AN ASSESSMENT OF EFFICIENCY OF HEAVY METALS REMOVAL BY A CONSTRUCTED WETLAND AT EGERTON UNIVERSITY, KENYA

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this thesis is my original work and has not been submitted or presented for examination in any other institution of learning to the best of my knowledge.

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Recommendation

We wish to confirm that this thesis was done under our supervision and has our approval to be presented for examination as per the Egerton University regulations.

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DEDICATION

This work is dedicated to my wife Rose and our children Chao, Andrew and Caleb for their encouragement, patience and prayers during my studies.

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ABSTRACT

An assessment of the efficiency of heavy metals removal by a constructed wetland system at Egerton University, Kenya, was conducted between August 2013 and January 2014. The aim of the study was to evaluate the physico-chemical characteristics of wastewater and investigate heavy metals retention in the wetland. Water samples were collected monthly in plastic bottles at the inlet, along the wetland, and at the outlet; sediment samples were collected from the gravel bed and the three wetland cells using a core sampler. Whole plants were randomly collected and pooled together to form composite samples for each species in every site. In every sampling occasion; temperature, pH, electrical conductivity (EC) and Dissolved Oxygen (DO) of water samples were measured *in situ*. In the laboratory the samples were processed and the concentrations of metals; lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) determined using Atomic Absorption Spectrophotometry (AAS). Minitab software was used to determine spatial variations of heavy metals concentrations and physico-chemical characteristics of water using Analysis of Variance (ANOVA). Further, correlation analyses were performed to establish relationships between the physico-chemical parameters and the retention of metals in the wetland. The study results showed significant variations in temperature and conductivity across the wetland ($p < 0.05$). On the contrary, there was no significant difference in DO across the wetland. Influent levels for lead, copper and zinc were 1.25 ± 0.75 mg/L, 1.09 ± 0.49 mg/L and 0.15 ± 0.11 mg/L respectively while the level of these metals in the effluent were 0.07 ± 0.07 mg/L and 0.32 ± 0.11 mg/L for lead and copper, respectively with zinc being below detection limit. Removal efficiencies of 94%, 70% and 100% for lead, copper and zinc respectively were observed. There was a significant negative correlation between zinc in sediment and pH in water ($r = -0.55$), and a moderate positive correlation between copper in plants and pH in water ($r = 0.47$). These findings indicate that the constructed wetland is efficient in removing heavy metals from the wastewater. The study recommends that the wetland should be rehabilitated to enhance and sustain its function of removing heavy metals from wastewater in order to safeguard human and environmental health.

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LIST OF ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
ATSDR	Agency for Toxic Substances and Disease Registry
BOD	Biochemical Oxygen Demand
DES	Department of Environmental Services
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GEF - SGP	Global Environment Facility - Small Grants Programme
NEMA	National Environment Management Authority
NGO	Non-Governmental Organization
RSC	Royal Society of Chemistry
SDGs	Sustainable Development Goals
UNEP/GPA	United Nations Environment Program/Global Programme of Action
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Children's Emergency Fund
USDOD	United States Department Of Defence
WC1	Wetland Cell 1
WC2	Wetland Cell 2
WC3	Wetland Cell 3
WHO	World Health Organization
WSPs	Waste stabilization Ponds
WWAP	World Water Assessment Programme

DEFINITION OF TERMS

Heavy metal: a general term given to the group of metals and metalloids with a specific density greater than 5g/cm^3.

Wetlands: lands transitional between terrestrial and aquatic systems where the water table is usually at/or near the surface or the land is covered by shallow water.

Wastewater: used water with dissolved or suspended solids, discharged from homes, commercial establishments, farms, and industries.

Constructed wetland: engineered systems designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters.

Phytoremediation: the direct use of living green plants for in situ removal, degradation, or containment of contaminants in soils, sludge, sediments, surface water and groundwater.

Sorption: the process in which one substance takes up or holds another (by either absorption or adsorption).

Absorption: soaking up; a process in which one substance permeates another; a fluid permeates or is dissolved by a liquid or solid).

Adsorption: surface assimilation (the adhesion of atoms, ions or molecules from a gas, liquid or solid to a surface).

Chemisorption: adsorption in which the adsorbed substance is held by chemical bonds.

Biosorption: a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure.

Precipitation: the formation of a solid in a solution or inside another solid during a chemical reaction or by diffusion in a solid.

Co-precipitation: the carrying down by a precipitate of substances which are normally soluble under the condition of precipitation.

Physico-chemical: joint action of both physical and chemical properties.

Biomagnification: the concentration of toxins in an organism as a result of ingesting other plants or animals in which the toxins are more widely disbursed.

Bioaccumulation: accumulation of substances, i.e. pesticides or heavy metals in an organism.

Redox potential: a measure of the tendency of a chemical species to acquire electrons and thereby be reduced.

Hydraulic loading rate (HLR): volume of water entering the wetland divided by the surface area of the wetland.

Hydraulic retention time (HRT): the average time that water remains in the wetland.

Metal removal efficiency: the ratio of the concentration of a metal at the outlet of the wetland to its influent concentration.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

The world's freshwater resource is scarce and vulnerable to pollution by waste streams generated by human activities. The decline of water quality across the world is constraining global freshwater supplies and millions of people lack access to adequate supply of drinking water (WHO/UNICEF, 2012). Hence most of the problems that humanity face in the twenty-first century are linked to water quantity and quality issues (UNESCO, 2009).

The major activities responsible for increase in water pollution are industrialization, urbanization and rapid growth in population. Pollutants, whether organic or inorganic, severely impact human health and the stability of natural ecosystems. It is projected that by the year 2050, untreated wastewater could reduce the world's freshwater resources by as much as 18,000 km^3 annually (Bao and Kuyama, 2013).

The practice of discharging wastewater into watercourses and natural wetlands was a common practice as a means of disposal in the past (McEldowney *et al.*, 1998). This method is effective where discharges do not exceed the capacity of the receiving system to assimilate, absorb or detoxify contaminants present (Bayes *et al.*, 1989). The major chemical pollutants in wastewater include ionic forms of nitrogen and phosphorus, heavy metals, detergents, pesticides, and hydrocarbons (Larsdotter, 2006). Unlike pollution associated with major plant nutrients in aquatic environment such as nitrogen and phosphorus, which can be assimilated to usable biomass at different trophic levels, heavy metals tend to accumulate and magnify at higher levels of the food chain. This is attributed to their non-biodegradability and persistence in the environment.

Heavy metal contamination can have devastating effects on human health and the ecological integrity of the receiving environment (Farombi *et al.,* 2007). Although living organisms require varying amounts of heavy metals such as Iron, cobalt, copper, manganese, molybdenum and zinc, excessive levels can have deleterious effect on organisms (Rainbow, 2007). Zinc, for example, when present in high levels inhibit many plant metabolic functions resulting to retarded growth and senescence (Ebbs and Kochian, 1997), while high concentration of copper may severely damage gills, adversely affect the liver and kidneys of fish or cause some neurological damage (Flemming and Trevors, 1989).

Removal of heavy metals from wastewater can be accomplished through various treatment options, including chemical precipitation, coagulation, complexation, activated carbon adsorption, ion exchange and reverse osmosis. Although chemical precipitation has been extensively practiced in the removal of heavy metals from wastewaters (McEldowney *et al.*, 1998), this technique is costly, requires continued supervision and sometimes present operational challenges (Upadhyay, 2004). Thus, effluents released from wastewater treatment plants may contain various heavy metals (Mapanda *et al.,* 2005).

The use of natural and artificial (constructed) wetlands for wastewater treatment has been proposed as an intermediate technological solution for handling wastewater (McEldowney *et al.*, 1998). These systems are economically attractive and relatively energy-efficient for wastewater treatment, especially for isolated populations, yet with capacity to improve aesthetic value of an area. The basic concept of these systems is deceptively simple and many constructed wetlands have not performed as expected.

Improvement in water quality in a wetland system may be assessed by monitoring and comparing a range of water-quality parameters at the inflow and outflow. Some of the parameters commonly used for monitoring wetland performance include dissolved oxygen content, biochemical oxygen demand, total phosphorus, total nitrogen, faecal coliform, pH, suspended solids, electrical conductivity, and heavy metal content. Corrective measures are then applied when results show that the system is not working according to the set objectives (Kayombo *et al.,* 2001*).*

The wastewater stabilization ponds at Egerton University were constructed several decades ago when the institution was an agricultural college with a few hundreds of students and staff. Upon upgrading to a fully-fledged University in 1987, the population on campus increased steadily and so did the volume, as well as the complexity of wastewater. In 2007, a wetland was constructed to polish the effluent from the waste stabilization ponds before discharging it into River Njoro.

1.2 Statement of the problem

The discharge of treated wastewater into River Njoro has been an issue of concern to the general public and other stakeholders. The river not only serves as a source of water for downstream communities but also provides the bulk of the total freshwater inflow into Lake Nakuru – a wetland of international importance. Previous studies have reported the working status of the WSPs at Egerton University as only moderate, with respect to nutrients, pathogens and traces of heavy metals in the effluent. Heavy metals have been detected in water, plants, fish and sediments in Lake Nakuru. Heavy metals persist in the environment, bio-accumulate in organisms and bio-magnify along the food chain. There have been episodes of flamingo deaths in Lake Nakuru and heavy metals were found accumulated in the organs of their carcases. This led to the listing of heavy metal toxicity as one of the possible causes for their death. Therefore, water contaminated with heavy metals may adversely affect human health for the River Njoro riparian populations and the Lake Nakuru biodiversity.

1.3 Objectives

1.3.1 General Objective

To assess the efficiency of Egerton University constructed wetland in removing heavy metals from wastewater.

1.3.2 Specific objectives

1. To evaluate the physico-chemical characteristics (pH, temperature, electrical conductivity and dissolved oxygen) of water across the wetland profile.
2. To determine the concentrations of lead, copper, cadmium and zinc in water, plants and sediments in the wetland.
3. To assess the effect of selected physico-chemical parameters on retention of heavy metals in the wetland.

1.4 Hypotheses

$H_0$1: The physico-chemical parameters of water have no significant variation along the wetland profile.

$H_0$2: The concentrations of lead, copper, cadmium and zinc in water, plants and sediments have no significant difference across the wetland profile.

$H_0$3: There is no significant relationship between selected physico-chemical parameters of water and the retention of heavy metals in the wetland.

1.5 Justification of the study

Egerton University is obligated to protect human health and environmental integrity as well as comply with regulations and related standards for effluent discharge into the environment. There is also the need to implement sustainable environmental management programmes that meet international standards (ISO 14001:2015) and actively participate towards the achievement of Vision 2030 of Kenya and the SDGs (Goal 6), (UN, 2015). It is also a moral/ethical obligation for the University to ensure that the water quality of River Njoro does not change to the detriment of downstream users. There is also need to protect the flora and fauna in Lake Nakuru, a Ramsar site, from the effects of heavy metals pollution. Effectively treated wastewater has great potential for re-use by humans and livestock as well as supplement existing freshwater supplies. Data obtained in this study will provide valuable information on the wetland and the entire wastewater treatment system at Egerton University.

1.6 Scope of the study

The study covered Egerton University constructed wetland only and investigated selected physico-chemical parameters of wastewater (pH, temperature, Electrical conductivity and dissolved oxygen) and the content of selected heavy metals (Pb, Cu, Cd and Zn) in water, dominant macrophytes (*P. stratiotes, C. alopecuroides*, *S. lacustris*, and *E. crassipes*) and sediments along the entire wetland profile. The study was conducted over a period of six months (August 2013 to January 2014).

CHAPTER TWO

LITERATURE REVIEW

2.1 Global water pollution

Water pollution is a major cause of the water crisis being experienced globally. The ever increasing population, industrialization, food production practices, improved living standards and poor water management approaches are responsible for this scenario. The water degradation risks impacts directly into our social and economic activities (UN, 2012). Most of the significant forms of water pollution worldwide are caused by inadequate sanitation infrastructure leading to contamination of watercourses (UNICEF /WHO, 2008). It is estimated that about 2 million tons of sewage and industrial waste are discharged daily into the world's water bodies. Thus, many important water bodies, which provide water for domestic use and irrigation, are displaying undesirable levels of potentially toxic substances (RSC, 2010).

Poor water quality is a risk to human and environmental health, escalates treatment costs and reduces availability of safe water (Palaniappan *et al.*, 2010). About 900 million people globally lack access to safe drinking water (UNDESA, 2009), while almost 2.6 billion do not have adequate sanitation (WHO/UNICEF, 2010). Consequently, polluted water is a major cause of disease and death in the developing world where about 80% of all diseases and over one third of deaths can be linked to use of polluted water (Pandey, 2006). In Kenya, about 43% of the population have no access to clean water and this severely affects many lives with many illnesses (Marshall, 2011). Worldwide, water related diseases are the number one killer of children below the age of five years (WHO, 2002). Although microbiological hazards form the largest contributor to waterborne diseases in the world, chemical pollutants can bring serious health problems irrespective of whether the source of chemicals are natural or anthropogenic (Terrence *et al.*, 2007).

2.1.1 Heavy metals occurrence in the environment

Environmental contamination with heavy metals is a growing problem globally and has worryingly increased in the last five decades due to the innumerable uses of metals in industrial processes and products (El-Safty, 2014). Metals contamination is one of the oldest environmental problems and still remains a serious health issue today. The widespread use of metals, the legacies of the past contamination and novel technologies continue to pose ecological risks to aquatic environments globally (Luoma and Rainbow, 2008).

Heavy metals are commonly defined as those having a specific density greater than 5 g/cm^3 and adversely affect the environment and living organisms (Järup, 2003). This group of metals are natural constituents of the earth crust and can be released into the environment through natural processes (e.g. weathering and volcanic eruptions) and various anthropogenic activities including sewage and storm water discharges, landfills, mining and smelting, electroplating, laboratories, tanning, battery manufacture, electronic waste disposal and many others (Morais *et al*., 2012). The most common heavy metals found in wastewater include arsenic, cadmium, chromium, copper, lead, nickel and zinc (Lambert *et al*., 2000). Heavy metals are significant environmental pollutants and their toxicity present a problem of increasing importance for ecological, evolutionary, nutritional and environmental concerns (Jaishankar *et al.*, 2014). Heavy metals cannot be degraded to harmless by-products by any biological, physical or chemical means but can be transformed from one oxidation state or organic complex to another.

2.1.2 Heavy metal toxicity to humans and other organisms

Generally, toxicity of heavy metals is attributed to the high affinity for ligands like sulphur and nitrogen, by which evolution exploits heavy metals as essential components of metabolism. This makes all heavy metals potentially toxic by binding to proteins or other molecules, consequently inhibiting them from functioning in their typical metabolic role (Louma and Rainbow, 2008). Heavy metals interfere with numerous enzymes and hormones function and disrupt many metabolic and physiological processes in the body (Mukke and Chinte, 2012). They compete with essential trace elements for binding sites on transport and storage proteins, metallo-enzymes and receptors. This leads to abnormalities in the metabolism of carbohydrate, protein/amino acids, lipids, neurotransmitters, hormones, and increase vulnerability to infections (El-Safty *et al.*, 2009). Heavy metals are particularly toxic when exposure occur early in life because they affect brain development and other organ systems at very low levels that are presumed to be safe for adults (ATSDR, 2005).

Among the best known heavy metal contamination are the episodes of the 1950s and 1960s that occurred around Minamata Bay, Japan, where about 3000 people, including many infants, suffered birth defects and severe nervous system damage from consuming shellfish and fish contaminated with mercury (Luoma and Rainbow, 2008). Another major occurrence of mercury poisoning happened in Northern Iran in 1972, where farmers consumed wheat seeds treated with mercurial fungicides instead of planting them (Freedman, 1989). Although human health episodes raised awareness of threats from heavy metal contamination, ecological damage is likely to be more persistent. In aquatic environments, heavy metals accumulate in upper sediment. The metals can be released by biological and geochemical mechanisms and become toxic to sediment-dwelling organisms and fish, leading to death, reduced growth, or reduced reproduction leading to lower species diversity (Praveena *et al*., 2007).

2.1.3 Lead pollution and toxicity

Lead is the commonest of the heavy metals, accounting for 13 mg/kg of Earth's crust (WHO, 2011). Lead has been widely used in construction, piping, in pigments, solders, shields for protection against radioactive materials, as an insecticide (lead arsenate), ceramic glazing, crystal tableware among other uses (Wright and Welbourne, 2002). Although most of these uses have been discontinued, their legacies of lead contamination in the environment still remain.

Lead is one of the most significant toxin of the heavy metals. Its toxicity causes significant changes in many biological processes. It is known to replace bivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+} leading to interference in the normal metabolism of the cell. Lead bio-accumulates in the body and this may lead to decrease in haemoglobin production, kidney, joint, reproductive and cardiovascular systems disorders as well as long-term damage to the central nervous system (Galadima and Garba, 2012). High lead concentration in a plant enhances the production of reactive oxygen species (ROS), causing lipid membrane damage which eventually interferes with chlorophyll and photosynthetic processes, hence reducing the overall growth of the plant (Srivastava *et al.*, 2015).

2.1.4 Zinc pollution and toxicity

Zinc, the 25th most abundant element in the earth's crust (78mg/kg), occurs only in the divalent state and does not occur as a metal in nature (Alloway, 2003). Zinc is used in dry batteries, in construction, galvanizing, making coins, alloys (e.g. brass and bronze), solder, paints, rubber products, cosmetics, printing ink, soap among others (Wright and Welbourne, 2002). Zinc is also used in dental, medical, and many household applications. Organo-Zn compounds are used as fungicides, topical antibiotics, and lubricants (Simon-Hettich *et al.*, 2001). It is a heavy metal of much interest since it is an essential element in living organisms as well as a potential contaminant in the environment. It is an essential plant micronutrient and a constituent of many metalloenzymes and transcription factors which are involved in many cellular processes such as gene expression, signal transduction, transcription, and replication (Berg and Shi, 1996).

Zinc is considered to be relatively non-toxic, especially when taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction (Nolan, 2003). The clinical signs of zinc poisoning have been reported as vomiting, diarrhoea, bloody urine, jaundice, liver failure, kidney failure and anaemia (Fosmire, 1990). Although the risk of excess Zn in humans from environmental sources is apparently low, zinc from anthropogenic sources spreads widely in the environment and adversely affect other organisims. In fish, for example, zinc causes decrease in oxygen consumption because it damages gills and also reduces growth and fertility (Pandey, 2006).

2.1.5 Cadmium pollution and toxicity

Cadmium is chemically similar to zinc and occurs naturally with zinc and lead sulphide. Cadmium forms organic complexes with organic ligands such as humic and fulvic substances. It is obtained as a by-product when refining zinc and other metals, especially copper and lead (Wright and Welbourne, 2002). Rocks mined to produce phosphate fertilizers contain varying amounts of cadmium. Occasionally the produced phosphate fertilizers have cadmium concentrations of up to 300 mg/kg hence the high cadmium content in agricultural soils (Grant and Sheppard, 2008). Sewage contains cadmium from human excretion, runoff from agricultural fields, industrial effluents and disposal of domestic waste containing zinc (Wright and Welbourne, 2002). Cadmium has many industrial uses including nickel-cadmium batteries, electroplating, pigments, plastic plasticizers, welding electrodes and alloys.

A very wide range of concentrations have been reported in foodstuffs from various parts of the world. Cadmium is mainly found in fruits and vegetables due to its high rate of soil-to-plant transfer (Satarug *et al.*, 2011). However, there have been a few cases of general population toxicity as a result of chronic exposure to cadmium in contaminated food and water. In the 1940s, Japanese mining activities contaminated the Jinzu River with cadmium and traces of other toxic metals. Rice crop grown by the river banks downstream accumulated cadmium and when local communities consumed the contaminated rice they developed *itai-itai* disease and renal abnormalities, including proteinuria and glucosuria (Nogawa *et al*., 2004). In fish, cadmium accumulates in the muscle, liver, gills and bones where it exerts deleterious effects in terms of nephrotoxicity, cytotoxicity, genotoxicity and immunotoxicity (Lippmann, 2000).

2.1.6 Copper pollution and toxicity

Copper is present in the earth's crust at a concentration of about 50 mg/kg (Emsley, 2003). The element has various roles in biological electron transfer and oxygen transportation, processes that exploit the easy inter-conversion of Cu (I) and Cu (II) (Lippard and Berg, 1994). The major activities that lead to mobilization of copper into the environment are extraction from its ores, agriculture and waste disposal. Humans are exposed to copper through inhalation of particulate copper, drinking water and eating food contaminated with copper. However, the toxicity of copper to humans is relatively low compared to other metals such as mercury, cadmium, lead, and chromium. When a person is exposed to copper levels above the essential levels needed for good health, the liver and kidneys produce metallothionein (low molecular weight) which binds with the copper to form a water-soluble complex which is then excreted (Bradl, 2005).

Chronic effects of copper exposure can cause liver and kidney damage. However, mammals have effective mechanisms of regulating copper and are hence generally protected from excess dietary copper levels (DES, 2005). High levels of free copper have been detected in people with Alzheimer's disease (Brewer, 2010). In this disease, copper and zinc are known to bind to the amyloid beta proteins (Faller, 2009). It is thought that the bound form of the protein mediates the production of reactive oxygen species (free radicals) in the brain (Hureau and Faller, 2009).

2.1.7 Potential sources of heavy metals at Egerton University

Egerton University has expanded rapidly in the last decade, and as described by Alshuwaikhat and Abubakar (2008), universities these days are 'small cities' because of their populations, and the various complex activities that take place in campuses. Thus wastewater generated from these institutions contain a wide range of contaminants that may include heavy metals, detergents, pesticides, pathogens, hydrocarbons, pharmaceuticals etc., which can prove difficult to treat effectively within existing systems.

Probable sources of heavy metals in the wastewater stream at Egerton University may include, but not limited to, teaching laboratories, agricultural demonstration fields and storm water. Storm water carries along with it run-off from paved areas, agricultural fields, cow sheds and lawns which may contain leached metals from carelessly disposed electronic waste, fungicides and soil. Some amounts of heavy metals are excreted through faeces and urine of human beings and animals. According to Davies and Nightingale, (1975), zinc is primarily excreted via the gastrointestinal tract and eliminated in the faeces after oral exposure in human beings; approximately 70-80% of an ingested dose is excreted in the faeces. Corrosive liquid waste from laboratories may cause solubilisation of heavy metals in the waste stream. The waste stabilization ponds may be a secondary source of heavy metals. Depending on the physico-chemical conditions of the ponds, the metals buried in the sludge may re-enter the water column.

2.2 Wastewater treatment options

Wastewater is 99.9% water and 0.1% solid matter, of which about half is suspended while the other half is in dissolved form (FAO, 2004). The major chemical pollutants in domestic and institutional wastewater include nitrogen, phosphorus, heavy metals, detergents, pesticides, and hydrocarbons (Larsdotter, 2006). Wastewater treatment is necessary in order to produce a disposable effluent which will not pollute and injure the receiving environment (Khopkar, 2004). There are various processes available for wastewater treatment systems with inherent advantages and disadvantages. Treatment can be done in decentralized systems (i.e. in septic tanks, bio filters or anaerobic treatment systems), or collection via a sewer network to a centralized system (e.g. a municipal treatment plant). Wastewater treatment can involve physical, chemical or biological processes or a combination of these processes depending on the required effluent standards (EPA, 1997).

2.2.1 Conventional waste stabilization Ponds

Waste stabilization ponds are popular for treating wastewater in tropical and subtropical regions because of the abundant sunlight and high ambient temperatures (Hodgson, 2007). They are man-made earthen basins and may include one or more series of anaerobic, facultative and maturation ponds depending on the quality of effluent required. Anaerobic and facultative ponds are designed for removal of Biochemical Oxygen Demand (BOD), and maturation ponds for pathogen removal, although some BOD removal also occurs in maturation ponds and some pathogen removal in anaerobic and facultative ponds (Mara, 1997). Wastewater stabilization ponds have been used to treat a variety of wastewaters, from domestic wastewater to complex industrial waters, and they operate under a wide range of weather conditions. They can be used alone or in combination with other treatment processes (USDOD, 2004).

WSPs are popular for municipal sewage purification, especially in developing countries, due to their cost-effectiveness and high potential of removing different pollutants. In addition, they are easy to operate and require minimal maintenance. They have been proved to be reliable, economic, flexible and adaptable, and able to meet the highest effluent standards (Kambole, 2003). Prior to treatment in the WSPs, wastewater is first subjected to preliminary treatment (screening and grit removal) to remove large and heavy solids.

2.3 Use of wetlands for wastewater treatment

Generally, wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at/or near the surface or the land is covered by shallow water. Saturation with water is the main factor determining the nature of soil development and the types of plant and animal communities living in the soil and on its surface (Cowardin *et al.*, 1979). Wetlands, both natural or constructed, are effective in lowering biochemical oxygen demand (BOD), suspended solids, nitrogen and phosphorus, as well as reducing concentrations of metals, organics and pathogens (Kadlec and Knight, 1996). Early history of wastewater treatment say that wastewater was collected and discharged into natural watercourses or wetlands devoid of any form of treatment (Rousseau, 2005). However, limits of assimilative capacity of natural receiving waters compelled the necessity to develop technologies that treat wastewater streams before they are discharged (Kadlec and Knight, 1996). It is the discovery of the purifying ability of natural wetlands that led to the development of constructed wetlands.

2.3.1 Constructed Wetlands

Constructed wetlands (CWs) are engineered systems that have been designed and constructed to utilize the natural processes comprising wetland vegetation, soils, and the associated microbial populations to assist in treating wastewaters (Vymazal, 2005). These systems utilize the physical, chemical, and biological processes existing in wetland ecosystems to remove and trap pollutants. Constructed wetlands for wastewater treatment have evolved during the last five decades into a reliable treatment technology which can be applied to all types of wastewater including sewage, industrial and agricultural wastewaters, landfill leachate and storm water runoff (Vymazal, 2010). Constructed wetlands have shown high effectiveness in removing heavy metals; both subsurface flow and surface flow systems having similar removal capability (Reed *et al.*, 1995).

2.3.2 Use of constructed wetlands for wastewater treatment in Kenya

The use of constructed wetlands for treatment of wastewater in Kenya is a fairly new idea and has not been well exploited, although CWs have almost limitless potential (Raymer, 2006). These systems have been successfully used to polish domestic wastewater (Nzengya and Witshitemi, 2001) as well as industrial wastewater from pulp and paper effluents (Abira *et al.*, 2003). The splash Carnivore wetland, which was established in 1994, handles up to 80 m^3 of wastewater daily with acceptable pollutants removal efficiencies (Nyakang‘o and van Bruggen, 1999). There are other operational constructed wetlands in Kenya which include: Nandi Hills Tea Factory CW that recycles agricultural waste and toilet effluent, The Kingfisher constructed Wetland at Naivasha, Shimo La Tewa Prison, and Mombasa – Kenya, Mara Simba lodge and Masai Mara National reserve.

2.3.3 Types of constructed wetlands

Constructed wetlands can be classified into two types according to the water flow mode and the dominant aquatic plant species (Kadlec *et al*., 2000):

1. Surface flow (SF) or Free-Water-Surface (FWS) Constructed Wetlands: where the water flows above ground and these can be further categorized into three subtypes according to the dominant plant species i.e. emergent aquatic macrophytes, floating aquatic macrophytes and submerged aquatic macrophytes.
2. Subsurface Flow (SSF) Constructed Wetlands: with the water flow below ground and can be categorized into two subtypes; horizontal and vertical subsurface flow according to the water current direction.

These types of wetland systems also differ from one another in layout, the removal efficiency of certain pollutants, area requirements, technical complexity, applications, and costs. The free water surface wetland technology closely resemble natural wetlands. They consist of large, shallow lagoons that contain submerged, emergent, or floating plant species. The microorganisms responsible for biological treatment of the wastewater form bio-films on the stems and leaves of the plants. The name “free surface” originates from the thin free water layers which are formed at the surface (Ulsido, 2014). These systems can be used for secondary treatment of wastewater and removal of heavy metals, but they are commonly used as tertiary treatment to remove nutrients to prevent eutrophication in the receiving water bodies (Gauss, 2008). Hybrid systems have also been developed and these combine different types of constructed wetlands so as to improve treatment efficiencies. They are useful when more stringent discharge limits for nitrogen are required and also more complex wastewaters are treated in constructed wetlands (Vymazal, 2013).

2.3.4 The role of vegetation in constructed wetlands

Vegetation is an essential component of constructed wetlands (Lim *et al*., 2001). Plants provide a large surface area for attachment and growth of microbes. The physical components of the plants stabilize the surface of the beds, slow down the water flow thus assist in sedimentation (Brix, 1994). However, not all wetland species are suitable for wastewater treatment because plants for constructed wetland must be able to tolerate a combination of incessant flooding and exposure to fairly high amounts of pollutants. According to Heers (2006), the three most essential criteria for choosing wetland plants include: availability in the climate zone, pollutant removal capacity (treatment efficiency) and tolerance ranges; and plant productivity and biomass utilization options.

Plants also have the ability to remove nutrients, trace elements, and organics from the water through biological uptake and surface adsorption. During photosynthesis, plants utilize carbon dioxide and release oxygen. Submerged aquatic plants growing within the water column elevate the dissolved oxygen level in the wetland surface water and deplete the dissolved carbon dioxide, leading to an increased pH. Rooted wetland macrophytes also actively transport oxygen from the atmosphere to the sediments thus supporting aerobic and facultative anaerobic microorganisms in the otherwise anaerobic sediments and soils (ITRC, 2003). However, plant senescence can lead to large nutrient and metal releases, compromising the treatment abilities of a constructed wetland (Kroger *et al*., 2007).

2.3.5 Macrophytes in the Egerton University Constructed Wetland

A free-water surface wetland was constructed to polish pre-treated wastewater effluent from the waste stabilization ponds. The wetland system consists of one vegetated gravel bed and a series of three connected, vegetated wetland cells. The dominant plants in the constructed wetland are *Eichhornia crassipes*, *Pistia stratiotes*, *Cyperus alopecuroides* and *Scirpus lacustris*.

1. *Eichhornia crassipes*

The *Eichhornia crassipes* (Water Hyacinth), (Plate1) is a member of the pickerelweed family (Pontederiaceae). It is a free-floating vascular plant, which adapts easily to various aquatic conditions and plays a significant role in extracting and accumulating metals from water (Weiliao and Chang, 2004). This ability is useful in removing toxic heavy metals and trace elements from contaminated soils and water in a process referred to as phytoremediation where plants are used to extract, sequester and/ or detoxify (Meagher, 2000).

Plate 1: *Eichhornia crassipes* (Photo by Mwanyika F.T 2013)

2. *Cyperus alopecuroides*

Cyperus alopecuroides (Foxtail flatsedge), (Plate 2) is a perennial herbaceous plant with a very short thick woody rhizome that grows up to 150 cm tall on shallow waters. The stems are erect, solitary, stout and trigonous with width of about 2-5 mm. They are leafy and thickened at base by sheaths and intravaginal shoots. Its leaves, generally shorter than the stems, are papery with blades 1-12 mm wide that are flat with prominent midrib and numerous lateral veins. It has 4-7 leaf-like bracts that can grow up to 70 cm long. The plant has a stem which carries a single inflorescence in the shape of an umbel with 5-10 primary rays, which can grow to 15 cm in length. It has 1-8 clustered; cylindrical spikes usually 5-10 mm wide and 15-40 mm long (Brullo and Sciandrello, 2006). Prusty *et al*. (2007) reported that *C. alopecuroides* showed capacity to adsorb copper.

Plate 2: *Cyperus alopecuroides* (Photo by Mwanyika F.T 2013)

3. *Pistia stratiotes*

Pistia stratiotes (Water lettuce), (Plate 3) is a free floating perennial aquatic plant native to South America. It is stoloniferous, forms colonies, and has rosettes up to 15 cm across. Leaves are light green and velvety - hairy with obvious parallel longitudinal veins, which are slightly broad and are widest at the apex (Ramey, 2001). It reproduces rapidly by vegetative offshoots formed on short, brittle stolons although it is also known to reproduce by seed. *Pistia stratiotes* has been included in the Global Invasive Species Database (GISD, 2005). Studies have shown that *Pistia stratiotes* is a bioaccumulator and a bioindicator of various heavy metals, including cadmium, chromium, copper and zinc (Mishra and Tripathi, 2008).

Plate 3: *Pistia stratiotes* (Photo by Mwanyika F.T 2013)

4. *Scirpus lacustris*

Scirpus lacustris (Bulrush), (Plate 4) is an emergent perennial macrophyte. It has shallow, dense root and rhizome system that extends near the surface and the plant grows up to a height of 2.5 – 3 m. It prefers soils that are slightly acidic or slightly alkaline and can tolerate permanent inundation up to 0.3 – 0.4 m and is sensitive to prolonged droughts. *Scirpus lacustris* shows a high adaptability to various wastewater conditions and potential to absorb, translocate and concentrate chromium in its tissues (Heers, 2006 and Yadav *et al*., 2005)

Plate 4: *Scirpus lacustris* (Photo by Mwanyika F.T 2013)

2.4 Heavy metals removal in wetland systems

When heavy metals enter a constructed wetland, they are distributed between the substrate, the water column and the vegetation (Sheoran and Sheoran, 2006). There are three main wetland processes that are involved in removal of heavy metals; namely, binding to soils, sediments and particulate matter, precipitation as insoluble salts, and uptake by bacteria, algae and plants (Kadlec and Knight, 1996).

The major physico-chemical removal processes of heavy metals occurring in CWs include (1) sedimentation and filtration, (2) sorption and (3) precipitation and co-precipitation. Sedimentation and filtration are important physical processes allowing the removal of heavy metals associated with particulate matter. Sedimentation has long been recognized as a principle process in the removal of heavy metals from wastewaters (Sheoran and Sheoran, 2006). Sorption of heavy metals on a surface may occur by processes of adsorption and precipitation. Heavy metals may be adsorbed either electrostatically, resulting in the formation of relatively weak complexes (physical adsorption) or chemically, resulting in the formation of strong complexes (chemisorption). Metals are more strongly bound by chemisorption than by physical adsorption (Evangelou, 1998). Metals that are adsorbed on the surface of the substrate can be exchanged by other cations by the process of cation exchange. Next to the sorption and sedimentation reactions, metals can also precipitate with (oxy-) hydroxides, sulphides, carbonates, etc. when solubility products are exceeded (Kadlec and Knight, 1996). Stability of metals precipitated as inorganic compounds is primarily controlled by pH. Redox potential (Eh) and pH in the wetland affect the reduction-dissolution of Fe and Mn oxides, which in turn affect the release of heavy metals (Nimirciag and Ajmone-Marsan, 2014).

Some wetland plants have proven to be good accumulators of heavy metals (hyperaccumulators). Once the heavy metals have been accumulated in the plant tissues via phytoextraction, the plants may be harvested for disposal or for incineration to retrieve the metals from the plants. Micro-organisms also affect the behaviour of heavy metals in constructed wetlands by their role in the biogeochemical cycles which affect metal speciation, biosorption, reduction and methylation of heavy metals (Kosolapov *et al*., 2004).

Wetlands have been used to effectively purify metal-contaminated mine drainage water (Weis and Weis, 2004), and can offer an alternative to conventional treatment of contaminated water. A substantial reduction of 99.6% of Pb, 94.4% of Cd and 94.8% of Zn was reported in constructed wetland in Sri Lanka (Jayaweera *et al*., 2006). Similarly, high removal efficiencies of 80.0%, 95.1% and 100% for Pb, Cu and Zn respectively have been reported at Nandi Hills tea estates wetland, Kenya (Gituku *et al*., 2015).

2.5 NEMA Standards for effluent discharge into the environment

The National Environment Management Authority (NEMA) came up with guidelines for discharge of effluent into the environment in 2006 and may add any other parameters as may be prescribed by the Authority from time to time. Table 1 shows the regulatory standards for some heavy metals and selected parameters in wastewater discharged into the environment.

Table 1: Partial NEMA standards for effluent discharge into the environment

Parameter	Max Allowable Limits
Arsenic and its compounds	0.1mg/L
Aluminum	0.02 mg/L
Cadmium and its compounds	0.1 mg/L
Chromium VI	0.0 mg/L
Copper	1.0 mg/L
Lead and its compounds	0.1 mg/L
Fluoride	1.5 mg/L
Zinc	0.5 mg/L
pH	6.5-8.5
Temperature (^{0}C)	± 3 ambient temp

(Source: Kenya Gazette supplement No 68, 2006)

2.6 Conceptual framework

Metals in wastewater are present in colloidal, particulate, and dissolved phases. When wastewater enter the wetland, the metals partition into three main compartments namely the substrate, water column and vegetation through interaction of different variables and prevailing geochemical conditions (Figure 1).

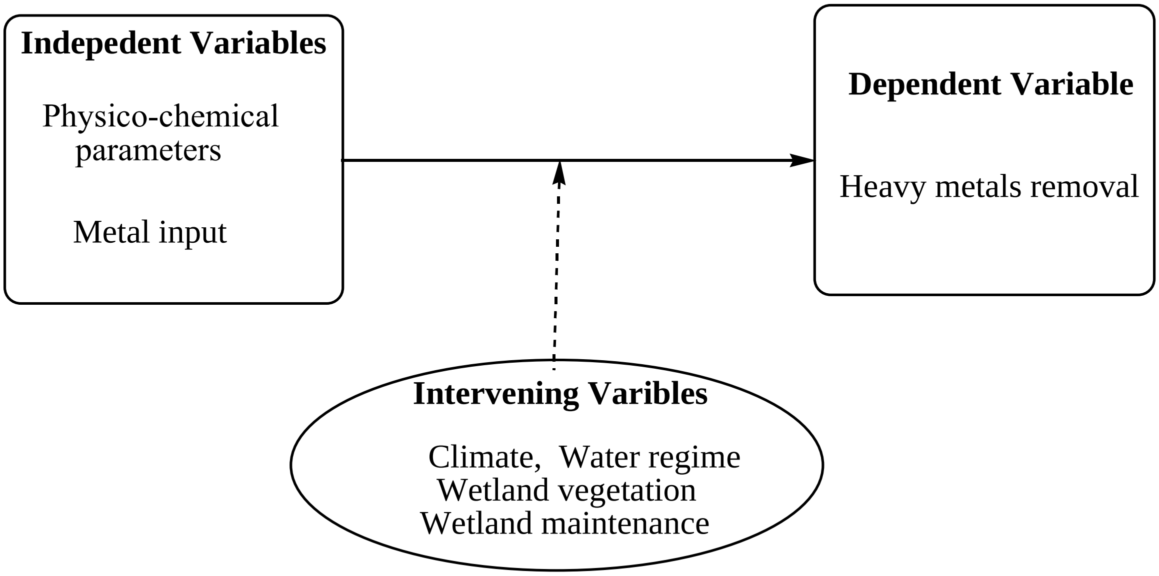


Figure 1: A conceptual framework showing some of the wetland processes and factors affecting metals retention in the wetland

There are several interconnected factors that influence heavy metals retention in a wetland which include: pollutant loading rate, hydraulic retention time (HRT), hydraulic loading rate (HLR), climatic conditions, temperature, pH, oxygen availability, wetland design components – substrate, vegetation and living organisms (DeBusk, 1999). According to Lesage *et al.*, (2007) there are four main mechanisms that affect metal retention in wetlands: (1) adsorption to fine sediments and organic matter, (2) precipitation as insoluble salts (such as sulphides and oxyhydroxides), (3) absorption and induced changes in biogeochemical cycles by plants and bacteria (Kaldec and Knight, 1996), and (4) deposition of suspended solids because of low flow rates. A decrease in pH will increase the competition between metals and hydrogen ions for binding sites and may dissolve metal complexes, releasing free metal ions into the water column. An increase in pH is generally accompanied by a decrease in the solubility of many toxic heavy metals in water (Ebrahimpour and Mushrifah, 2008). Temperature influences most of the processes taking place in the wetland. Roots of wetland plants provide surface for plaque formation which limits the movement of metals in the water column for a period of time until there is a change in physicochemical conditions which can release the metals back into the water column (Hansel *et al.*, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

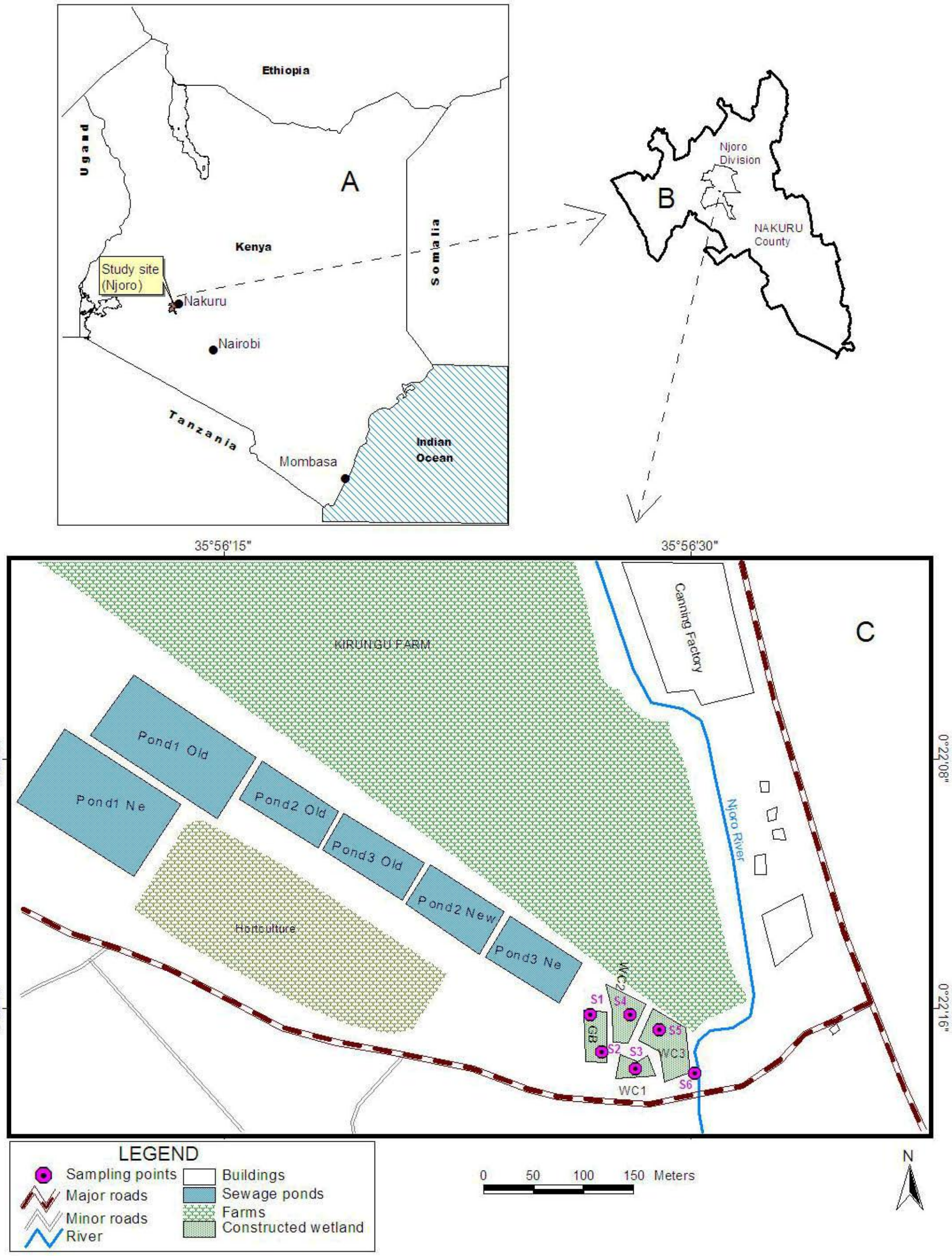
The study was conducted on a constructed wetland located at Njoro campus of Egerton University, Kenya. The Campus, is located about 25 Km south-west of Nakuru town in Nakuru County. It stands on about 1580 hectares of land and lies within the River Njoro watershed at an altitude of about 2238 m above sea level. This is an agricultural area characterized by bimodal precipitation pattern ranging between 760 and 1270 mm per annum (Appendix 1). The long rains fall from March – May while the short rains occur in September - November. The average maximum and minimum temperatures range from 18 to 22℃ and 5 to 8℃, respectively. The soils are pre-dominantly andosols with a pH of 6.0 to 6.5 (Gogo *et al*., 2012).

With a population of about 18,000 people, the campus generates about 800 m^3 /day of wastewater which is treated within the University using waste stabilization ponds (lagoons) that comprise of two anaerobic ponds, two facultative ponds and two maturation ponds. The wastewater treatment system was designed to handle wastewater generated by a smaller population when the institution was still an agricultural college. Deterioration of effluent quality (Murage, 2003 and Mokaya *et al.*, 2004) necessitated the construction of the wetland in series with the waste stabilization ponds and next to the banks of River Njoro.

Consequently, through *Maji na Ufanisi* (an NGO), the GEF-SGP funded the development of a constructed wetland to purify the wastewater from the WSPs in 2007. The wetland was designed to purify about 100 m^3/day of wastewater. Entering the wetland from the WSPs the water runs through a gravel bed into three cells planted with water loving (hydrophilic) plants which act as a biological filters before the water is finally discharged into River Njoro. The wastewater retention time in the wetland is estimated to be 10 to 14 days. The wetland was also supposed to serve as biodiversity conservancy, a nature trail and a recreation park and thereby offer eco-tourism activities. The sedimentation/gravel bed is rectangular in shape and measures approximately 50×21 m with alternating wall embankments within, which ensures that the wastewater flow is slowed down before percolating through a section (about 100 m^2) filled with graded ballast before discharging into the wetland cells whose areas are shown in Table 2.

Table 2: Main Components of the Constructed Wetland and their sizes

Section of the Constructed Wetland	Maximum Possible Area (m^2)
Sedimentation/gravel bed	1050
wetland cell 1	900
wetland cell 2	1250
wetland cell 3	2000
Total Area	5200



Source: Google Map (Modified by G. Maina, Environmental Science Dept., Egerton University)

Figure 2: Map of the study area showing the location of the constructed wetland at Egerton University in, (A) Kenya, (B) Nakuru County/Njoro Division and (C) Sampling points

3.2 Sampling points

Six sampling points (S1-S6) were chosen along the wetland system as shown on Figure 2. Sampling point S1 was located at the inlet to the wetland (discharge point from the WSPs) and was therefore not influenced by the wetland. S2 represents the sedimentation/gravel bed and the dominant aquatic plant species was the *Pistia stratiotes*. Other plant species included *Typha* sp., *Cyperus alopecuroides* and *Scirpus lacustris* (Plate 5). S3 is wetland cell 1 and the dominant aquatic plant was *Eichhornia crassipes*. Other plants were *Cyperus alopecuroides*, *Scirpus lacustris*, *Typha* sp. and *Cyperus papyrus* (Plate 6). S4 is wetland cell 2 which was almost completely covered by Water hyacinth with a few tufts of *Cyperus alopecuroides* on the fringes (Plate 7). S5 represents Wetland cell 3 which was basically an open pond with scanty vegetation, mainly *Cyperus alopecuroides* at the edges (Plate 8) while Point S6 was the outlet of wetland 3 where effluent discharged into River Njoro (Plate 9).

Plate 5: Sedimentation/gravel bed (**S2**) (Photo by Mwanyika F.T 2013)

Plate 6: Wetland cell 1(**S3**) (Photo by Mwanyika F.T 2013)

Plate 7 : Wetland cell 2(**S4**) (Photo by Mwanyika F.T 2013)

Plate 8 : Wetland cell 3(**S5**)   (Photo by Mwanyika F.T 2013)

Plate 9: River Njoro at the wastewater discharge point   (Photo by Mwanyika F.T 2013)

3.3 Sample collection

Sampling was carried out every four weeks over a period of six months (August 2013 to January 2014).

3.3.1 Collection of water samples

Water samples were collected in one litre plastic bottles which were pre-cleaned with detergent, rinsed with tap water, soaked in 1M HNO_3 overnight, rinsed with deionized water and finally with the water samples from respective sampling. Three one litre samples of water were collected from the sampling points between 9:00 am – 12:00 noon during all sampling occasions. The water samples were labelled and acidified to $pH < 2$ using concentrated nitric acid (APHA, 2005), then transported to the laboratory for analysis. During each sampling session the following parameters were determined *in situ*; water temperature, pH, DO and electrical conductivity which were measured using a calibrated electrochemical analyzer (Jenway 3405) fitted with probes for each parameter.

3.3.2 Collection of plant samples

For each sampling point, six plants were randomly collected and pooled together to form a composite sample for each species. The whole shoot and root of each species were collected. Freshly collected plant samples were stored separately in labelled polythene bags and transported to the laboratory for processing.

3.3.3 Collection of sediment samples

Sediment samples were collected in triplicate from the sedimentation/gravel bed and the three wetland cells respectively using a cylindrical core sampler inserted up to a depth of 15 cm. The samples were then placed in clean labelled plastic bags and transported to the laboratory for processing and heavy metals determination.

3.4 Samples preparation and analysis

3.4.1 Heavy metals analysis in wastewater samples

Duplicate samples of 30 mL each of the water samples were measured into 100 mL conical flasks followed by 10 mL of Hydrochloric acid : water (1:1) and 2 mL Nitric acid: water (1:1) solutions. The samples were digested on a hot plate until the volume reduced to about 25 mL. The samples were cooled to room temperature, filtered and diluted up to 50 mL in volumetric flasks using deionized water (APHA, 2005). The samples were then analysed for metals using Flame Atomic Absorption spectrophotometer. The Direct Air-Acetylene Flame Method (APHA, 2005) was used to analyse the samples for Pb, Cu, Cd and Zn using a Flame Atomic Absorption Spectrophotometer (model S11 from Thermo Jarell Ash Cooperation of Waltham, MA, USA.). Standard solutions were serially diluted from stocks of 1000 mg/L concentrations of each metal.

3.4.2 Heavy metals analysis in plant samples

Plant samples were washed with tap water then rinsed with deionized water to remove soil particles and any aerial particulate deposition. They were allowed to drain and then transferred to a regulated oven set at 60^{o}C and kept there until much of the water had evaporated. The temperature was increased to 80^{o}C and the samples kept at this temperature until they became crispy ready for grinding. They were ground using a hammer mill and sieved using a 0.5 mm mesh size sieve (Radojević and Bashkin, 1999), then finally packed in labelled polythene bags. Duplicate samples of 0.5 gram each of the ground plant material were weighed and transferred into 100mL conical flasks followed by 10 mL of Hydrochloric acid: water (1:1) and 2 mL Nitric acid: water (1:1) solutions. The samples were digested on a hot plate at until the volume reduced to about 5 mL. The samples were cooled to room temperature, filtered and diluted up to 50 mL in volumetric flasks using deionized water. The samples were then analysed for metals using the Flame Atomic Absorption spectrophotometer as described in sections 3.4.1.

3.4.3 Heavy metals analysis in sediment samples

Sediment samples were placed in evaporating dishes and oven dried at 105°C for 24 hours, followed by grinding and sieving through 0.5 mm sieve (Radojević and Bashkin, 1999). Duplicate samples of one gram each of dry sediment samples were weighed and placed in 100 mL conical flasks followed by 10 mL of Hydrochloric acid: water (1:1) and 2 mL Nitric acid: water (1:1) solutions. The mixtures were digested on a hot plate until the volume reduced to about 5 mL. The samples were cooled to room temperature, filtered and diluted up to 50 mL in volumetric flasks using deionized water. The samples were then be analysed for metals using the Flame Atomic Absorption spectrophotometer as described in sections 3.4.1 and 3.4.2.

3.5 Data analysis

Minitab statistical software was used to manage the data. The means for various parameters were calculated and comparisons of variables performed using one-way analysis of variance (ANOVA). The difference among means was tested using Tukey’s Honesty Significant Different test (HSD test). The relationships between physico-chemical variables of water and heavy metals concentrations in the wetland were determined using Pearson’s correlation analysis. All the tests were carried out at 95% significance level.

The heavy metals removal efficiency of the wetland was determined by considering influent and effluent concentrations of the metals and calculations were done using equation (1),

$$\%\ \text{Metal removal} = \frac{C_1 - C_2}{C_1} \times 100 \qquad \text{(Leta } \textit{et al.}\text{, 2004)} \tag{1}$$

Where, C_1 is the concentration of the metal in the influent and C_2 is the concentration of the metal in the effluent (units for metal concentrations are given in mg/L)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physico-chemical characteristics of wastewater in the wetland.

Values recorded for water temperature, conductivity, dissolved oxygen and pH during the study period are presented in Table 3. Mean water temperature ranged between 17.3 ± 0.31 - 18.6 ± 0.24 °C and showed significant difference across the wetland (ANOVA: F = 3.65, p = 0.029). The lowest water temperature of 14.8 °C was recorded in S4 while the highest was 20.7 °C in S1. There was a significant difference in conductivity along the wetland cells (ANOVA: F = 5.54, p = 0.005). The highest concentration of dissolved oxygen was recorded in S5 (12.0 mg/L) while the lowest concentration in the wetland was 0.1 mg/L, in S4. However, there was no significant difference in oxygen concentration across the wetland cells (ANOVA: F = 1.48, p = 0.233). The highest pH recorded was in S1 (8.5) while the lowest was in S4 (6.7).

Table 3: Mean values and ranges of physico-chemical parameters at different sites in the wetland (values in parenthesis are the ranges).

Site	Temp (°C) (Mean ± SE)	EC(μS/cm) (Mean ± SE)	DO (mgL^{-1}) (Mean ± SE)	pH (Range)
S1	18.6 ± 0.24 (17.7 – 20.7)	781.2 ± 9.33 (725 - 836)	4.7 ± 0.82 (1.2 – 9.6)	7.9 - 8.5
S2	18.4 ± 0.24 (16.5 – 20.2)	797.4 ± 10.00 (718 - 847)	2.2 ± 0.33 (0.4 – 5.1)	7.1 - 7.8
S3	17.9 ± 0.26 (15.3 – 19.5)	802.3 ± 7.91 (729 - 842)	2.4 ± 0.43 (0.5 - 6.4)	7.0 - 7.8
S4	17.3 ± 0.31 (14.8 -19.4)	781.7 ± 8.25 (715 - 841)	2.2 ± 0.4 (0.1 - 6.2)	6.7 -7.7
S5	18.1 ± 0.29 (16.4 - 20.4)	771.4± 11.79 (690 - 885)	4.3 ± 0.66 (1.4 - 12)	6.9 - 8.1
S6	18.2 ± 0.64 (16.7 - 19.8)	776.2 ± 0.11 (752 - 807)	3.2 ± 0.11 (2.7 - 3.4)	7.4 - 7.6
NEMA (MPL)	-	-	-	6.5 - 8.5
ANOVA	F = 3.65 p = 0.029	F = 5.54 p = 0.005	F = 1.48 p = 0.233	-

4.2 Heavy metals concentration in the wetland.

4.2.1 Heavy metals concentration in water samples

Results for metal concentrations as determined in water samples are summarized in Table 4 and their variations along the constructed wetland wastewater treatment pathway are shown in Figure 3. The highest mean lead concentration (1.25 ± 0.75 mg/L) was in S1 and lowest in S6 (0.07 ± 0.07 mg/L). There was no significant difference in lead concentration across the wetland (ANOVA: F = 0.54, p = 0.705). Similarly, highest copper concentration (1.09 ± 0.49 mg/L) was in S1 and lowest in S6 (0.32 ± 0.11 mg/L). There was no significant difference in copper concentration across the wetland (ANOVA: F = 1.27, p = 0.282). The highest zinc concentration (0.15 ± 0.11 mg/L) was in S1 and was not detected at S6. There was significant difference in zinc concentration across the wetland (ANOVA: F = 10.69, p = 0.000). Lead concentration was highest in S1 then dropped almost by half in S2, rose again in S3 then reduced consistently up to S6. Although copper metal followed a similar trend as lead up to S3, its concentration continued to rise up to S5 before dropping in S6. Zinc concentration was also highest in S1 but reduced in subsequent sites until it was not detected in S6. Cadmium was below detectable limit of 0.01 mg/L in all samples in the wetland.

Table 4: Mean values of selected metals concentration in water samples at different sites in the wetland (Data presented as Mean ± SE).

Site	Pb (mg/L)	Cu (mg/L)	Zn (mg/L)
S1	1.25 ± 0.75	1.09 ± 0.49	0.15 ± 0.11
S2	0.69 ± 0.85	0.79 ± 0.40	0.09 ± 0.08
S3	0.97 ± 0.85	0.87 ± 0.56	0.08 ± 0.08
S4	0.83 ± 0.87	0.96 ± 0.60	0.05 ± 0.06
S5	0.69 ± 0.85	0.99 ± 0.65	0.03 ± 0.04
S6	0.07 ± 0.07	0.32 ± 0.11	ND*
NEMA MPL⁺	0.1 mg/L	1.0 mg/L	0.5 mg/L
ANOVA	F = 0.54 p = 0.705	F = 1.27 p = 0.282	F = 10.69 p = 0.000

* ND - not detected, ⁺MPL - Maximum Permissible Limits

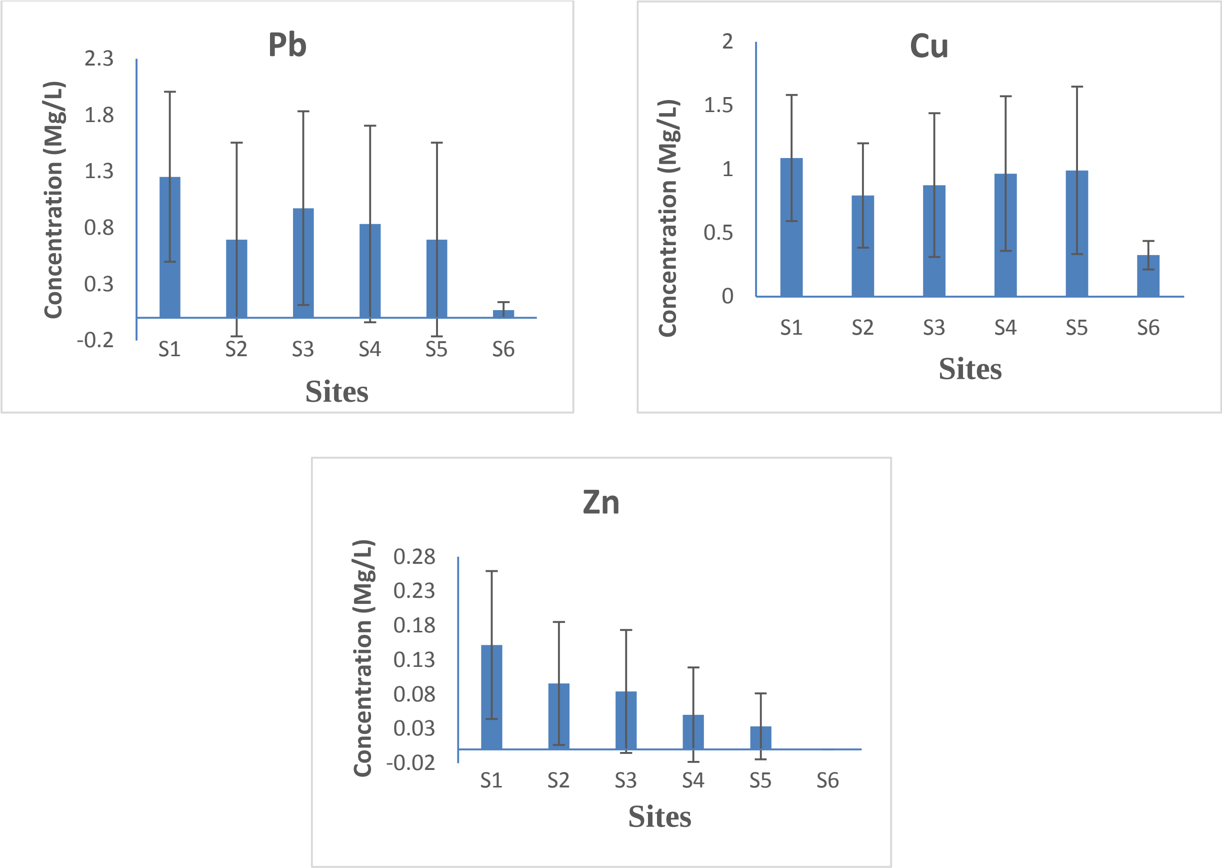


Figure 3: Variation of metal concentrations along the wetland profile. Vertical bars: ± SE

4.2.2 Heavy metals concentration in sediment samples

The concentrations of heavy metals in sediments for each sampling site are shown in Table 5. The highest concentration of lead was in S5 (49.99 ± 0.01 mg/Kg) and the lowest in S4 (25 ± 27.38 mg/ Kg). There was no significant difference in lead concentration along the wetland (ANOVA: F=1.75, p = 0.159). Copper concentration was highest in S2 (42.14 ± 18.63 mg/ Kg) and reduced gradually along the wetland cells to a low of 27.97 ± 16.55 mg/ Kg in S5. There was no significant difference in copper concentration along the wastewater flow path in the constructed wetland (ANOVA: F=0.97, p = 0.408). Zinc concentration was highest in S3 (116.16 ± 13.32 mg/ Kg) and lowest in S2 (84.21± 22.19 mg/ Kg). There was significant difference in zinc concentration across the wetland cells (ANOVA: F = 29.61, p = 0.000).

Table 5: Mean values of selected metals concentrations in sediment samples at different sites in the wetland (Data presented as Mean ± SE).

Site	Pb (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)
S2	41.64 ± 20.40	42.14 ± 18.63	84.21 ± 22.19
S3	41.66 ± 20.41	39.50 ± 23.00	116.16 ± 13.32
S4	25.00 ± 27.38	31.73 ± 12.96	109.77 ± 13.69
S5	49.99 ± 0.01	27.97 ± 16.55	95.38 ± 12.05
ANOVA	F=1.75 p = 0.159	F=0.97 p = 0.408	F = 29.61 p = 0.000

4.2.3 Heavy metals concentration in plant samples

The results of the metal concentrations as determined in plants are summarized in Table 6. *P. Stratiotes*, found in S2 only, contained 84.37 ± 21.93 and 25.67 ± 10.16 mg/Kg copper and zinc respectively, but lead was below detection limit. *C. alopecuroides* was found in all sites in the wetland. Lead, copper and zinc were present in varying concentrations with exception of S5, where lead was not detected. *S. lacustris*, present only in S2 and S3, showed the presence of lead, copper and zinc. *E. crassipes*, in S3 and S4, was found to have lead, copper and zinc in varying concentrations. Lead varied significantly in plants along the wetland cells (ANOVA: F = 2.58, p = 0.015). There was no significant difference in copper concentration in plants across the wetland cells (ANOVA: F = 1.06, p = 0.399). Zinc concentration differed significantly in plants along the wetland cells (ANOVA: F = 3.20, p = 0.003).

Table 6: Mean values of metals concentration in plant samples at different sites in the wetland (Data presented as Mean ± SE).

Site	Plant(s)	Pb (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)
S2	*P. stratiotes*	ND*	84.37 ± 21.93	25.67 ± 10.16
	C. alopecuroides	19.99 ± 42.15	82.00 ± 18.36	13.80 ± 7.93
	S. lacustris	5.00 ± 15.80	68.68 ± 29.02	26.07 ± 11.25
S3	*E. crassipes*	40.00 ± 51.64	71.39 ± 30.76	27.44 ± 13.42
	C. alopecuroides	44.99 ± 49.71	86.98 ± 35.34	26.87 ± 13.48
	S. lacustris	20.00 ± 42.16	95.31 ± 35.49	18.25 ± 7.54
S4	*E. crassipes*	15.00 ± 33.74	85.61 ± 33.44	11.66 ± 10.15
	C. alopecuroides	49.99 ± 52.70	62.99 ± 33.58	29.03 ± 17.32
S5	*C. alopecuroides*	ND*	78.65 ± 37.90	19.26 ± 6.31
	ANOVA	F = 2.58 p = 0.015	F = 1.06 p = 0.399	F = 3.20 p = 0.003

4.3: Relationship between physico-chemical parameters and heavy metals in the wetland.

The Pearson correlation coefficient analyses between physico-chemical parameters of wastewater and the selected heavy metals in water, sediments and plants is presented in Tables 7 - 10.

Table 7: Correlation coefficients between temperature and heavy metals in water ($_w$), sediment ($_s$) and plants ($_p$).

	Temp	**Zn_p**	**Zn_w**	**Pb_s**	**Pb_p**	**Pb_w**	**Cu_w**	**Cu_p**	**Cu_s**	**Zn_s**
Zn_p	-0.024	1								
Zn_w	-0.024	1.000	1							
Pb_s	0.006	-0.059	-0.059	1						
Pb_p	0.006	-0.059	-0.059	1.000	1					
Pb_w	0.075	0.001	0.010	0.273	0.275	1				
Cu_w	-0.125	-0.116	-0.116	-0.063	-0.063	0.058	1			
Cu_p	0.101	-0.042	-0.042	0.152	0.152	0.259	-0.090	1		
Cu_s	0.080	-0.118	-0.118	0.320	0.320	0.299	0.043	0.394	1	
Zn_s	-0.311	-0.262	-0.262	-0.103	-0.103	-0.121	0.326	-0.329	0.111	1

$r \geq 0.5$ indicates existence of a strong correlation between parameters.

$r < 0.5$ indicates existence of a weak correlation between parameters.

The results of the Pearson correlation analysis between temperature and heavy metals in the wetland (Table 7) show existence of weak correlation between temperature and metals concentration in the wetland.

Table 8: Correlation coefficients between DO and heavy metals in water ($_w$), sediment ($_s$) and plants ($_p$).

	DO	**Zn_p**	**Zn_w**	**Pb_s**	**Pb_p**	**Pb_w**	**Cu_w**	**Cu_p**	**Cu_s**	**Zn_s**
Zn_p	0.111	1								
Zn_w	0.111	1.000	1							
Pb_s	0.169	-0.059	-0.059	1						
Pb_p	0.169	-0.059	-0.059	1.000	1					
Pb_w	0.061	0.010	0.010	0.275	0.275	1				
Cu_w	-0.032	-0.116	-0.116	-0.063	-0.063	0.058	1			
Cu_p	0.026	-0.042	-0.042	0.152	0.152	0.259	-0.090	1		
Cu_s	-0.175	-0.118	-0.118	0.320	0.320	0.299	0.043	0.394	1	
Zn_s	-0.317	-0.262	-0.262	-0.103	-0.103	-0.122	0.326	-0.329	0.111	1

$r \geq 0.5$ indicates existence of a strong correlation between parameters.

$r < 0.5$ indicates existence of a weak correlation between parameters.

The results of the Pearson correlation analysis between DO and heavy metals in the wetland (Table 8) show existence of weak correlation between dissolved oxygen and metals concentration in the wetland.

Table 9: Correlation coefficients between pH and heavy metals in water ($_w$), sediment ($_s$) and plants ($_p$).

	pH	Zn_p	Zn_w	Pb_s	Pb_p	Pb_w	Cu_w	Cu_p	Cu_s	Zn_s
Zn_p	0.158	1								
Zn_w	0.158	1.000	1							
Pb_s	0.111	-0.059	-0.059	1						
Pb_p	0.111	-0.059	-0.059	1.000	1					
Pb_w	0.077	0.010	0.010	0.275	0.275	1				
Cu_w	-0.236	-0.116	-0.116	-0.063	-0.063	0.058	1			
Cu_p	0.468^*	-0.042	-0.042	0.152	0.152	0.259	-0.090	1		
Cu_s	0.175	-0.118	-0.118	0.320	0.320	0.299	0.043	0.394	1	
Zn_s	-0.552^*	-0.262	-0.262	-0.103	-0.103	-0.122	0.326	-0.329	0.111	1

Significant levels are indicated by * at $p < 0.\ 05$.

$r \geq 0.5$ indicates existence of a strong correlation between parameters.

$r < 0.5$ indicates existence of a weak correlation between parameters.

The Pearson correlation analysis between pH and heavy metals in the wetland showed that copper in plants was positively correlated to pH while zinc in sediments was negatively correlated to pH (Table 9). However pH showed weak correlations with metals in the other sample matrices in the wetland.

Table 10: Correlation coefficients between EC and heavy metals in water ($_w$), sediment ($_s$) and plants ($_p$).

	EC	**Zn_p**	**Zn_w**	**Pb_s**	**Pb_p**	**Pb_w**	**Cu_w**	**Cu_p**	**Cu_s**	**Zn_s**
Zn_p	-0.019	1								
Zn_w	-0.019	1.000	1							
Pb_s	0.302	-0.059	-0.059	1						
Pb_p	0.302	-0.059	-0.059	1.000	1					
Pb_w	0.081	0.010	0.010	0.275	0.275	1				
Cu_w	-0.040	-0.116	-0.116	-0.063	-0.063	0.058	1			
Cu_p	0.034	-0.042	-0.042	0.152	0.152	0.259	-0.090	1		
Cu_s	0.066	-0.118	-0.118	0.320	0.320	0.299	0.043	0.394	1	
Zn_s	-0.029	-0.262	-0.262	-0.103	-0.103	-0.121	0.326	-0.329	0.111	1

$r \geq 0.5$ indicates existence of a strong correlation between parameters.

$r < 0.5$ indicates existence of a weak correlation between parameters.

The results of the Pearson correlation analysis between EC and heavy metals in the wetland (Table 10) show existence of weak correlation between electrical conductivity and metals concentration in the wetland.

4.4 Heavy metals removal efficiency in the wetland

The efficiency of heavy metals removal from the wastewater in the wetland is summarized in Table 11.

Table 11: Metal removal efficiency in the wetland

Metal	Influent concentration (mg/L)	Effluent concentration (mg/L)	% Removal
Lead (Pb)	1.25 ± 0.75	0.07 ± 0.07	94.4
Copper Cu)	1.09 ± 0.49	0.32± 0.11	70.64
Zinc (Zn)	0.15 ± 0.11	ND*	100

ND* - Not Detected

In this study, metals removal efficiencies of 94.4%, 70.64% and 100% for lead, copper and zinc respectively were observed.

4.5 Discussion

4.5.1 Physico-chemical characteristics of water in the wetland

Physicochemical parameters play an important role in determining the water quality and greatly influence the functioning of the wetland. The accumulation of metals from the wastewater to the sediment and plants is dependent on a number of external environmental factors such as pH, dissolved oxygen, electrical conductivity and the available surface area for adsorption (Davies *et al*., 2006).

In this study, the highest temperature was recorded in S1 (18.6 ± 0.24 ^{o}C) and could be attributed to the direct sunlight into the last lagoon of the waste stabilization pond system. The mean temperature drop in S2, S3 and S4 may have resulted from the vegetation mats over the wetland cells coupled with low flow rate in the system. The temperature rise in S5 may be attributed to the fact that the wetland cell was basically an open pond with limited vegetation cover. Generally, wetland water temperature is assumed to be close to mean daily air temperature (Kadlec and Knight, 1996). Temperature is an essential factor in life processes, including biological, chemical and physical processes that occur in the environment. It influences growth of macrophytes, therefore, it also affects the accumulation effect of heavy metals. When the temperature is high, the growth and the metabolism of aquatic plants are high, leading to an improvement of metal accumulation.

The pH values in the wetland were found to be within the limits set by NEMA (6.8 – 8.5) for effluent discharge into the environment. The highest pH value was recorded in S1 (8.5) while the lowest was in S4 (6.7). Kadlec and Wallace, (2009) reported that pH does not fluctuate significantly throughout the year in most natural and constructed wetlands. Decrease in pH may be attributed to organic substances generated within a wetland through growth, death and decomposition cycles of wetland vegetation that are a source of natural acidity (Kadlec and Knight, 1996). pH affects the solution chemistry of the metals, the activity of functional groups in biomass and competition of metallic ions (Li *et al*., 2005). Redox potential and pH of the sediment–water system are the major factors that influence the mobility of heavy metals in wetlands (Koretsky *et al*., 2008).

Electrical conductivity is a measure of the ability of water to conduct an electric current and it is related to the amount of dissolved ions in the water. It is a good and rapid method to measure the total dissolved ions and is directly related to the total solids in the water sample (Singh *et al.,* 2010). Electrical conductivity in the wetland ranged between 690 – 885 µS/cm. Variation of EC in the wetland cells can be attributed to the physico-chemical processes occurring there, which include removal of ions through sedimentation, precipitation, adsorption and uptake by aquatic plants. Although NEMA (Kenya) has not set the maximum permissible levels for conductivity in the discharge, the South African guideline for conductivity in effluent discharged into the receiving water bodies is 250 µS/cm (Government Gazette, 1984). NEMA should gazette maximum permitted levels for EC since it is a very important parameter in water to be used for irrigation.

The mean concentration of dissolved oxygen at the inlet (S1) was 4.7 ± 0.82 mg/L but decreased in S2, S3 and S4 before rising slightly in S5 then drop again in S6. Decrease of oxygen levels, especially in S2 and S3, may have been due to high oxygen demand by decomposing organic matter and in the wetland cells. Dissolved oxygen concentrations above 1.5 mg/L are required for most treatment processes (Ding *et al*., 2012). In unpolluted water, dissolved oxygen concentrations normally range between 8 and 10 mg/L and concentrations below 5 mg/L adversely affect aquatic life (Rao, 2005). The near-total cover by plant mats over the wetland cells; low water velocity and settling of dead plants at the bed of the cells impede uniform flow of water thereby creating channels and dead-ends within the system.

4.5.2 Heavy metals concentration in water samples

Metals in wastewater are present in colloidal, particulate, and dissolved phases. However, more than 90% of heavy metals load in aquatic systems are found on suspended particles and sediments while only a small portion of free metal ions remain dissolved in water (Zahra *et al.*, 2014). The decrease in mean concentrations of the metals in S2 is mainly attributed to sedimentation. However, the variation in concentrations of the metals in water across the wetland may be attributed to the various processes taking place in the wetland. Short-circuiting and preferential flow paths were observed in the wetland, as demonstrated by the distribution patterns of metal concentrations in the water sampled from each site. The decomposition process of higher organic matter may lead to the lower value of pH due to the production of humic acid (Nobi *et al*., 2010), which in turn increase metal release back to the water column. The wetland is located next to horticultural fields and application of fertilizers and herbicides/fungicides could be contributing copper and zinc into the wetland. The constructed wetland showed removal efficiencies of 94.4%, 70.64% and 100% for lead, copper and zinc respectively. Gituku *et al.* (2015) reported removal efficiencies of 80.0%, 95.1% and 100% for Pb, Cu and Zn respectively from a study on Nandi Hills tea estates constructed wetlands and the results of these wetlands compare favourably.

4.5.3 Heavy metals concentration in sediment samples

Sediments form the most significant component of water ecosystems with respect to metal accumulation in wetlands (Willow and Cohen, 2003). Sediment quality is an important indicator of water pollution and has been used as a tool to assess the health status of aquatic ecosystems and is therefore, an integral component for functioning of ecological integrity (Zahra *et al.,* 2014). In this study Lead, copper and Zinc were present in sediments at much higher concentrations than those observed in water. The metals concentrations in sediments across the wetland ranged between 25 – 50 mg/Kg for lead, 28 – 42 mg/Kg copper and 84 – 116 mg/Kg zinc. The accumulation process involves complex chemical and physical mechanisms which depend on the nature of the sediment matrix and the properties of the adsorbed compounds (Machate *et al.,* 1999). pH and redox potential are the most important variables that govern heavy metal solubility (Delaune *et al.,* 1997). The high levels of metals found in the sediments indicate that the wetland is quite good at retaining these metals by capturing sediments and allowing for sedimentation of suspended metals. Wetlands provide anaerobic conditions that promote the growth of sulfate-reducing bacteria. Lead, copper, cadmium and zinc form highly insoluble sulfide compounds in contact with low concentrations of hydrogen sulfide. Sulfide minerals have been reported in a number of wetland sediments (Sobolewski, 1999). However the metal ions may renter the water column should the conditions e.g. pH and redox potential change in the sediments or when there is perturbation in the wetland.

4.5.4 Heavy metals concentration in plant samples

All the four plant species investigated exhibited some phytoremediation characteristics towards the selected metals. The levels of detected metals varied widely between/within plants species and sites. This may be attributed to the intervening variables such as water volume and flow as well as the prevailing physico-chemical conditions in each site. Wetland plants may indirectly facilitate removal processes by providing surfaces for active and diverse microbial communities (Rossmann *et al*., 2012), and preventing re-suspension of sedimented pollutants. They not only assimilate pollutants directly into their tissues, but they also catalyze purification reactions by increasing the environment diversity in the root zone and supporting a variety of chemical and biochemical reactions that improve purification. Macrophytes alter the redox conditions, pH and organic matter content of sediments and so affect the chemical speciation and mobility of metals (Jacob and Otte, 2003).

In some cases metals get adsorbed onto plaques of iron and manganese oxides that form on the roots of wetland plants (Batty *et al*., 2000). Root plaques have been observed to contain a variety of metals and metalloids including aluminium, arsenic, cadmium, chromium, mercury, nickel, lead and zinc (Sundby *et al*., 1998). This means that if conditions at any site favour the formation of these plaques on the roots and submerged stems, then high concentrations of metals will be observed.

4.5.5 Relationship between physico-chemical parameters of water and heavy metals concentrations in the wetland.

The Pearson correlation analysis between physicochemical parameters of water and heavy metals concentration in the wetland generally showed weak correlations (Table 6 - 9). However, pH was positively correlated to copper in plants and negatively correlated to zinc in sediments (Table 8). This implies that as pH increased in the wetland, copper concentration also increased in the plants while zinc concentrations reduced in sediments. Haiyan *et al*. (2013) reported that the effect of pH on Zn and Cu in sediments was such that the release of the metals increased under both acidic and alkaline conditions. This may possibly explain the decrease of Zinc level in sediments as pH increase; and the availability of copper for plant uptake. The physicochemical parameters investigated, to some extent, do influence the concentrations of heavy metals in the compartments of the wetland.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the study findings, the following conclusions were made:

1. The physico-chemical parameters of wastewater varied spatially along the constructed wetland wastewater treatment pathway.

2. The concentrations of lead, copper and zinc in wastewater generally declined along the constructed wetland wastewater treatment pathway.

3. The dominant macrophytes *(P. stratiotes, C. alopecuroides, S. lacustris,* and *E. crassipes*) exhibited good uptake of the metals and therefore aided in the removal of the metals from wastewater.

4. The heavy metal concentrations in the sediments were higher than the recommended EPA's Effects Range Low (ERL) values for sediment-dwelling organisms and thus likely to elicit adverse lethal and sub-lethal effects to the benthic organisms.
5. The physico-chemical characteristics of wastewater influenced the distribution of heavy metals in the three components of the constructed wetland – water, plants and sediments.

5.2. Recommendations

The following recommendations were made with the aim of enhancing the efficiency of heavy metals removal from wastewater by the constructed wetland:

1. The proportion of the floating macrophytes (*P. stratiotes* and *E. crassipes*) to that of emergent macrophytes (*C. alopecuroides, S. lacustris*) should be regulated through periodic harvesting so as to improve the physico-chemical characteristics of wastewater in the wetland.
2. Macrophytes harvested from the constructed wetland should disposed off properly to prevent recirculation of metals into the wetland upon plants' decomposition.

5.3. Further Research

It is recommended that further studies be conducted in the following areas:

1. The distribution of heavy metals in macrophyte tissues (root, stem and shoot) and the growth stage where metal uptake is at the peak.
2. Bio-monitoring as a tool for assessing water quality improvement within the wetland. The suggested bio-indicators include algae, macrophytes, zooplanktons, aquatic insects, mollusks, amphibians, fish and birds.
3. Speciation and potential mobility of heavy metals in the sediment.
4. Nutrient removal by floating and emergent macrophytes in the constructed wetlands.

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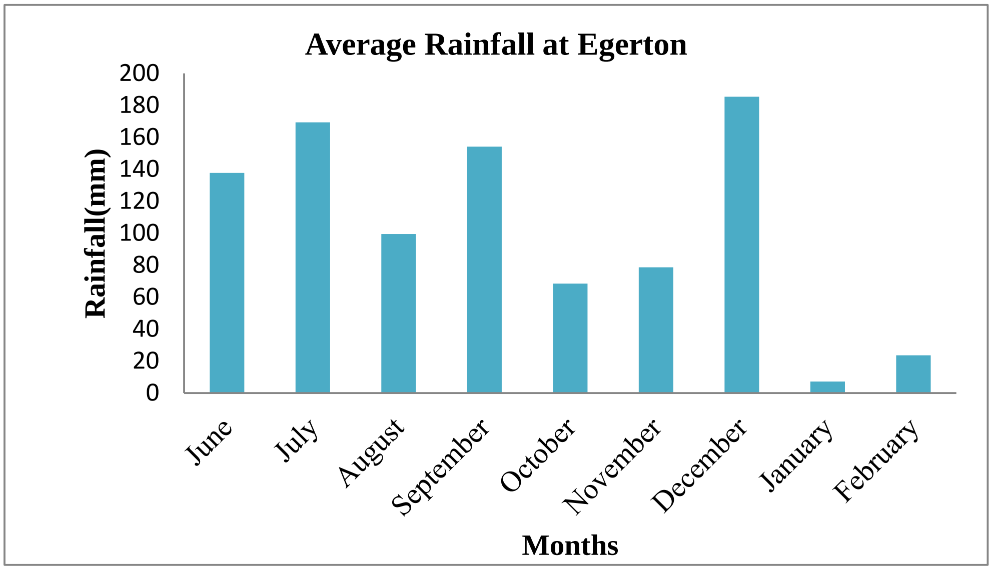
APPENDIX

Appendix 1: Average rainfall at Egerton University between June 2013 and February 2014.



(**Source:** Department of Civil and Environmental Engineering, Egerton University, 2014)