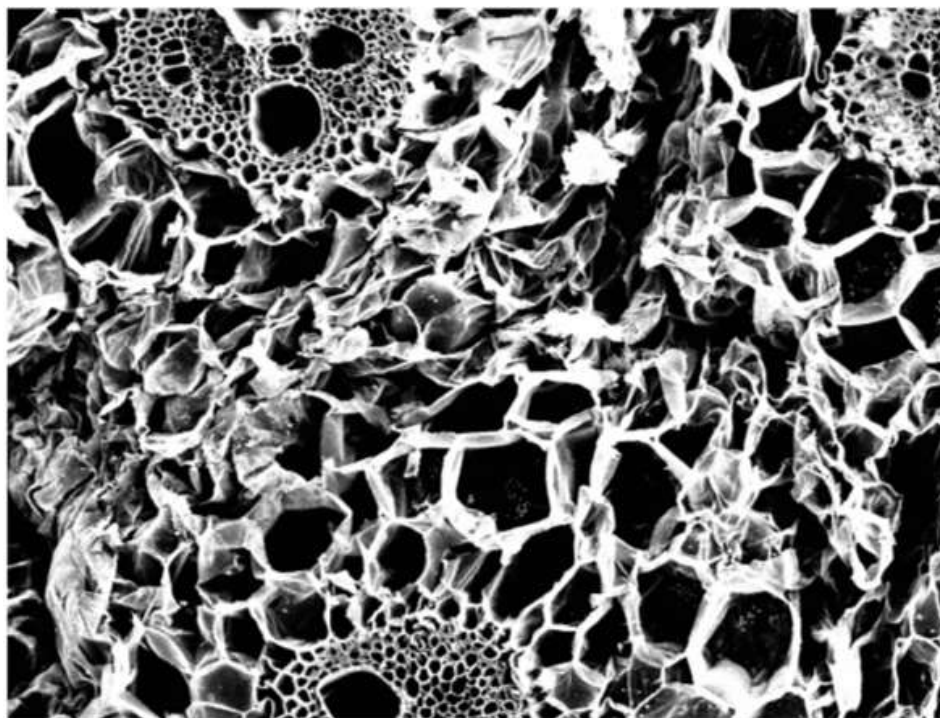


Figure 3.22 Scanning electron micrograph of normal stem of *P. australis* without metal deposits. (142 x magnification).

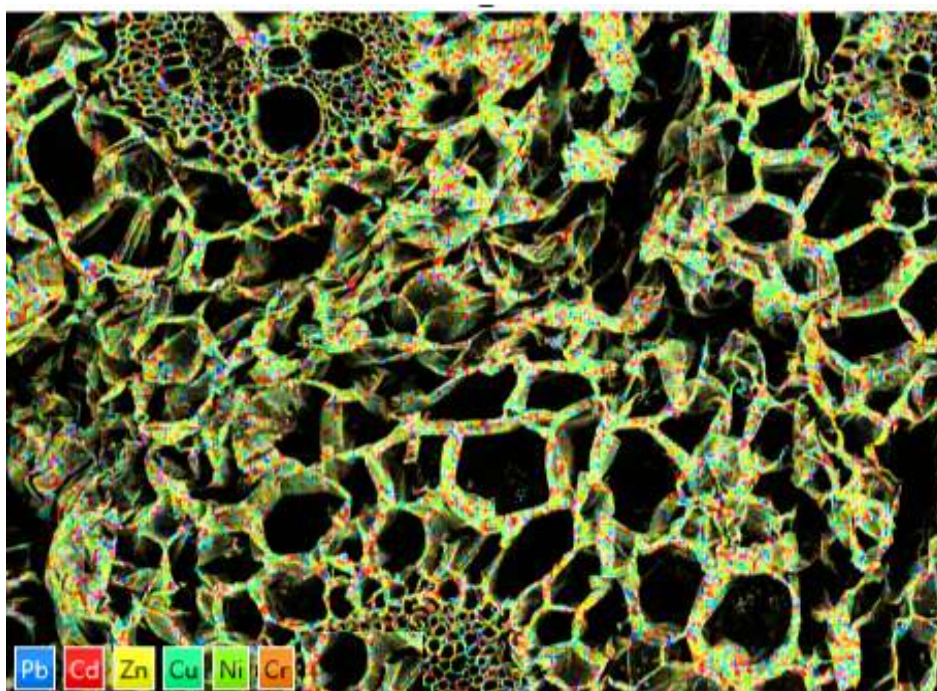


Figure 3.23 EDS layered image of the stem of *P. australis* showing metal deposits on the circular waxes denoted by the various colours. (142 x magnification).

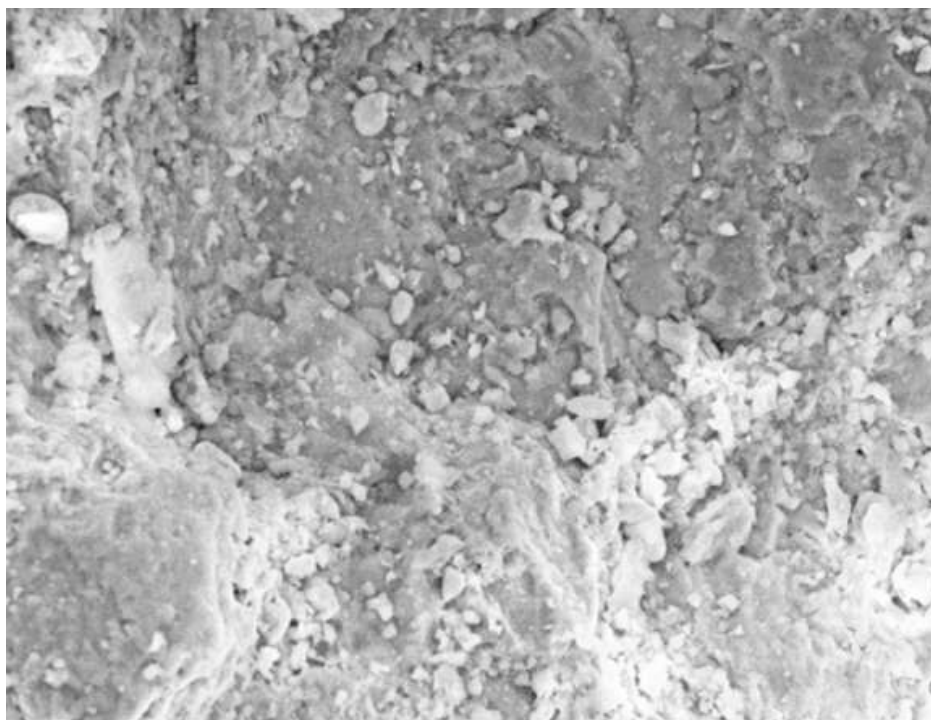


Figure 3.24 Scanning electron micrograph of the normal structure of sediment in the reference section of rhizofilter without metal deposits. (100 x magnification).

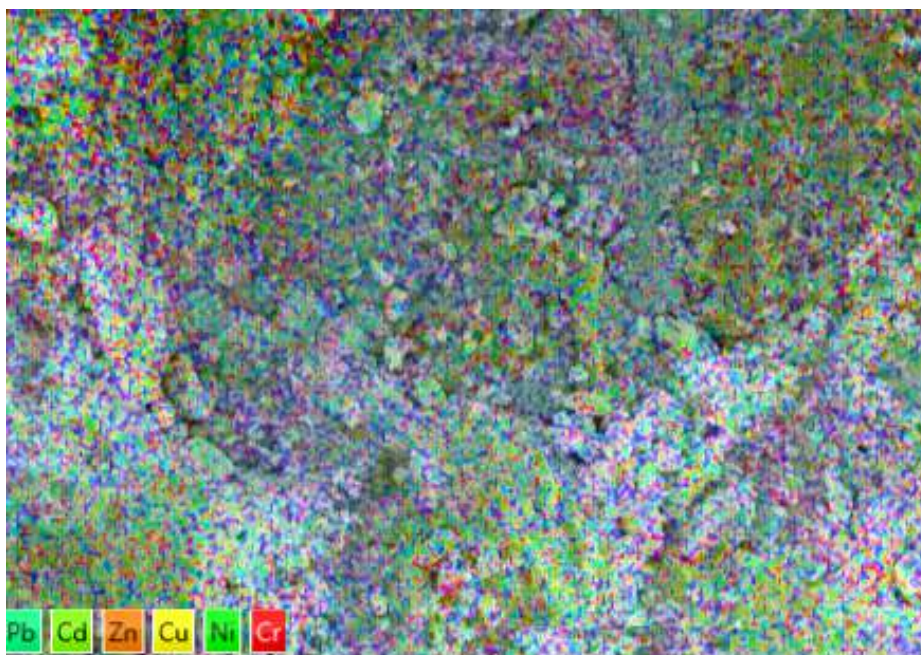


Figure 3.25 EDS layered image of sediment showing metal deposits as denoted by the various colours. (100 x magnification).

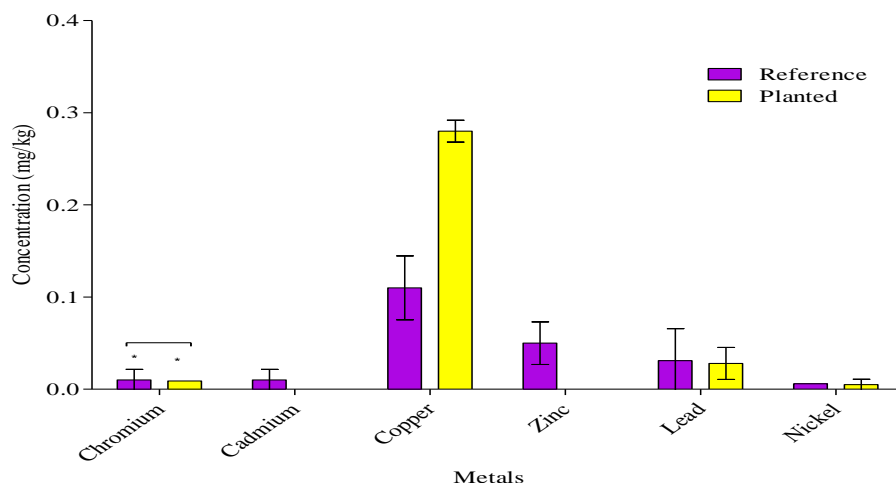


Figure 3.26 Metals accumulation on sediment from planted and reference sections of the rhizofilter as seen on SEM. Whiskers represent standard deviations of 12 means.

Comparatively large amounts of Cu were accumulated on the sediment (Figure 3.26) of the planted section which recorded  $0.28 \pm 0.22$  mg/kg against  $0.11 \pm 0.03$  mg/kg in the reference section. The variances of means between planted and reference were significant ( $p=0.0417$ ) F- test. This is also seen by the abundant yellow colouration on the layered image of sediment in the reference section of the rhizofilter (Figure 3.24). Figure 3.23 shows the binding sites for metals on the sediment when viewed under the electron microscope. Chromium ions (Cr) and Cd were negligible in the planted section but were each available at  $0.01\text{mg/kg} \pm 0.01$  in the reference section (Figure 3.25). Zinc ions (Zn) were negligible in the planted section but were available at  $0.05 \pm 0.02$  mg/kg at reference, while Pb and Ni recorded  $0.025 \pm 0.01$ ,  $0.03 \pm 0.03$  and  $0.005 \pm 0.001\text{g/kg}$  against  $0.0 \pm 0.0\text{mg/kg}$  in the planted and reference sections respectively.



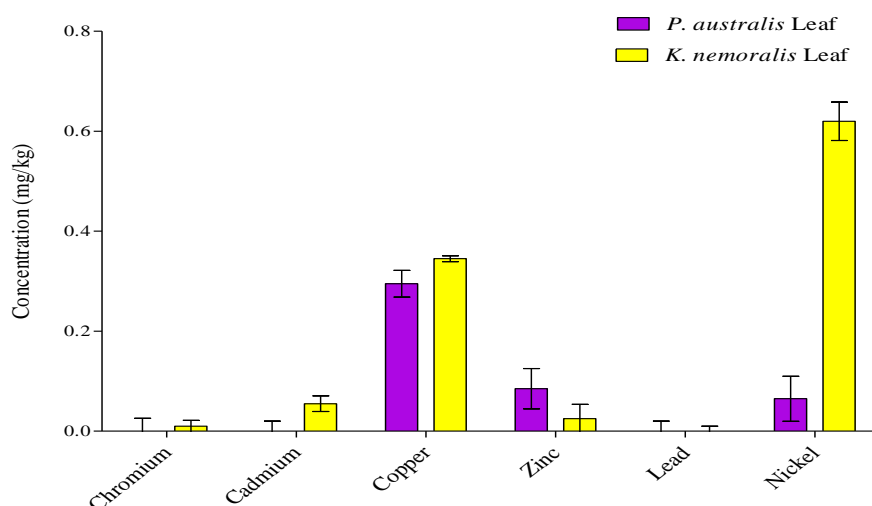


Figure 3.27 Variations in metals concentration on the leaf of *P. australis* and *K. nemoralis* as seen on Electron microscopy. Whiskers represent standard deviations of 12 means.

*Kyllinga nemoralis* accumulated more Cr, Cd, Cu, Zn and Ni (at 0.01 mg/kg  $\pm$  0.012, 0.345 mg/kg  $\pm$ 0.01, 0.025 mg/kg  $\pm$ 0.03 and 0.629 mg/kg  $\pm$ 0.139 respectively) in the leaf than the *Phragmites australis*, while Pb was accumulated an equal amount by the leaf of both plants (Figure 3.26). *Phragmites australis* leaf only accumulated more Zn (at 0.085 mg/kg  $\pm$ 0.04 against *K. nemoralis* at 0.025 mg/kg  $\pm$ 0.03) than *K. nemoralis* leaf. F test to compare mean variances between the leaves of both plants was not significant ( $p= 0.1054$ ). However, the accumulation (%) of metals in the leaf was, therefore, higher in *K. nemoralis* than in *P. australis*.

There was some relationship (Figure 3.27) between the accumulation of metals in the stems of both plants as the variations were proved to be insignificant ( $P<0.05$ ) when subjected to the t-test. Chromium (Cr) was higher in the stem of *P. australis* (0.01) as compared to 0.005 mg/kg on the *K. nemoralis* stem. Cadmium was 0.02 mg/kg on the *K. nemoralis* stem and nil on the *P. australis*. Copper ions (Cu), Zn, Pb, and Ni were 0.305 mg/kg and 0.30 mg/kg, 0.020 mg/kg and 0.04 mg/kg, 0.005 mg/kg and nil, 0.035 mg/kg and nil for *K. nemoralis* and *P. australis* respectively.

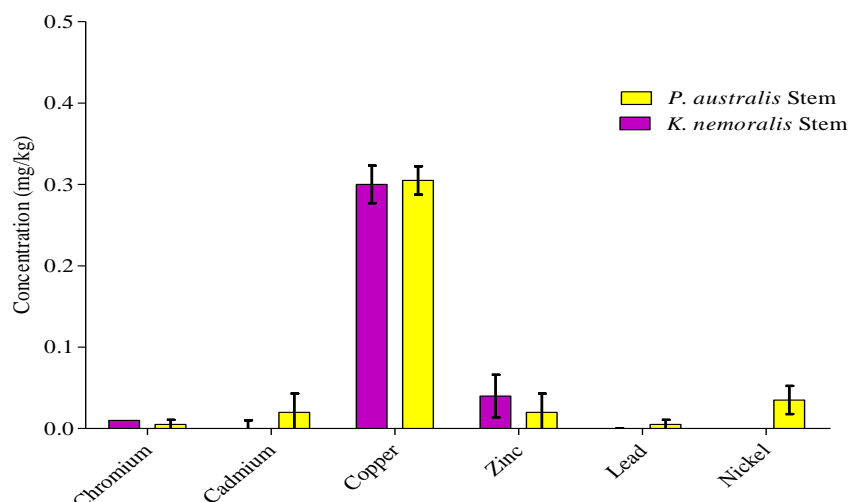


Figure 3.28 Variations in metals concentration in stem of *K. nemoralis* and *P. australis* as seen on Electron microscopy. Whiskers represent standard deviations of 12 means.

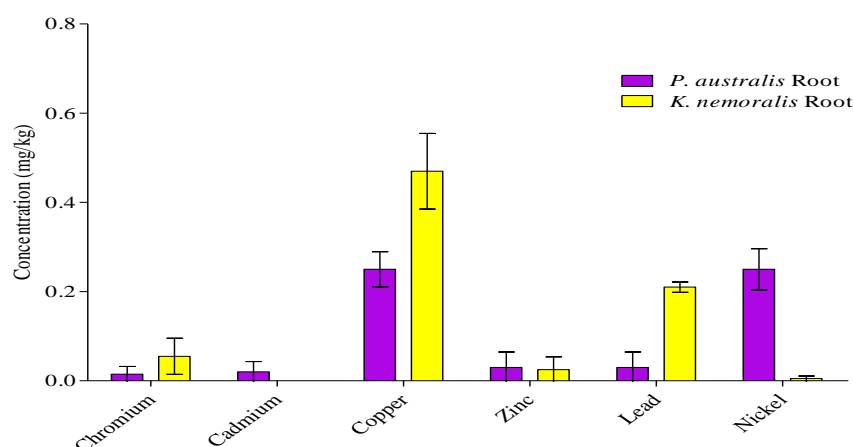


Figure 3.29 Metals concentration on roots of *P. australis* and *K. nemoralis* as seen on Electron microscopy. Whiskers represent standard deviations of 12 means.

Higher levels of Cu were found in the roots of *K. nemoralis* ( $0.470 \pm 0.18$  mg/kg against  $0.25 \pm 0.69$  mg/kg in *P. australis*) according to analysis by the electron microscopy (Figures 3.28 and 3.29). The calculated means between the two plants were not significantly varied ( $p = 0.9245$ ) t-test. Cadmium was in negligible amounts in *K. nemoralis* while *P. australis* recorded a root accumulation of  $0.02 \pm 0.02$  mg/kg of cadmium. Also noted were greater amounts of Pb ( $0.210$

mg/kg) and Cr (0.055 mg/kg) deposits on the root of *K. nemoralis* against root of *P. australis* at 0.030 mg/kg and 0.015 mg/kg respectively (Figure 3.30). The calculated means were not significantly varied ( $p=0.7917$ ).

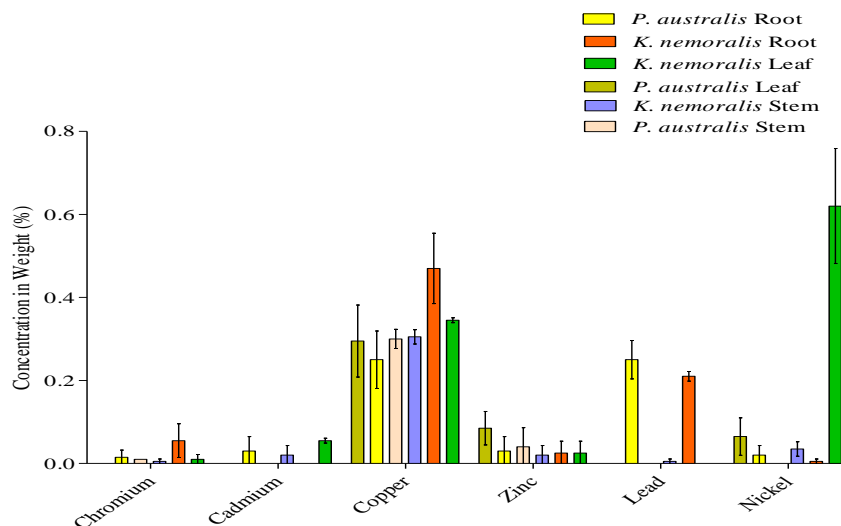


Figure 3.30 Average concentration of metals in leaf, stem and root of the macrophytes as seen by electron microscopy. Whiskers represent standard deviations of 12 means.

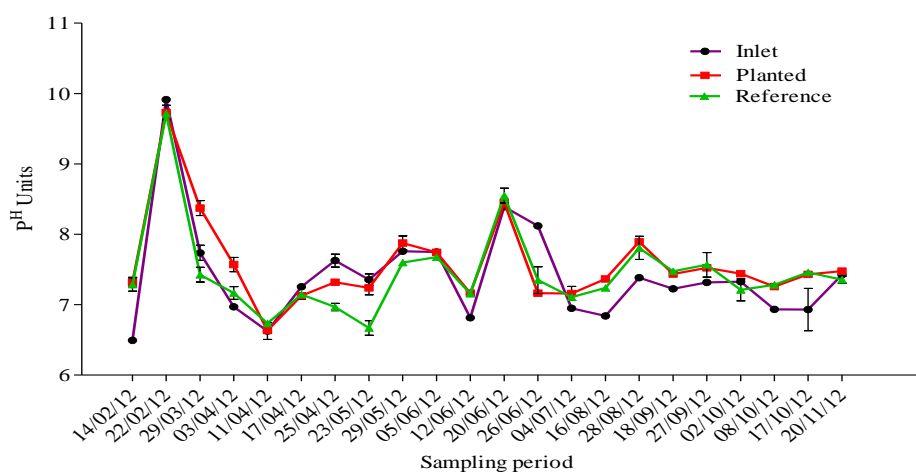


Figure 3.31 pH variations at the inlet, planted and reference sections covering hot and cold seasons in 2012. Whiskers represent standard deviations of 12 means.

Comparatively large amounts of Ni and Cu (0.345 mg/kg and 0.620 mg/kg respectively) were found in the leaf and root of *K. nemoralis* against 0.295 mg/kg and 0.065 mg/kg in the leaf of *P. australis* (Figure 3.30). In the stem of *K. nemoralis*, Cr was 0.005%, Nickel was 0.035%, Cu 0.305%, Zn 0.02%, Cd 0.02% and Pb 0.005%. In the root Cr was 0.055%, Nickel 0.005%, Cu 0.47%, Zn 0.025% and Pb 0.21%. In the leaf Cr was 0.01%, Ni 0.055%, Cu 0.345%, Zn 0.025%, Cd 0% and Pb 0.62%.

Concentration (%) of metals in the sediment in the reference section varied with Cr recording about 0.01%, Ni was 0%, Cu 0.11%, Zn 0.05%, Cd 0.01% and Pb 0.03%. Accordingly, the concentration of metals in sediment of the planted section recorded Cr at 0%, Ni was 0.005%, Cu 0.28%, Zn 0%, Cd 0% and Pb 0.025%. The remaining percentage was distributed in varying amounts between aluminium, /potassium, calcium, silicon, tin, manganese and selenium.

Comparatively higher pH values were recorded during the months of February and March (Figure 3.31). This was during part of the warmer months that also witnessed higher removal efficiencies of metals (Zinc and Lead) as seen in figure 3.3. The means were however not significantly varied ( $p = 0.7025$ ) according to Tukey's multiple comparison tests. The high metals removal efficiency of the rhizofilter could have been as a result of low metal ions concentration in the influent, low hydraulic loading of the influent (2000 litres per day), the minimum retention period of 6 hours and channelling of the inflow which also contributed to the low retention period.

### 3.5 DISCUSSION

Plants have the potential to naturally accumulate various metal ions in their tissues for their growth requirements (Figure 3.5). Aquatic plants have been used for phytoremediation of wastewater and have demonstrated their potential as effective treatment mechanisms in detoxifying wastewater from municipalities and industries worldwide according to studies by Adhikari *et al.*, (2011) and Pedescoll *et al.*, (2011). The macrophytes, *Kyllinga nemoralis* and *Phragmites australis*

selectively accumulated metals in their leaves, roots and stem (Figure 3.29). This could have resulted from the various functional and environmental processes within the rhizosphere. The ability to accumulate metal ions in tissues of the experimental plants could have been dependent on the bioavailability and distribution of the metal ions within the plants, plant species of choice (*K. nemoralis* and *P. australis*), cation exchange capacity, root exudates, temperature variations during the study period, and levels of dissolved oxygen. For example uptake of Cr by plants was found to be concentrated in the roots and was dependent on its oxidation state and availability of binding sites (Figure 3.19 and 3.23) for colloids. In this study, the metals uptake by plants may have been favoured by the age of plants at the initial stage of the study and the application of young rhizomes which required a substantial amount of growth nutrients in concurrence with findings of Schreck *et al.*, (2012). A wetland plant, *K. nemoralis* (which has not been investigated as a potential biosorbent and in wastewater treatment) was successfully used in this study and proved its potential in bioremediation of wastewater (Figure 3.6).

The functionality and efficiency of the rhizofiltration system were realised when the pH of effluent was stabilized to near neutral in all seasons during the study from the initial acidic conditions (Figure 3.31). Appropriate pH levels play major functional roles in phytoremediation according to Fibbi *et al.*, (2012). The temperature was stabilized by the plant roots to enhance growth in the planted section while in the reference section, the effluent temperature was slightly raised due to the exposure of the substrate to direct ultraviolet light. A slight positive correlation (Spearman  $r$ ) was observed between temperature and metals removal as more Zn was removed in the cold season (Figure 3.2) while the hot season had a higher removal rate for Pb, but no clear seasonal removal trends could be concluded without further research.



### 3.5.1 Determination of Metals in Plant Tissues and Sediment (SEM)

Heavy metals uptake, transfer pathways, and distribution within macrophytes (mainly vascular plants) has not been exhaustively investigated (Schreck *et al.*, 2012). Heavy metals absorbed and adsorbed by plant tissues was found to be distributed in varying amounts within the plant tissues (Figure 3.7). The distribution within tissues of *Phragmites australis* and *Kyllinga nemoralis*. (Figure 3.28) was investigated using scanning electron microscopy incorporating energy dispersive X-ray microanalysis (SEM-EDX). The SEM methods were considered because of the ability to display metal distribution using few micrometre resolutions. Cryofixation method applied in this study protected the tissues from damage to the ultrastructure of the specimen by the rapid freezing (at  $10^4$  to  $10^6$  /s). Cryofixation also prevented tissue damage due to the failure of formation of ice crystals normally associated with tissue damage. Cryofixation is a preferred method when preservation of natural ‘life-like’ structure of cells is also desired. Cryofixation was also preferred because it enhanced tracking of elements on plant surfaces due to its rapid freezing design, a phenomenon that was also reported by Sharma *et al.*, (2014).

### 3.5.2 Detection of Heavy Metals using Inductively Coupled Plasma Emission (ICP).

The choice of Inductively Coupled Plasma Emission (ICP) was based on the sensitivity of the method as it gave good precision to concentrations as low as  $0.01\mu\text{g/L}$ . High temperatures of the ICP source in an ICP-AES are advantageous as compared to other types of emission analyzers. Some of these advantages included good precision and excellent accuracy, the ability to excite the rare elements and high performance as compared to cost ratio. The ICP displayed the ability to detect low limits and low chemical interferences with minimal inter-element effects (Figure 3.8) in concurrence with reports from a study by Marin *et al.*, (2011). During the ICP processes, ions produced were detected easily after the sample was fed to the nebulizer using a peristaltic pump. Here the liquid sample was converted to a fine aerosol and transported to the plasma where it dissociated,

atomized and evaporated. The ions (produced ions which are positive) were then measured by an electron multiplier according to a method by Kaur and Mehra, (2012).

### 3.5.3 Metals in Wastewater

Among the heavy metals studied in this research, Pb, Zn and Cu were found in varying concentrations as shown in Figures 3.1 and 3.30. Varied amounts were found in the plant tissues, wastewater and sediment of the rhizofiltration system's planted and reference sections (Figure 3.26). However the values obtained were found to fall within acceptable discharge standards of Cr at 0.11mg/l, Cu at 0.002 mg/l, Pb at 0.009 mg/l and Zn at 0.05 mg/l of according to USEPA, (2003) and National Water Act waste discharge standards – DWA, (2010).

The mean concentration of metals in wastewater within the rhizofilter had some seasonal variations as well (Figure 3.2). For example in summer, Pb was not efficiently absorbed or adsorbed by the rhizospheric mechanisms and was found in slightly higher quantities in the effluent of the planted section (Figure 3.3). Consequently in winter Zn uptake by the sediment and plants was low and explains why there was a slightly higher concentration of Zn in the effluent from the planted section (Figure 3.2). Chromium and Ni were mainly accumulated in the roots. According to this study, there was an insignificant accumulation of Ni in the reference section. The initial metals concentration in the influent was too low such that their removal or reduction at the effluent cannot be exclusively on account of uptake by plant tissues, but rather adsorption onto the rhizofiltration sediment. This may be supported by observations from a study by Forbes *et al.*, (2011) that low concentration of contaminants in the influent contributes to high in removal efficiencies.

### 3.5.4 Metals Bioavailability in Sediment

The actual interaction of the metals and rhizofilter substrate is the key issue in the concept of phytoremediation according to a study by Ali *et al.*, (2013). The

bioavailability of metals was assessed in order to also determine the effect of biofilm in the removal of metal ions from wastewater. This process may have been stimulated by the competition of  $H^+$  for binding sites as reported by Schreck *et al.*, (2012). Slightly acidic sediment (occasioned by the pH of the influent), could have played a major role in the uptake of Cd and Mn. Accordingly, Cu chemically bound with organic material present in the water and settled on the substrate. This scenario possibly explains why Cu was heavily accumulated in the sediment of the rhizofilter (Figure 3.4). This observation was consistent with reports by Luca *et al.*, (2011) and Maine *et al.*, (2004) indicating that a high percentage of metals was retained in the sediment of the constructed wetland. The raised levels of Cu in the sediment could also have occurred as a result of cumulative effect with time. According to risk assessment code as adopted by Singh *et al.*, (2012), data from the sediment analysis by electron microscopy was fitted well in the code. All the metals tested were found to be below 10% wt for the planted and reference sections of the rhizofilter and were thus classified as under low risk at the time of this study.

### 3.5.5 Metals Uptake by Macrophytes

Results from SEM analysis showed metal deposits in normal sediment and plant tissues as presented in figures 3.12, 3.14, 3.16, 3.21, 3.23 and 3.25. The transport of these nutrients into other plant parts was possibly mediated by proteins which are responsible for transport functions in the plant tissues in agreement with a study by Vymazal, (2011). The process involved trans-membrane transport of some specific metal ions. For example, Cd transport into plant roots may have occurred as a result of Zn intake because of the structural analogue of Cd against Zn. These membrane transporters are distinguished by affinity for the particular ion ( $K_m$ ), transport capacity ( $V_{max}$ ) whereby  $V_{max}$  potentially measures the rate of ion transport while  $K_m$  estimates affinity towards a specific metal ion as was also reported by Jin *et al.*, (2012). Some metal ions were possibly adsorbed onto the root surface (extra-cellular binding sites) and these could not be translocated to the shoot biomass. The metals (metal sap) translocation (phytoextraction) from

the root to shoot of the macrophytes was probably controlled by leaf transpiration and pressure of the metal sap on the root cavity. After this translocation process, the metal ions may reabsorb into leaf cells (Figure 3.9). Figure 3.7 and 3.1 show initial metals concentration in the various plants' tissues and at the inflow, while figures 3.4 and 3.8, show the increase in the concentration of metals into various plant parts and rhizofiltration sediment. This increase in concentration at the various plant tissues, sediment and effluents could have resulted from uptake through diffusion and mass flow. The difference in physiology and morphology of the 2 plants of study may have contributed to the difference in metals uptake and adsorption as seen in figure 3.27. This is consistent with observations in a study by Schreck *et al.*, (2012). By convention, metal ions (soluble) transpose from sediment to root surface. In this study, the plant roots may have re-absorbed water within the rhizosphere to replace the water that had been transpired through the leaves (Ali *et al.*, 2013). During this process, a zone of depletion was possibly created which produced a concentration gradient enhancing the movement of metal ions in solution to the root surface and finally to the other parts of the plant. This observation was consistent with findings of a study by Kim *et al.*, (2010). The root exudates from plants regulate the solubility of certain metal ions by complexing the ions in solution into forms available for root uptake. This phenomenon affirms that the root exudates of the grass family (*K. nemoralis* and *P. australis*) played a role in the metal uptake (through siderophores). Both *P. australis* and *K. nemoralis* were found to be efficient bio-accumulators of metals from wastewater. Moreover, more metal uptake was witnessed on the *K. nemoralis* against *P. australis* (Figure 3.30).

### 3.5.6 Mechanisms of Metals Removal

#### 3.5.6.1 Physical mechanisms

Heavy metals removal from rhizofilters occurs through processes such as filtration, plant uptake by absorption, adsorption onto plant root system and

materials forming the substrate, sedimentation by attachment on the suspended matter and chemical transformation (Khan *et al.*, 2009). Sedimentation is normally dependent on the particle size and shape, settling velocity, type of metal and temperature. The coalescing together with suspended matter within the rhizosphere and subsequent sedimentation of the particles may have been the primary metals removal process in this system in concurrence with a study by Song and Li, (2014). The seasonal variations in temperature during the study period contributed to the varied metals removal capacities by the rhizofilter. The comparatively higher levels of metals in the sediment (above 50% increase) of the rhizofilter confirm that some metals may have coalesced with suspended matter and settled on the sediment (Figure 3.4). This compares well with sentiments by Zhang *et al.*, (2014a). Formation of the biofilm on the plant roots and on sediment enhanced the metal ions uptake from the water phase into the plant tissues by the action of microorganisms present.

#### 3.5.6.2. Biological removal processes

Uptake of metals by plants through absorption is one of the major removal mechanisms in rhizofilters. This process is efficiently achieved by the transfer and exchange of metal ions which are normally in solution phase within the rhizofilter to a solid phase (Vymazal, 2010; Ali *et al.*, 2013). In this study, metals were potentially removed through absorption by the wetland plants and the interaction of metal ions with sediment and plant roots (Appendix 4). The inorganic matter within the rhizosphere may have also acted as bio solvents for the metal ions exchange thereby relocating or attaching them to the microorganisms and the plants. The microbes as platforms for attachment may have also been responsible for metals removal. A similar observation was made by Saeed *et al.*, (2014) in their study on pollutant removal from municipal wastewater employing baffled subsurface flow and integrated surface flow-floating treatment wetlands. The pH increased from 5.9 to 7.1 pH units. Means were different between the inlet and planted and inlet and reference, but the difference was not significant ( $P < 0.05$ ), Tukey's test. This situation may have reduced the mobility of the metal ions and

therefore did not favour metals reduction through the rhizosphere especially during the warm seasons from January to March and October to December (Figure 3.31). increased oxygen levels within the rhizosphere may have been caused by the plant roots. These raised levels may have increased the oxidation rate of metal sulphides and the release of metal ions adsorbed into solution and possibly was rebound to other mobile compounds (Hartley and Dickinson, 2010). The high levels of zinc on plant roots occurred possibly due to the co-precipitation of zinc with Mn and Fe oxides forming iron plaques which easily attach to the roots of plants (Figure 3.21).

Metals removal by plants depends on a number of factors such as type and form of metals of concern, type of plant and physical, biological conditions. Results from the SEM and EDX (Figure 3.10) analysis revealed that the macrophytes of study *K. nemoralis* and *P. australis* have the ability for metals binding and are thus suited for metals removal from polluted waters (Putra *et al.*, 2014). The SEM analysis was used to investigate the metal deposits on the various compartments of the study macrophytes. The normal morphology of the various plant components was first observed under the SEM as seen in figures 3.11, 3.13, 3.15, 3.17, 3.18, 3.20, 3.22 and 3.24. The layered images showed the metals deposits having various colours as was observed in SEM (Figures 3.12, 3.14, 3.16, 3.19, 3.21, 3.23 and 3.25). In this research, *K. nemoralis* was found to store more of the metals in the roots than *P. australis*.

#### 3.5.6.3. Chemical mechanisms of metals removal

Metal ions within the rhizosphere are known to form complexes with the organic matter and other compounds leading to the trapping, sedimentation and precipitation of the metal compounds (Adhikari *et al.*, 2011). By chemisorption processes, Zn, Pb, Cu and Cr may have formed such complexes bound by organic matter within the rhizosphere. Consequently, Cu and Cr also created chemical bonds with sediment and (USEPA, 1999) oxides within the rhizosphere and eventually settled or sedimented out as metals removed. Conversions of metals

from one form to the other assists in metals removal from wastewater. For example, Cr removal may have occurred through the change from hexavalent form to acquire a trivalent form which accumulated and settled in the rhizosphere through processes involving oxygenation of the rhizosphere. Studies by Fibbi *et al.*, (2012) and Shelef *et al.*, (2013) reported similar observations. Metals occasionally form unstable metal carbonates within wetlands which are envisaged to be a trapping mechanism for some metals. Lead ions (Pb) and Ni were possibly removed from the wastewater by this mechanism. The rise in pH as seen in figure 3.31 and presence of carbonate and calcium ions may have favoured metals accumulation within the rhizosphere. This concurs with findings from a study by Marchand *et al.*, (2010).

Figure 3.26 shows higher metals uptake by sediment on the planted section of the reference section. Figure 3.3 shows higher metals concentration on the effluent from planted against unplanted section during the warm season. Figure 3.2 shows a higher concentration of metals on effluent from planted section against reference during the cold season. Metal uptake from wastewater was found to be comparatively higher on the planted section as compared to the reference section. From the study, the planted section adsorbed, absorbed and accumulated more metal ions as compared to the reference section of the rhizofilter.

### 3.6. CONCLUSIONS

- The below-ground biomass had a higher metals storage capacity than above ground for *K. nemoralis* and *P. australis*.
- Metals removal in the system was mostly dependent on the rate of uptake by the macrophytes and absorption/adsorption efficiency.
- The study suggests that there are variations in the metals uptake by the various plants and each plant *K. nemoralis* and *P. australis* have different uptake capacities.
- The uptake of metal ions by potential bacteria and fungi in rhizofiltration system also form vital areas for further research.

## CHAPTER 4

### IN-VITRO ASSESSMENT OF METALS BIOACCUMULATION BY WETLAND PLANTS-*Kyllinga nemoralis*. AND *Phragmites australis*

#### 4.1 INTRODUCTION

Different forms of inorganic substances including heavy metals, enter the environment through various natural and human activities (Schreck *et al.*, 2012). When levels of heavy metals exceed certain set limits or occur where they are not wanted, they are considered as environmental or water pollutants (Singh *et al.*, 2011). When the release of heavy metals into the environment surpasses their allowable threshold, efficient removal through natural processes cannot be effectively achieved. This causes metals accumulation in the environment (Wang *et al.*, 2014). Conventional methods for wastewater treatment cannot efficiently remove heavy metals from wastewater and are therefore not recommended as best treatment options for metals (Otte and Jacob, 2006). This limitation has led to the research and development of new alternative technologies in-cooperating rhizofiltration systems which are cost effective in comparison to other methods such as electrolysis, adsorption, chemical precipitation, reverse osmosis and ion exchange (Adhikari *et al.*, 2011). Moreover, rhizofiltration is a form of phytoremediation that concentrates, precipitates, absorbs and adsorbs metal ions from aqueous environments.

There is, therefore, a need to separate the metals from the sorbent in order to efficiently remove metal pollutants from the environment. Economically, biosorbent regeneration lowers treatment costs and increases profits for biosorbent producers according to Hegazi, (2013).

Many studies have used metal chelating agents such as the Ethylene-diamine-tetraacetic acid (EDTA) to increase solubility, but this is not encouraged in real applications due to added toxicity to the soil (January 2006). Other studies have reported a reduction of metal uptake by plants, and growth with the addition of EDTA (Turan and Esringü, 2007, Ebrahimi *et al.*, 2013). Consequently, other



studies have also been undertaken in order get solutions to heavy metals removal from aquatic environments. For example, leaves of the purple plant have been used to evaluate removal of Cu, Ni and Pb from water and conclusions were drawn to the effect of removal order as  $Pb > Cu > Ni$  according to Einollahipeer and Paksadtoochaei, (2013). Hegazi, (2013) conducted a study on metals (Pb, Fe, Ni, Cd and Cu) removal using rice husks and concluded that such cost-effective adsorbents can efficiently remove heavy metals up to the range of 20-60 mg/l. Bakar *et al.*, (2013) and Salam *et al.*, (2011) assessed the potential of the macrophytes *Egeria densa*, *Cabomba piauhyensis* and *Hydrillia verticillata* in the removal of Zn, Al, and As and found that *H. verticillata* had the highest potential (84.5%) to accumulate the heavy metals.

Plants used in phytoremediation are able to naturally or adopt some features that tolerate high toxicities without significant disintegration and at the same time accumulate metal ions through phytoextraction (Ebrahimi, 2014). Their potential to remedy wastewater is enhanced by the ability to break down organic pollutants via microbial activities in the rhizosphere (Akpore and Muchie, 2010). South Africa experiences about 497 mm of rainfall annually. This is way below the required range of about 860 mm (Mthembu *et al.*, 2014). Consequently the need to prioritise water recycling and reuse.

Possible routes for metals uptake by plants is through (1) Root absorption. The plant takes water from the substrate through the roots. Therefore as the water is absorbed into the plant through the root, it takes up the possible contaminants at the same time. The carboxyl groups normally present in the root system are responsible for the induction of cation exchange which happens via the plant cell membranes (Odinga *et al.*, 2013). This is a possible way by which the root system absorbs metal ions. The root system harbours aerobic bacteria due to the suitable environment provided by the introduction of  $O_2$  by the plant root system. (2). Absorption through Folia. The stoma cells and tiny cracks found in the cuticle are possible routes for passive absorption of metals. (3). Adsorption. The fibrous root network trap suspended matter which provides attachment for bacteria and the growth of fungus which play a key role in the adsorption of metals on the root

surface and sediment through ion exchange (Appendix 4). After plants adsorb the metals, the process of desorption may render the plants less toxic in the environment as by-products.

EDTA was not used in this experiment as there was enough metal concentration in the test water and EDTA may have introduced toxicity to the plant biomass, a situation that potentially decreases plant biomass thereby minimizing their metals uptake and translocation capacity to other plant parts (January 2006).

The aim of this study was to assess the heavy metal accumulation and adsorption by the wetland plants *K. nemoralis* and *P. australis* and sediment and to determine the potential of the two plants to accumulate metals. Ion – exchange potential and metals desorption by the plants were also assessed. Desorption potential of the plants was also investigated to determine whether the adsorbed metal ions were recoverable.

#### 4.1.1. Aim

To determine metals accumulation and adsorption by the wetland plants *K. nemoralis* and *P. australis*

#### 4.1.2. Objectives

- To determine the metal adsorptive capabilities of *P. australis* and *K. nemoralis* under controlled laboratory conditions in order to establish the maximum holding capacity of each genus and the relative distributions of metals.
- To establish the mechanisms of uptake of metals by the two plants to determine the plant affinities for the various metals and their order of uptake.
- To assess desorption capacity of the plants by immersing the roots of the adsorbents in neutral solution in order to recover the adsorbed metal ions.

## 4.2. METHODS

### 4.2.1. Preparation of Solutions

The metal ions of Cr, Cd, Cu, Ni, Pb and Zn were used in the experiment. The glassware used during the solutions preparation was of “A” grade and were washed in 50% (v/v) nitric acid (HNO<sub>3</sub>) and 50% (v/v) hydrochloric acid (HCl). This was followed by thorough rinsing of the same with tap water and finally triple deionised water. The glassware was then air -dried ready for use. A stock solution of 1000mg/l of each metal was prepared and stored in 1L volumetric flasks. This was used to prepare lower concentrations for the experiment. Analar grades of ZnSO<sub>4</sub>.7H<sub>2</sub>O (BDH, England), CuSO<sub>4</sub>. 5H<sub>2</sub>O (BDH, England), NiCl<sub>2</sub>. 6H<sub>2</sub>O (Hopkins and Williams, England), CrK (SO<sub>4</sub>)<sub>2</sub>.12H<sub>2</sub>O (BDH, England), Pb (NO<sub>3</sub>)<sub>2</sub> (BDH, England) and Cd (NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O (BDH, England) were used to prepare the metal solutions. The pH of the mixed metals solution was maintained between 2.0 to 6.0 with HNO<sub>3</sub> or NaOH during the experiments.

Triplicate number of young plants of *K. nemoralis* and *P. australis* were carefully uprooted from the rhizofilter. Sediment samples (100 g in triplicate) were also obtained by the plastic scoop from a depth of 5cm, The samples were kept in a cooler box with ice and transported to the laboratory for analysis. The plant roots were washed with distilled water to remove soil particles. The plants were weighed and the heights also measured according to the method by Bakar *et al.*, (2013). The sediment material was stored at 4°C until analysis. Initial metals concentration in the plants was determined as shown in figure 4.1.

The biosorption procedure for both plant material and sediment was done following a method by Luca *et al.*, (2011). About 50 g of sediment from each batch was weighed and transferred into 100 ml ceramic crucibles and dried for 24 hours in the oven at 70°C following the method by Saeedi *et al.*, (2004). Initial metals concentration in the sediment was determined following the procedure outlined in section 3.2.3. About 5 g of the sediment was weighed and placed in the glass jars for each triplicate batch. About 180 ml of mixed metal solution was

added to the jars. The jars were capped and agitated at 150 rpm at 20°C, 30°C and 40°C for a maximum period of 18 days to ensure efficient mixing. Sediment samples were taken for analysis by decanting of the supernatant. Metals concentration was determined by ICP, according to the method described in section 3.2.3. Consequently, the roots of the plants were exposed to metal ions in 180 ml of individual and mixed solutions of varying concentrations of 5mg/l, 10 mg/l and 20 mg/l in 1 L glass jars. The jars were maintained at temperatures ranging from 20°C – 40°C. One glass jar did not have plants and was used as the control. The plants were harvested from the metal solutions at 0, 3, 5, 8 and 15 days for analysis of heavy metals in solution and in plant parts. After adsorption of the metals experiment, the plants were removed from the metal solutions and placed in 180 ml of distilled water according to a method by Ndlovu *et al.* (2012). Desorption of metals study was also done by immersing the roots laden with metal ions into 180 ml deionised water according to a method by Silva and Brunner, (2006). Similar blanks were also run for each procedure.

#### 4.2.2. Sample Preparation for metals Analysis by Inductively Coupled Plasma membrane spectrophotometer.

Water, plant specimen and sediment samples were obtained from the glass jars and prepared according to the methods described chapter 2.2.10.1, 2.2.10.2 and 3.2.3 respectively.

### 4.3 DATA ANALYSIS

Statistical data analysis packages such as GraphPad Prism software, version 5.01. (GraphPad Software Inc., San Diego, CA USA; 2005) and Microsoft Excel were used to analyse the data. Variations in the means were determined by one-way ANOVA and Tukey's post-tests.

The adsorption of metals by the plants was calculated using the formula:

$$q_e = \frac{V(C_i - C_e)}{m}$$

(1)

as described by Ndlovu *et al.* (2012), where  $q_e$  is the metal uptake (mg metal /g biosorbent),  $V$  is the sample volume (solution) in ml,  $C_i$  denotes the initial metal concentration in solution (mg/L),  $C_e$  denotes the final concentration in solution (mg/L), while  $m$  denotes the amount of dry weight (g) of the biosorbent.

The % increase of metals in dry biomass was calculated using the formula:-

$$= 100 \frac{(C_i - C_e)}{C_i}$$

Where:-  $C_i$  denotes the initial metal concentration in solution (mg/L) and  $C_e$  denotes the final concentration in solution (mg/L). The measure of bioaccumulation of metals (Bioaccumulation factor - BCF) was calculated using the formula suggested by Rezvani and Zaefarian, (2011) as:-

$$BCF = \frac{\text{Metal in plant biomass (mg kg}^{-1}\text{)}}{\text{Metal in solution (mg)}} \quad (2)$$

The equation 3 as shown below was used to calculate the amount of metal adsorbed onto plant tissue.

$$q_t = q_e - c_t (v/m) \quad (3)$$

Where  $q_t$  and  $q_e$  are the biomass phase metal ion concentration (mg/g) and  $c_t$  solution phase metal ion concentration (mg/l) at time  $t$  (d) respectively. In addition, kinetic of desorption, as described by a pseudo-first order rate equation which was adopted by Wankasi *et al.*, (2005) and modified by Hegazi, (2013) as shown below in equation 4 was used to account for the bound metal ion resistance to desorption.

$$\ln \frac{q_t}{q_o} = \ln \theta - k_{des} t + (1 - \theta) \quad (4)$$

Where  $q_t$  is the metal concentration in the biomass phase (mg/g) at time  $t$  (d).  $q_o$  is the total desorbable metal concentration in the biomass at the beginning (mg/g)

and  $k_{des}$  is the pseudo-first order desorption rate constant per day ( $\text{day}^{-1}$ ).  $\theta$  is a desorption fraction in the initial metal loading ( $q_o$ ) and  $(1 - \theta)$  is fraction resistance to desorption. The pseudo – first order kinetic of desorption  $k_{des}$  and values of desorbable fraction  $\theta$ , and fraction resistance desorption  $(1 - \theta)$  was obtained from a plot of  $\ln \frac{q_t}{q_o}$  against time  $t$  (d). It was necessary to consider the kinetics of desorption for each metal ion to assess the overall performance of desorbing reagent, which in this case was deionised water.

#### 4.4. RESULTS

##### 4.4.1. Metals Absorption in Plant parts

This section describes the biosorption of metal ions into the different sections of the plants when they were exposed to solutions of compounds with varied metal concentrations, at different temperatures over time. Biosorption, which is dependent on growth, is a fairly active process though some studies have reported efficient metal binding capacities on inactive biomass according to a study by Bakar *et al.*, (2013). The metal solutions were prepared by diluting standard solutions of the various compounds of analar grades.

Results from this study indicated that the macrophytes and sediment from the rhizofilter, were capable of biosorbing metal ions (Figures 4.1, 4.4, 4.8, 4.9, 4.11-4.13). Bakar *et al.*, (2013) reported 90% removal for nickel, iron, zinc, copper and lead during his study of Arsenic, Zinc, and Aluminium removal from Goldmine wastewater effluents and accumulation by Submerged Aquatic Plants.

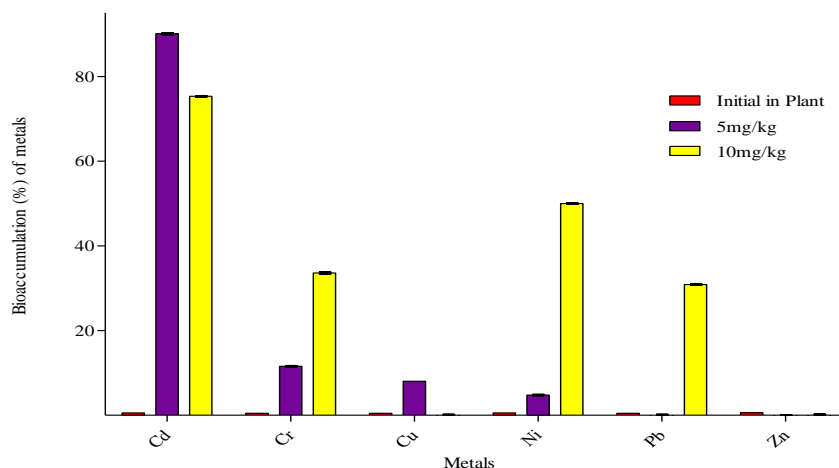


Figure 4.1 Removal of metals by bioaccumulation in the leaf of *K. nemoralis*. Whiskers represent standard deviations of three means.

In this study, the leaf of *K. nemoralis* showed varied patterns of accumulation based on different metals concentration in the test solution. The calculated means were not significantly varied ( $p = 0.1544$ ). The initial concentration of all the metal ions in the solution was an average of 0.41 mg/l each (Figure 4.1). Cadmium had a 90% accumulation on plant leaf when the plant was exposed to a solution of 5 mg/l, and a 75% accumulation when exposed to a solution of 10 mg/l. Similarly, there was 11.6% at 5 mg/l and 33.6% at 10 mg/l for Cr accumulation. Copper was accumulated at 8.04% on 5 mg/l and had no significant accumulation at 10 mg/l. There was 4.7% accumulation for Ni on 5 mg/l test solution and 50% accumulation of 10 mg/l test solution. Lead had a 30.8% accumulation when exposed to the test solution at 10 mg/l while there was an insignificant accumulation of 5 mg/l test solution. There was no significant accumulation for Zn when exposed to test solution at 5 mg/l and 10 mg/l respectively (Figure 4.1). Comparison of means from exposure to 5 mg/l and 10 mg/l was not significant with ( $P < 0.05$ ).

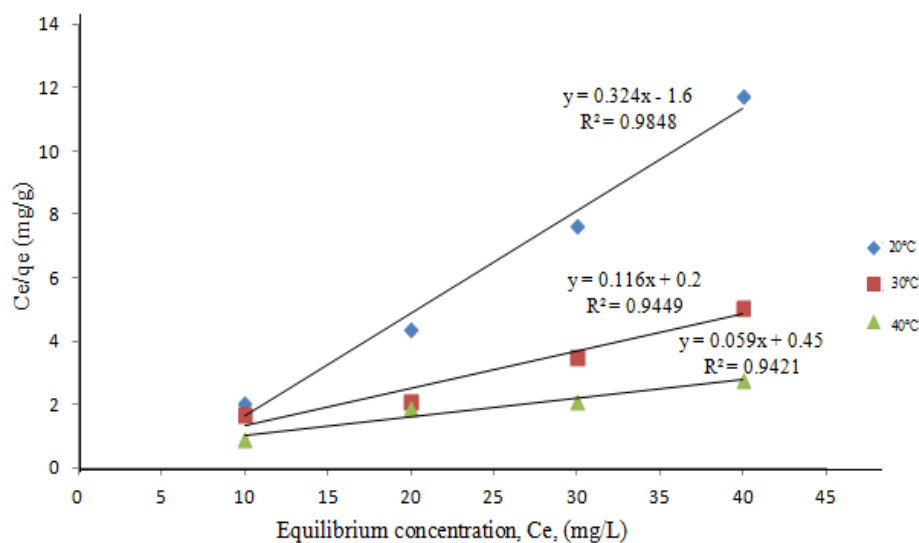


Figure 4.2 Adsorption isotherms for Cu sorbed in aqueous solution by *K. nemoralis*.

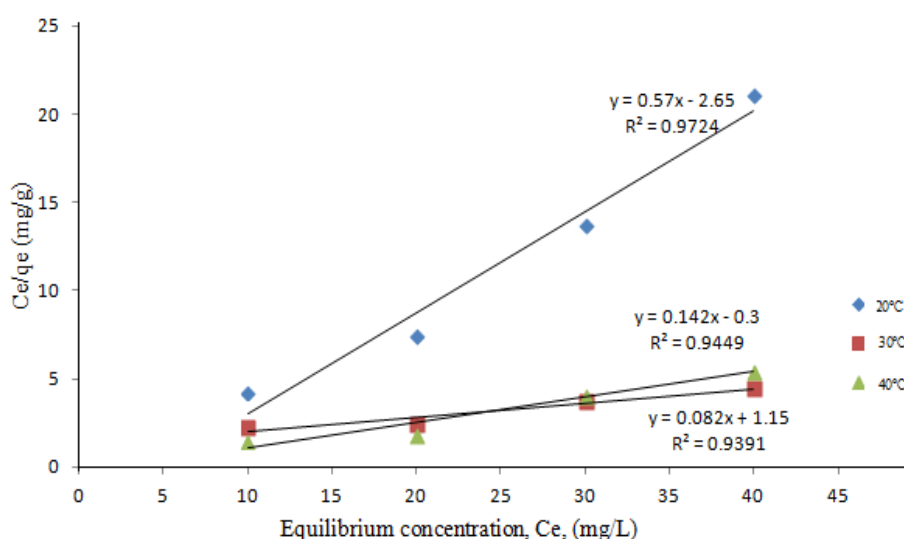


Figure 4.3 Adsorption isotherms for Cr uptake from aqueous solution by *K. nemoralis*.

Chromium attained a maximum adsorption of 0.8 mg/g after three days of treatment while Cd and Cu were maximum at 0.28 mg/g and 0.53 mg/g respectively after eight days (Figure 4.2). Contact time is a significant factor in the reaction processes that result in metals sorption. The time factor gives the



required information on the metal ion movement from the treatment solution into the adsorbent matrix according to Luca *et al.*, (2011).

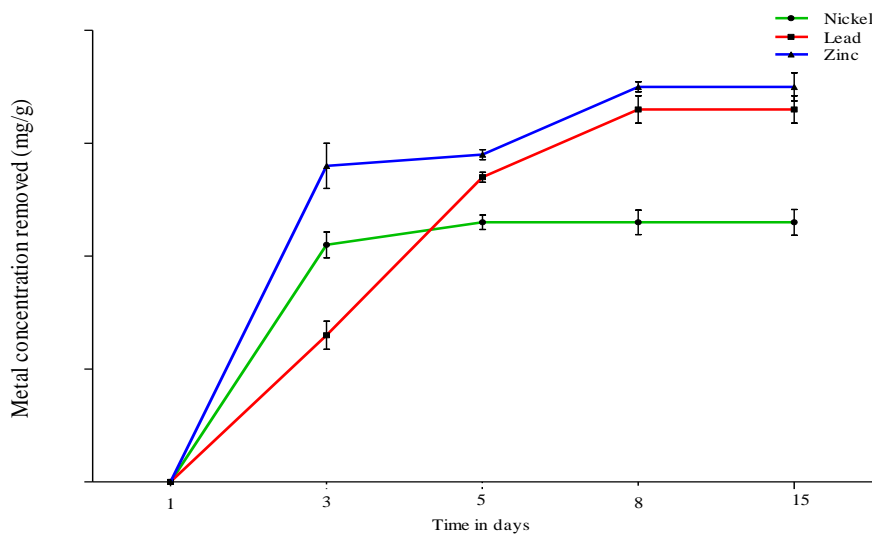


Figure 4. 4 Absorption of Ni, Pb and Zn by *K. nemoralis* from the aqueous solution containing 5 mg/l of each metal ion. Whiskers represent standard deviations of 12 means.

Adsorption of Ni reached a maximum within four days, while Pb (at 0.35 mg/kg) and Zn attained maximum adsorption after eight days of exposure (Figure 4.4). Maximum adsorption for Zn (0.3349.95 mg/g ) occurred within eight days of treatment. Nickel showed the highest adsorption capacity of 0.51 mg/g by *K. nemoralis* and *P. australis* as the biosorbents.

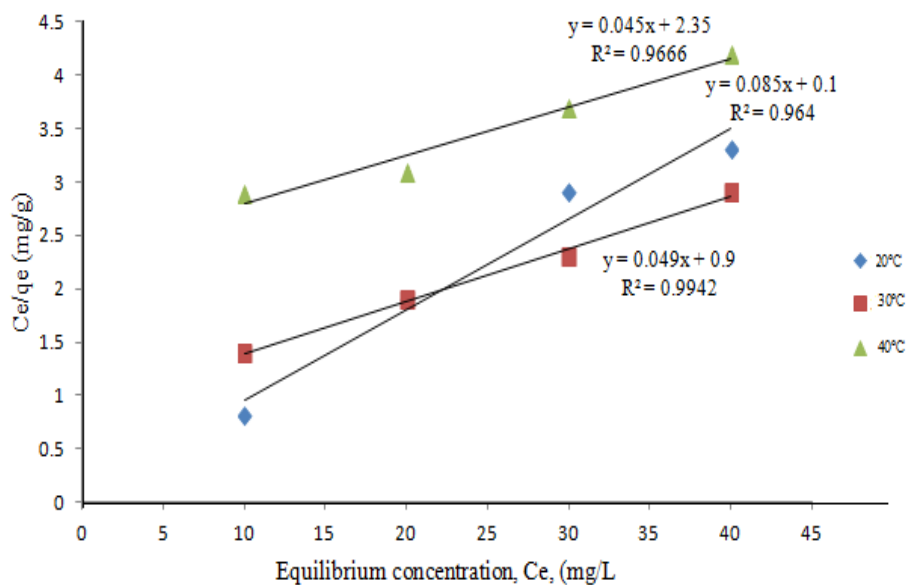


Figure 4.5 Adsorption isotherms for Zn uptake from aqueous solution by *K. nemoralis*.

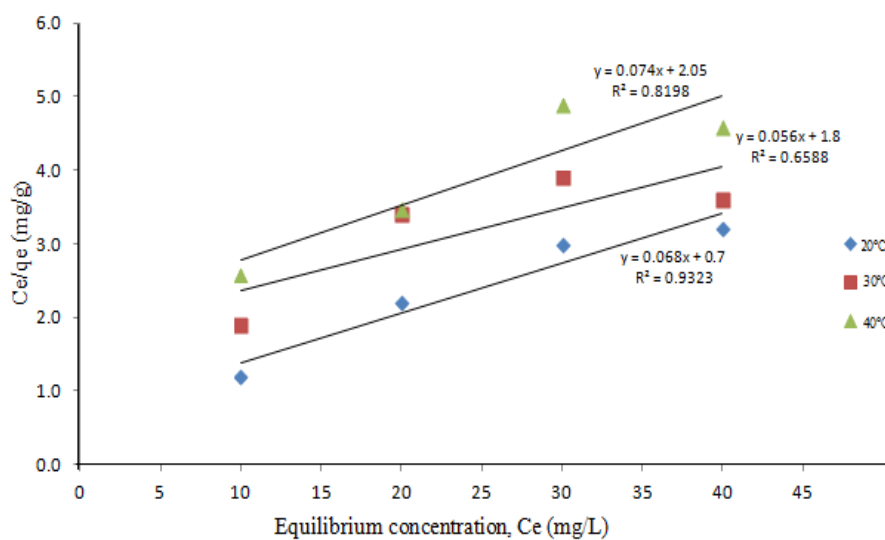


Figure 4.6 Adsorption isotherms for Pb uptake from aqueous solution by *P. australis*.

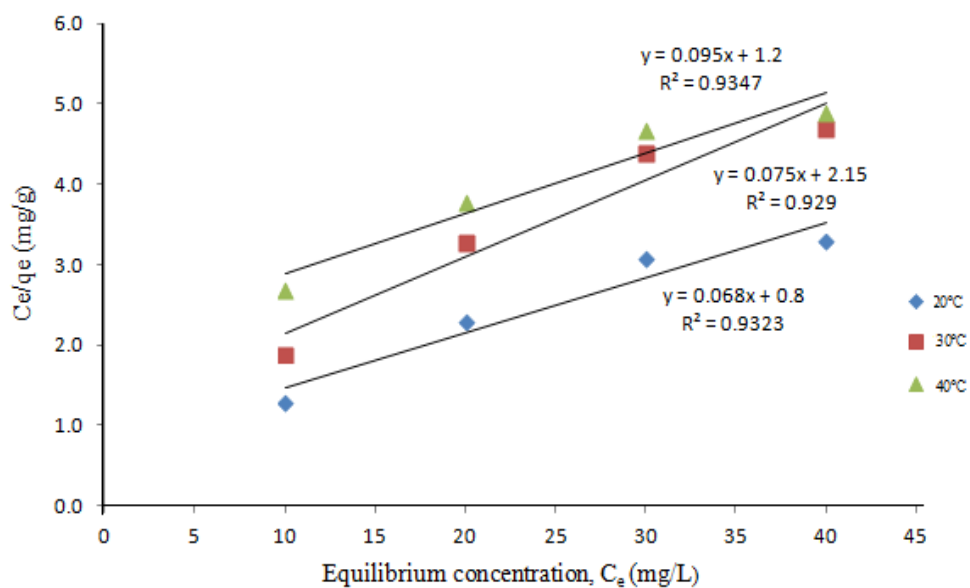


Figure 4.7 Adsorption isotherms for Ni uptake from aqueous solution by *P. australis*.

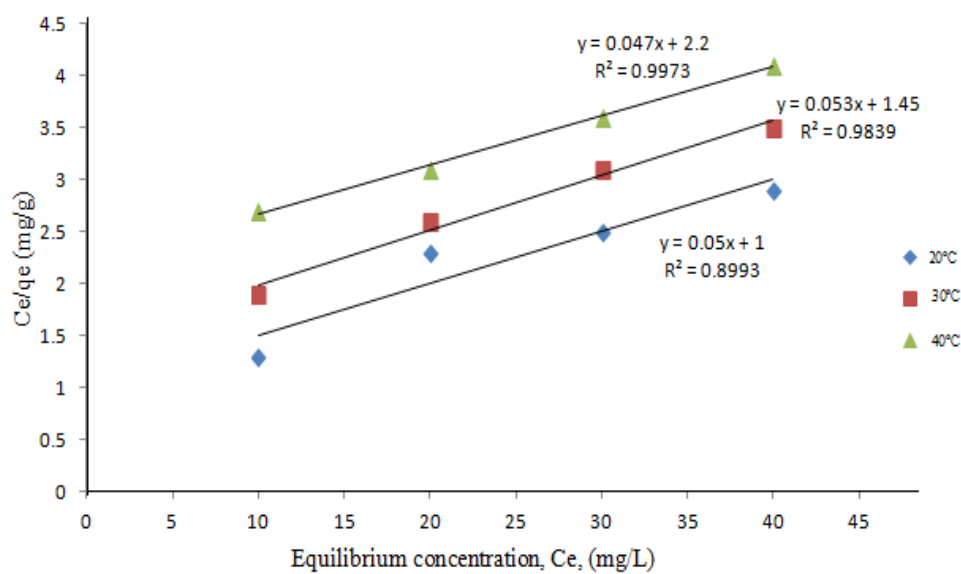


Figure 4.8 Adsorption isotherms for Cd uptake from aqueous solution by *P. australis*.

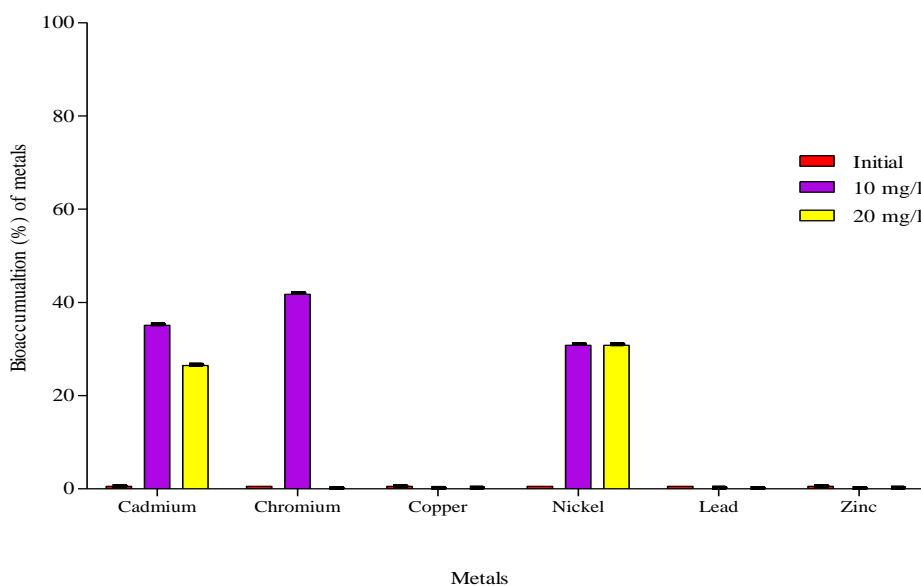


Figure 4.9 Bioaccumulation (%) of heavy metals in the leaf of *P. australis* after exposure to solutions having 10 mg/l and 20 mg/l of compounds of Cu, Cd, Cr, Ni, Pb and Zn. Whiskers represent standard deviations of three means.

There was a 30.7% and 30.7% uptake of Ni respectively at both the 10 mg/l and 20 mg/l concentration of the test solution with statistically varied means ( $p=0.0349$ ) t-test. Cadmium uptake was 35% at an exposure to 10 mg/l while at 20 mg/l the uptake was at 26.5%. Chromium experienced an accumulation by 41% when exposed to a solution of 10 mg/l while at 20 mg/l there was no noted accumulation. There was also no notable accumulation of Zn, Pb and Cu when exposed to both solutions in the leaf of *P. australis* (Figure 4.9).

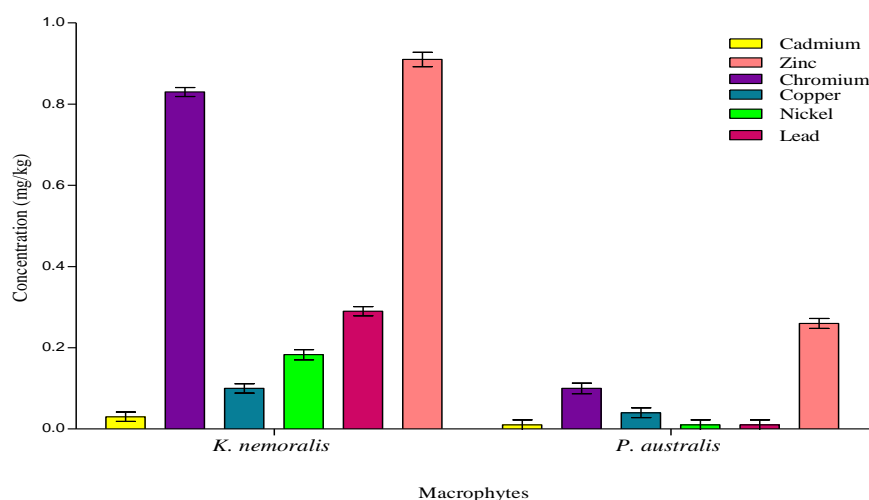


Figure 4.10 Concentration of heavy metals in the root of *K. nemoralis* and *P. australis* five days after exposure to a solution containing 10 mg/l each of Pd, Cr, Cd, Zn, Ni and Cu. Whiskers represent standard deviations of 12 means.

The root of *K. nemoralis* had comparatively higher metal accumulation than the root of *P. australis* (Figure 4.10). Copper was accumulated at 0.1 mg/kg for *K. nemoralis* and 0.04 mg/kg for *P. australis*. There were significant variances between the mean concentration for the various metals. For example, means for Cd and Cr between the two plants varied significantly ( $p = 0.0349$ ) t-test. Recorded values for the other metals were Cd at 0.03 mg/kg and 0.01 mg/kg, Cr at 0.83 mg/kg and 0.10 mg/kg, Ni at 0.183 mg/kg, and 0.010, Pb at 0.29 mg/kg and 0.01 mg/kg, Zn at 0.91 mg/kg and 0.26 mg/kg for *K. nemoralis* and *P. australis* respectively when exposed to a solution of 10 mg/l metal ion concentration. The highest concentration was recorded for Zn (0.91 mg/kg) in *K. nemoralis* and Cr (0.83 mg/kg) in *K. nemoralis*.

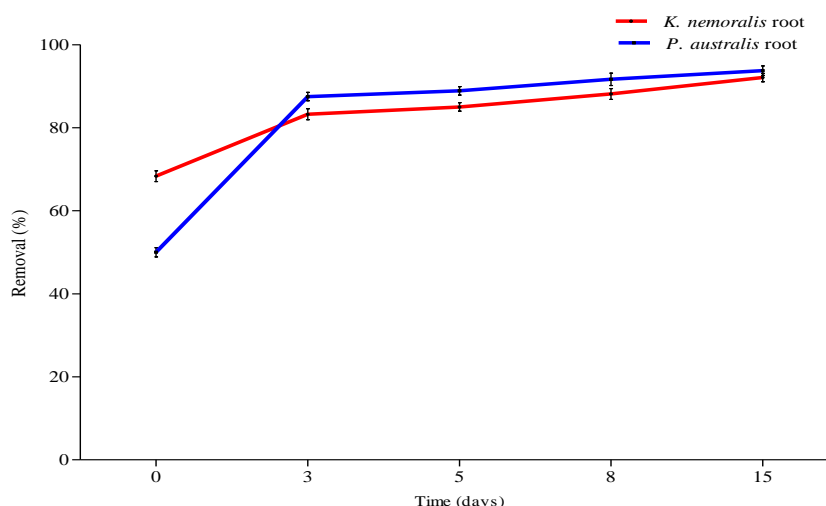


Figure 4.11 Effect of contact time on removal (%) of Cd by the root of *K. nemoralis* and *P. australis* after 5 days. Whiskers represent standard deviations of 12 means.

The highest removal of Cd was observed on the 15<sup>th</sup> day of the experiment at 93.8% for *P. australis* and 92.1% for *K. nemoralis* (Figure 4.11). The highest accumulation (%) of 92% and 93% for *K. nemoralis* and *P. australis* respectively, was observed the 15<sup>th</sup> day of the experiment. This concurs with reports from a study by Wankasi *et al.*, (2011), that time plays a key role in accumulation and retention of metal ions in plant tissues. At an exposure of 20 mg/l, the shoot of *K. nemoralis* accumulated more of Pb at 0.05 mg/kg and Cd at 0.05 mg/kg. The least accumulated were Zn at 0.01 mg/kg and Cu at 0.01 mg/kg (Figure 4.7). At an exposure of 20 mg/l, Zn at 0.325 mg/kg and Cr at 0.04 were mostly absorbed by the shoot of *P. australis* while Pb and Cu were least absorbed (Figure 4.8). Chromium retention could be related to prevailing charges in ions and the components and properties of the macrophytes. Similar sentiments were recorded by Covelo *et al.*, (2011) in their study of competitive adsorption and desorption of cadmium, chromium, copper, nickel, lead, and zinc by humic umbrisols.

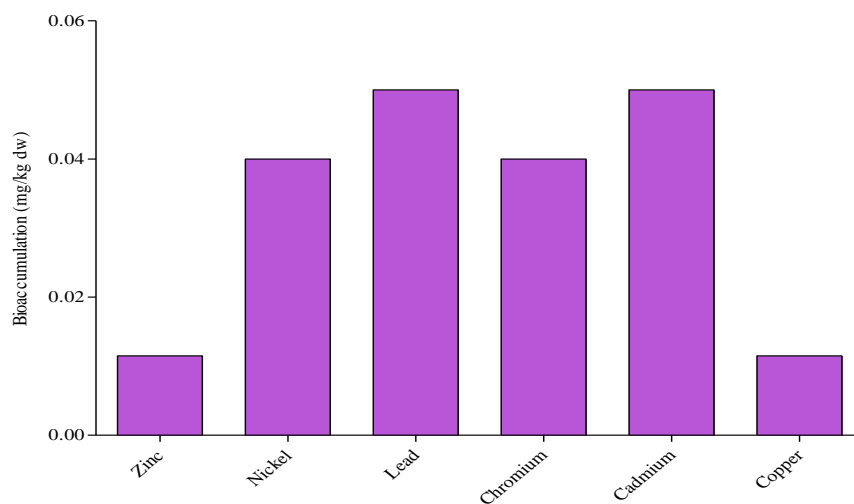


Figure 4.12 Heavy metals accumulation (mg/kg dw) by the shoot of *K. nemoralis* when exposed for five days in a 20 mg/l mixed solution of Zn, Pb, Cr, Cd, Cu, and Ni.

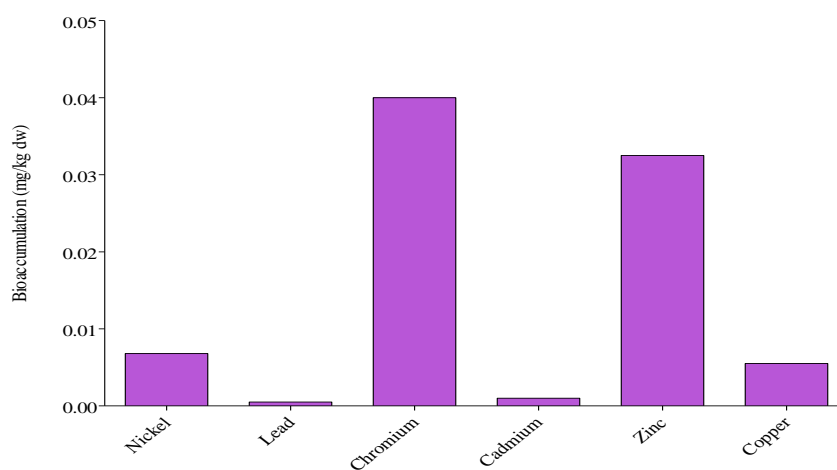


Figure 4.13 Heavy metals accumulation (mg/kg dw) by the shoot of *P. australis* when exposed for 5 days in a 20 mg/l mixed solution having compounds of Zn, Pb, Cr, Cd, Cu, and Ni.

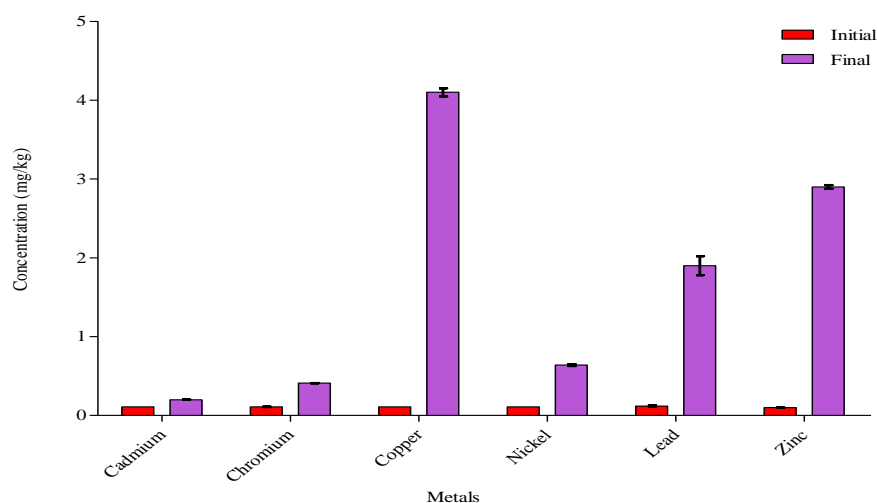


Figure 4.14 Heavy metals accumulation (mg/kg) on the sediment when exposed for 5 days in a mixed solution having compounds of Zn, Pb, Cr, Cd, Cu, and Ni. Whiskers represent standard deviations of 12 means.

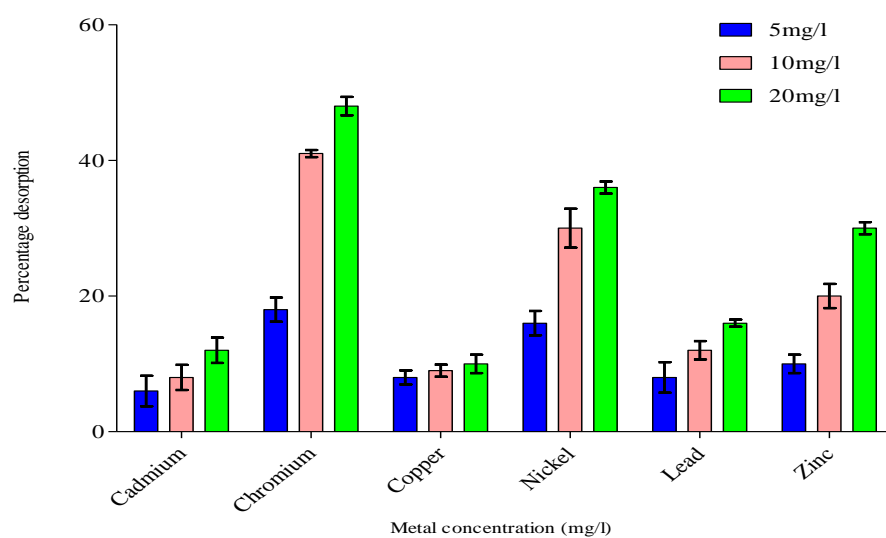


Figure 4.15 Percentage desorption of metals from roots of *K. nemoralis* by deionised water. Whiskers represent standard deviations of 12 means.



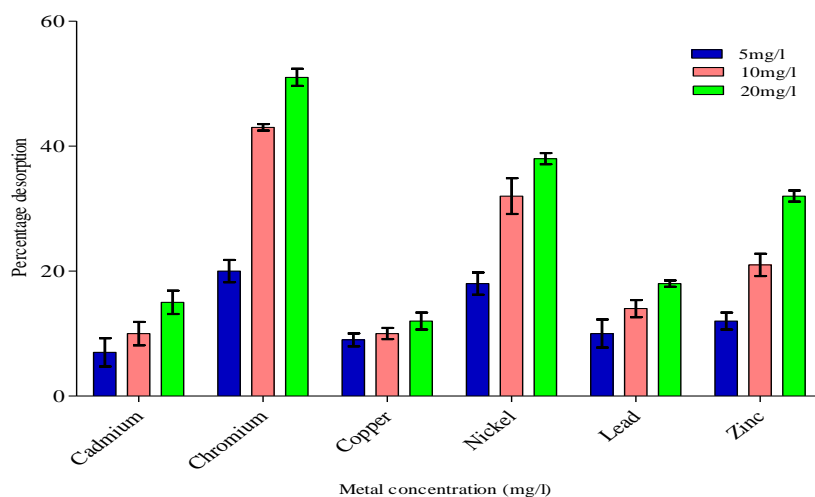


Figure 4. 16 Percentage desorption of metals from roots of *P. australis* by deionised water. Whiskers represent standard deviations of 12 means.

The process of desorption involves the change of bulk phase and an adsorbing phase. This is the total desorbable metal concentration in the biomass at the beginning of the experiment. Figures 4.15 and 4.16 describe the percentage desorption on the metal ions from the desorbent. The result shows that chromium is the most retained metal in the tissues of both *K. nemoralis* and *P. australis*.



Figure 4.17 Growth of new root (new roots are clean white) of *K. nemoralis* after 15 days in metal ion solution.

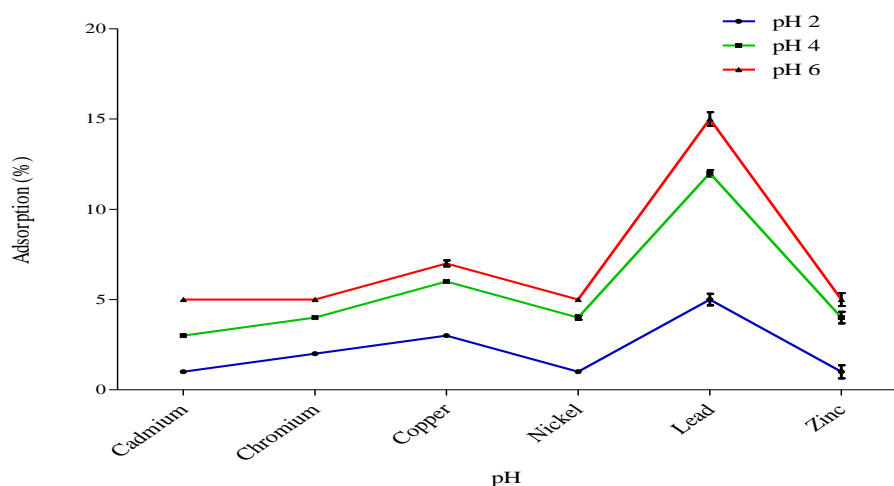


Figure 4.18 Effect of pH on adsorption of metals on plant tissues. Whiskers represent standard deviations of three means.

The effect of pH on adsorption of the various metal ions is shown in figure 4.18. Results indicate that an increase in the solution pH yielded an increase in adsorption (%). The highest percentage of adsorption was seen at pH of 6 for all the metal ions. This, therefore, means that the optimum pH for the adsorption of metal ions in this study was at pH 6.

#### 4.4.2 Discussion and Conclusions

Results from this study show that the macrophytes *K. nemoralis* and *P. australis* were adapted to metal biosorption and significant amounts of metals were efficiently biosorbed onto the plant organs. A significant amount of metal ions ( $p = 0.0349$ ) were accumulated in the root structures than the above-ground tissues of the wetland plants (Figure 4.10). With the exception of Zn, results also show that there was a decrease in adsorption (%) with an increase in the concentration of metal in solution for Cd and Cu while the reverse was witnessed for desorption of the metal ions (Figures 4.14 and 4.15). The low adsorption of metal ions at the initial exposure to adsorbent could have been due to the attainment of saturation adsorption point at day three (Figure 4.4) according to Ebrahimi, 2014). In a study of metals adsorption using activated sludge, it was observed that nickel, zinc, copper, iron and lead were adsorbed by almost 90% in agreement with Luca *et al.*,

(2011) and Ahmadpour *et al.*, (2012). The type II adsorption was predominantly witnessed in all the metals of examination. This means that the adsorption of their ions was in the monolayer to multilayer. The straight lines witnessed were indicative of continuous adsorption capacity with an increase in the metal concentration load. This phenomenon may have arisen due to the sizes of the metal binding sites on the root surface of the macrophytes that were available for attachment of the metal ions. Also, the larger pore sizes were likely accommodative of the second layer of accumulation of metal ions. Another likely scenario to explain the initial higher metals adsorption is a possibility of a high collision occurring between the biosorbent surface and metal ions which caused increased diffusion rate of metal ions onto the surface of the adsorbent. This is consistent with the results from a study by Wang *et al.*, (2014) that when the initial metal concentration is high, it accelerates the ability to bind but the resistance to mass transfer. Higher adsorption of Cd, Pb Zn and Ni occurred at 40°C, while Cr and Cu had higher adsorption potential at 20°C (Figures 4.2, 4.3, 4.5, 4.6, 4.7 and 4.8).

*Kyllinga nemoralis* has a network of intertwined rhizomes which contributed heavily to metals uptake (Appendix 4, Figure 4.18). Metals uptake could have been through microbes attachment onto the rhizomes and subsequent absorption of metals by the microbes. The decrease in adsorption of nickel and zinc metals may have been caused by a rise in pH (Figure 4.18) of the metal solution, saturation in the functional groups where metal ions attached themselves, especially during the warm months (Figure 3.31). At pH 2-3, it is reported that abundant  $H_3O^+$  ions will likely compete with Zn, Pb and Cu ions for binding sites on the biosorbents. Also when the solution pH is low, more protons ( $H^+$ ) will be added to the ions of the functional groups (Putra *et al.*, 2014). The tendency of higher adsorption at higher pH could be attributed to the loose inhibitory sequel of  $H_3O^+$  ions. The metal ion precipitation was assumed to occur at optimum between pH ranges of 2-6. *Kyllinga nemoralis* was able to withstand a high concentration of metal ions and was observed to grow new roots during the 15 days of the batch experiments (Figure 4.17). Similar observations were made by Walraven *et al.*, (2015) in their study of factors controlling the oral bioaccessibility of anthropogenic Pb in polluted soils.

Figures 4.12 and 4.13 display the metals accumulation of the two experimental plants. *Kyllinga nemoralis* was found to accumulate more metal ions in its tissues as compared to *Phragmites australis*. This brings us to a conclusion that *K. nemoralis* has more metal binding capacity than *P. australis*. Considering the two experimental macrophytes, *K. nemoralis* was found to adsorb more metal ions (Zn 0.02 mg/kg, Ni 0.04 mg/kg, Pb 0.05 mg/kg, Cr 0.04 mg/kg, Cd 0.05 mg/kg and Cu 0.01 mg/kg) than *P. nemoralis* (0.03 mg/kg, 0.01 mg/kg, 0.00 mg/kg, 0.04 mg/kg, 0.00 mg/kg and 0.00 mg/kg respectively) (Figure 4.12 and 4.13).

Desorption studies are normally carried out as they expound on the removal mechanisms of metal ions and their recovery from the biosorbent for sustainable, operational and environmental management (Wankasi *et al.*, 2005). In order to assess the absorbency of *K. nemoralis* and *P. australis*, neutral desorption reagent was used in various metal ion concentrations against contact time. Desorption of metals by plants is largely dependent on ionic phase and organic acids in soil according to Di Luca *et al.*, (2011). In this study, the desorption of metals was found to be dependent on the bonding capacity of each metal ion and the initial concentration in the adsorbent.

The application of the Langmuir model in the interpretation of the data in this section may account for the kinetic reactions and nonlinear equilibrium to describe the kinetics of metal ions sorption and release from the metal ionic phase and vice versa. For example, the desorption of Cd (Figures 4.14 and 4.15) may have been affected by its slow release against time. This was evidenced by the reductions from day three (at -0.646 mmol/l) to the eighth day (at - 1.029 mmol/l) when an equilibrium was reached. The isotherms constants including their correlation coefficients R<sup>2</sup> are shown in figures 4.2, 4.3, 4.5, 4.6, 4.7 and 4.8. Cadmium desorption may have been influenced by the carbonate fraction and the exchangeable oxides in the ionic phase. The concentration of metal ions in the plant tissues is an important factor to consider when assessing the efficiency achieved through bioremediation. Similar observations were recorded from a study by Rezvani & Zaefarian, (2011). This is because the biosorbent determines the

equilibrium of the system which is a function of the initial metal ion concentration and the quantity of the biomass. The initial concentration of metal ions in the biosorbent determines the resistance of the mass ion transfer from the aqueous phase to the biosorbent (Kumar *et al.*, 2010).

The adsorption capacities of the test metals decreased after 8 days. This could be attributed to the degradation of plant biomass which caused the decrease in the number of metal binding sites according to Ndlovu *et al.*, (2012).

*Kyllinga nemoralis* roots accumulated large amounts of Zn, Cr and Pb and still new roots were able to grow despite the high metal concentration in the test solution. This suggests that *K. nemoralis* could be a hyperaccumulator for Cr, Pb, Ni, Cd, Cu and Zn ions. These findings concur with the results recorded by Mazzei *et al.*, (2013) when he investigated the accumulation of metals in *M. verticillatum* using a hydroponic setup and concluded that *M. verticillatum* has high potential to accumulate metals.

The uptake of heavy metals by plant tissues in aqueous solution is highly dependent on the concentration of the metal ions. For example, Cd had a 90% accumulation when exposed to a solution of 5 mg/l while at an exposure of 10 mg/l the accumulation was at 75%. The maximum removal time was only achieved between the 14<sup>th</sup> and 15<sup>th</sup> day. This also suggests that the removal of some metal ions is dependent on the time or period of exposure to the solution. According to Ebrahimi, 2014, the major sorption mechanism could be the interaction of metal ions and the functional groups on the surface of the macrophytes.

The removal of metals from the solution was based on the initial concentration of the metal ions in solution. It is also an ion exchange process which is pH dependent. The optimum contact time for adsorption in this experiment was estimated as one day with the optimum adsorption capacity was at 509.6 mg/l, 349.9 mg/l, mg/l, 230.3 mg/l 32.98 mg/l 270 mg/l 80.0 mg/l for Cu, Zn, Pb, Ni, Cd and Cr respectively. The optimum pH for adsorption of metals onto the plant parts was in the range of 5.8-6.9 pH units which is in agreement with the report of Lukman *et al.*, (2013) in their study of adsorption and desorption of heavy metals onto natural clay material. Influence of pH on adsorption sites is dependent on the

prevailing charge in the binding site. More sites are usually occupied by hydrogen ions ( $H^+$ ) which makes the surface to be positively charged. This scenario may have favoured the attraction of negative charge of the metal ion to accomplish the binding on the surface of the adsorbent according to the discussion of Walli, (2015).

Copper and chromium were adsorbed better at 20°C (Figures 4.2 and 4.3) while Zn, Pb, Ni and Cd (Figures 4.5, 4.6, 4.7 and 4.8 respectively) had higher adsorptions at 40°C. This was attributed to increasing mobility of the metal cations with temperature. This may have also increased the number of molecules with enough energy to interact with the active binding sites on the adsorbent in agreement with Ebrahimi, (2014).

#### 4.4.3 CONCLUSIONS

- The macrophytes of study *K. nemoralis* and *P. australis* have the potential to accumulate metal ions.
- The adsorption of metals ions was dependent on temperature, pH and the strength of ions in solution.
- The Langmuir model was used to adequately describe adsorption of the metals.
- The successful application of distilled water as the desorbent suggests that the adsorption process most likely was through physical mechanisms such as attachment or adherence on the root surface.
- Low concentration of heavy metals in the influent supported high percentage removal of the metal ions.

## CHAPTER 5

### APPLICATION OF A CONSTRUCTED RHIZOFILTRATION SYSTEM IN THE REMOVAL OF ENTERIC PATHOGENS FROM MUNICIPAL WASTEWATER

#### 5.1 INTRODUCTION

Wastewater contains a large number of microorganisms, part of which are pathogenic to humans (Bates, 2012; Charles *et al.*, 2014). In order to assess the quality and maintain discharge standards of wastewater, the occurrence, of common enteric micro-organisms such as enterococci and coliforms is normally investigated (Abreu-Acosta and Vera, 2011). However, the addition of bacteriophages to the list of investigative microorganisms in assessing the quality of effluent discharged into water bodies has been proposed (Miernik, 2003; Jofre *et al.*, 2014).

Coliforms are indicators of the presence of faecal material from warm-blooded animals including human beings; thus the presence of these organisms in irrigation water or river water, indicates pollution from human or animal waste (Molleda *et al.*, 2008; Cheng *et al.*, 2012). Prevalence of such microorganisms in the environment and domestic water is an indication of poor hygiene and/or potential contamination from the sewerage system (Leon *et al.*, 2013).

Bacterial pathogens such as *Shigella* spp. and *Salmonella* spp. are only recovered in wastewater during epidemics or outbreaks of disease and are not, therefore, good indicators of pathogen pollution (Oakley, 2004; Charles *et al.*, 2014).

The removal of helminth ova from wastewater should be a priority as this is the form in which the pathogen is infective (Bitton, 2010). Their eggs (up to 200,000 may be produced by female *Ascaris lumbricoides* per day) are excreted in faeces and therefore are prevalent in wastewater and soil (Calheiros *et al.*, 2010; Rani *et al.*, 2014). Enteric viruses from human excreta occur in treated wastewater and can potentially contaminate recreational waters and rivers (Zhang *et al.*, 2014a). Their

presence in wastewater is normally associated with the presence of bacteriophages in wastewater, though a study by Jebri *et al.*, (2012) shared a contrary view that there is no clear interrelationship between the presence of bacteriophages and enteric viruses. Reed beds in rhizofilters are known to harbour a number of fungal species such as *Candida* especially the potentially pathogenic *Candida albicans* (Biedunkiewicz and Ozimek, 2009).

The presence of plants in rhizofiltration systems provides a conducive atmosphere for oxygen transfer within the rhizosphere and a medium for attachment of microorganisms which play a significant role in biological treatment of wastewater (Agunbiade *et al.*, 2009; Cabral, 2010).

Rhizofilters are considered to be a viable alternative for effective wastewater treatment through bioremediation and phytoremediation as compared to the high-cost conventional microbiological treatment systems (Campbell and Ogden, 1999; Calheiros *et al.*, 2010). However, effective pathogens removal is dependent on the design of the rhizofilter, filtration media, composition, structure of media and the plant species that is employed in the CRS (Haarstad *et al.*, 2012; Sukumaran, 2013). The systems' efficiency in bacterial removal is attributed to the bacterial attachment on the large surface area offered by roots of the aquatic plants (Calheiros *et al.*, 2009; Sukumaran, 2013). Consequently, root exudates from certain macrophytes, and the ability to supply more oxygen into the rhizosphere, filtration processes by the soil, adsorption onto the soil matrix and plant roots, natural die-off and predation are some of the rhizofiltration processes involved in the removal of microorganisms from wastewater (Torrens *et al.*, 2009; Lee *et al.*, 2014).

Plants contribute many bio-physical effects towards pollutant removal from constructed or natural wetlands (Tangahu *et al.*, 2011). The plants are known to stabilise the substrate thereby providing a good surface area for potential microbial biofilm attachment as was observed and reported by Brix, (1994) and Vymazal, (2011). Other functions of wetland plants include reduction of water flow velocity into the wetland, oxygen supply into root system which is necessary for aerobic



degradation by microorganisms. Various researchers have reported significant high pathogens removal from wetlands as positively correlated with the type of macrophytes in the wetland. For example, Boutilier *et al.*, (2011) used cattails (*Typha*) in a constructed wetland and achieved 95% removal of *E. coli*. Mairi *et al.*, (2012) reported removals by *Phragmites australis* as, 72% - 98% removal of *E. coli* while Chang – Xing *et al.*, (2010) conducted a study using *Canna flaccid*, *Iris versicolor* L., and *Juncus effusus* L. and concluded that the wetland planted with *C. flaccid* achieved higher removal rates of 99.98%, and 100% for faecal coliforms and *E. coli* respectively.

The use of macrophytes with potential antimicrobial agents as one of phytoremediation mechanisms for water treatment has been investigated by a number of research groups (Gruyer *et al.*, 2013; Yang *et al.*, 2013). Medicinal plants have been used and continue to be used in traditional practices to treat various ailments in animals. Extracts obtained from various plants including mangroves are believed to possess varied medicinal properties. For example, *E. agallocha*, *A. illicifolias* and *A. marina* are reported to display significant analgesic properties (Song *et al.*, 2011)

These may be of high medicinal value to human pathogenic isolates (Abeyasinghe, 2010). A major breakthrough in this field will be a much welcome discovery considering the cost of treatment, availability of the plant, side effects and environmental impacts.

The antioxidant composition of medicinal plants is responsible for the treatment they give for various ailments (Sindhu *et al.*, 2014). *Kyllinga nemoralis*. has not been studied on its potential to remove pollutants from wastewater but has been proved to have antimicrobial qualities and has been used in medicine while *Phragmites australis* was chosen as a control with the previous record of pollutant reduction in constructed wetlands (Ramankutty, 1994; Duhan *et al.*, 2013).

*Phragmites australis*, on the other hand, is considered as one of the aquatic plants with very high pollution tolerance potential. Since it is an invasive plant, its spread was more likely to occur within a short period of time after system establishment.

*Phragmites australis* is not susceptible to attack by pests in their new environments and is also resistant to floods and fire. These characteristics make them rapid re-colonizers especially during wetlands restoration according to Baudart *et al.*, (2000) and Vymazal, (2011).

There exist some knowledge gaps on the investigation of the fate of protozoa, fungi and bacteria pollution indicators in a subsurface flow rhizofiltration system planted with *Kyllinga nemoralis*. This study investigated the potential of a constructed rhizofiltration system in the removal of enteric pathogens from municipal wastewater.

#### 5.1.2 Aim

To investigate the potential of the rhizofiltration unit in the removal of pathogens and to further identify and confirm the presence of bacteria (*Salmonella* spp.) in the wastewater using genetic level screening.

The objectives of this study was to:-

- Assess the removal efficiency of the rhizofilter for nematodes, bacterial, fungal pathogens and indicators.
- Evaluate antimicrobial properties of extracts of *K. nemoralis* and *P. australis* against selected pathogens.

### 5.2 MATERIALS AND METHODS

#### 5.2.1 Physical and Chemical Analyses

Samples were collected as described in chapter two. Methods for physicochemical parameters are described in chapter two, section 2.2.5.

#### 5.2.2 Microbiological Analyses

Influent from the pre-treated wastewater and effluent samples from the rhizofilter were prepared and analysed for *Salmonella* spp. using the selective plating methods

as reported by Hench *et al.*, (2003) and Hassanein *et al.*, (2011). *Shigella* spp. were enumerated according to APHA, (2005). Totalcoliforms and *Escherichia coli* were detected following the Colilert<sup>®</sup> method while the modified Bailenger method was used for the enumeration of ova of *Ascaris lumbricoides* according to methods by Duarte *et al.*, (2011) and Jagals *et al.*, (2013). *Candida* species were enumerated using the pour- plate method according to Anaemene, (2012), while coliphage enumeration was done using the practical direct plaque assay method as described by Baker *et al.*, (2003).

### 5.2.3 The Direct Plaque Assay for the Enumeration of Coliphages

The direct plaque method as described by Cliver, (1997) was applied in the enumeration of coliphages. The water sample was collected in 100 ml sterile glass bottles, kept in a cool box with ice and transported to the laboratory for analysis. Three serial dilutions of the sample were made ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ). Two controls (one of the positive T4 inoculated in sterile water and one negative of sterile water) were also prepared. Approximately 1.5 ml of each of the serially diluted samples were transferred into 2 ml micro-centrifuge tubes. About three drops of chloroform were added to the solutions and centrifuged at 11,752 g for five minutes. Approximately 1 ml of supernatant was removed and into the remaining solution, 0.1 ml of a solution of *E. coli* which had been grown in Tryptic Soy broth was added. The solution was vortexed for about one minute. The tubes were then incubated at 25°C for five minutes. Each incubated mixture was transferred into separate tubes containing 5 ml of melted Luria-Bertani top agar. The tubes were mixed gently to ensure homogeneity and poured onto prepared agar plates of Luria-Bertani. The plates were incubated for 18 hours at 37°C. Plaque forming units (PFU) were counted (clear regions within *E. coli* lawn growth) (Figure 5.9) and reported as PFU/ml according to the formula:-

$$\frac{\text{Phage}}{\text{ml}} = \left( \text{number of } \frac{\text{plaques}}{\text{plate}} \right) \times \left( \frac{1}{\text{ml}} \text{ plated} \right) \times \text{dilution factor}$$

#### 5.2.4 Determination of *Candida* Species

Using the method by Jacob *et al.*, (2013), 100 ml of sample was filtered through 0.45 µm pore cellulose nitrate filter disks (Sartorius Stedim Biolab Products Augbanc, France). The disks were transferred onto Sabouraud Dextrose agar plates and were then incubated for 48 hours at 37°C (Baker *et al.*, 2003; Jacob *et al.*, 2013). Visible cream colonies of *Candida albicans* were enumerated before any growth of filamentous fungi was detected. Colonies were reported as colonies /100 ml.

Sabouraud Dextrose agar is usually used to culture fungi and has a low pH that inhibits the growth of most bacteria; it also contains the antibiotic gentamicin which is specific in inhibition of growth of Gram-negative bacteria.

#### 5.2.5 Determination of *Ascaris lumbricoides* and the coliform Bacteria

Methods for analysis are described in chapter two, section 2.2.11 and 2.2.12.

#### 5.2.6 Enumeration of *Salmonella* species

Serial dilutions were prepared (90 ml/10 ml of 0.1% peptone water) and 100 µl aliquot of the diluted sample was carefully plated out on Salmonella *Shigella* agar plate and incubated at 37°C ±2 for a period of 24 hours (Popko *et al.*, 2006; Hassanein *et al.*, 2011). Enumeration of the colonies was done after visual identification using a magnifying glass fitted onto a colony counter as colourless colonies with a black centre.

#### 5.2.7 Determination of *Shigella* species

Serial dilutions were prepared and 100 ml of the diluted sample was carefully filtered through 0.45 µm pore cellulose nitrate filter disk (GN-6 metric grid 47 mm Ø) according to APHA, (2005). The filter disks were carefully placed onto

MacConkey agar plates while avoiding air bubbles and incubated at 35°C for a period of 24 hours. The colourless non – lactose fermenting colonies of *Shigella spp.* were enumerated (CFU/100 ml) using a colony counter fitted with a magnifying glass.

#### 5.2.8 Antibacterial Potential of *P. australis* and *K. nemoralis*

Fresh plant material consisting of leaves, stem and root (*P. australis* *K. nemoralis*) was obtained from the field and authenticated by Prof. H. Baijnath of the Department of Biotechnology and Food Technology. The material was washed using sterile water and air-dried on the workbench in the laboratory (between 25°C -28°C) for 14 days according to methods by Ogu *et al.*, (2012) and Garg *et al.*, (2013). The dried plant material (leaves, stem and roots) was pulverized using a Milestone Start D Mellerware 50 g capacity blender to a fine powder. The working bench was sterilized using 70% alcohol prior to performing the experiment. A pure culture of *E. coli* was obtained from the Department of Biotechnology, Durban University of technology.

An aqueous extract of the plant stem, leaf and root were obtained through cold water extraction by weighing 200 g of the powder and mixing with 500 ml of cold sterile distilled water in a 1-litre flask according to a method by Ogu *et al.*, (2012). The mixture was shaken several times, allowed to stand for 48 hours and filtered using Whatman No 1 filter paper. The filtrate was dried in a water bath at about 50°C and kept for use in the experiment for antimicrobial potential. Subsequently, extraction with hot water was done by soaking 200 g of the dry material in 500 ml of hot water which had been boiled for 30 minutes. The mixture was stored for 48 hours, dried in an oven at 50°C and stored at 4°C for use in antibacterial determination (Ogu *et al.*, 2012; Majumder, 2013).

An ethanolic extract was also done by weighing 200 g of the dry material and soaking in 500 ml of 70% ethanol for a period of 48 hours with agitation (Orbishake, Labotec) for 24 hours at 200 rpm. The solution was filtered using

Whatman No. 1 filter paper. The filtrate was dried using a rotary vacuum evaporator (Buchi R 110-Switzerland) at 50°C -60°C (Majumder, 2013). The dried material was stored and used for antibacterial potential determination. To confirm that the extracts for antimicrobial determination were sterile, the extracts were each inoculated on Mueller Hinton agar for 24 hours at 37°C. Lack of any growth on the extracts after incubation indicated their sterility. All chemicals used in this experiment were of analytical grade. Other apparatus included sterile discs, Mueller Hinton agar, the standard antibiotic and Whatman No.1 filter paper (Bio-rad, South Africa).

#### 5.2.9 Antibacterial Activity Determination

The antibacterial activity was determined using the disc diffusion method as described by Garg *et al.*, (2013). The dried plant extracts (leaf, stem and root) were added to 100 ml sterile water and mixed (Ogu *et al.*, 2012). Approximately 6 mm Ø sterile discs were prepared using Whatman filter paper and placed to blot 10 ml of each extract solution till all the solution was absorbed by the discs according to the procedure by Ogu *et al.*, (2012). The filter discs with extracts were allowed to dry and then carefully placed on the surface of prepared Muller-Hinton agar plates containing colonies of gram-negative *E. coli*. The negative control was sterile water while the positive control was 10 mg/ml of Streptomycin according to a method by Palaksha *et al.*, (2010) and Vu *et al.*, (2016). Triplicate experiments were performed and each time, plates were incubated at 37°C ± 0.1°C for a period of 18-24 hours. After incubation, clear zones of inhibition were measured in millimetres using a transparent ruler according to the Kirby-Bauer procedure.

Consequently, to estimate tubes with the minimum bactericidal concentration (MBC), a few loopfuls from the tubes that had the lowest bacterial growth were inoculated on Nutrient agar plates and incubated for 24 h at 37°C. The plates that had no growth were matched to the tube dilutions and those dilutions were taken as the minimum bactericidal concentrations. The activity of extracts was

expressed in a varying sequence of <2 mm zone no activity, <4 mm – 8 mm active zone and >10 mm as very active according to procedures by El-Mahmood *et al.*, (2008) and Ogu *et al.*, (2012).

#### 5.2.10 Pathogens Detection in the rhizofiltration Sediment

Sediment samples analysis was done according to a traditional microbiological method of ISO-6579:2003 described by Oltuszek-Walczak *et al.*, (2009). Samples were carefully scooped from the depth of about 10cm from the surface, placed in sterile glass bottles and transported to the laboratory in a cool box for analysis. About 25 g of sample was weighed and placed in a sterile flask. Approximately 225 ml of BPW (Buffered Peptone Water) was added and vigorously mixed. The supernatant was carefully syphoned out immediately for the evaluation of the bacteria using the method for *Salmonella* spp. About 100 µl of samples were taken and transferred onto agar plates of *Salmonella Shigella* media. The prepared sample was incubated for 18-24 hours at 37°C after which typical *Salmonella* colonies (with a black spot) were identified and counted.

### 5.3 RESULTS

#### 5.3.1 Physical and Chemical Analyses

Results for the physical and chemical analyses are described in chapter two sections 2.4.2 and 2.5.1. Salinity levels were within the acceptable limits and did not significantly affect the macrophyte growth. Results for the organic load were also lower than many reported values regarding secondary treatment in wetlands.

#### 5.3.2 Microbiological Analysis

The coliphage colonies appeared as plaques in the Luria-Bertani agar (Figure 5.1) The coliphage bacteria had varying concentrations and reduction levels across the

nine sampling points of the rhizofilter when the system was subjected to various concentrations of the wastewater. Since coliphage concentration is normally varied in wastewater, the study recommended the use of 100% raw settled sewage for the determination of coliphage removal from the system according to a method by Abdulla *et al.*, (2007). The variances differed significantly at ( $P < 0.05$ ) from sampling point 'A' to point 'J'. The removal (%) from the planted section was an average of 94.637% while in the reference section was 93.6667%. The removals were not significantly varied ( $p = 0.6395$ ) across the sampling points though points 'A' and 'B' had the highest removal rates of 96% as compared to an average of 94% on points 'I' and 'J' of the reference section (Figure 5.2).

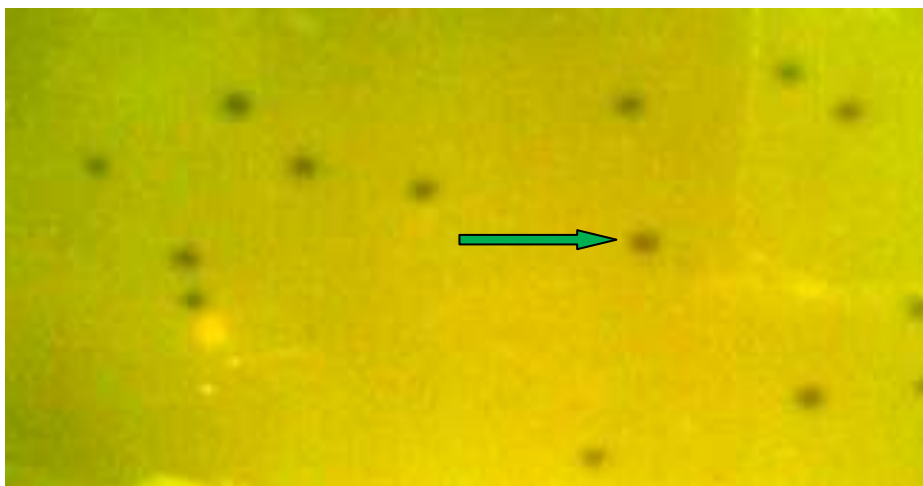


Figure 5. 1 Coliphage colonies (shown by the green arrow) as clear sections on Luria-Bertani agar after  $24 \pm 2$  hour incubation.



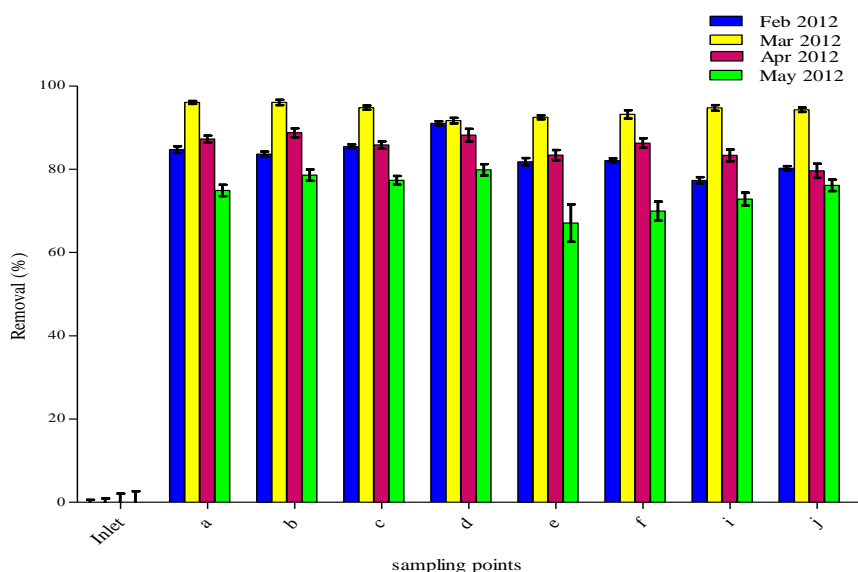


Figure 5.2 Average removal (%) of coliphage on effluents from planted and reference sections of rhizofiltration system over a four-month period in 2012. Whiskers represent standard deviations of 12 means.

### 5.3.3 Antibacterial properties of *K. nemoralis* and *P. australis*

The modified Kirby Bauer method was used to determine the antibacterial properties of the macrophytes. There were variations on the zones of growth inhibition as displayed by extracts from roots of both plants. The calculated means were not significant ( $P < 0.05$ ). Zone of inhibition of extract from leaf was found to be larger than that from the stem extract for both plants. The inhibition zones at a concentration of 25 mg/ml for *K. nemoralis* were measured at 4-10 mm (ethanol), 3-7 mm for the cold extraction and 4-8 mm for the hot water extraction in summer. In winter, the inhibition zones were 4-6 mm for ethanol, 2-6 mm for the cold extraction while hot extraction measured 3-6 mm (Table 5.1). The situation in spring was 3-6 mm for ethanol, 3-6 mm for the hot water extraction while cold water zones ranged between 2-6 mm. The largest zones of inhibition were witnessed with the ethanol extract, while water (as a universal solvent) achieved a wider zone with extract at a higher temperature. *Kyllinga nemoralis* leaf had the highest extracted value of 6.7 mm with ethanol extract in summer while the smallest zone was at 4.33 mm with cold water extraction in winter. The root of *K. nemoralis* had the highest zone at 9.97 mm with ethanol extract and the lowest zone

at 6.17 mm in cold water extraction during the winter season. *K. nemoralis* root recorded a much higher inhibition zone than the root of *P. australis* which recorded a zone of 8.63 mm with ethanol extract.

Table 5.1 Variations in Ø of zones of inhibition of *E. coli* growth on extracts from *K. nemoralis* and *P. australis* using cold water, hot water and ethanol extractions of leaves, roots and stems.

	Summer			Winter			Spring		
	Cold	Hot	Ethanol	Cold	Hot	Ethanol	Cold	Hot	Ethanol
KL	4.87±0.19	5.4±0.09	6.7±0.09	4.33±0.1	4.57±0.05	4.87±0.05	4.57±0.05	4.63±0.05	4.87±0.05
KR	7±0.09	8.2±0.09	9.97±0.19	6.17±0.14	6.43±0.14	6.63±0.14	6.57±0.05	6.63±0.05	6.8±0.09
KS	4.17±0.2	4.3±0.09	4.9±0.09	4.07±0.05	4.4±0.09	4.63±0.10	4.03±0.05	4.13±0.05	4.37±0.05
PL	4.2±0.09	4.6±0.18	4.7±0.09	4.28±0.06	4.57±0.05	4.7±0.09	3.93±0.05	4.07±0.10	4.3±0.09
PR	6.47±0.21	7.63±0.10	8.63±0.22	5.38±0.06	5.63±0.14	5.8±0.09	6.23±0.05	6.37±0.05	6.57±0.05
PS	3±0.09	4.13±0.14	4.7±0.09	2.9±0.09	3.5±0.09	4.07±0.14	2.83±0.05	3.1±0.09	3.47±0.05
PC	12.17±1.71	12.17±1.7	12.17±1.71	11.37±0.19	11.3±0.09	11.5±0.09	12.13±0.14	12.07±0.05	12.23±0.14
N	-	-	-	-	-	-	-	-	-

Zones of inhibition (mm) ±SD of *Kyllinga nemoralis*.- KL=leaf, KR = Root, KS = Stem and *Phragmites australis*- PL=leaf, PR= Root, PS=Stem, PC = positive control and N= Negative control, No inhibition. Means not significant at P < 0.05 (Tukey's test)

#### 5.3.4. Enumeration of Candida Species

The concentration of *Candida* spp. in the rhizofilter was found to vary significantly ( $\alpha = 0.05$ ) with a p-value of 0.0005 (two tailored) across the sampling points though point 'j' had the highest mean concentration of 7.66 CFU/100 ml. The average means were 5.16 CFU/100 ml and 5.5 CFU/100 ml in the planted and reference sections respectively (Figure 5.3). On average, the removal of *Candida* spp. was 64.7% in the planted section and 62.5% in the reference section. The highest removal point was at sampling point 'G' in the reference section of the rhizofilter (Figure 5.4).

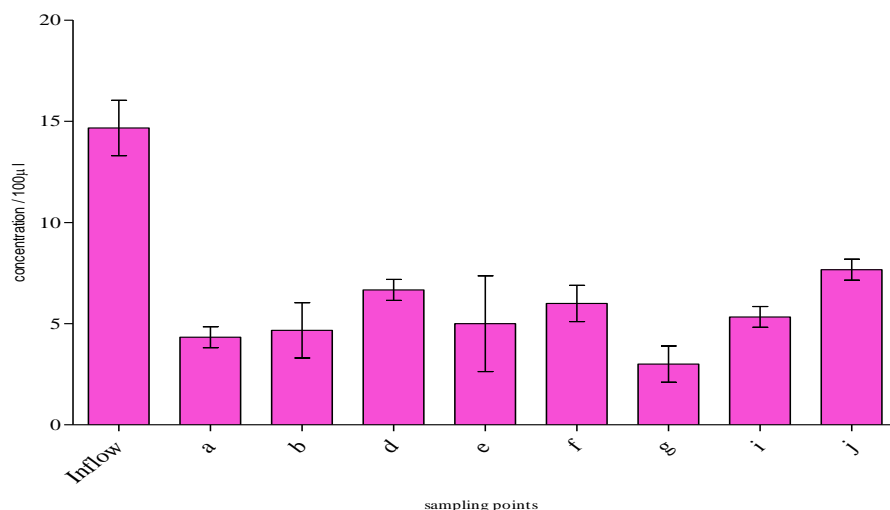


Figure 5.3 Average concentration of *Candida* spp. on inflow and effluents from both of means. Whiskers represent standard deviations of three means.

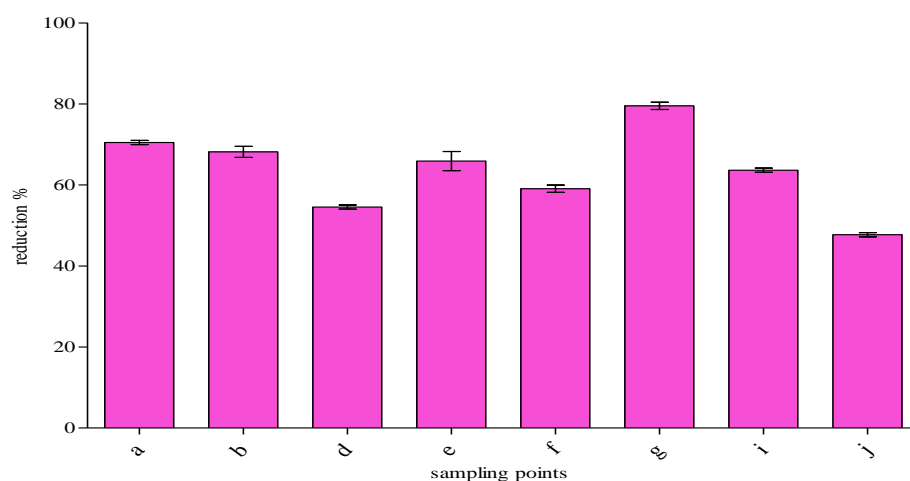


Figure 5.4 Reduction (%) of *Candida* spp. in effluents from the planted and reference sections of the rhizofilter. Whiskers represent standard deviations of 12 means.

### 5.3.5 *Ascaris lumbricoides* , *Salmonella* spp. *Shigella* spp. and Coliform bacteria

Helminth ova was seen in various stages of its life cycle. Notably was the hatching stage as seen in figure 5.5. The abundance of *A. lumbricoides* ova was significantly reduced by up to 98 %, in the planted section of the rhizofilter while the reference section had an average reduction of 92.4%

(Figure 5.6). The reduction (%) of ova of *A. lumbricoides* varied significantly between the planted and reference section of the rhizofilter ( $p = 0.0177$ ), unpaired t-test. The concentration of ova of *A. lumbricoides* (Figure 5.7) from the rhizofilter effluent was low during the months of July, August and September (cold season) and high in the months of March and April. The mean values recorded were 3.5 ova/l and 5.8 ova/l in the planted and reference sections respectively. The highest concentrations in the influent were in March (warm month) with mean removal rates of 84.2% in the planted section and 63.4% in the reference section of the rhizofilter.

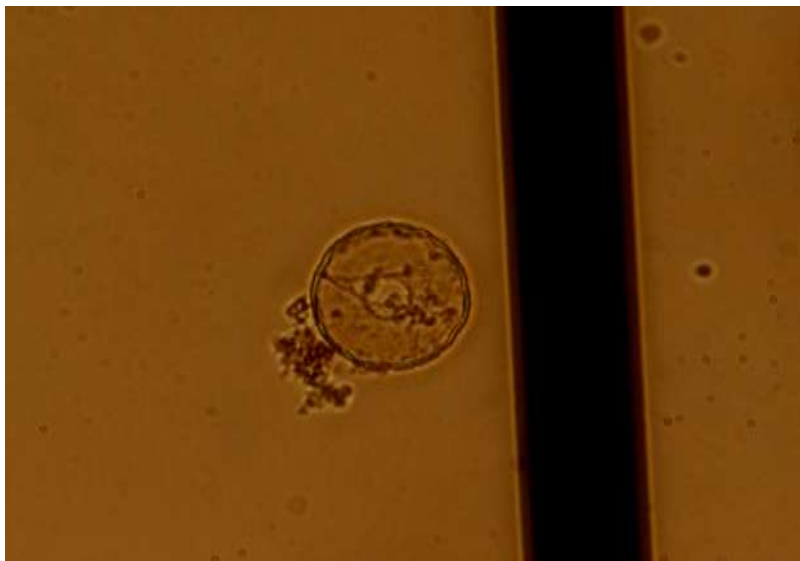


Figure 5.5 Ovum of *A. lumbricoides* (*hatching*) as seen under light microscopy at 400 x magnification (Nikon 81, Japan). The dark line represents a section of the Mac master slide.

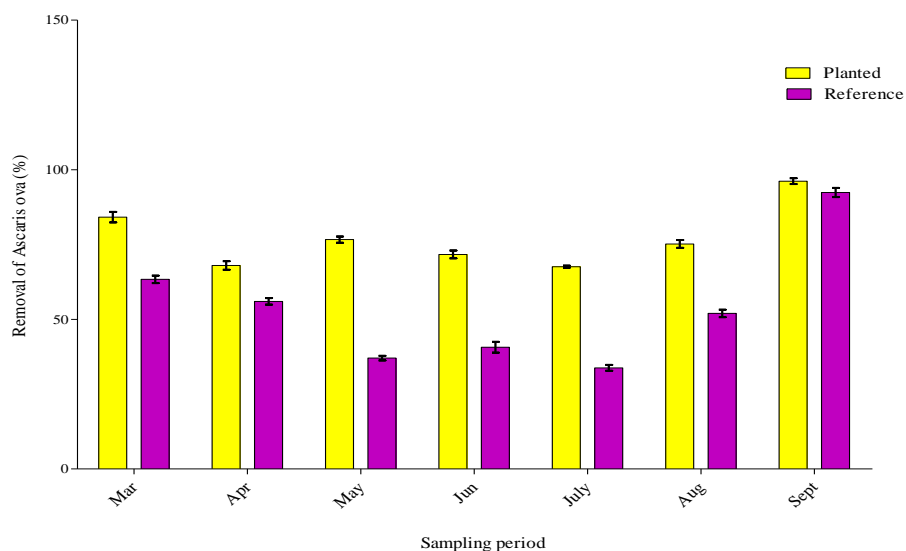


Figure 5.6 Removal (%) of ova of *Ascaris lumbricoides* covering warm and cold seasons in 2012. Whiskers represent standard deviations of three means.

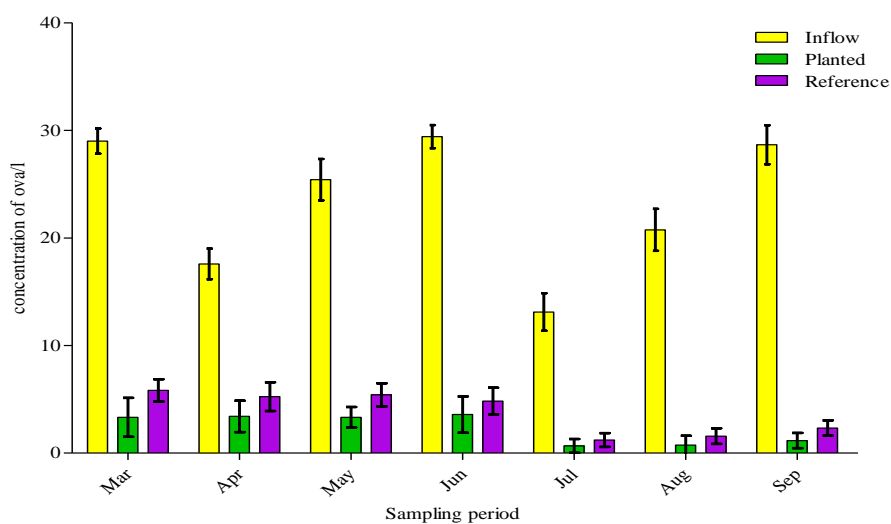


Figure 5. 7 Average concentration of ova of *Ascaris lumbricoides* after seven months of study. Whiskers represent standard deviations of 12 means.

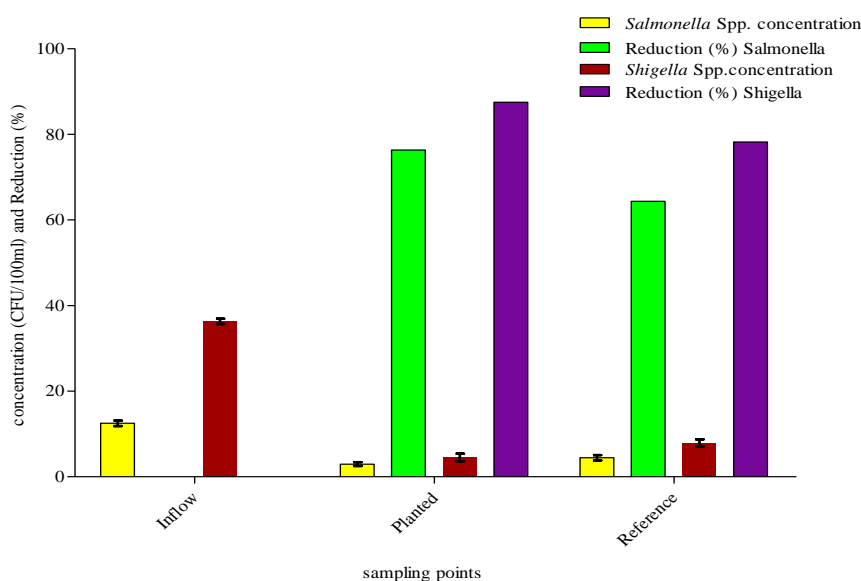


Figure 5.8 Average reduction (%) of *Salmonella* spp. and *Shigella* spp. in the sediment of the planted and reference sections of the rhizofilter. Whiskers represent standard deviations of three means.

The concentration of *Shigella* spp. was reduced by 91.8 % in the planted section and 73.6% in the reference section (Figure 5.8). The mean reduction between the various sampling points was significant (ANOVA  $p < 0.05$ ). *Salmonella* spp. was reduced by a maximum of 94 % in the planted section and 81.6% in the reference section (Figure 5.8). Average concentrations of *Salmonella* spp. in the sediment was found to be 22 CFU/100ml in the planted section and 89.66 CFU/100ml in the reference section. The removal rate was also found to be higher in the planted section which recorded a mean removal of 94.673% while the reference section saw a 78.28% removal. Variations of the calculated mean reduction of *Salmonella* between the two sections was statistically significantly (ANOVA  $p = 0.0007$ ). This was consistent with results of Vymazal, (2011) in his study of waste stabilization ponds.

The average concentrations of *E. coli* were 3-5 MPN /100ml in the planted section and 5-6 MPN /100ml in the reference section (Figure 5.9). *Escherichia coli* was

reduced by 60.95% in the reference section and 81.7% in the planted section with lowest reductions being witnessed during the month of November (Figure 5.9).

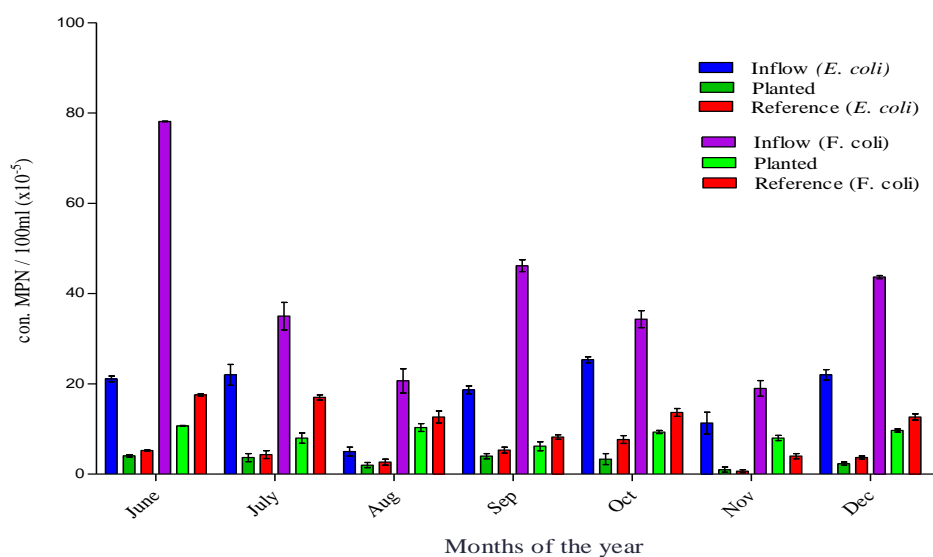


Figure 5.9 Concentration of *E. coli* and *F. coli* in the planted and reference sections of the rhizofilter. Whiskers represent standard deviations of three means.

There were variations in the concentrations of the *E. coli* and *F. coli* within the various sampling points of the rhizofiltration unit. This compares well with Reinoso *et al.*, (2008) who found corresponding results during their study on *E. coli* removal using a constructed wetland facility. They attributed the removal pattern to variations in unit design, filtration matrix, plant cover and root network development.

Table 5.2 summarizes results for totalcoliforms (FC) *Escherichia coliforms* (EC), *Salmonella* spp., *Shigella* spp. and helminth ova. The means were significantly varied ( $P \leq 0.001$ ) between the inflow and reference and between inflow and planted section when subjected to 1way ANOVA with Tukey's comparison test. Totalcoliforms were reduced by an average of 68.1% in the reference section and 80.52% on the planted section.

Table 5.2 Summary of microbial analysis of wastewater from influent and effluent of the rhizofiltration system over a period of one year.

Parameter /unit	Concentration			Maximum Removal %		Discharge limits WHO (Vivien <i>et al.</i> , 2012), (UNEP/WHO/HABITAT/WS SCC, 2004), DWAF, (1998)
	Inflow Mean $\pm$ S.D	Planted Mean $\pm$ S.D	Reference Mean $\pm$ S.D	Planted	Reference	
<i>Ascaris lumbricoides</i> (eggs/1L)	37.809 $\pm$ 2.8	6.1904 $\pm$ 2.0	11.916 $\pm$ 2.1	96.4	92.4	1 egg/kg
<i>Salmonella</i> spp. (CFU/100ml)	12.5 $\pm$ 2.06	2.95 $\pm$ 0.87*	4.46 $\pm$ 0.95*	94	96	0
<i>Shigella</i> spp. (CFU/100ml)	36.3 $\pm$ 3.82*	4.54 $\pm$ 1.27*	7.92 $\pm$ 1.56*	97.5*	86.5	0
<i>Escherichia coli</i> x 10 <sup>-5</sup> (CFU/100ml)	13.3 $\pm$ 1.37	3.25 $\pm$ 0.89	5.67 $\pm$ 1.13	98	97	0 cfu/100 ml
Total coliform x 10 <sup>-5</sup> (CFU/100ml)	80.9 $\pm$ 0.36	32.37 $\pm$ 0.86	34.5 $\pm$ 2.81	98	96	400-1000/100ml after disinfection
Coliphage (PFU/100ml)	9 $\pm$ 1.2*	2 $\pm$ 0.52*	2 $\pm$ 0.89*	96	92	Nil coliforms/100ml
<i>Candida</i> spp. x 10 <sup>-5</sup> (CFU/100ml)	18.6 $\pm$ 1.71	7.3 $\pm$ 1.32	6.1 $\pm$ 0.5	79	86	0

\*p<0.05 significance was detected.

## 5.4 DISCUSSION

One of the major concerns in the design of a wastewater treatment system is the removal of pathogenic microorganisms from the final effluent. The occurrence and removal of the selected wastewater pathogens were monitored over a period of four sampling seasons. Significant differences were noted in the quantity at influent and removal rates of the microorganisms that were detected during the various seasons. The system showed average pathogens removals between 45% and 98% on both the planted and reference sections during the various seasons as shown in Table 5.2, figures 5.2, 5.4, 5.6, 5.7, 5.8, and 5.9. These percentages were observed especially on removals of *Salmonella* spp., *Shigella* spp. and *Ascaris lumbricoides*.

The high removal percentages could also have been as a result of comparatively low pathogens load in the influent to the rhizofilter (Figures 5.3, 5.7, 5.8, 5.9). These findings concur with Calheiros *et al.*, (2010) in their study of Bacterial community dynamics in horizontal flow constructed wetlands. Temperature variations within the cold and warm seasons may have played a role in the



dynamics of removals of microbes by influencing the performance of ammonium oxidising bacteria (AOB) and nitrite oxidizing bacteria during periods of reduced oxygen levels as was also reported by He *et al.*, (2012).

Comparatively higher removal of *A. lumbricoides* (Possibly in the hatching stage – Figure 5.5) was achieved in summer due to elevated atmospheric temperatures of the substrate (Figure 5.7). During winter there was a slowdown in the treatment of organic load which may have contributed to the reduced treatment efficiency by the aerobic and anaerobic bacteria (Figure 2.9). This observation compares well with findings in a study by Garcia *et al.*, (2010).

#### 5.4.1 Pathogens Removal

According to Cai and Tong, (2013), the main pathogens removal mechanisms and processes in constructed wetlands rhizofilters include solar irradiation, sedimentation, aggregation, filtration, oxidation, predation, antibiosis and competition.

In this study, reduction of *E. coli* in the rhizosphere from 80% to > 99% may have been achieved through exposition to antibacterial agents found in the root extracts from the macrophytes and natural die off (Figure 5.1). This relationship was found to relate favourably to reports from a study by Hench *et al.*, (2003). There was some correlation ( $P \leq 0.001$ ) between the increase in the levels of coliforms and increase in the levels of COD, BOD (Figure 2.9) and total suspended solids when subjected to Dunnett's multiple comparison tests. This could be attributed to the increase in organic load into the rhizofilter through the influent. This observation is in agreement with reports in studies by Kayyali and Jamrah, (1999) and Solano *et al.*, (2004).

Similarly, there was some positive correlation between the concentration of *E. coli*, temperature and coliphage (Figure 5.9). For example, lower temperatures recorded low removal percentages of *E. coli* and coliphages which agrees with reports in studies by Kayyali and Jamrah, (1999) and Jurzik *et al.*, (2010). The constructed rhizofilter effectively reduced the levels of totalcoli and *E. coli* by 65%-85% which

is the range reported by most studies on the performance of constructed wetland systems for wastewater treatment in agreement with a report by Zhang *et al.*, (2014a). In this study, however, the percentage reduction of both *E. coli* and total coliforms did not meet the WHO guidelines for discharge of effluents (Table 5.2).

Figure 10 shows the variations in the concentration (MPN/100mlx10<sup>5</sup>) of *E. coli* in the influent and effluent of the planted and reference sections during the warm months. The variations were noted from 24 x 10<sup>5</sup> cfu/100ml in the influent to 1.0x10<sup>5</sup> in the planted and 2.0x10<sup>5</sup> in the reference section. These values translate to 93% ± 3.1 removals from the planted section to an average 88%± 3.3 removals from the reference section. One – way analysis of variance showed that the means were significantly different (P<0.0001) indicating that the planted section removed more *E. coli* than the reference section as shown in figure 5.9 in concurrence with studies by Sleytr *et al.*, (2009), This also confirms that the macrophytes played a key role in the removal of the *E. coli*.

Removal of *E. coli* might have been achieved through the biophysical processes and effects of solar radiation, filtration and adsorption of microorganisms onto root-substrate complexes and the associated biofilm (Figure 5.9). This result compares well with studies by Sleytr *et al.*, (2009) and Jagals *et al.*, (2013).

In this study, the batch hydraulic loading design which was applied at 2000 litres per batch was found to be optimum for the pilot system. Optimum retention period as reported by McCarthy *et al.*, (2011) is 20 days but in this study, the 6 hour retention period was found to effectively remove pollutants to some acceptable levels as shown in the overall recorded results.

Design parameters of the rhizofiltration unit (subsurface constructed system) may have been responsible for the significant removal of pathogens such as coliphages since they were potentially retained in the sediment/substrate (Figure 5.2). This compares well with results from a study by Zhang *et al.*, (2014a), that significant reduction of the coliphages is achieved through factors relating to the design of the rhizofilter.

The intensity of Sunlight/ solar radiation on the filter matrix has some bactericidal effect on certain microorganisms including *Salmonella* spp. Some bacteria are known to survive well under high-temperature conditions according to a study by Boutilier *et al.*, (2011), Garcia *et al.*, (2012) and Mthembu *et al.*, (2013). In this study, the temperature rise due to sun rays on the exposed reference section of the rhizofilter may have contributed to the multiplication of or higher survival rates for *Salmonella* spp. which may be the reason higher levels were isolated from the reference section (Table 5.2). *Shigella* spp. may have been removed through physical processes such as filtration, attachment on the plant root structure or natural die off (Figure 5.8).

#### 5.4.2 Rhizofiltration macrophytes

Macrophytes play a key role in pollutant removal through complex mechanisms and the root network within the wetland rhizosphere (Garcia *et al.*, 2010). Removal mechanisms are based on the type and concentration of the pollutant, the season of the year, the root network and growth patterns as reported by Tanner and Headley, (2011).

In this study, pathogens such as *E. coli* may have been removed by the antibacterial action of the root exudates from *K. nemoralis* and *P. australis* (table 5.1), after the two plants displayed zones of inhibition of growth of *E. coli* inhibition at  $9.97 \pm 0.19$  mm and  $8.63 \pm 0.22$  mm at  $37 \pm 2^\circ\text{C}$  respectively. Since *K. nemoralis* had a wider zone, it can be concluded that it has a higher potential to remove *E. coli* than *P. australis*. This is an indicator these plants exhibit some in - vitro antimicrobial characteristics. Results also indicate that the zone of inhibition was dependent on the type of the extract that was used for both plants (Table 5.1). Ethanol extract may have dissolved significant portions of the ingredient that is bioactive in the plants than the aqueous extracts. Larger inhibition zones were witnessed in the summer season when temperatures were comparatively higher. This observation seems to explain why high removal of pathogens especially *E. coli* was witnessed

majorly during the hot months. These results compare well with those of Abeysinghe, (2010) and Duhan *et al.*, 2013 ). The slightly elevated levels of antibacterial potential by hot aqueous extracts confirm that a large percentage of the bio-active element was obtained at elevated temperatures (Figure 5.1). This phenomenon further explains the reason for high treatment efficiency during summer months when it is assumed there was a high availability of extracts for phytoremediation (Figure 5.9). This observation is in agreement with what Ogu *et al.*, (2012) reported in his study of antimicrobial potential in *Cyathula prostrate* in which ethanol extract yielded the highest inhibition zone. The root of the two wetland plants displayed larger zones of inhibition than the stem and leaves. This also is an indicator that more of the bioactive components were present in the root sections of the plant. Similarly, highest inhibition zone of  $12.17 \pm 1.17$  mm was seen on the streptomycin against the plant tissues since this was an antibiotic in its pure state. Similar observations were made after a study by El-Mahmood *et al.*, (2008). Further research calls for investigation of growth patterns for *E. coli*, *Salmonella* spp., *Shigella* spp. and other pathogens at varied temperatures as may be optimum for the rhizofilter when subjected to extracts of the two plants.

Mechanisms involved in the removal of helminths, bacteria, coliphages and yeast in this study were possibly sedimentation since a high percentage removal was seen on the extract from sediment. A study by Wu *et al.*, (2014) reported similar observations. In rhizofiltration systems, accumulation of large amounts of coliforms has been reported to occur in the substrate and also river mud has been found to contain about 100-1000 times more faecal coliforms than the surface water above it according to Singh *et al.*, (2014). About 90% of *Salmonella* spp. accumulated in the sediment (Figure 5.8) of the rhizofiltration system. This may imply that removal of *Salmonella* spp. was supported by sedimentation, accumulation and adsorption onto the filtration matrix. A similar scenario was also witnessed in a study by Ogu *et al.*, (2012). This study also reports that the highest percentage (94%) removal of *Salmonella* spp. was found in the sediment in the planted section against 74% in the reference section as the conditions in sediment give the pathogens longer survival rates (Figure 5.8). *Shigella* spp. was potentially removed by (1) sedimentation onto

the rhizofiltration substrate. (2) Attachment to colloidal matter. The planted section of the rhizofilter performed better in the removal of *Shigella* spp. than the reference section. Enteric viruses are reported to attach themselves to colloidal material which eventually settles down on the sediment. This is possibly the main mechanism by which the coliphages may have been removed from the wastewater. According to a study by Karim *et al.*, (2004), when the underlying sediment is disturbed, possibly by some human or natural action such as storm, the pathogens may find a re-entry path to the water. This observation seems to explain why there were raised levels of *Ascaris lumbricoides* in September due to runoff occasioned by high rainfall at that time (Figure 5.6). (3) Root structure. The root structure of wetland plants also filters out a number of pathogenic microorganisms. For example, in this study, the network and intertwined formation of the root of *K. nemoralis* potentially played a major role in filtering out eggs of helminths and thus their removal from the wastewater. *Candida* species (Figure 5.3) may have been attached to the root structure which reduced their concentration in the planted section of the rhizofilter, and therefore their subsequent removal as shown in figure 5.4. (4) Predation. *Escherichia coli* may also have been removed through predation by the protozoa (Figure 5.9) within the oxygen-rich zones of the rhizofilter especially in the planted section that experienced comparatively increased dissolved oxygen levels than the reference section. This observation was in agreement with reports from a study by Saeed *et al.*, (2014). High pollutant removals as seen in this study may have been as a result of comparatively low pollutant load in the influent, the batch loading of 2000 l/d and the size of the rhizofiltration system as mentioned in chapter two.

Thus the rhizofiltration system was found to be effective in removal of pathogens. The planted section experienced higher removal capacity than the reference section. Also, *K. nemoralis* was found to potentially remove more pollutants than *P. australis*.

## 5.6 CONCLUSIONS AND RECOMMENDATIONS

- The constructed rhizofiltration system with vertical flow system had a high potential to eliminate pathogenic microorganisms (Table 5.2). Thus, the presence of macrophytes in the system contributed to the reduction of the pollutants.
- The evolution of CWs rhizofiltration systems designed to remove specific pollutants brings hope into the wastewater treatment systems that have experienced the major challenges, especially in the developing world today.
- The rhizofiltration system for treatment of municipal wastewater removed quite significant amounts of organic and pathogenic microorganisms, though these reductions were not enough to render the final effluent suitable for reuse.
- Design of the rhizofilter had a direct effect on the efficiency of pollutant removal by the microorganisms, the rate of decay and filtration effects.
- Total suspended solids (SS) was not efficiently reduced by the system possibly due to design limitations on the rhizofiltration unit. A much better system in terms of SS reduction would require an additional settling tank for the inflow before releasing water into the rhizofilter.
- Microorganisms including indicator bacteria were effectively removed from the rhizofiltration system despite the short intervals of batch loading.
- Higher pollutant removal efficiencies are thus envisaged with low influent loading rates.
- The abundance of helminth eggs in the sediment confirms that the matrix played a major role in media removal and retention of the eggs.
- Pathogens removal was higher on the planted section as compared to the reference section of the rhizofilter as seen in table 5.2. thus confirming the effectiveness of the rhizofilter, planted section and macrophytes in pollutant removal.
- When considering indicator organisms as a measure of pollution levels, much emphasis should be laid on the helminth eggs as potential indicators alongside *Escherichia coliforms*. This is because coliforms tend to re-grow again when subjected to favourable conditions even after disinfection of the effluent.

- It is recommended that the final effluent be subjected to chlorination and also the design of wetlands should include multiple sedimentation tanks before subjecting the wastewater into the wetland for biological, physical and chemical treatment.

## CHAPTER 6

### 6.1 CONCLUSIONS

The rhizofiltration system that was used in this study was weeded once in a month to remove opportunistic macrophytes in reference to a study by Srivastava *et al.*, (2016). Whenever sampling was done, the plants also received water for their growth requirements. For these reasons, the rhizofiltration system was found to be a cheap and efficient treatment facility for wastewater. The system proved to be self-sustaining and pollutant removal may have been achieved through physical, biological and chemical processes.

### 6.2 PHYSICAL AND CHEMICAL REMOVAL PROCESSES

Physico-chemical parameters were possibly reduced or stabilised through filtration and sedimentation facilitated by the plant roots within the rhizosphere. Some of the results obtained for the various parameters were influenced by the flow rate of the influent and effluent from the rhizofiltration system in agreement with reports from a study by Vymazal and Kropfelova, (2005). This was occasioned by the pumps location at the sump of the raw wastewater collection point. The rate of flow from the sump into the Jojo tanks was influenced by the viscosity of the wastewater. This situation created some head over the filter material which assisted in the establishment of a constant head for the sampling at the various points (Figure 2.6). In this study, attempts to prevent channelling was done by application of layers of sand and gravel in reference to a study by Huang *et al.*, 2010.

The results obtained for the physicochemical parameters may have been influenced by the high temperatures and high rainfall experienced in this Subtropical type of climate.

According to figure 2.7 significant variations in temperature are seen in the influent, planted and reference sections in the rhizofilter. The temperature rise in the influent may have resulted from storage in the black Jojo tanks during the wastewater pre-settling. The decrease in temperature within the rhizofilter and the



effluent may have been a result of interaction with the cool filtration matrix, the presence of water and microclimate caused by the macrophyte root network in the rhizofilter. This result compares well with reports from a study by Vymazal, (2011). The average inflow concentration of DO in the rhizofilter was 6.9 mg/l, and was noted to vary compared to the more stable DO levels at the outlet, which was 7.7 mg/l on average. The rise in DO could be attributed to raised air-water interface from the inflow and available organic matter in the rhizosphere. The decrease in dissolved oxygen levels in the rhizofilter could be attributed to some contaminants that may have been in the composted manure that was added into the system to aid in acclimatizing the system. This contributed to the growth of microorganisms which played a major role in the breakdown of organic matter. Similar observations were reported by Bouasria *et al.*, (2012) and Srivastava *et al.*, (2016). The system recorded an increase in DO in samples from the planted section than the reference section.

The warmer seasons recorded the highest electrical conductivity levels while the lowest levels were recorded during the cold seasons. This rise could be attributed to the addition of the compost that was added into the system to aid in macrophyte growth. Elevated levels of EC could be attributed to the contamination brought by the compost that was added into the system to aid macrophyte growth. The planted section recorded higher COD reduction than the reference section of the rhizofilter with a statistically significant difference ( $p < 0.05$ ) especially during the period of system establishment (Figure 2.9).

Turbidity and suspended solids were reduced by sedimentation and filtration of organic matter present in the rhizosphere. The initial increase in TDS within the rhizosphere may have been caused by the fine sand particles which were attached to plant roots. This compares well with reports from a study by He *et al.*, (2012).

The pattern of removal of phosphate by the rhizofiltration system is represented in figure 2.22. the highest removal was seen during the month of September. There were significant variations in removals between the dry and rainy seasons ( $p = 0.02$ ) and consequently between the reference and planted sections of the rhizofilter ( $p = 0.007$ ). Removal of nitrates was highest during the month of June which was the peak of the rainy season (Figure 2.21), while ammonia removal was highest

during the months of May, June and July (Figure 2.23). Ammonia removal could be attributed to the fact that significant amount of ammonia may have been converted to nitrite and further to nitrate which may have been absorbed by the plants in the planted section of the rhizofilter. Reduction in the levels of ammonia in the system during the subsequent months is indicative of efficiently functioning system.

### 6.3 HEAVY METALS REMOVAL PROCESS

Sources of heavy metals, their effects on the environment and conventional treatment methods were reviewed in this study. Metals removal, for example, cadmium, could have been achieved through filtration by the rhizofiltration matrix, plant uptake by absorption, adsorption onto plant root system and chemical transformation from one state to another. Some metal ions may have coalesced together with suspended organic matter and settled on the sediment. This was evidenced by the comparatively raised levels of metals in the sediment (Figure 4.4). Similar findings were reported in a study by Lee *et al.*, (2014).

Another metals removal mechanism may have been through absorption by the wetland plants and the interaction of metal ions with the filtration sediment and plant roots. The inorganic matter within the rhizosphere acted as biosolvents for the metal ions exchange thereby relocating or attaching them to the microorganisms and the plant tissues.

The high levels of zinc on plant roots occurred possibly due to the co-precipitation of zinc with manganese and iron oxides forming iron plaques which easily attached on the roots of plants. Metal ions within the rhizosphere could have formed complexes with organic matter leading to sedimentation, and precipitation of metal compounds through chemisorption. Chibuike and Obiora, (2014), in their study also observed and reported that metal ions form complexes with organic matter.

Copper and chromium may also have been removed by chemical bonding leading to sedimentation and adsorption onto the sediment. Chromium may have been removed by changing their oxidative state from the hexavalent to the trivalent form

(plant nutrient form) which accumulated and settled in the rhizosphere. Metals (copper and lead) occasionally form unstable metal carbonates within wetlands. This could have been the trapping mechanism for these metal ions. Lead ions (Pb) and nickel (Ni) were possibly removed from the wastewater by this mechanism. Similar observations were made in a study by Mthembu *et al.*, (2013).

Heavy metal accumulation in plant tissues was not confined to a particular part of the plant but was found to be distributed between the roots, stem and leaves of *K. nemoralis* and *P. australis*. Zinc, for example, may have been readily absorbed by plant tissues while Cu was absorbed or attached to sediment. The optimum removal pH was found to be between pH 3 – pH 6 while adsorption equilibrium was achieved after three days (Figure 4.4).

Metal ionic forms depend on many factors such as pH, the presence of organic matter, ionic strength and redox potential. When organic matter is low, the cationic forms diffuse across the cell wall of the macrophyte tissues and bind to carboxyl and hydroxyl groups. In general, macrophytes possess epidermal hairs which are responsible for absorption of ions from the surrounding water. Some of these hairs accumulate metal ions in their stems and leaves. Passive water movement and interaction with metal ions in the aqueous phase through the cracks found in the cuticle or through the stomata and cell wall of the macrophytes may form other removal mechanisms that reduced metal ions within the rhizofilter. These ions were possibly attached to the active sites on the surfaces of the macrophyte tissues and sediment. Further removal could have been through the plasmalemma and the phytochelatin cells. This observation was also reported from a study by Putra *et al.*, (2014). The complex root network of the macrophytes possibly translocated oxygen from the aerial plant parts to the rhizosphere through the aerenchyma cells. This oxidation process possibly promoted the precipitation hydroxides of metal ions. Lee *et al.*, (2013) reported similar findings in his study. When plant root exudates such as phenolics are released by decaying cells, they change the metal status and speciation causing protons to be released. The release of protons caused acidification of the rhizosphere which may have promoted the transport of metal

ions and enhanced their bioavailability. The concentration of metal ions in the plant tissues was distributed in the order root > leaf > stem in agreement with a study by Putra *et al.*, (2014).

*Kyllinga nemoralis* was found to absorb higher amounts of the metal ions in the roots than in the roots of *P. australis* as shown in figure 3.2, 3.4, 4.10. However, distribution of the ions into other plant parts was varied between the two plants. For example, slightly more ions were absorbed in the stem of *P. australis* than the stem of *K. nemoralis* as seen in figure 3.6. The planted section of the rhizofilter was found to remove/ accumulate comparatively higher amounts of metal ions than the reference section as seen in figure 2.17.

#### 6.4 PATHOGENS REMOVAL PROCESS

The efficiency of the pollutant removal improved when the system achieved fully functional stage at about 80% plant cover as seen in appendix 1. The planted section removed comparatively more pathogens than the reference section. This phenomenon proved that the macrophytes played a major role in the removal and reduction of pathogens in the system (Figure 5.8). This concurs with the findings of a study by Vymazal, (2011). *Kyllinga nemoralis*. was found to have removed more pollutants than *P. australis*. This may have occurred due to the presence of fibrous root network of *K. nemoralis* as seen in appendix 4. The design and construction of the rhizofilter (allowing vertical flow) also optimized the pathogens removal processes. The aeration channels as shown in appendix 3 potentially allowed more air carrying extra oxygen to penetrate the filtration matrix. This may have contributed to enhanced biodegradation of the organic pollutants. The pre-settlement of the raw sewage in Jojo tank 1 (5,000 L) then overflow to Jojo tank 2 (10,000 L) allowed for removal of some pollutants through settling and sedimentation in both tanks (Appendix 2). Pathogens may have been removed from the rhizofilter through chemical, physical and microbiological processes. The physical mechanisms that may have positively removed pathogens from the rhizofilter include adsorption of bacteria onto rhizospheric surfaces, temperature,

aggregate formation, filtration and sedimentation. The immobilization of microbial cells in the system was positively influenced by adsorption onto the porous media in the rhizofiltration system and the physical straining and sedimentation through the rhizofiltration substrate. This observation was also reported in a study by Achenbach *et al.*, (2013).

Major factors that may have affected the straining process in the rhizosphere were the bacterial size, media particle size, the rate of hydraulic loading of the system and a head loss potentially caused by system clogging and channelling of effluent which may have been a result of the design and construction of the rhizofiltration unit. Another factor that may have contributed to the straining process in the rhizofilter may have been the type of macrophyte that was used in the treatment process. To note is that the study used *K. nemoralis* as a treatment macrophyte and from literature, this plant has not been used in any other study for this kind of process. Pollutant removal through adsorption on media was also influenced by biofilm layer development and antibiosis, organic matter content and electrostatic charges between the strength of ions of the solution and the particles of the rhizofiltration matrix (Srivastava *et al.*, 2016). Faecal coliforms have a high adsorption tendency under equilibrium conditions. The coliforms removal may have occurred through adsorption onto the rhizofiltration matrix and plant roots. The moisture content in the matrix and temperature fluctuations influenced bacterial survival as significant die-off was witnessed during the months of summer when the moisture content in the matrix was low (Table 5.1) and root exudates from the macrophytes were concentrated. However, lower reduction rates of bacteria were mainly affected by sedimentation on the matrix and vegetation cover. This phenomenon removed the bacteria from affluent but retained it on the matrix in agreement with Boutilier *et al.*, (2011).

Oxygenation of the rhizosphere through the macrophytes root network played a key role in the elimination of bacteria within the first 30 cm of matrix layer since the microbial community is mainly concentrated between 0-10 cm. This reflected a balanced supply of nutrients for the growth, symbiosis and predation by free-living protozoa, mainly ciliates within the microbial community in the rhizosphere.

Similar observations were made from a study by Abbadi *et al.*, (2012). Hydraulic retention period in constructed wetlands is reported to play an important role in pathogens reduction. In this study, long water retention periods was not achieved because the system was designed for batch experiments and could not hold water for long periods of time due to leakages observed on the structure when it was first commissioned for the commencement of the study. The maximum retention time obtained in this study was 6 hours. The optimum retention period according to McCarthy *et al.*, (2011) is 20 days. However, the minimum retention period for this study yielded significant pollutant removals. With this observation, it can be concluded that the constructed rhizofilter has a high potential to remove pollutants from wastewater.

Solar radiation especially on the reference section of the rhizofilter enhanced removal of bacteria especially the *Salmonella* spp. that was significantly removed during the summer months (Figure 5.8).

## 6.5 ROLE OF MACROPHYTES IN METALS AND PATHOGENS REMOVAL

In this study, heavy metals removal was primarily through adsorption, absorption and the interaction with microorganisms and wetland plants/sediment while pathogens were removed by physical, chemical and biological mechanisms such as filtration, die-offs, chemical emissions from plant roots and adsorption by the biofilm. Aguila and Woods (2014), reported similar observations in their study on cell nutrition.

The macrophytes of study, *Kyllinga nemoralis*. and *Phragmites australis*, proved to be potential biosorbents and were also able to remove some specific pathogens such as *E. coli* from the wastewater. Selection of the most appropriate macrophyte for systems efficiency remained a challenge but considering the study by Vu *et al.*, (2016) the antimicrobial characteristics of the two plants contributed to the decision to choose them as the plants of study. This was seen through the growth inhibition displayed by the root extracts from the plants (Table 5.1). The root system of *K. nemoralis* may have enhanced the filtration of pathogens through the rhizosphere.

However, a comparatively higher percentage of *Candida* spp. was removed from the reference section than the planted section (Figure 5.4). This suggests that the removal mechanism of *candida* spp. was possibly through filtration by the matrix and not the macrophytes.

The plants also stabilized the filter matrix and reduced erosion of the substrate which would otherwise have eroded down with the speed of water flow. The vegetation cover of the planted section prevented the growth of algae which would have hampered the treatment process by reducing microbial activity within the root zone.

There was minimal system clogging due to the extensive root network of the macrophytes which also played a role in the reduction of turbidity (Figure 2.13). The plants also influenced the macroclimate experienced in the rhizofilter. This was seen in the reduction and stabilization of the temperature during the study. A similar report was given in a study by Abdel-Raouf *et al.*, (2012). Stabilization of Alkalinity could have been partially through evaporation from the plants.

The plants also provided the rhizosphere effect which enhanced the growth of microorganisms responsible for metals and pathogen removal. Comparatively higher removal efficiencies were seen on the *K. nemoralis* against *P. australis*. This was evidenced by the metals removal efficiencies as seen in figure 4.10. The high removal efficiency displayed by *K. nemoralis* against *P. australis* may have been influenced by extensive root network as displayed by *K. nemoralis*.

The relatively new Rhizofiltration technology in the form of constructed wetlands may be used as on-site, secondary wastewater treatment facility adjacent to the main conventional treatment systems so that it remedies the final effluent through the removal of residual metal ions and pathogens.

Successful and efficient pollutant removal as witnessed in this study supports the use of rhizofiltration technology as an efficient, cost-effective and environmentally friendly treatment facility. The system design which included controlled flow regimes, well- distributed filter matrices and macrophytes positively influenced the removal of the pollutants.

Wetland plants *K. nemoralis* and *P. australis* accumulated metals in their tissues and could potentially be used as wastewater treatment macrophytes despite final disposal and management of used wetland plants (having a significant amount of absorbed metal ions) would be a challenge to all authorities. The macrophytes played a major role in pollutants removal through their well-developed rhizosphere which enhanced the supply of oxygen into the system.. This aerated the environment in favour of protozoa population which possibly formed bacterial predators. *K. nemoralis* and *P. australis* were the macrophytes of choice since they were easily available in the environs of the study site and also due to their root development which forms horizontal rhizomes that easily penetrated the filter depth. A similar observation was reported by Vymazal and Kropfelova, (2011). Consequently, the filter media played a major role in the removal of solids while the thick intertwined macrophytes root systems contributed to the filtration of the pollutants while maintaining the permeability of the wastewater. The planted section of the rhizofilter performed better than the reference section on pollutants removal.

This study successfully performed a maiden investigation of *K. nemoralis* as a biosorbent. This study reveals that apart from treating various animal and human ailments, extracts from plants can also be used to treat wastewater. A conclusion can also be drawn to the effect that *K. nemoralis* has a huge potential of bioaccumulation and should be considered a wastewater treatment macrophyte in constructed wetlands.

## 6.6 RECOMMENDATIONS

- This study recommends that pilot plants of rhizofilters should be established in institutions of higher learning to study the viability in wastewater treatment. This may involve the use of alternative macrophytes, filtration material, varied design characteristics in regard to population size. National, local administration and institutions of higher learning are also encouraged to promote and support studies on wastewater treatment and management using phytoremediation.



Further studies to determine the water balance of wetland macrophytes such as *K. nemoralis* and *P. australis* (their hydraulic kinetics and growth patterns) needs to be carried out in order to determine pollutant removals in relation to evapotranspiration. It still remains unclear whether the final effluent according to this study can be used for irrigation of food crops since some parameters tested (*E. coli* and *F. coli*) did not meet the accepted discharge limits. Results from this study are a clear demonstration that the leaf, stem and root extracts of *K. nemoralis* and *P. australis* exhibit antimicrobial properties and can be used for phytoremediation of wastewater using constructed wetland rhizofilters.

Further research on the potential of rhizofilters in the treatment of effluents from large manufacturing sectors such as battery manufacturing, textile industry, tea and oil processing is recommended. This should involve seasonal variations in their treatment performance under determined optimum conditions. Desorption of treatment plants containing metal ions remains a major challenge which, when well investigated and managed, will promote the use of constructed rhizofiltration systems as a holistic efficient wastewater treatment option. The planted section of the rhizofilter witnessed higher pollutant removals than the reference section as seen in Figure 2.17 and table 5.2. However, rhizofiltration technology may be suited for small communities with primary treatment facilities as it requires pre-treatment of the raw wastewater in order to achieve effective treatment.

- Further research may involve the investigation of the metals binding sites, whether the ions were bound to the adsorbent sites through film formation or by intra particle diffusion. In connection with this, studies on adsorbent metal desorption are encouraged, as this is likely to yield a solution to environmentally safe adsorbent disposal.

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## APPENDICES

Appendix 1. Fully functional rhizofiltration unit constructed at Kingsburgh conventional wastewater treatment plant.



Appendix 2. Raw sewage settling tanks. The raw sewage was first pumped into the 5000L settling tank sitting on the brick slab. The settled sewage then flowed to the adjacent 10,000L tank through the top overflow channel (arrowed).

Overflow channel



Appendix 3. Effluent sampling points placed on opposite sides of the rhizofiltration unit.





Appendix 4. The fibrous root network of *Kyllinga nemoralis*. One of the treatment macrophytes used in this study.



Appendix 5. National WATER ACT waste discharge standards for selected parameters. DWA 2010.

<b>Variables and substance</b>	<b>Existing General Standards</b>	<b>All Discharges</b>
pH	Between 5.5 and 9.5	5.5 and 7.5
Chemical Oxygen Demand	75mg/l	65mg/l
Nitrate as (N)	15	15mg/l
Total Cadmium (as Cd)	0.005	0.001 mg/l
Total Chromium 111 (as Cr III)	-	0.11 mg/l
Total Copper (as Cu)	0.01	0.002 mg/l
Total Lead (as Pb)	0.01	0.009 mg/l
Total Zinc (as Zn)	0.1 mg/l	0.05 mg/l
Faecal coliforms per 100ml	1000 CFU/100ml	1000 CFU/100ml

## Appendix 6. Publications from this study.

World Academy of Science, Engineering and Technology  
International Journal of Civil and Environmental Engineering Vol:3, No:4, 2016

### Removal of Heavy Metals from Municipal Wastewater Using Constructed Rhizofiltration System

**Authors :** Christine A. Odinga, G. Sanjay, M. Mathew, S. Gupta, F. M. Swalaha, F. A. O. Otieno, F. Bux

**Abstract :** Wastewater discharged from municipal treatment plants contain an amalgamation of trace metals. The presence of metal pollutants in wastewater poses a huge challenge to the choice and applications of the preferred treatment method. Conventional treatment methods are inefficient in the removal of trace metals due to their design approach. This study evaluated the treatment performance of a constructed rhizofiltration system in the removal of heavy metals from municipal wastewater. The study was conducted at an eThekweni municipal wastewater treatment plant in Kingsburgh - Durban in the province of KwaZulu-Natal. The construction details of the pilot-scale rhizofiltration unit included three different layers of substrate consisting of medium stones, coarse gravel and fine sand. The system had one section planted with *Phragmites australis* L. and *Kyllinga nemoralis* L. while the other section was unplanted and acted as the control. Influent, effluent and sediment from the system were sampled and assessed for the presence of and removal of selected trace heavy metals using standard methods. Efficiency of metals removal was established by gauging the transfer of metals into leaves, roots and stem of the plants by calculations based on standard statistical packages. The Langmuir model was used to assess the heavy metal adsorption mechanisms of the plants. Heavy metals were accumulated in the entire rhizofiltration system at varying percentages of 96.69% on planted and 48.98% on control side for cadmium. Chromium was 81% and 24%, Copper was 23.4% and 1.1%, Nickel was 72% and 46.5, Lead was 63% and 31%, while Zinc was 76% and 84% on the on the water and sediment of the planted and control sides of the rhizofilter respectively. The decrease in metal adsorption efficiencies on the planted side followed the pattern of Cd>Cr>Zn>Ni>Pb>Cu and Ni>Cd>Pb>Cr>Cu>Zn on the control side. Confirmatory analysis using Electron Scanning Microscopy revealed that higher amounts of metals was deposited in the root system with values ranging from 0.015mg/kg (Cr), 0.250 (Cu), 0.030 (Pb) for *P. australis*, and 0.055mg/kg (Cr), 0.470mg/kg (Cu) and 0.210mg/kg (Pb) for *K. nemoralis* respectively. The system was found to be efficient in removing and reducing metals from wastewater and further research is necessary to establish the immediate mechanisms that the plants display in order to achieve these reductions.

**Keywords :** wastewater treatment, *Phragmites australis* L., *Kyllinga nemoralis* L., heavy metals, pathogens, rhizofiltration

**Conference Title :** ICEET 2016 : 18th International Conference on Civil, Environmental Engineering and Technology

**Conference Location :** Los Angeles, USA

**Conference Dates :** April 05-06, 2016

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## Investigating the efficiency of constructed wetlands in the removal of heavy metals and enteric pathogens from wastewater

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Constructed wetlands (CWs) are low-cost, eco-technologically engineered systems, used for sustainable and efficient treatment of wastewater. These systems are popular due to their high pollutant removal efficiencies and low-management costs. CWs reduce pollution loads by removing suspended matter, organic material, heavy metals and enteric pathogens from wastewater through their expansive rhizosphere and the filtration matrix. Previous reports indicate reductions by 71.2% for biochemical oxygen demand, 75.1% chemical oxygen demand, 24.5% total phosphates, 87.3% suspended solids and 23.5% total nitrogen. Heavy metals removal has been reported at 42% for manganese, 75–99% for cadmium, 26% for lead, 75.9% for silver and 66.7% for zinc. Heavy metals are removed by adsorption and absorption into the filtration matrix and the leaves, shoots and rhizomes of the wetland plants. CWs are also able to remove 97.6% of protozoan pathogens such as *Cryptosporidium parvum* 94.8% of *Giardia lamblia* and 52% and 99.9% of *Escherichia coli*. In order to appreciate the current intense research on the use and management of CWs for wastewater treatment, a detailed review of previous work on these systems was done. The focus was on the different design techniques, choice of macrophytes, treatment efficiencies, applications and models applied to the technology.

**Keywords:** constructed wetlands; wastewater; phytoremediation; pathogens; heavy metals

### 1. Introduction

Population increase, increased demand for potable water and the trend in industrial revolution have led to environmental degradation especially by the release of partially treated or untreated wastewater into aquatic systems. Reports from recent research indicate that conventional biological treatment systems are labour intensive and prone to system failure and the addition of residual pollutants such as sludge to the environment.[1–3] Conventional systems, depending on flow rates, are also known to be inefficient in retaining large amounts of suspended solids (SS), a situation which eventually lowers their overall treatment efficiency.[4–6]

In the developing countries, there are limited wastewater treatment and recycling facilities due to high maintenance costs and ineffective water policies management strategies. Unsustainable use of fresh water resources, such as unplanned settlements in urban localities, industrialization, modern agricultural practices and forest destruction, have led to increased water demand and pollution of surface and ground water reserves.[5] Therefore, there is need to conserve the available water resources and recycle wastewater in order to reduce the water stress in areas that are experiencing water scarcity.

Conventional wastewater treatment methods such as activated sludge processes and trickling filters [7,8] are cost-effective in chemical and energy supply and have potential safety hazards mainly associated with chemical handling, delivery, operation and by-products associated with disinfection. Conventional systems are also reported to be less efficient in the removal of pathogens and parasites as compared with CWs and usually require additional treatment through the addition of disinfectants such as chlorine, ozone and ultraviolet radiation in order to achieve acceptable discharge limits.[7,9] Stabilization ponds which are cost-effective in installation and management are systems that have also been successfully used for wastewater treatment in developing countries.[10] The stabilization ponds, however, cannot eliminate pollutants such as heavy metals and pathogens, which is the goal to efficient wastewater treatment in developed countries. Another treatment system that can achieve maximum wastewater treatment is wetlands (natural or constructed) technology. Wetlands are known to achieve better treatment of wastewater, including the removal of heavy metals, SS and wastewater pathogens, unlike the conventional treatment systems which are reported to only partially remove the above pollutants.[10]

Wetlands are areas where many biological transformations driven by soil, water, wind, natural energies,

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Microorganisms

## 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  $86.1 \pm 0.6\%$  than when the section was planted with one plant as well as the reference section  $12.6 \pm 0.4\%$ . 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  $46\% \pm 0.9$  than in the reference section  $40 \pm 0.2\%$  ( $P=0.01$ ). 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 and were analyzed for phosphate, orthophosphate, ammonia, nitrate and nitrite using spectrophotometric methods. For the analysis of nitrogen and phosphorus content in plant biomass, roots, stems and leaves were harvested and analyzed using automated combustion method and tri-acid digestion and ascorbic



## 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 cause 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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**From:** Ram Bharagava [mailto:bharagavadem.bbau@gmail.com]  
**Sent:** Sunday, 12 November 2017 4:41 AM  
**To:** Mathews Simon Mthembu <MthembuMS@unizulu.ac.za>  
**Subject:** Re: Invitation for contribution of Book Chapter in Edited Book

Consent to Publish Form: Bioremediation of Industrial Wastes for Environmental Safety

**Respected Researcher,**

A very good morning to you and your co-authors and I'm very sorry for delay reply.

I'm writing to you regarding the book chapter entitled: **Constructed treatment wetlands: An emerging phytotechnology for degradation and detoxification of industrial wastewaters** written by Mthembu MS<sup>1\*</sup>, Odinga CA<sup>2</sup>, Bux F<sup>2</sup> and Swalaha FM<sup>2</sup> has been accepted for publication in the edited book entitled: **"Bioremediation of Industrial Wastes for Environmental Safety"** which has been contracted and signed with one of the world leading publisher **"Springer Nature, Singapore"** and ahead of publication.

I'm happy to inform you that the book will be published in two edited volumes i.e. **Volume I & II** as stated below:

**1. Gaurav Saxena and Ram Naresh Bharagava (Eds.): Bioremediation of Industrial Waste for Environmental Safety - Volume I: Industrial waste and its management**

**2. Ram Naresh Bharagava and Gaurav Saxena (Eds.): Bioremediation of Industrial Waste for Environmental Safety - Volume II: Biological agents and methods for industrial waste management**

It is informed to you that both the volumes of the book will be published at the same time and your **"Book Chapter"** will be published in **"Volume II"**.

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With my kindest regards,

**Dated:** November 12, 2017

**Dr. Ram Naresh Bharagava**  
 (Corresponding Editor)

Manuscript Submitted

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**Rhizofiltration system consisting of *Phragmites australis* and *Kyllinga nemoralis***  
**efficiently removes metals and pathogenic microorganisms from wastewater**  
 --Manuscript Draft--

Manuscript Number:	
Full Title:	Rhizofiltration system consisting of <i>Phragmites australis</i> and <i>Kyllinga nemoralis</i> efficiently removes metals and pathogenic microorganisms from wastewater
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Abstract:	A rhizofiltration system planted with <i>Phragmites australis</i> L. and <i>Kyllinga nemoralis</i> L. was constructed at eThekweni municipal wastewater treatment plant in KwaZulu-Natal, Republic of South Africa and evaluated for its efficiency in removing heavy metals and enteric pathogens from municipal wastewater. Influent and effluent, plant tissue and sediment from the system were sampled bi-monthly for a period of six months and assessed for physicochemical parameters (pH, temperature, electrical conductivity, salinity, turbidity, biochemical oxygen demand, chemical oxygen demand) and removal of <i>Escherichia coli</i> , faecal coliforms, trace heavy metals (cadmium, chromium, copper, nickel, lead and zinc). There was an increase in pH in the planted and reference sections, respectively. The BOD and COD values reduced as compared to the influent sample. Suspended solids, turbidity and alkalinity were reduced in the planted section and in the reference sections. Considering the entire rhizofilter, heavy metals were accumulated at varying concentrations in the planted and reference section of the rhizofilter. Planted section showed a greater potential to remove heavy metals from wastewater than the reference section. The entire rhizofiltration system was found to