Phytoremediation Potential of *Cyperus Alternifolius, Cyperus Dives* and *Canna Indica* in Flamingo Farm Constructed Wetland, Naivasha Sub-County, Kenya

By

Josephat Otieno Oluoch (BSc. Forestry)

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DECLARATION

This thesis is my original work and has not been presented for a degree or award in any other University.

……………………………… …………………………..
Signature Date

Josephat Otieno Oluoch
N50/CTY/PT/23970/2011
Department of Environmental Sciences
Kenyatta University

Declaration by Supervisors

I/We confirm that the work reported in this thesis was carried out by the student under our supervision.

………………………………. …………………………..
Signature Date

Dr. Esther Kitur
Department of Environmental Sciences
Kenyatta University

……………………………… …………………………….
Signature Date

Dr. Paul Obade
Department of Environmental Sciences
Kenyatta University

DEDICATION

To my family: Beryl, Fidel and Faith for being a constant source of support and encouragement especially during low moments in the course of undertaking this research.

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It will be unfair to forget SGS Kenya Limited who allowed me to conduct the analysis of heavy metals in their laboratories and Kenyatta University especially for the degree award that is on the way. Lastly, I am very grateful to Almighty God.

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ACRONYMS AND ABBREVIATIONS

ANOVA	:	Analysis of Variance
ATP	:	Adenosine Triphosphate
BCF	:	Bioconcentration Factor
BOD	:	Biological Oxygen Demand
CEC	:	Cation Exchange Capacity
DNA	:	Deoxyribonucleic Acid
EPS	:	Extracellular Polymeric Substances
EU	:	European Union
FAO	:	Food and Agriculture Organisation
GBH	:	Gravel Bed Hydroponics
HCL	:	Hydrochloric Acid
IAA	:	Indole-3-Acetic Acids
IARC	:	International Agency for Research on Cancer
ICP-OES	:	Inductively Coupled Plasma Optical Emission Spectrometer/ry.
KPHC	:	Kenya Population and Housing Census
NRMED	:	Natural Resource Management and Environment Department
PGPB	:	Plant growth-promoting bacteria
ROS	:	Reactive Oxygen Species
TF	:	Translocation Factor

ABSTRACT

Contamination of fresh water sources with heavy metals found within the agricultural areas is largely contributed by amongst other factors, application of inorganic fertilizers and pesticides. Removal of heavy metals from the contaminated waters using conventional methods is cost prohibitive and more importantly less effective especially when concentration of heavy metals is low. As a result, use of alternative technologies such as phytoremediation is being promoted. Phytoremediation uses plants called phytoremediators and in order to achieve successful remediation of wastewater, proper selection of the phytoremediators is important. This study, therefore, aimed at evaluating phytoremediation potential of *Cyperus alternifolius*, *Cypersu dives* and *Canna indica* plants which grow in Finlays Flamingo Farm constructed wetland. The study was conducted between February 2014 and August 2014. The objectives were to determine bioconcentration and translocation factors of Arsenic, Cadmium and Lead metals in *Cyperus alternifolius, Cyperus dives* and *Canna indica*, and evaluate the phytoremediation potential of the study plants. Eighteen plants of each species were planted on the Gravel Bed Hydroponics and left to grow for four months. Six plants of each species and wastewater were then sampled at the end of the fifth, sixth and seventh months. Roots and shoots of each species were put together to form composite samples and analyzed for Arsenic, Cadmium and Lead metals using the Inductively Coupled Plasma Optical Emission. Wastewater samples were also analyzed for Arsenic, Cadmium and Lead metals using the American Public Health Association procedures. Data were presented in tables and graphs, and analyzed using Analysis of Variance and Tukey tests at p=0.05. The findings showed that biocentration factor values for Arsenic, Cadmium and Lead metal in the study plants were greater than one (ranging from 7 to 1208) while translocation factor values were less than one (ranging from 0.069 to 0.934). These results confirmed that the study plants were effective phytostabilizers of Arsenic, Cadmium and Lead metals and can be used for phytoremediation. However, for effective removal of these heavy metals from wastewater, harvesting of the study plants should include removal of their roots.

CHAPTER 1.0: INTRODUCTION

1.1 Background to the Study

Wastewater treatment and disposal in developing countries has been a major environmental challenge due to shortcomings associated with conventional wastewater treatment systems. These systems require high construction and operational that include cost of chemicals and skilled personnel required. As a result, the systems have been poorly maintained and consequently, freshwater sources have been polluted. The water pollutants have included heavy metals which are associated with serious public health problems (Sundaramoorthy *et al.,* 2010). For instance, a correlation has been found to exist between high concentrations of dissolved heavy metals in drinking water with damage of nerves, liver, bones, blocking of functional groups of vital enzymes and have been thought to be possible human carcinogens (Zhang *et al.*, 2012). Therefore, there is need to find an alternative and or complementary technology to the conventional wastewater treatment system.

There is evidence that constructed wetlands can offer solutions to the challenges associated with conventional wastewater treatment systems. This technology uses aquatic plants to remediate wastewater. Aquatic plants are preferred because of their high growth rates that enable them to achieve high level of heavy metals removal (Rahman and Hasegawa, 2011) when they are harvested. They have capability of absorbing, degrading, transforming and stabilizing contaminants within substrates using physical, chemical and biological processes (Zhang *et al.*, 2010). The plants can also tolerate, absorb and translocate heavy metals that would otherwise be toxic to most organisms in the environment (Zhang *et al.,* 2010). This technology is widely accepted as a cost-effective way of treating wastewater (Mojiri, 2012) and because aesthetically its pleasing and appropriate for sites with low to moderate levels of heavy metal contamination (Setia *et al.,* 2008).

The plants that are used in constructed wetlands can either be naturally occurring heavy metal hyperaccumulators or those that have been genetically engineered (Setia *et al.*, 2008) to enhance their metal accumulation capability. Once the plants are identified, they can be used to remediate a variety of polluted waters including agricultural runoff (Olguín and Galván, 2010). In this technology, heavy metals are generally removed through harvesting of plants that have been grown in wastewater and have accumulated heavy metals in their tissues (Yadav and Chandra, 2011). By so doing, plants renovate wastewater by stripping it of its pollutants and this feature is often referred to as phytoremediation.

1.2 Statement of the Problem and Justification

In 1980s, there was emergence of intensive horticultural farming in Kenya that resulted in indiscriminate use of inorganic fertilizers and pesticides (Ndungu *et al*., 2014). These inorganic fertilizers and pesticides have different types of heavy metals as part of their formulations (Sadhana, 2014) and if not managed well end up in wastewater. Due to challenges associated with conventional wastewater treatment systems, for instance, incomplete heavy metal removal and generation of substantial amounts of toxic sludge that would require further appropriate disposal (Sekhar *et al.*, 2003), these conventional wastewater treatment systems have not been effective in wastewater treatment. They become ineffective particularly when the concentration of heavy metals is very low (Ahluwalia and Goyal, 2007) and because the heavy metals are soluble in water, it is impossible to remove them from wastewater using physical separation methods (Hussein *et al*., 2004) that these systems employ. As a result, fresh water sources are contaminated, and environmental perturbations are experienced. Moraa, 2010 reports that the death of fish in Lake Naivasha in February 2010 was due to lack of oxygen which was a pointer to the fact that the health of Lake Naivasha ecosystem was deteriorating. This leads to the decline of the availability of freshwater resources and may cause ‘water stress’ that may trigger fierce competition for water resources better known as ‘water war’ (Postel and Wolf, 2001).

There is evidence that aquatic plants can be used in constructed wetlands to address the challenge of incomplete heavy metals’ removal from wastewater through the process of phytoremediation. However, for this process to be successful, the constructed wetland must be planted with aquatic plants with desirable qualities. The plants should be able to develop resistance towards heavy metals toxicity and should be able to accumulate heavy metals in their tissues at high levels (Yoon *et al.*, 2006). Therefore, selection of plants to be used in a constructed wetland is critical in this process. This study therefore sought to select aquatic plants that can be used for heavy metals removal from wastewater by evaluating their ability to uptake from wastewater, accumulate in their roots and translocate to their aerial parts.

1.3 Research Questions

The questions that this study sought to address therefore were:

1) Can *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland accumulate Arsenic, Cadmium and Lead metals in their roots?
2) Can *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland translocate Arsenic, Cadmium and Lead metals to their shoots?
3) Do *C. alternifolius*, *C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland have phytoremediation potential for removing Arsenic, Cadmium and Lead metals from wastewater?

1.4 Research Objectives

The overall objective of the study was to evaluate phytoremediation potential of *C. alternifolius, C. dives* and *C. indica* in removing heavy metals from wastewater. Therefore, the specific objectives of the study were:

1) To determine if *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland can accumulate Arsenic, Cadmium and Lead metals in their roots.
2) To determine if *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland can translocate Arsenic, Cadmium and Lead metals from their roots to their shoots.
3) To determine if *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland have phytoremediation potential for removing Arsenic, Cadmium and Lead metals from wastewater.

1.5 Hypotheses

1) *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland can accumulate Arsenic, Cadmium and Lead metals in their roots.
2) *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland can translocate Arsenic, Cadmium and Lead metals to their shoots.
3) *C. alternifolius, C. dives* and *C. indica* plants growing in Flamingo Farm constructed wetland have phytoremediation potential for removal of Arsenic, Cadmium and Lead metals from wastewater.

1.6 Significance of the Study

Aquatic plants can be used in constructed wetlands to address the challenge associated with conventional wastewater treatment systems in removing heavy metals from wastewater particularly when the concentrations are low. However, for these constructed wetlands to be efficient in heavy metals removal, aquatic plants to be used should be selected based on the desired qualities, that is, developing resistance to heavy metals' toxicity and the ability to accumulate heavy metals in the tissues. Currently, this information is lacking for some aquatic plants, and the findings of this study will be used in selection of aquatic plants to be used in constructed wetlands. The information generated will be particularly useful to horticultural farms which use inorganic fertilizers and pesticides and are likely to have heavy metals in wastewater.

The study will also make an important contribution towards an improved understanding of phytoremediation in terms of which parts of the plants, roots or shoots, should be harvested as a way of removing heavy metals from wastewater management. This study will generate information that will determine whether it is more efficient to harvest the above ground biomass or below ground biomass or both to achieve optimum heavy metals' removal from wastewater.

This study will also contribute to knowledge in wastewater management and will and will serve as reference and or source of information for future related studies.

1.7 Conceptual Framework

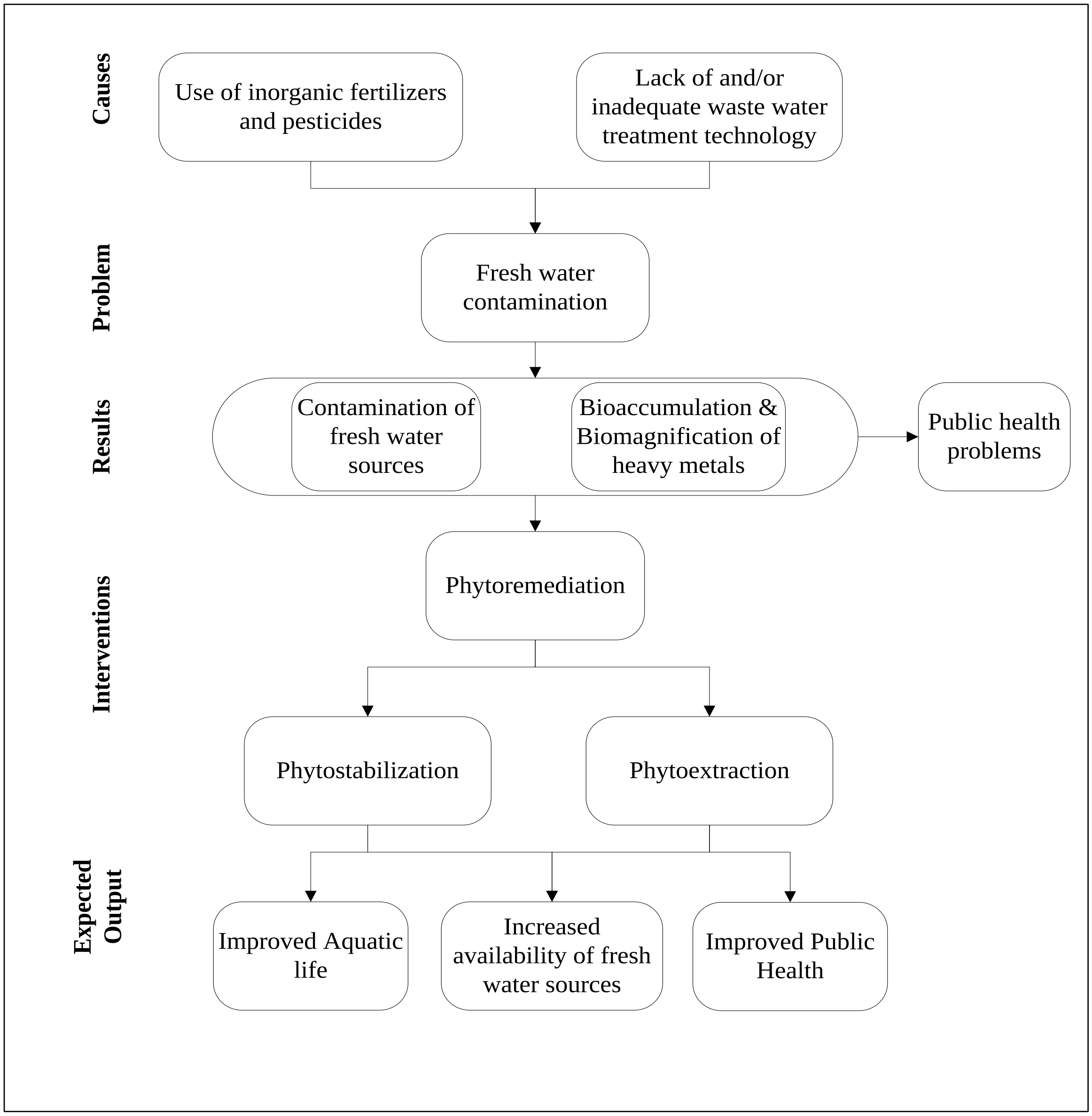

Figure 1.1: Conceptual framework for the study

1.8 Definition of Terms

1) Phytoextraction is the uptake of heavy metals from the substrate by plants using their roots. The heavy metals, in soluble form, are then translocated to other plant tissues for storage in plant parts that can be harvested like stems and leaves.
2) Phytostabilization is the process by which plants are used to reduce the bioavailability of heavy metals in the substrate.

3) Bioconcentration Factor (BCF) is a ratio used to show ability of a plant to accumulate a particular heavy metal in its roots with respect to the heavy metal concentration in the substrate (Ghosh and Singh 2005). It is used to determine the amount of heavy metals absorbed and accumulated by the plant from the substrate.
4) Translocation Factor (TF) is a ratio that is used to show the ability of a plant to translocate heavy metals from its roots to its above ground biomass (Chakroun *et al.*, 2010). The translocation factor shows the efficiency of the plant in translocating heavy metals from roots to above ground biomass.

CHAPTER 2.0: LITERATURE REVIEW

2.1 Heavy Metals and the Environment

Heavy metals can be defined as metallic elements with relatively higher densities. They cannot be degraded or destroyed and occur naturally in the earth's crust. They enter our bodies through food, water and air. Some of these heavy metals such as copper, selenium, zinc are essential elements that are required for various biochemical and physiological functions in plants and animals. Copper, for example, is an essential co-factor for several oxidative stress-related enzymes (Stern, 2010). However, these essential metals are required at very small quantities and at higher concentrations, they can lead to tissue damage and may cause a variety of adverse effects that may include human diseases (Tchounwou *et al.*, 2008). Copper, for example, has the ability to cycle between oxidized and reduced state which makes it potentially toxic and excessive exposure to Copper can lead to Wilson disease in humans (Tchounwou *et al.*, 2008).

Exposure to heavy metals also affect organelles and other cell components, and enzymes that are involved in metabolism, detoxification and repair of damages (Wang and Shi, 2001). These metal ions interact with cell components such as deoxyribonucleic acid (DNA) and nuclear proteins thereby causing damage to DNA and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis (Beyersmann and Hartwig, 2008). Studies have shown that reactive oxygen species (ROS) production and oxidative stress play an important role in the toxicity and carcinogenicity of heavy metals like arsenic (Yedjou and Tchounwou, 2007), cadmium (Tchounwou *et al.*, 2001), chromium (Patlolla *et al*., 2009), lead (Yedjou and Tchounwou, 2008), and mercury (Sutton and Tchounwou, 2007). Some heavy metals like arsenic, cadmium, chromium, lead and mercury are of great public health importance due to their high toxicity and can cause damage to organs even at lower exposures. Epidemiological and experimental studies have also shown an association between cancer incidences (in animals and humans) and exposure to these metals. This has led to the classification of these metals as either probable or known carcinogens (Wang and Shi, 2001).

Nonetheless, there has been tremendous increase in the use of heavy metals in industrial, agricultural and domestic applications. This has increased human exposure to heavy metals and as a result, there have been both ecological and public health concerns related to these metals (Bradl, 2002) and thus the need to address the problems associated with heavy metals.

2.2 The Wastewater Problem

UN-Water, 2014 reported that the world has been facing a water quality crisis that is as a result of the ever-increasing world population. The report continued to say that the crisis is also as a result of environmental challenges related to urbanization, industrialization, food production practices and more importantly poor water use practices and wastewater management strategies. Poor wastewater management or simply the lack of it has had a direct impact on the biological diversity particularly of aquatic ecosystems. This has caused disruption of fundamental integrity of life support systems on which a wide range of sectors, ranging from urban development to food production and industry, rely on (Corcoran *et al.*, 2010). Therefore, it is essential that wastewater management is appreciated and considered as part of an integrated eco-system-based management system that would operate across social, economic and environmental dimensions (Corcoran *et al.,* 2010).

Environmental challenges related to water quality and ultimately water quantity were echoed by World Water Forum, 2012. This forum resolved that there is an urgent need to bring wastewater to the fore of world politics. Although there have been efforts geared towards improving sanitation, these efforts have largely focused on increasing service coverage in terms of access to improved toilet facilities. Sadly, far less efforts have been directed towards ensuring wastewater is collected and appropriately treated before discharging it to the natural environment. UN Water, 2014 reports that worldwide wastewater treatment systems are failing and as a result, wastewater, septage and faecal sludges continue to be discharged into the natural environment without any form of treatment and consequently causing diseases to humans and damaging key ecosystems. Naivasha.

United Nations, in their report UN-Water for 2014 opined that water crisis has been largely viewed as a water quantity problem. However, water quality as an aspect of water problem is now increasingly been recognized as a major factor in the water crisis equation in many countries. Previously, degraded water quality were mainly associated with public health problems which are still major concerns in less developed and developing countries, particularly in Africa. But more importantly, contribution of degraded water towards water crisis in terms of loss of beneficial water that can be used for agricultural and ecological uses is currently being appreciated (UN-Water, 2014).

Many national economies incur a multitude of economic losses due to fresh water pollution. These costs are for the expansion of water treatment facilities, developing alternative potable water sources, loss of fish for commercial purposes, loss of other biodiversity, degradation of habitats and loss of tourism revenues. There are also other direct and indirect losses such as cost of diseases, loss of agricultural production as well as loss (or increased cost) of industrial production due to impaired water quality, and cost of social unrest and population migration associated with extremely degraded aquatic environments (NRMED, 2015).

The situation in Africa is highly variable, from moderately developed to very under-developed countries. Although not all countries are facing a water crisis, all to some extent have had serious problems associated with degraded water quality. This is particularly evident in highly eutrophic lakes and reservoirs, and in increased incidences of gastro-enteric diseases especially in young children (NRMED, 2015). In Kenya, for instance, water scarcity is a limiting factor to development activities. The current water availability is estimated as $650m^3$/year per capita and could drop to $350m^3$/year by the 2020 (Ngigi and Macharia, 2006). With dropping per capita freshwater availability, there is increasing dominance of wastewater in the water balance and this makes wastewater a very important source of irrigation water for urban agriculture (Githuku, 2009).

2.3 Wastewater in Lake Naivasha

Lake Naivasha was one of the ecosystems that was known and treasured for its preference as a tourist attraction site. This was attributed to its richness in biological diversity that led to its designation as a Ramsar site in 1995 (Ndungu *et al*., 2014). However, because of the growing population in Naivasha, as informal settlements and also as horticultural farms in 1980s, pollution pressure was exerted on the lake ecosystem leading to its deterioration (Ballot *et al.,* 2009). The Lake has transformed from a clear state dominated by macrophytes to one that is turbid and dominated by algae. Several environmental perturbations that include species invasion, soil erosion and hydrological changes and excessive water abstractions have degraded the ecosystem. Consequently, aquatic plants that included water lilies and submerged macrophytes started and continued to fluctuate between absence and presence due to the increased turbidity of the lake (Britton *et al.,* 2007) and a shift in biological diversity, particularly in the phytoplankton communities, has been observed (Hubble and Harper, 2002).

2.4 Conventional Wastewater Treatment

There are three levels in conventional wastewater treatments systems. These levels include primary treatment which is also called mechanical treatment, secondary treatment which is also called biological treatment, and tertiary treatment. Primary treatment level is a mechanical process that is designed to remove solids from wastewater. This is achieved through physical screening and sedimentation of suspended solids by gravity. In this process, chemicals can sometimes be used to accelerate the process of sedimentation. The end result of this mechanical treatment is that Biological Oxygen Demand (BOD) and suspended solids are reduced by about 50-60% and 20-30% respectively (NRMED, 2015).

Biological treatment such as constructed wetland is the second phase of wastewater treatment in a conventional wastewater treatment system. At this level, dissolved and suspended organic matter is consumed by microorganisms who feed on them. During the process, carbon dioxide, water and energy are produced as by-products. Sedimentation for suspended solids also continues and BOD as well as suspended solids are reduced by about 85% (NRMED, 2015).

Tertiary level of wastewater treatment can be described as an additional treatment beyond the secondary or biological treatment. At this stage, up to 99% of impurities is removed from wastewater improving its quality to almost the quality of drinking water. However, the technology is expensive, requires skilled and well trained labour, constant supply of energy and chemicals, and equipment which are very specific. Therefore, these may not be readily available which makes this level of treatment not preferred. The final step in conventional wastewater treatment process involves disinfection with chlorine before final discharge into the natural environment or reuse. The use of chlorine is however discouraged by some authorities because of its residual ability in the environment (NMRED, 2015).

2.5 Wastewater Treatment using Constructed Wetlands

The existence of natural wetlands and the recognition of the role they play in water purification process led to the development of constructed wetlands. Use of constructed wetlands as an alternative way for treating wastewater is rapidly increasing due to the low construction and operational costs as well as performance associated with them (Kadlec *et al.,* 2000). They are being accepted as the final step in a conventional wastewater treatment system to finally ‘polish’ wastewater before discharge into natural environment. The technology is simple, and sustainable economically and ecologically but very effective and contributes towards achieving compliance with effluent discharge standards (EU 2000/60). The greatest advantage of constructed wetlands is that benefits associated with natural wetlands can also accrue through constructed wetlands.

The use of constructed wetlands in Africa is a relatively new concept and the technology is rapidly spreading in many countries. In Kenya, for example, there are constructed wetlands that are fully operational across the country. These wetlands are in Nairobi, Nandi Hills, Kericho, Naivasha, Maasai Mara and Timau areas. They are used for treating wastewater from various establishments that include restaurants, horticultural farms and tea factories. None of these constructed wetlands are, however, owned or managed by the communities.

Constructed wetlands have both vascular plants (the higher plants) and non-vascular plants (algae) which are important in many ways. Photosynthesis by algae increases the dissolved oxygen content of the water which in turn affects nutrient and metal reactions. Vascular plants contribute to the treatment of wastewater through stabilization of contaminants in the substrates thereby limiting channelized flow, slowing down velocities and allowing suspended root systems to settle, taking up carbon, nutrients and heavy metals, and incorporating them into plant tissues.

2.6 Using Plants in Wastewater Treatment

2.6.1 Distribution of Heavy Metals in Plants

Plants take up heavy metals from substrates and accumulate them in their biomass. Some heavy metals are translocated to stems and leaves (Woranan *et al*., 2010) but relatively larger portions of heavy metals are stored in the roots of the plants as compared to the above ground biomass (Cheng, 2003). This makes heavy metal concentration being higher in roots, followed by leaves and least in fruits. Lateral roots also accumulate more heavy metals than main roots while older leaves accumulate more heavy metals than younger leaves (Cheng, 2003). Weis and Weis, 2004 also reported that there are higher concentrations of heavy metals within cell walls than inside the cell walls. This distribution and accumulation of heavy metals in plants is affected by the species of the plant, type of heavy metal, and heavy metal bioavailability. Other factors that influence heavy metal distribution in plants include environmental factors such as redox reactions, pH of the substrate, cation exchange capacity, dissolved oxygen and temperature (Woranan *et al*., 2010).

Tolerance to heavy metal toxicity by plants is controlled by physiological factors like root cation exchange capacity (CEC), phytochelation, antioxidative stress, production and utilization of carbohydrates (Woranan *et al*., 2010). Transportation of heavy metals in the plants largely depends on the chemical status of the plants themselves. Heavy metals have the ability to combine with inorganic compounds such as sulphides and small-molecular organic substances such as glutathione and it is this kind of metal-ion interactions that have been thought to be responsible for metal uptake and translocation in plants (Chantiratikul *et al.*, 2008).

2.6.2 Metal-Inducible Proteins

Once the metals have been taken up by the roots into the root symplasm, there are usually three processes that take place for their movement into the xylem. These processes include sequestration of the metals inside root cells, symplastic transport of metals into stele, and release of metals into xylem which is mediated by membrane transport proteins (Woranan *et al*., 2010). Chelation of metals by organic acids, amino acids and metalloproteins also takes place to detoxify heavy metals and ensure plants' tolerance to the heavy metals (Callahan *et al.*, 2006). This mechanism plays a very critical role in transport, translocation and detoxification of heavy metals in plants (Cobbett and Goldsbrough, 2002).

2.6.3 Microorganism in Rhizosphere

There are heavy metals that are very important components of microbial cells. These heavy metals are, for instance, sodium and potassium which control gradient across cell membranes; copper, iron and manganese which are very key in metalloenzymes used in photosynthesis and electron transport. Therefore, heavy metals which are not essential are probably the most toxic and they include cadmium, mercury, lead, etc. Microorganisms have developed mechanisms that enable them tolerate toxicity presented by non-essential heavy metals. These mechanisms include the ability of cells to prevent entry of heavy metals into the cell and the ability to pump out heavy metals that have entered the cells. This is accomplished through processes such as active transport, chemical transformation through redox reactions and sequestration (Woranan *et al*., 2010).

In sequestration, toxic heavy metals are complexed using microbial products that include extracellular polymeric substances and metallothione. Heavy metals once complexed are transported out of the cell or stored as a granule intracellularly. In active transport, toxic heavy metal ions that have entered the cell are pumped out using adenosine triphosphate-dependent efflux pumps that are metals-specific.

In the rhizosphere, there are also plant growth-promoting bacteria (PGPB) that are known to promote the growth of plants. As plants grow rapidly due to the effect of PGPB, their biomass increases very fast and consequently their ability to tolerate toxic heavy metals also increases. PGPB promotes rapid growth of plants through nitrogen fixing, phosphate solubilization, sulphate oxidation and synthesis of phytohormones (Zhuang *et al.*, 2007). There are also other mechanisms that PGPB uses to promote plants' tolerance to heavy metals toxicity. These mechanisms include releasing of antibiotics, releasing of extracellular enzymes, and releasing of chemicals and volatile compounds. These mechanisms allow respiration to take place in the roots and that leads to increase in size of the plants (Mukerji, 2006). PGPB also aid phytoremediation by increasing availability of heavy metals and their mobility to plants (Jing *et al.*, 2007).

2.7 Phytoremediation Potential

Phytoremediation is a technology that uses plants and associated microorganisms to remove, degrade and isolate contaminants from the environment (Dickinson, *et al.*, 2009). There are many contaminants that can be subjected to phytoremediation for removal from the environment. They include heavy metals, metalloids, inorganic compounds, radioactive chemical elements, petroleum hydrocarbons, pesticides and herbicides, explosives, chlorinated solvents and industrial organic wastes (Ensley, 2000).

2.7.1 Phytoremediation Strategies

There are various mechanisms that phytoremediation uses to remediate contaminated substrates. The mechanism employed is largely dependent on the chemical nature and properties of the contaminant. It also depends on the characteristics of the plant that is being used in phytoremediation. Therefore, there are six different strategies that phytoremediation employs, and more than one strategy can be employed simultaneously by a plant. These strategies include phytodegradation, phytostabilization, phytovolatization, phytoextraction, phytofiltration and rhizodegradation.

Phytodegradation involves organic contaminants being degraded through metabolization in plant cells. Specific enzymes such as nitroreductases, dehalogenases and laccases are involved in this process (Rylott, 2008). Phytostabilization involves contaminants, organic or inorganic, being incorporated in the cell wall of root cells or humus. The contaminants are then precipitated in insoluble forms using root exudates and they finally get trapped in the substrate. The process prevents mobility of contaminants thus limiting their diffusion in the substrate (Ali, 2013).

Some plants have the ability to absorb and volatilize metals or metalloids through a strategy called phytovolatilization. The plants are able to absorb these element ions by their roots, convert them into forms that are non-toxic and then release them into the atmosphere (Pilon-Smits and LeDuc, 2009). Phytoextraction takes place when heavy metals are absorbed from the substrate into the roots of plants and transported and accumulated in the aerial parts of the plant. For its success, plants referred to as hyperaccumulators are preferred since they have the ability to accumulate high concentrations of specific heavy metals in their above ground biomass (Van der Ent *et al.*, 2013).

Phytofiltration is another strategy that plants employ to remediate wastewater. This is actualized by the roots of plants which filter the water as effluent pass through the roots (Ali, 2013). Plants that have high root biomass and tolerance to the heavy metals achieve the best results (Prasad, 2004). In rhizodegradation, growing roots promote the proliferation of rhizosphere microorganisms which then degrade heavy metals. The microorganisms utilize exudates and plant metabolites as source of carbon and energy in this process. Additionally, plants themselves also exude biodegrading enzymes (Prasad, 2004). The application of this strategy is limited to organic contaminants and microbial species of the genus *Pseudomonas* are the predominant organisms associated with roots (Ali, 2013).

2.7.2 Phytoextraction and Phytostabilization

Phytoextraction and phytostabilization are the most important phytoremediation strategies in removal of heavy metals and metalloids from contaminated substrates. However, there has been immense interest on phytoextraction due to its relatively high efficiency as a strategy in phytoremediation and the possibility that there can be economic value in metal recovery and end energy production (Pedron *et al.,* 2009) from the process. There are desirable characteristics in plants that make them preferred for use in phytoextraction. The characteristics include the ability of the plant to tolerate high concentration of metals, the ability of the plant to accumulate high amounts of metals in the above ground biomass and the ability of the plant to grow rapidly and produce high biomass (Shabani and Sayadi, 2012). Other desirable characteristics include having a profuse root system and ability to be cultivated and harvested easily (Shabani and Sayadi, 2012).

Effectiveness of phytoextraction as a phytoremediation strategy is highly dependent on whether the contaminant can be removed through harvesting. This can only be possible if accumulated heavy metals are translocated to the easily harvestable parts of the plant, that is, the aerial parts. The timing of harvesting is critical as it should be done before the plants fall, die and before they decompose (Blaylock and Huang, 2000). Phytomining can be done after harvesting to recover metals from the plants absorbed during phytoremediation. When the plants are incinerated, energy produced can be harnessed and used in other economic activities. The residual ash can also be further processed to extract metals.

Phytostabilization, aims to reduce mobility of contaminants in the substrate. Vegetation that is tolerant to toxic contaminants is grown and covers the substrate, and consequently limits soil erosion and leaching of the contaminants. This phytoremediation strategy reduces mobility of heavy metals through adsorption of the heavy metals and also through precipitation of the contaminants into the atmosphere. It can be improved by inducing pH changes or by oxidizing the root environment. (Domínguez *et al.*, 2009). It can also be enhanced by using plant species that have the ability to produce high amounts of chelating substances. Plants with phytostabilization potential are of great value for vegetation of mine tailings and contaminated areas (Antosiewicz *et al.*, 2008).

2.7.3 Metal Transfer Coefficients

2.7.3.1 Bioconcentration Factor (BCF)

Bioconcentration Factor (BCF) is an index that shows the ability of the plant to accumulate a particular metal in its biomass with respect to the metal concentration in the substrate (Ghosh and Singh, 2005). It is used to determine the quantity of heavy metals absorbed by the plant from the substrate. According to Zhuang *et al.*, 2007, the bioconcentration factor (BCF) is defined as the ratio of the total concentration of an element in the roots of a plant to its concentration in the substrate in which the plant is growing.

2.7.3.2 Translocation Factor (TF)

The efficiency of phytoremediation can be quantified by calculating translocation factor (TF). The TF expresses the capacity of a plant to store an element in its above ground biomass. It is defined as the ratio of metal concentration in the upper part to that in the roots (Chakroun *et al.*, 2010). The translocation factor indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots (Padmavathiamma and Li, 2007).

2.8 Literature Review Summary and Research Gaps

There is considerable evidence that constructed wetlands can offer solutions to challenges being associated with conventional wastewater treatment systems. Aquatic plants can be used through the process of phytoremediation to remove soluble heavy metals from wastewater even when the metals are in low concentrations. However, plants must have the desired attributes that allow them to aid in wastewater treatment and these attributes vary greatly between plant species (or even ecotypes) (Haakensen et al., 2015). Therefore, to have predictable and effective treatment in a constructed wetland, it is imperative to do proper selection of plants and the selection should made on a site-specific basis and guided by the treatment objectives (Haakensen et al., 2015).

CHAPTER 3.0: METHODOLOGY

3.1 Introduction

The chapter focuses on the location, climatic conditions, type of soils, biodiversity, economic activities and population of the study area. Selection of the study area and study plants including how the propagation was done is described. Sampling and processing of the study plants and wastewater, analysis of the heavy metals, and data presentation and analysis are also described.

3.1.1 Study Area

3.1.1.1 Location

The study was carried out in Finlays Flamingo Farm constructed wetland. The farm is about 13 kilometres south of Naivasha Town. It lies within latitude 01^{o}10'S and longitude 36^{o}02'E along Moi South-Lake Road in Naivasha District, Nakuru County (Figure 3.1). The farm covers a total area of 80ha. The establishments that neighbour the farm are Sumuni Farm, Sawela Lodge and Nini Farm to the North, and Sopa Lodge and Longonot Farm to the South, Longonot DEB School to the East and Lake Naivasha to the West.

Finlays Flamingo Farm was selected because of its well-established constructed wetland that the farm uses to treat its wastewater and because of the good rapport that the researcher had with the farm's management. In addition, the farm was also interested in identifying areas of improvement to ensure effective wastewater treatment in their farm. Review of wastewater quality reports revealed that concentration of some heavy metals was above the effluent discharge standards as stipulated by the Environmental Management (Water Quality) Regulations, 2006, Kenya. It was therefore important to establish the if the plants used in the constructed wetland were efficiently removing heavy metals from the wastewater.

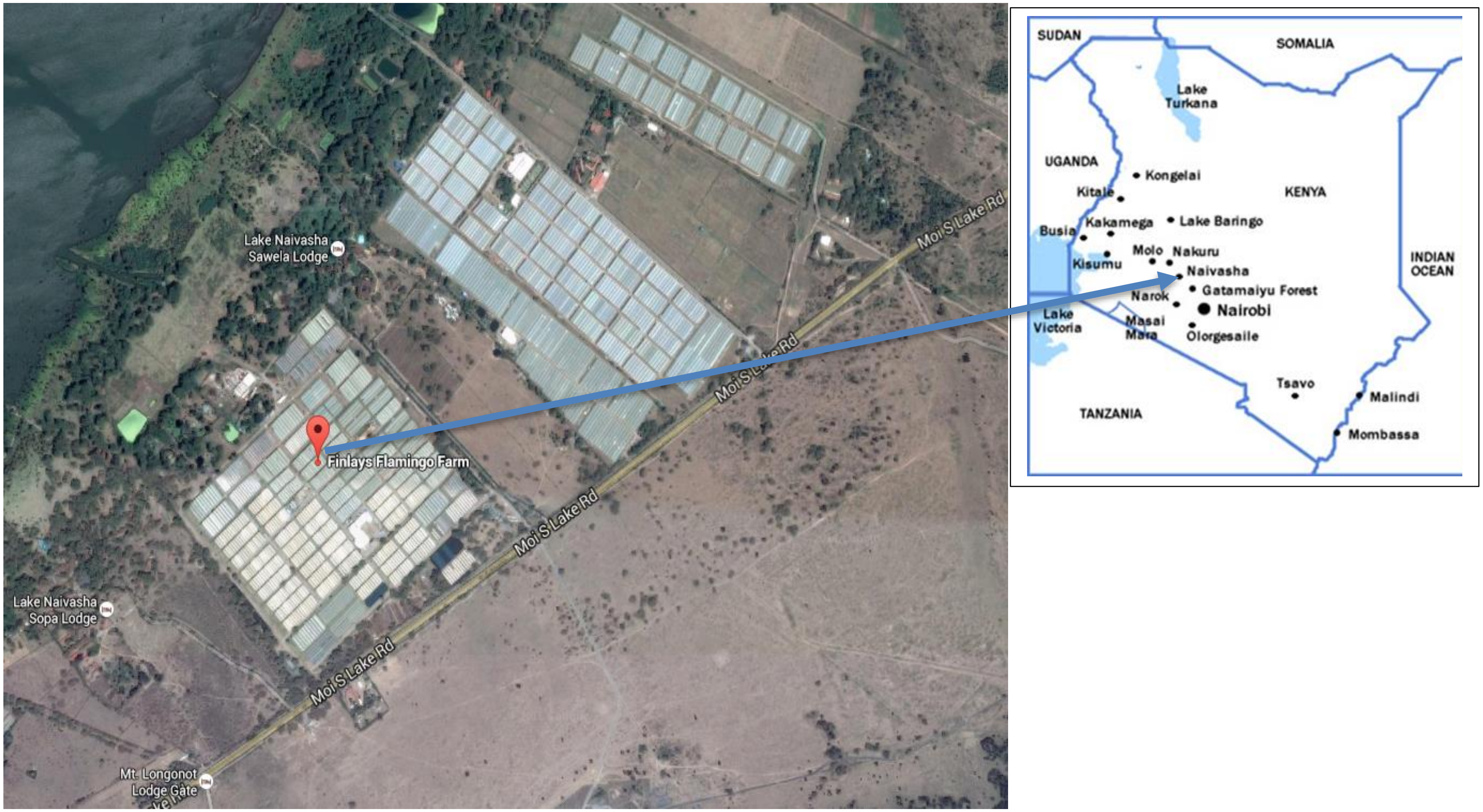

Figure 3.1: Map of the study area showing location of the study area where experiment was done (Source: Google Maps)

3.1.1.2 Soils

The underlying soils have volcanic origins, extremely fertile and as a result attractive to floriculturists and agriculturalists (Harper and Mavuti, 2004). The soils have high sodium and pumice content, the latter making the soils permeable with a low water-holding capacity (Becht *et al.*, 2006). Towards Lake Naivasha is mud which is loose, dark coloured and consisting of the remains of benthic and planktonic algae. This mud washes away through wave action, grazers, and crayfish burrowing. The black organic soil is fine-particled and rich in detritus but because they are exposed they tend to become extremely dry (Becht *et al.*, 2006).

3.1.1.3 Climatic Conditions

The climate of the study area is influenced by altitude. In the lake area where Finlays Flamingo Farm is located, semi-arid conditions dominate and annual precipitation averages range from ~650mm around Lake Naivasha to ~1300mm in the mountain forests of the Aberdares. Precipitation distribution is typically bimodal with long rains occurring during the periods of March - May and short rains in October - November. Mean monthly minimum temperatures range from 6 degrees Celsius to 10 degrees Celsius while mean monthly maximum temperatures range from 26 degrees Celsius to 31 degrees Celsius. Average monthly temperatures range from 15.9 degrees Celsius to 17.8 degrees Celsius (De Jong, 2011).

3.1.1.4 Economic Activities

The economic activities in the area include commercial flower and vegetable growing, smallholder farming and tourism. Pastoralism and fishing are also important aspects of social and economic activities of the area. The local horticulture and tourism activities are substantial foreign exchange earners and Naivasha is well known as the hub of flower production for export in Kenya. There are also several informal settlements that have sprung up around the lake, mainly to house workers from the neighbouring flower farms. Geo-thermal industry is also rapidly developing in the area.

Fisheries was once one of the most important economic activities supporting livelihoods in Naivasha. However, the introduction of crayfish, *Procambarus clarkia,* in the lake for commercial purposes created an ecosystem in which it was the only introduced endemic carp species that could remain (Harper and Mavuti, 2004). Although fish for subsistence still continues, commercial fishery collapsed in 1970s due to the damage that was caused by the introduction of this invasive species (Hickley *et al.*, 2002).

3.1.1.5 Population

The arrival of the labour-intensive horticultural industry to the area brought with it a number of employment opportunities. Because of this, the human population of Naivasha and the lake hinterland experiences very high population growth. In 1999 census, the population of Naivasha was 147,000 and in 2009 the population increased to 376,000 and it is projected that in ten years, the population in Naivasha will increase by five-fold (KPHC, 2010).

3.1.1.6 Biodiversity

Lake Naivasha ecosystem boasts diverse flora with about 108 plant species falling in 43 families. The most dominant species in the lake ecosystem are *Cyperus papyrus, Cyperus digitatus* and *Cyperus immensus* (Harper and Mavuti, 2004). The lake is surrounded by a woodland of *Acacia xanthophlea* which provides a very important habitat for a wide range of animals including water bucks, giraffes, monkeys, etc. Grazers, largely consists of cattle and hippos, which prefer the Kikuyu grass (*Pennisetum clandestium)* growing on the high grounds behind the papyrus fringe swamps. To reach their preferred feeding, the grazers plough paths through the fringe swamps. The papyrus plants are most pressured and vulnerable to grazing when they are very young and tender. On the surface of the lake water, there are many floating and submerged aquatic plant species including *Cyperus papyrus* along with *Pistia stratiotes, Wolffia arrhiza* and *Nymphaea* (Harper and Mavuti, 2004).

3.2 Study Design

3.2.1 Flamingo Farm Constructed Wetland

Finlays Flamingo farm constructed wetland was selected because it is one of the well-established constructed wetlands in the area. It comprises of a Sedimentation Tank (ST), Gravel Bed Hydroponics (GBH), Day Cell (DC) and two Surface Cells (SC). The ST has an inlet through which wastewater enters the constructed wetland (CW). At ST, sedimentation takes place by allowing sediments with relatively high densities to settle down. The wastewater then moves through sieves to the GBH section of the constructed wetland. Treatment of the wastewater using plants starts at the GBH and only soil-rooted plants are grown for the uptake and accumulation of heavy metals. There are no floating plants at this section of the wetland.

From the GBH, wastewater moves to the DC which is designed to retain wastewater up to a certain level before flowing to the SCs. The DC ensures that wastewater is retained for approximately 21 days for proper treatment of the wastewater. At the DC, both soil-rooted and floating plants are grown for treatment of wastewater through uptake and accumulation of heavy metals and other nutrients. From the DC, wastewater moves to the first and second SCs. At the SCs, treatment of wastewater happens through exposure to sunlight as well as uptake and accumulation of heavy metals and other nutrients by both soil-rooted and floating plants. Soil-rooted plants are grown on the edges (embankments) of the SCs while floating plants are grown on the wastewater surface. In the last SC, there is an outlet through which wastewater leaves the CW into a water reservoir for reuse in the Farm (Figure 3.2).

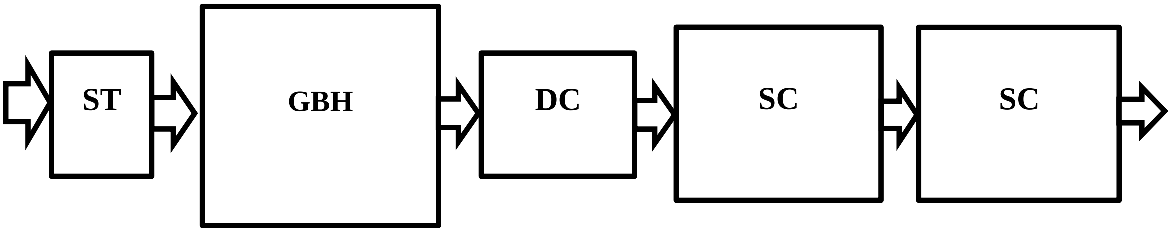

Figure 3.2: Layout of Finlays Flamingo Farm Constructed Wetland.

3.2.2 Selection of GBH

Soil-rooted and floating aquatic plants are grown in the constructed wetland. However, the GBH section of the constructed wetland does not have floating plants. The soil-rooted plants in the GBH are *Cyperus alternifolius*, *Cyperus dives* and *Canna indica*. The DC has floating *hydrocotile* plants, and the SCs have floating *hydrocotile* plants and water lettuce (Nile cabbage). *Canna indica* plants and edible yams are also grown on the edges (embankments) of the SCs. GBH part of the constructed wetland was purposely selected for the study to guarantee exclusion of the effects of non-experimental plants since this was the only section of the constructed wetland that had soil-rooted plants only. All the other sections of the constructed wetland had both soil-rooted and floating plants, and therefore, excluding effects of floating plants would have been extremely difficult.

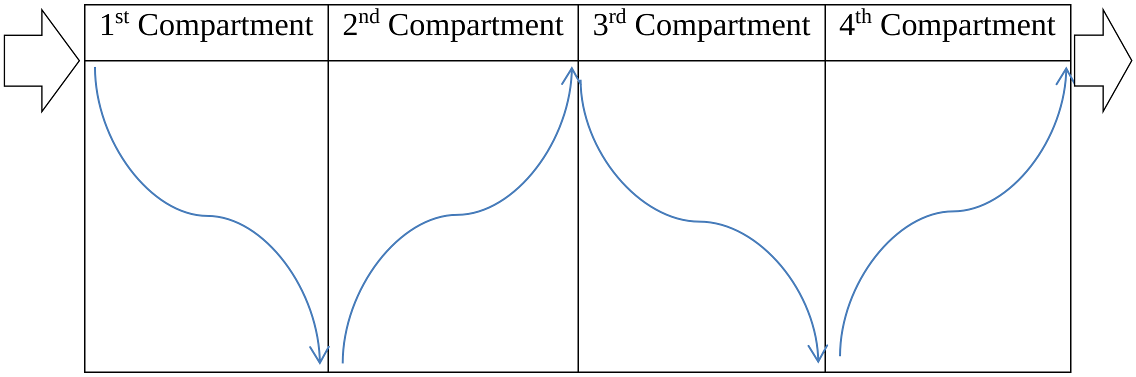

Figure 3.3: Figure showing the direction of wastewater movement through GBH compartments.

The GBH has four compartments which are designed such that wastewater enters the GBH and flows through the first, second, third and fourth compartments before it exits the GBH and enters the DC. Based on flow of wastewater through the GBH, the first two compartments were purposely selected for the study to ensure study plants receive wastewater before removal of the heavy metals by the aquatic plants takes place (Figure 3.3).

3.2.3 Selection of Plants for the Study

The plants selected for the study were *Cyperus alternifolius*, *Cyperus dives* and *Canna indica*. These plants were selected because they were the only plants growing in the GBH section of the Flamingo farm constructed wetland at the time of the study.

3.2.4 Preparation of the GBH for Planting

The first two GBH compartments were prepared for propagation of the selected plants by uprooting all plants that were growing in the two compartments at the time. The uprooting was done carefully by pulling up the plants ensuring that the whole plant (roots and shoots) are removed.

3.2.5 Planting Selected Plants

Young seedlings shooting from the selected plants growing in the GBH at that time were collected. All seedlings were selected in a manner that ensured uniformity in size, age (shooting at the same time) and free from disease symptoms (through physical examination). The young seedlings of the study plants were then planted in the GBH (Table 3.1 and Table 3.2). The seedlings were planted in nine (9) rows and in each row two (2) plants of each species were planted making it a total of 18 plants per species. This design was chosen to ensure that each of the three species was evenly distributed across the GBH and that plants that received wastewater first and those that received wastewater last were sampled at the same time to randomize effects and reduce variability. The plants were then left to grow for a period of five months i.e. February 2014, March 2014, April 2014, May 2014 and June 2014 before the first sampling was done.

Table 3.1: Distribution of study plants in the GBH

Rows	Plants					
R1	CD	CA	CI	CD	CA	CI
R2	CA	CI	CD	CA	CI	CD
R3	CI	CD	CA	CI	CD	CA
R4	CD	CA	CI	CD	CA	CI
R5	CA	CI	CD	CA	CI	CD
R6	CI	CD	CA	CI	CD	CA
R7	CD	CA	CI	CD	CA	CI
R8	CA	CI	CD	CA	CI	CD
R9	CI	CD	CA	CI	CD	CA

Where: CA – *C. alternifolius*; CD – *C. dives*; CI – *C. indica*

R1 – Row 1; R2 – Row 2; R3 – Row 3; R4 – Row 4; R5 – Row 5; R6 – Row 6; R7 – Row 7; R8 – Row 8; R9 – Row 9

3.2.6 Plants and Wastewater Sampling

The first, second and third sampling of plants and wastewater was done after 20, 24 and 28 weeks respectively after planting. During each sampling, two (2) plants of each species were harvested from three (3) rows making a total of 6 plants of each species, At the same time wastewater was also sampled at the inlet to the GBH. The plants' sampling design used ensured that during each sampling, the study plants were harvested from all sections of the GBH (Table 3.2). This was to randomize effects and reduce variability.

Table 3.2: Showing how harvesting of study plants was done

Rows	**Plants**					
S1	CD	CA	CI	CD	CA	CI
S2	CA	CI	CD	CA	CI	CD
S3	CI	CD	CA	CI	CD	CA
S1	CD	CA	CI	CD	CA	CI
S2	CA	CI	CD	CA	CI	CD
S3	CI	CD	CA	CI	CD	CA
S1	CD	CA	CI	CD	CA	CI
S2	CA	CI	CD	CA	CI	CD
S3	CI	CD	CA	CI	CD	CA

Where: CA – *C. alternofolius*; CD – *C. dives*; CI – *C. indica*
S1- First sampling; S2 - Second Sampling; S3 - Third Sampling

During each sampling, fresh plants' samples were harvested before mid-morning of the sampling day. The harvesting was done by pulling the plants very carefully from the substrate to avoid damage to the roots and shoots of the plants. At the same time, wastewater was sampled at the inlet to the GBH for determining heavy metals' concentration in wastewater. The inlet to the GBH was chosen to ensure that wastewater is sampled before any treatment. The wastewater sampling was done by putting a one-litre sampling bottle under GBH inlet pipe. The wastewater was allowed into the sampling bottle until the bottle was completely full. One (1) litre of wastewater was sampled each time study plants were harvested. Wastewater samples were then put in cool boxes and transported to SGS Kenya Limited laboratories for analysis of heavy metals.

Sampled plants were, however, washed under a stream of tap water to remove soil and other foreign particles from the plants. They were then rinsed with distilled water. Plants belonging to each species were put together to form composite samples. Study plants of the same species were then separately placed in brown paper bags and transported to SGS Kenya Limited laboratories for analysis of heavy metals.

3.2.7 Processing of Plant Samples

The plant samples were processed in accordance with Ryan *et al.,* 2001. Each group of species was divided into roots and shoots using a guillotine. Roots and shoots of each plant species were then air-dried and later placed in a dehydrator for 3 days. This was then followed by oven drying for 4 hours at 100^{o}C. Portions of 1.0g of ground plant material were weighed into a porcelain crucible. The porcelain crucible was then placed into a cool muffle furnace where the temperature was increased gradually up to 550^{o}C. The ashing was continued for 5 hours after attaining the temperature of 550^{o}C. The porcelain crucibles were then taken out and cooled in the desiccator. The white ash was then taken up in acid determination of elements (1:1 hydrochloric acid) and made to volume (50ml) with de-ionised water. Inductively Coupled plasma Optical Emission Spectrometer (ICP-OES) was then used to analyse concentration of Arsenic, Cadmium and Lead metals in the roots and shoots.

3.2.8 Processing of Wastewater Samples

Processing of wastewater samples was done in accordance with APHA, 1999. Wastewater samples of 5ml were measured into 250 VF beakers and 3ml of concentrated nitric acid was added and the samples were evaporated to less than 5ml volume without boiling. To the sample, 5ml of concentrated nitric acid, 10ml of 1:1 hydrochloric acid and 15ml of de-ionised water were added. The mixture was then heated for 15 minutes and removed from the hot plate. The mixture was then cooled and later topped up to 100ml volumetric flask. ICP-OES was then used to analyse the concentration of Arsenic, Cadmium and Lead metals in wastewater.

3.2.9 Preparation of Reagents and Standards

Calibration blank was used to check calibration validity after every calibration, every 10 analyses and at the end of analyses. Laboratory reagent blank was used to check for possible contamination of the reagents and apparatus. Quality control standard was used post calibration to check the accuracy of the calibration by analyzing a second source standard. Instrument check performance was done to check the accuracy and drift by analyzing a standard as a sample after every 10 samples and at the end of analyses.

Quality control standards for ICP-OES were used as stock standards for preparing working standards. The calibration standard concentration for Arsenic, Cadmium and Lead used was 1.0mg/l. All standards were prepared in deionised water and acidified with nitric acid. Deionised water acidified with nitric acid was also used as the calibration blank (HNO_3, 1+1: Add 500 mL conc. HNO_3 to 400 mL water and dilute to 1 L).

3.2.10 Determination of Heavy Metal Concentration

Each sample run was begun with an analysis of the calibration blank followed by analysis of the method blank. This permitted a check of the sample preparation reagents and procedures for contamination. Samples were analyzed alternating them with analyses of calibration blank and rinsing for 60 seconds with dilute acid between samples and blanks. After introducing each sample or blank, the system was let to equilibrate before starting signal integration.

The analytical wavelengths used for Arsenic, Cadmium and Lead were 193.70, 226.50 and 220.35 respectively. Selection of the analytical wavelengths used ensured freedom from spectral interferences, the different sensitivities and expected concentration in the samples.

3.3. Determination of Metal Transfer Coefficients

Plants’ ability to uptake heavy metals from wastewater and accumulate them in their roots was estimated using bioconcentration factors. The Bioconcentration Factor (BCF) was calculated using the formula:

$$BCF = \{[Metal]\ Root\}/\{[Metal]\ Wastewater\}$$

Where: BCF = Bioconcentration Factor
[Metal] Root = Metal concentration in the roots
[Metal] Wastewater = Metal concentration in the wastewater

The plants’ ability to translocate heavy metals from their roots to their shoots was estimated using translocation factors. The Translocation Factor (TF) was calculated using the formula:

$$TF = \{[Metal]\ Shoot\}/\{[Metal]\ Roots\}$$

Where: TF = Translocation Factor
[Metal] Shoots = Metal concentration in the shoots
[Metal] Roots = Metal concentration in the roots

By comparing BCF and TF, the ability of the study plants in taking up metals from wastewater, accumulating the metals in their roots and translocating them to their shoots was evaluated. Plants with BCF and TF values greater than one (BCF>1and TF>1) were considered to have potential for phytoextraction while plants with BCF greater than one and TF less than one were considered to have potential for phytostabilization.

3.4 Data Analysis and Presentation

The data obtained were concentration of Arsenic, Cadmium and Lead metals in wastewater, and roots and shoots of the study plants. From these concentrations, bioconcentration factors (BCF) and translocation factors (TF) were calculated using the formulae shown below:

BCF = {[Metal] Root}/{[Metal] Wastewater} and TF = {[Metal] Shoot}/{[Metal] Roots}

Where: [Metal] Root = Metal concentration in the roots
[Metal] Wastewater = Metal concentration in the wastewater
[Metal] Shoots = Metal concentration in the shoots

Differences in BCF and TF values among the study plants were detected using One-way ANOVA test and Tukey test. A significance level of $p=0.05$ was used throughout the study. The results were presented in tables and bar graphs.

CHAPTER 4.0: RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents findings on concentration of Arsenic, Cadmium and Lead metals in wastewater, roots and shoots of study plants, metal transfer coefficients, and comparisons with other similar studies.

4.2 Bioconcentration Factor (BCF) of Arsenic, Cadmium and Lead Metals

4.2.1 Arsenic Metal

Arsenic concentration in wastewater was 0.005mg/l in week 20, 0.050mg/l in week 24 and 0.050mg/l in week 28. These concentrations were above the maximum allowable limit (0.020mg/l) for Arsenic concentration in wastewater as stipulated by the effluent discharge standards in Kenya (Water Quality Regulations, 2006). Therefore, treatment of the wastewater to reduce Arsenic concentration before discharging into the natural environment was required.

Arsenic concentration in roots of *C. alternifolius* was 0.360mg/kg in week 20, 0.880mg/kg in week 24 and 0.760mg/kg in week 28. In the roots of *C. dives,* Arsenic concentration was 2.720mg/kg in week 20, 5.850mg/kg in week 24 and 6.040mg/kg in week 28. In the roots of *C. indica,* Arsenic concentration was 2.750mg/kg in week 20 and week 24, and 3.210mg/kg in week 28.

BCF values of the study plants varied during the study period. They ranged from 7 to 152 in *C. alternifolius,* 54 to 1,208 in *C. dives* and 55 to 642 in *C. indica* (Table 4.1). These BCF values were all greater than one showing that the study plants have the ability to uptake from wastewater and accumulate considerable amounts of Arsenic metal in their roots. This compared with Li *et al.*, 2011 who studied Arsenic metal uptake in aquatic plants also found that the highest Arsenic accumulation was in the roots.

Table 4.1: BCF for Arsenic in the study plants sampled in week 20 – 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	7	18	152
C. dives	54	117	1,208
C. indica	55	55	642

ANOVA test showed that there was no significant difference (p=0.547) in BCF values for Arsenic metal in the roots of study plants. This indicated that the study plants uptake and accumulate Arsenic metal in their roots in a similar way. Therefore, the processes, that is, release of root exudates and microbial activity associated with the roots, that are thought to regulate the uptake and accumulation of heavy metals in the roots of plants worked in a similar way in the study plants.

The highest BCF values for Arsenic metal in the study plants (*C. alternifolius* - 152, *C. dives* - 1208, *C. indica* – 642) were recorded in week 28 (Figure 4.1). This indicated that uptake and accumulation of Arsenic metal in the roots of the study plants appears to be initially restricted but later picks up with time. This trend shows that the study plants are able to uptake and accumulate more Arsenic metal in their roots with time (Figure 4.1). There is a sharp increase of Arsenic metal accumulation in week 28 week and this suggests that the study plants should be allowed to grow beyond week 28 if maximum removal of Arsenic metal is to be achieved.

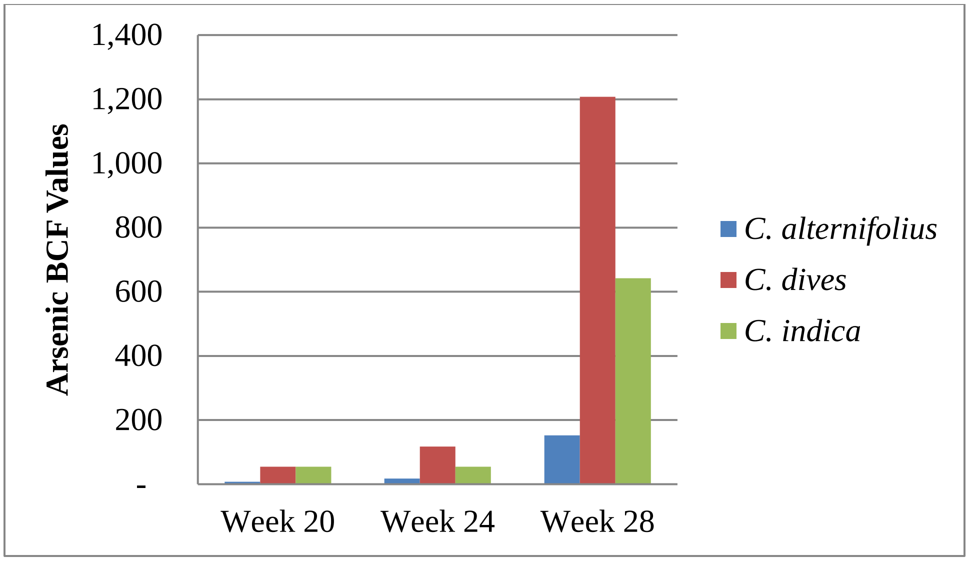

Figure 4.1: Temporal variations of BCF for Arsenic in the study plants sampled in week 20 - 28

C. dives recorded the highest BCF values (20th week - 54, 24th week - 117, 28th week – 1,208) while *C. alternifolius* the lowest BCF values (20th week - 7, 24th week - 18, 28th week - 152) throughout the study period (Figure 4.1). This indicated that *C. dives* was the most efficient while *C. alternifolius* was the least efficient in uptake and accumulation of Arsenic metal in roots throughout the study period.

4.2.2 Cadmium Metal

Concentration of Cadmium in wastewater remained the same (0.004mg/l) throughout the study period. This concentration was lower than the allowable maximum limit (0.010mg/l) for Cadmium metal in wastewater as stipulated by effluent discharge standards in Kenya (Water Quality Regulations, 2006). Nonetheless, removal of Cadmium from wastewater was still critical because cumulatively even relatively lower concentrations of heavy metals can cause public health and environmental related problems.

Cadmium concentration in roots of *C. alternifolius* was 0.080mg/kg in week 20, 0.290mg/kg in week 24 and 0.310mg/kg in week 28. In *C. dives* the concentration was

0.110mg/kg in week 20, 2.390mg/kg in week 24 and 3.210mg/kg in week 28. In *C. indica* the concentration was 0.310mg/kg in week 20, 1.020mg/kg in week 24 and 1.320mg/kg in week 28.

BCF values varied from 20 to 78 in *C. alternifolius*, 28 to 803 in *C. dives* and 78 to 330 in *C. indica* (Table 4.2). All BCF values for Cadmium metal in the three study plants were greater than one. This finding compared with those of Subhashini *et al*, 2013 who found that BCF values for selected plants were more than one. In this study, the highest BCF (803) was in *C. dives* and was significantly greater than one. This revealed that rhizomes for *C. dives* are preferable in stabilizing Cadmium metal in wastewater. This conclusion agreed with the finding of Zhang *et al.*, 2014 who found that *Athyrium wardii* which is a mining ecotype had a greater ability of stabilizing Cadmium from the mobile fraction to immobile fractions. Similar results had also been reported in *Thlaspi caerulescens* and durum wheat (Luo *et al.*, 2000; Matthieu *et al.*, 2012).

Table 4.2: BCF for Cadmium in the study plants sampled in week 20 - 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	20	73	78
C. dives	28	598	803
C. indica	78	255	330

ANOVA test showed no significant differences (p=0.187) in BCF value of Cadmium metal in the study plants. This points to similarity in Cadmium metal accumulation in the roots of the study plants. Therefore, processes such as release of root exudates and microbial activity associated with the roots of these plants may be working in a similar way. This is supported by Li *et al.*, 2011 who found that decrease in pH and increase of dissolved organic matter in the rhizosphere of the Cadmium hyperaccumulator *Sedum alfredii* significantly reduced the sorption and increased the desorption of Cadmium.

BCF values for Cadmium metal increased with time during the study period in all the study plants. The highest BCF values for Cadmium metal in the study plants were recorded in week 28 of the study period (*C. alternifolius* - 78, *C. dives* - 476, *C. indica* – 221) (Table 4.2) and consequently, the more the study plants are allowed to grow, the more they absorb and accumulate Cadmium metal in their roots.

C. dives recorded the highest BCF values (28 in week 20, 598 in week 24) while *C. alternifolius* recorded the lowest BCF values (20 in week 20, 73 in week 24, 78 in week 28) (Figure 4.2). This indicated that amongst the study plants, *C. dives* was the most efficient over time in acumulating Cadmium metal in roots. Accumulation of Cadmium metal in *C. indica* steadily and continouosly increased throughout the study period. This suggests it could the most efficient over time and should therefore be given a longer period of time of growth. *C. alternifolius* was the least efficient in accumulating Cadmium metal in the roots (Figure 4.2).

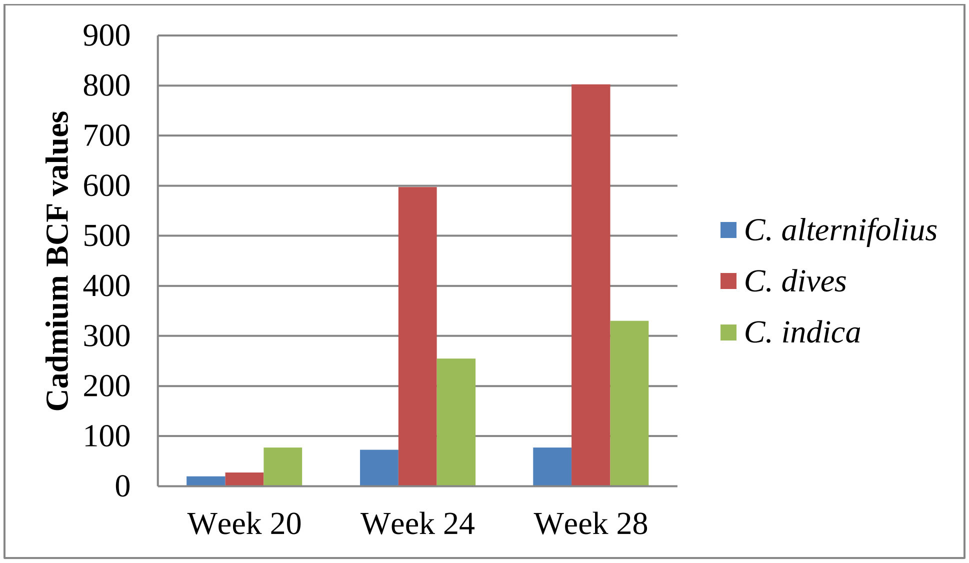

Figure 4.2: Temporal variations of BCF for Cadmium in the study plants in week 20 - 28

4.2.3 Lead Metal

Lead concentration in wastewater remained the same (0.040mg/l) throughout the study period. The concentration (0.040mg/l) was however above the maximum limit (0.010mg/l) for discharge into the natural environment as stipulated by the effluent discharge standards in Kenya (Water Quality Regulations, 2006). Thus, the wastewater required treatment to reduce the concentration of Lead before discharging into the natural environment.

Lead concentration in roots of *C. alternifolius* was 0.900mg/kg in week 20, 0.480mg/kg in week 24 and 0.610mg/kg in week 28. In *C. dives* Lead concentration was 0.450mg/kg in week 20, 8.790mg/kg in week 24 and 6.820mg/kg in week 28. In *C. indica* the concentration was 2.240mg/kg in week 20, 3.260mg/kg in week 24 and 4.320mg/kg in week 28.

Table 4.3: BCF for Lead in the study plants sampled in week 20 - 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	23	12	15
C. dives	11	220	171
C. indica	56	82	108

BCF values of Lead metal in the study plants varied during the study period. BCF values for Lead metal varied from 15 to 23 in *C. alternifolius,* 11 to 171 in *C. dives* and 56 to 108 in *C. indica* (Table 4.3). All the BCF values for Lead were substantially greater than one. This finding compared with those of Subhashini *et al*, 2013 who found BCF value of Lead in *C. indica* to be greater than one. This suggests that the study plants are very promising for phytostabilization of Lead metal and their rhizomes are critical for accumulation of Lead.

ANOVA test showed no significant difference (p=0.165) in BCF values of Lead metal in the study plants. This indicated that the study plants accumulate Lead metal in their roots in a similar way and thus there is similarity in how the processes that regulate uptake and accumulation of heavy metals in the roots of these plants work.

BCF for Lead metal in *C. alternifolius* decreased in week 24 before marginally increasing in week 28 (Figure 4.3). This can be attributed to the possibility that *C. alternifolius* initially translocated to its aerial parts considerable amount of Lead metal. This translocation then reduces in week 28 and accumulation of Lead metal in its roots increases. In *C. dives* the BCF sharply increases in week 24 before sharply decreasing in week 28. This can be attributed to the possibility that the plant starts to approach its Lead toxicity level and if exposure to Lead continues then the plant is likely to die. In *C. indica*, the BCF steadily and continuously increases during the study period (Figure 4.3) indicating that *C. indica* can accumulate more Lead metal in its roots with time and should therefore be given time to grow before its harvested.

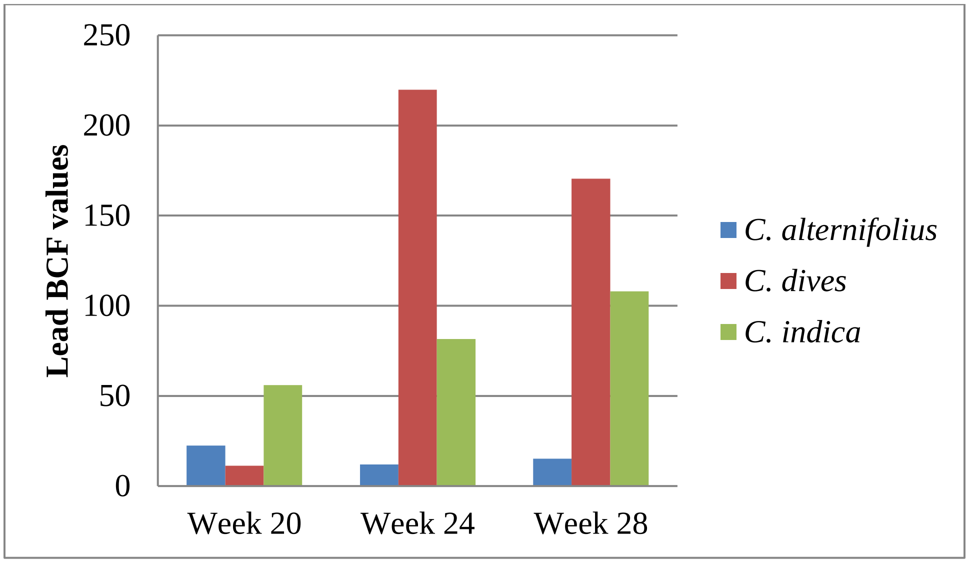

Figure 4.3: Temporal variations of BCF for Lead in the study plants in week 20 - 28

4.3 Translocation Factor (TF) of Arsenic, Cadmium and Lead Metals

4.3.1 Arsenic Metal

TF values varied from 0.667 to 0.934 in *C. alternifolius,* 0.060 to 0.169 in *C. dives* and 0.069 to 0.150 in *C. indica* (Table 4.4). This compares to Yadav *et al*., 2012 who found TF value for Arsenic metal to be 0.52 for selected plants growing in wastewater. All the TF values for Arsenic metal were less than one (TF<1) indicating that study plants did not translocate substantial amounts of Arsenic metal to their shoots as compared to the Arsenic metal accumulated in their roots. This finding agrees with Li *et al*., 2011 who studied Arsenic uptake in aquatic plants and deduced that less Arsenic metal was present in the stem and leaves as compared to roots.

Table 4.4: TF for Arsenic in the study plants sampled in week 20 - 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	0.667	0.739	0.934
C. dives	0.169	0.060	0.079
C. indica	0.069	0.135	0.150

ANOVA test indicated significant differences (p=0.001) in TF values of Arsenic metal in the study plants. Tukey test confirmed that significant differences existed between C. *alternifolius* and *C. dives* (p=0.000), and between *C. alternifolius* and *C. indica* (p=0.000). It showed that *C. alternifolius* translocated about 67.7% and 66.2% of Arsenic metal more than *C. dives* and *C. indica* respectively. However, Tukey test confirmed no significant difference between *C. dives* and *C. indica* (p=0.978).

TF values for Arsenic metal in *C. alternifolius* were the highest across the study period (Figure 4.4). This indicated that *C. alternifolius* was the most efficient in translocating Arsenic metal to its aerial parts. The finding can be attributed to the increased expression of genes that control systems which transport and store heavy metals in *C. alternifolius*. Transport of heavy metals from roots to shoots is strongly regulated by genes that are expressed in both hyper-accumulator plants and non-hyper-accumulator plants. However, genes known to code for the transport systems of heavy metals are constantly over-expressed in hyper-accumulating plants when they are exposed to heavy metals.

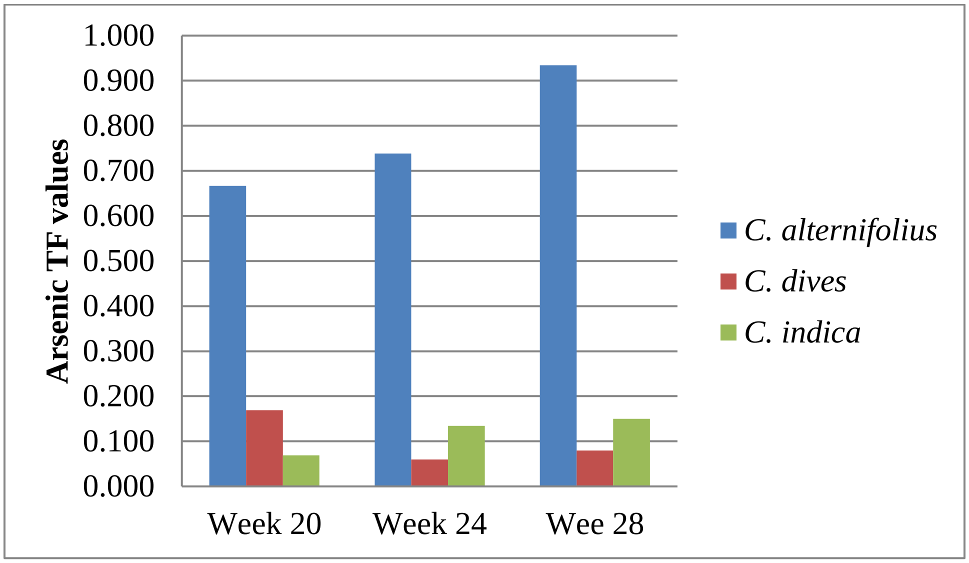

Figure 4.4: Temporal variations of TF for Arsenic in the study plants in week 20 - 28

In *C. alternifolius*, TF values increased from week 20 to week 28 while in *C. indica*, the margin of increase substantially declined between week 24 and week 28. In *C. dives*, TF values for Arsenic metal decreased in week 24 before minimally increasing in week 28 (Figure 4.4). These trends show that over time, *C. alternifolius* can translocate relatively more Arsenic metal to its shoots and therefore harvesting of shoots to remove Arsenic metal from the wastewater should be done after a relatively longer period (beyond week 28 after planting) to allow translocation of more Arsenic metal to its shoots. In both *C. indica* and *C. dives*, translocation of Arsenic metal seems to be greatly restricted and even declines with time.

4.3.2 Cadmium Metal

TF values varied from 0.375 to 0.581 in *C. alternifolius*, 0.038 to 0.273 in *C. dives* and 0.065 to 0.326 in *C. indica* (Table 4.5). This compares to the finding of Subhashini *et al.*, 2013 who found that TF index for Cadmium metal was in the range of 0.2674 to 0.7984 in *Acalypha indica, Abutilon indicum, Physalisminima, Cleome viscosa, Catharanthus roseus, Ruellia tuberosa, Canna indica, Perotis indica, Echinocloa colona, Cyperus rotundus*.

Table 4.5: TF for Cadmium in the study plants sampled in week 20 - 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	0.375	0.448	0.581
C. dives	0.273	0.038	0.044
C. indica	0.065	0.314	0.326

ANOVA test showed significant differences (p=0.042) in TF values for Cadmium metal in the study plants. Tukey test confirmed that significant difference existed between C. *alternifolius* and *C. dives* (p=0.038) and that *C. alternifolius* translocated about 35.0% of Cadmium metal more than *C. dives*. There was, however, no significant difference between *C. alternifolius* and *C. indica* (p=0.150) and between *C. dives* and *C. indica* (p=0.548).

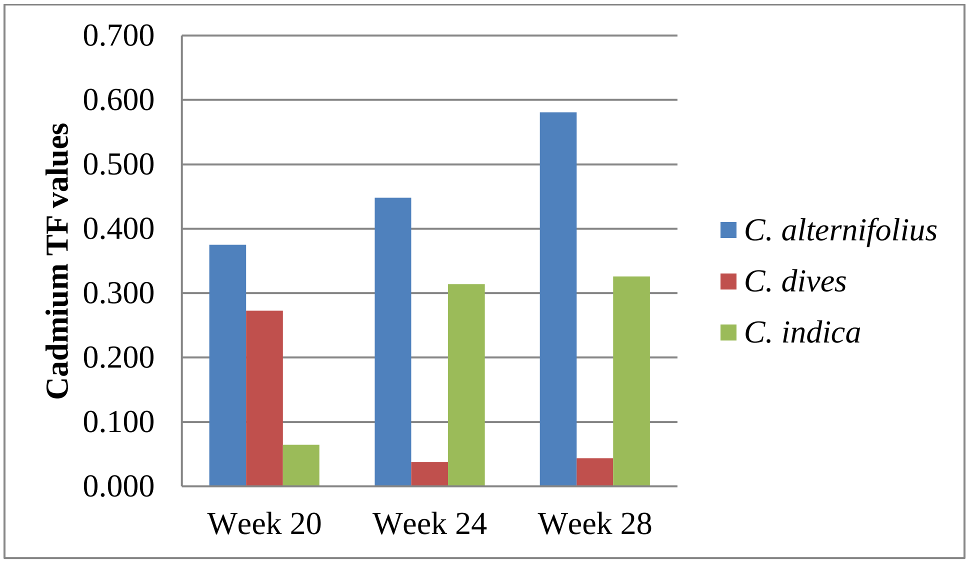

Figure 4.5: Temporal variations of TF for Cadmium in the study plants in week 20 - 28

In comparing the study plants, *C. alternifolius* had the most efficient Cadmium transport systems that translocated the Cadmium metal to the aerial parts. The relatively more efficient transport system can be attributed to the expression of genes that code for Lead metal transport in plants. TF values for Cadmium in *C. alternifolius* were highest and increased across the study period (Figure 4.5).

In *C. alternifolius*, and *C. indica*, TF values increased from week 20 to week 28 while in *C. dives*, TF values for Cadmium metal were high in week 20 and sharply decreased in week 24 before minimally increasing in week 28 (Figure 4.5). The observed trends of Cadmium translocation in *C. indica* and *C. dives* compares to findings of Subhashini *et al.*, 2013 that found accumulation of Cadmium metal in stem and leaves is marginal. Translocation of Cadmium metal in *C. indica* declined in weeks 24 and 28 (Figure 4.5) This finding informs the harvesting time for optimal removal of Cadmium metal. Harvesting of shoots should be done not later than week 24 after planting.

4.3.3 Lead Metal

TF values varied from 0.167 to 0.705 in *C. alternifolius*, 0.027 to 0.689 in *C. dives* and 0.045 to 0.129 in *C. indica* (Table 4.6). This compared to the findings of Yadav *et al.*, 2012 who found TF values for Lead metal to be 0.71 for plants growing in wastewater. Subhashini *et al.*, 2013 however found a translocation factor for Lead in *C. indica* to be 3.13 suggesting that *C. indica* was able to translocate considerable amounts of Lead metal to its shoots.

Table 4.6: TF for Lead in the study plants sampled in week 20 - 28

Plant species	**Week 20**	**Week 24**	**Week 28**
C. alternifolius	0.167	0.667	0.705
C. dives	0.689	0.027	0.045
C. indica	0.045	0.129	0.123

ANOVA test showed no significant differences (p=0.263) in TF values for Lead metal in the study plants and thus, the study plants translocated Lead metal in a similar way. The trend showed that translocation of Lead metal was restricted in the study plants. This could be attributed to the less expression of genes that code for transport of Lead metal in the study plants.

TF values for Lead metal in *C. alternifolius* were highest (0.167 in week 20, 0.667 in week 24, 0.705 in week 28) throughout the study period (Figure 4.6). In *C. dives*, the TF values declined substantially in week 24 before minimally increasing in week 28. This is comparable to Subhashini *et al*., 2013 who found that the highest absorption of Lead in *C. indica* is during the first twenty days and after that decreases slowly. These trends also showed that *C. alternifolius* was the most efficient in translocating Lead metal as compared to both *C. dives* and *C. indica*. However, the translocation declines substantially from week 24 which informs the optimum time for harvesting of plants as part of wastewater remediation.

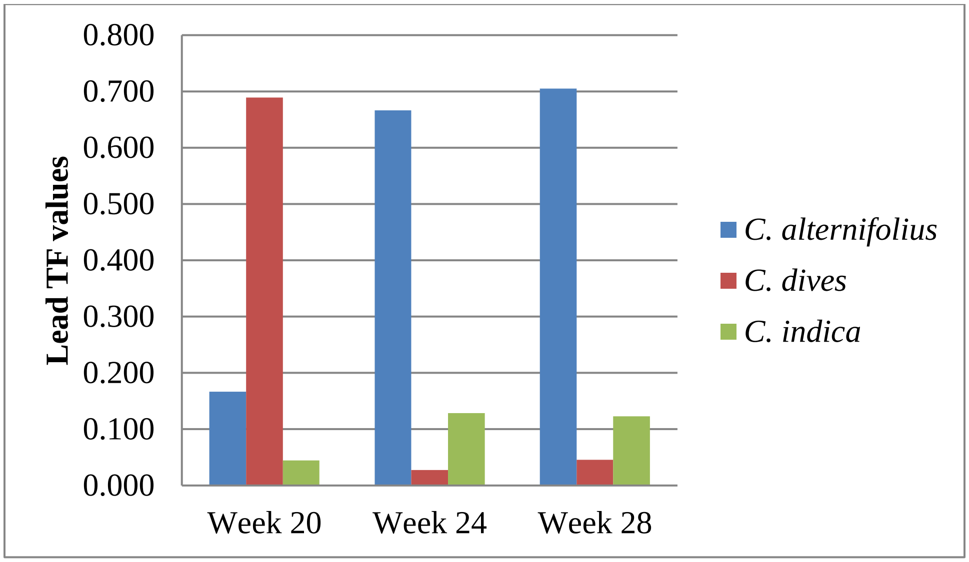

Figure 4.6: Temporal variations of TF for Lead in the study plants in week 20 - 28

4.4 Potential of *C. alternifolius, C. dives* and *C. indica* as Phytoremediators of Arsenic, Cadmium and Lead Metals

4.4.1 Arsenic Metal

BCF values in week 20, week 24 and week 28 for Arsenic metal in the study plants were substantially greater than one (BCF>1) indicating that all the study plants can accumulate substantial amounts of Arsenic metal in their roots. However, TF values throughout the study period for Arsenic metal in all the study plants were less than one (TF>1). According to Yoon *et al.*, 2006, plants with BCF values greater than one and TF values less than one (BCF>1 and TF<1) are effective phytostabilizers. This indicates that the study plants can accumulate Arsenic metal in their roots but its translocation from roots to shoots of the study plants is restricted. This finding agreed with those of Rahman and Hasegawa, 2011 who indicated that there are few plants that can translocate high amounts of Arsenic metal. This find finding confirms that the below-ground biomass of these plants is very critical in Arsenic metal removal from wastewater.

Arsenic metal translocation into shoots of the study plants appears to be very restricted so that harvesting the shoots of the plants is not an effective way of removing Arsenic metal from wastewater. However, in view of toxicology, this is a desirable property because the metal is not translocated to the shoots and thus would not pass into the food chain through herbivores. The low metal translocation from roots to aerial parts indicates a possible limitation for the utilization of the study plants in Arsenic phytoextraction. However, the confinement of these metals in the roots opens perspectives for utilization of the study plants for rhizofiltration of metal-polluted waters.

4.4.2 Cadmium Metal

BCF values across the study period for Cadmium metal in all the study plants were greater than one (BCF>1). All the BCF values were substantially greater than one indicating that the study plants can accumulate considerable amounts of Cadmium metal in their roots. On the other hand, TF values for Cadmium metal were substantially less than one (TF>1) during the study period. Consequently, the study plants are effective phytostabilizers of Cadmium metal. This means that translocation of Cadmium metal from roots to shoots is restricted in the study plants. This finding agrees with a similar study by Cheng *et al*., 2002 who concluded that the highest content of heavy metals occurred in the roots with the lowest found in the shoots. This phenomenon is explained by Lux *et al*., 2011 who says that translocation of Cadmium is often restricted due to the ability of the element to create Cd-phytochelatin complex by sequestration in the vacuole. Cadmium movement from root to shoots probably occurs within the xylem and the levels of free Cadmium in the symplast can be influenced highly by cellular sequestration of Cadmium and thus affecting the movement of Cadmium throughout the plants (Niu *et al.*, 2007). This find finding confirms that the below-ground biomass of these plants is also very critical in Cadmium metal removal from wastewater.

4.4.3 Lead Metal

BCF values for Lead metal in all the study plants were significantly greater than one (BCF>1) during the study period with the lowest BCF being 11. TF values across the study period for Lead metal in all the study plants were however less than one (TF>1) with the highest TF being 0.542. This showed that the three study plants accumulated substantial amounts of Lead metal in their roots but translocation to the shoots was greatly restricted. This finding shows that the study plants are phytostabilizers of Lead metal and this compares with similar studies, Marchand *et al.,* 2010 and Soda *et al*., 2012, who evaluated phytoremediation potential of other *Cyperus* species.

CHAPTER 5.0: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

5.1.1 Bioconcentration Factor

BCF values for Arsenic, Cadmium and Lead metals in *C. alternifolius*, *C. dives* and *C. indica* across the study period were all greater than one (BCF>1). ANOVA test revealed that there were no significant differences in BCF values for the three metals in the study plants.

5.1.2 Translocation Factors

TF values for Arsenic, Cadmium and Lead metals in *C. alternifolius*, *C. dives* and *C. indica,* were all less than one (TF<1). ANOVA test showed that there were significant differences in TF values of Arsenic metal in the study plants. Tukey test however showed that *C. alternifolius* translocated 67.7% and 66.2% of Arsenic metal more than *C. dives* and *C. indica* respectively. This finding confirmed that *C. alternifolius* was the most efficient and *C. dives* the least efficient in translocating Arsenic metal to the shoots.

ANOVA test showed significant difference in TF values for Cadmium metal in the study plants. Tukey test however revealed that *C. alternifolius* translocated 35.0% of Cadmium metal more than *C. dives*. However, there was no significant difference in TF values for Cadmium metal between *C. alternifolius* and *C. indica*. Therefore, this finding showed that *C. dives* was the least efficient in translocating Cadmium metal to the shoots.

For Lead metal, ANOVA test showed no significant difference in TF values for Lead metal in *C. alternifolius*, *C. dives* and *C. indica*.

5.1.3 Phytoremediation Potential

BCF values were greater than one while the TF values were less than one (BCF>1 and TF <1) for Arsenic, Cadmium and Lead metals in all the studied plants. This indicated that the study plants were able to accumulate Arsenic, Cadmium and Lead metals in their roots but were not able to translocate these metals from their roots to their shoots.

5.2 Conclusions

1. BCF values of Arsenic, Cadmium, and Lead metals in *C. alternifolius*, *C. dives* and *C. indica* were greater than one throughout the study period. Therefore, the three study plants can absorb from wastewater and accumulate in their roots the three metals and consequently, the hypothesis was accepted.
2. TF values of Arsenic, Cadmium, and Lead metals in *C. alternifolius*, *C. dives* and *C. indica* were less than one throughout the study period. Therefore, the three study plants cannot translocate substantial amounts of the three metals from their roots to their shoots and consequently, the hypothesis was rejected.
3. BCF values and TF values of Arsenic, Cadmium, and Lead metals in *C. alternifolius*, *C. dives* and *C. indica* were greater than one and less than one respectively throughout the study period. Consequently, these plants are Phytostabilizers of Arsenic, Cadmium and Lead metals and can therefore be used for phytoremediation of the three metals. The hypothesis was therefore accepted.

5.3 Recommendations

5.3.1 Recommendations for Management Actions

1. *C. alternifolius*, *C. dives* and *C. indica* are effective phytostabilizers of Arsenic, Cadmium and Lead metals. Consequently, their roots (below ground biomass) are very significant in phytoremediation and therefore, for optimum removal of heavy metals from wastewater, whole plants should be harvested, and fresh ones planted.

2. For maximum removal of Arsenic and Cadmium metals through harvesting of *C. alternifolius*, *C. dives* and *C. indica* biomass, the plants should be allowed to grow for a relatively longer period, that is, beyond six months after planting. For optimum removal of Lead metal from wastewater, harvesting of *C. alternifolius* should be done not later than week 24.
3. *C. alternifolius* is the most effective phytoextractor of Arsenic, Cadmium and Lead metals. Therefore, if removal of the heavy metals is through harvesting of the shoots (above ground biomass) then *C. alternifolius* is most promising. However, for Lead metal removal, the above ground biomass should be harvested not later than week 24.

5.3.2 Recommendations for Further Research

1. Further study should be conducted to determine heavy metal toxicity level for *C. alternifolius*, *C. dives* and *C. indica* to determine the best time for harvesting of the plants in order to achieve optimal results in removal of the heavy metals from the wastewater.
2. Further study should be carried out to determine how the rhizosphere factors affect the uptake and accumulation of the heavy metals by the plants studied and how they can be enhanced to increase uptake and accumulation.
3. Further study should be carried out to identify genes that are over-expressed in plants that translocate heavy metals to above ground biomass. Those genes can then be introduced in plants that are not hyperaccumulators to enhance their phytopremediation potential.

5.3.3 Recommendation for Policy

1. Aquatic plants with phytoremediation potential should be incorporated to compliment conventional wastewater treatment systems for removal of heavy metals from wastewater which are usually in low concentrations but in most cases above the allowable maximum limit for discharge into the natural environment.

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APPENDICES

Appendix 1: Concentration of Heavy Metals in Wastewater, Roots and Shoots

Concentration of Heavy Metals (mg/l) in Wastewater				
Metal	**Aug-14**	**Jul-14**	**Jun-14**	**Average**
Arsenic	0.050	0.050	0.005	0.035
Cadmium	0.004	0.004	0.004	0.004
Lead	0.040	0.040	0.040	0.040

Arsenic Concentration (mg/kg) in Roots				
Plants	**June**	**July**	**August**	**Mean**
C. indica	2.750	2.750	3.210	2.903
C. alternifolius	0.360	0.880	0.760	0.667
C. dives	2.720	5.850	6.040	4.870
Cadmium Concentration (mg/kg) in Roots				
C. indica	0.310	1.020	1.320	0.883
C. alternifolius	0.080	0.290	0.310	0.227
C. dives	0.110	2.390	3.210	1.903
Lead Concentration (mg/kg) in Roots				
C. indica	2.24	3.26	4.32	3.273
C. alternifolius	0.9	0.48	0.61	0.663
C. dives	0.45	8.79	6.82	5.353

Arsenic Concentration (mg/kg) in Shoots				
Plant Species	**June**	**July**	**August**	**Mean**
C. indica	2.75	2.75	3.21	2.903
C. alternifolius	0.36	0.88	0.76	0.667
C. dives	2.72	5.85	6.04	4.870
Cadmium Concentration (mg/kg) in Shoots				
C. indica	0.31	1.02	1.32	0.883
C. alternifolius	0.08	0.29	0.31	0.227
C. dives	0.11	2.39	3.21	1.903
Lead Concentration (mg/kg) in Shoots				
C. indica	2.24	3.26	4.32	3.273
C. alternifolius	0.9	0.48	0.61	0.663
C. dives	0.45	8.79	6.82	5.353

Appendix 2: ANOVA Tables

1=*C. alternifolius*, 2=*C. dives* and 3=*C. indica*

BCF – Arsenic Metal

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	241192.507	2	120596.253	.667	.547
Within Groups	1084423.093	6	180737.182		
Total	1325615.600	8			

BCF – Cadmium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	267676.389	2	133838.194	2.242	.187
Within Groups	358175.000	6	59695.833		
Total	625851.389	8			

BCF – Lead metal

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20709.125	2	10354.563	2.469	.165
Within Groups	25162.750	6	4193.792		
Total	45871.875	8			

TF – Arsenic metal

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.897	2	.448	55.160	.000
Within Groups	.049	6	.008		
Total	.946	8			

TF - Cadmium metal

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.191	2	.095	5.651	.042
Within Groups	.101	6	.017		
Total	.292	8			

TF – Lead metal

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.263	2	.131	1.680	.263
Within Groups	.469	6	.078		
Total	.731	8			

Appendix 3: Tukey Test Tables

TF – Arsenic metal

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	1.00					
	2.00	.6770322	.0736243	.000	.4511323942	.90293204878
	3.00	.66211482361*	.07362481864	.000	.43621550963	.8880146759
2.00	1.00	-.6770322480*	.07362181864	.000	-.90293234878	-.45113602082
	2.00					
	3.00	-.014917351119	.073624181864	.978	-.24081867517	.21092965279
3.00	1.00	-.66211487361*	.073622181864	.000	-.888014983759	-.436215150963
	2.00	.014917381119	.073624381864	.978	-.21098265279	.24081867517
	3.00					

*. The mean difference is significant at the 0.05 level.

TF – Cadmium metal

					95% Confidence Interval	
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1.00	1.00					
	2.00	.34997437405*	.10601618605	.038	.02468759025	.67526113786
	3.00	.23330727459	.10601618605	.150	-.09197928922	.55859477839
2.00	1.00	-.34997437405*	.10601617605	.038	-.67526113786	-.02468759025
	2.00					
	3.00	-.11666715947	.10601618605	.548	-.44195388327	.20861967434
3.00	1.00	-.23330724459	.10601618605	.150	-.55859407839	.09197958922
	2.00	.116667103947	.106016189605	.548	-.20861967434	.441953889327
	3.00					

*. The mean difference is significant at the 0.05 level.

TF – Lead metal

					95% Confidence Interval	
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1.00	1.00					
	2.00	.25886800191	.228237425288	.530	-.441410794768	.95914135150
	3.00	.414029987985	.22823425288	.243	-.286249976974	1.11430952944
2.00	1.00	-.258869170191	.228237425288	.530	-.959146135150	.441416794768
	2.00					
	3.00	.155161517794	.22823425288	.783	-.545119147165	.855440782754
3.00	1.00	-.414025987985	.22823225288	.243	-1.11430952944	.286248976974
	2.00	-.155161817794	.228232425288	.783	-.855442782754	.545116947165
	3.00					

Appendix 4: Photolog

 Plate 1: Preparation of GBH before planting of the study plants.	 Plate 2: Study plants just before the first sampling after five months.
 Plate 3: Harvesting of study plants by carefully pulling the plants.	 Plate 4: Sampled study plants ready for packing in brown paper bags.