Assessment of selected plants growing along Nairobi River for uptake of copper, zinc and cadmium, Nairobi County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

I dedicate this work to my family whose words of encouragement and push for tenacity ring in my ears. To my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. To my mother, who taught me that even the largest task can be accomplished if done step by step

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ACRONYMS AND ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
Al	Aluminium
AOAC	Association of Official Analytical Chemists
As	Arsenic
Cd	Cadmium
CF	Concentration factors
CO_2	Carbon dioxide
Cr	Chromium
Cu	Copper
DTPA	Diethylenetriamine pentaacetate
DW	Dry weight
EDL	Electrodeless discharge lamp
EDSS	Ethylenediamine-N,N'-disuccinic acid
EDTA	Ethylenediaminetetra acetic acid
FIMS	Flow injection mercury systems
H_2O	Water
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HCL	Hollow cathode lamp
Hg	Mercury
HNO_3	Nitric acid
IUPAC	International Union of Pure and Applied Chemists
KEBS	Kenya Bureau of Standards
N_2	Nitrogen
Ni	Nickel
PAHs	Polyaromatic hydrocarbons
Pb	Lead
SD	Standard deviation
Zn	Zinc

ABSTRACT

Nairobi River is polluted with heavy metals emanating mainly from discharge of waste and waste wastewaters from industrial and domestic activities from the drainage basin. Plants have tremendous potential for removal and remediation of heavy metals from polluted waters. Phytoremediation is the use of plants and plant processes to remove, degrade or render harmless hazardous materials present in the soil or water. The objective of this study was to investigate the potential of selected plants to uptake copper (Cu), zinc (Zn) and cadmium (Cd). The plants selected were *Polygonum senegalensis (P. senegalensis), Amaranthus hybridus* (*A. hybridus*) and *Eichhornia crassipes (E. crassipes)*. The study was conducted along Nairobi River in six sites; Kikuyu, Kawangware, Chiromo, Gikomba, Njiru, and Fourteen falls. The concentrations of the heavy metals in water, soil and plants were determined using Shimadzu AA7000 atomic absorption spectrometer available at the Kenya Bureau of Standards laboratory. The values obtained were used to evaluate the bio-concentration factors (BCF) of *P. senegalensis, A. hybridus* and *E. crassipes.* The concentration of Cu (26.6±0.5µg/Kg), Zn (64.7±0.8µg/Kg) and Cd (37.7±0.3µg/Kg) in soil was higher than the values recorded in water (Cu-23.1±0.5µg/L, Zn-57.9±0.8µg/L and Cd-37.7±0.3µg/L). This difference may be attributed to dilution effect of the water due to flow and pre-concentration of the metals in soil. The concentration of the heavy metals in the plants were Cu 8.73±0.08, 7.27±0.05, 7.23±0.05 µg/Kg, Zn 21.79±0.04, 17.42±0.2, 16.32±0.1µg/Kg and Cd 2.91±0.01, 4.11±0.03, 2.15±0.01 µg/Kg in *P. senegalensis, A. hybridus* and *E. crassipes* respectively. The BCF values for *P. senegalensis, A. hybridus* and *E. crassipes* ranged from 0.27-0.29, 0.24-0.26 and 0.21-0.23 respectively which indicated that the plants have a potential to uptake Cu, Zn and Cd. The study, thus, demonstrates the potential of *P. senegalensis, A. hybridus* and *E. crassipes* in phytoremediation of Cu, Zn and Cd polluted waters.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study area

Nairobi River and its tributaries traverse through the Nairobi County which is the Kenyan Capital. It is the main river of the Nairobi River Basin, a complex of several parallel streams flowing eastwards. All of them join east of Nairobi and meet the Athi River, eventually flowing to the Indian Ocean. These rivers are mostly narrow and highly polluted. The main stream, Nairobi River, bounds the northern city center and is partly canalized.

Nairobi River lies between 1° 11′ 59″ S and 37° 9′ 26″ E (Figure 1-1)

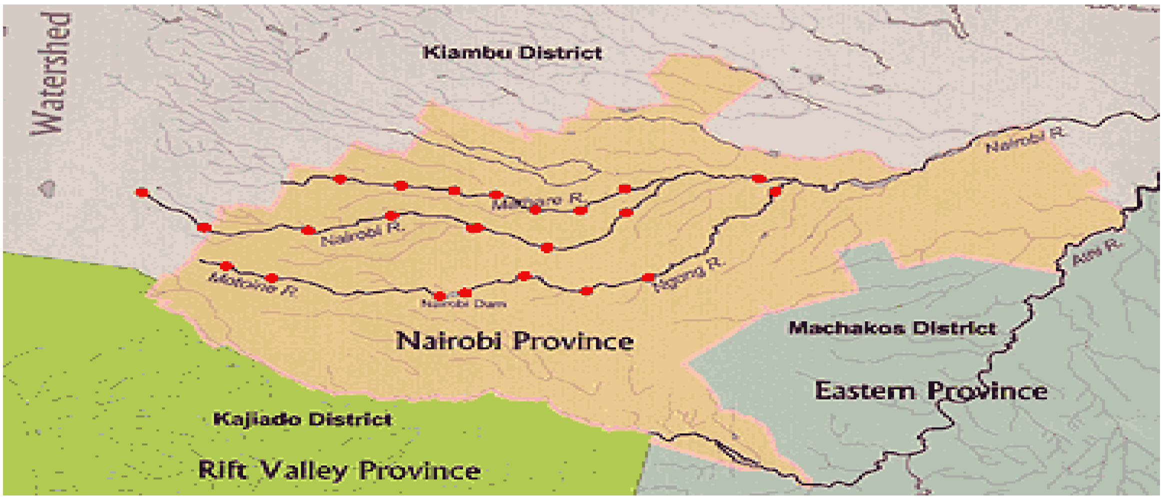

Figure 1-1: Location of Nairobi River

Ecologically, the study area lies within agro-ecological zones which range from humid, through semi-humid to semiarid lands. However, land-use systems are highly influenced by rainfall patterns, topography and human activities. The area has two distinctive land-use systems, comprising agriculture, which is the main land use in the Kiambu area, and industry, which is the predominant land use in Nairobi city and its environs. Population growth and industrial production has increased the volume of domestic waste and effluent load discharged into the rivers passing through the city and has caused a serious deterioration in water quality. The source of Nairobi River is the Kikuyu springs at an altitude of 2000 m above sea level (Otieno, 1995). From Kikuyu the river flows eastwards through Dagoretti, Kawangware, Chiromo, the central business district, Eastleigh and Kariobangi sewage treatment works. After Kariobangi the Nairobi River runs through barren Njiru quarry sites where the Gitathuru and Ruaraka Rivers join it. The Nairobi River then flows past the Nairobi Falls and Fourteen Falls. The river joins the Athi River and eventually the Sabaki River which discharges its water into the Indian Ocean at Malindi on the East African coast.

1.2 Water pollution

Water pollution is associated with population explosion and industrialization consequently making it unfit for human consumption, industrial use and aquatic biota. The city has experienced rapid industrialization and growth in population during the last 100 years (Okoth and Otieno, 2001). This rapid growth has not been matched by development of infrastructure to deal with waste disposal. As a result problems have arisen with regard to garbage, human and industrial waste disposal leading to pollution of the water resources.

Sources of pollution of the Nairobi River include industrial effluent, effluent from petrol stations and motor vehicle garages, surface run off, factories and other business premises, raw sewage from broken or overloaded sewers as well as raw sewage from informal settlements (Ndwaru, 1994; Otieno, 1995; Okoth and Otieno, 2001).

Water pollutants mainly consist of heavy metals, microorganisms, fertilizers and toxic organic compounds (WHO, 2010). Copper and zinc are essential elements which are toxic in high concentrations. Cadmium is one of the most toxic elements, has an accumulative effect and is an environmental priority pollutant (Chena *et al*., 2005).

Heavy metal pollution in water systems has seriously influenced the quality of life, especially in developing countries. They may accumulate in tissues and may cause serious health problems for humans and animals. Toxic elements like cadmium inhibit biosynthesis, and affect the kidney, brain cells and the permeability of the liver membrane, therefore reducing some of their functions. They can be accumulated in the body and can promote disturbance such as nausea, vomiting, diarrhea, sweating and in some cases convulsions and coma (Maranhao *et al*., 2005; Sharma *et al*., 2009)

The U.S. Environmental Protection Agency (USEPA) has classified cadmium as a Group B2 (probable) human carcinogen. The World Health Organization (WHO) has established the maximum allowable limit of 1 mg/L for copper, 5 mg/L for zinc and 0.05 mg/L for cadmium in drinking water. It is, therefore, important to monitor the lead level in the environmental samples (WHO, 2010)

1.3 Water pollution remediation studies

Pollution of rivers and streams is one of the crucial environmental problems. Although some kinds of water pollution can occur through natural processes, it is mostly as a result of human activities. Direct discharge or wet and dry depositions of contaminants increase the concentration of trace elements in aquatic systems, thus resulting in their accumulation in sediments (Dunbabin and Bowmer, 1992; Sinicrope *et al*., 1992).

Aquatic plants absorb elements through roots and/or shoots (Pip and Stepaniuk, 1992; Jackson, 1998). Various species show different behavior regarding their ability to accumulate elements in roots, stems and/or leaves. Therefore, it is useful to identify the plant organ that absorbs the greatest amount of trace elements (St-Cyr and Campbell, 2000; Baldantoni *et al*., 2004).

In aquatic ecosystems, where pollutant inputs are discontinuous and pollutants are quickly diluted, analyses of plant components provide time-integrated information about the quality of the ecosystem (Baldantoni *et al*., 2005). Phytoremediation, the ability of plants to uptake heavy metals, has several advantages and is the most significant one in study of sub-lethal levels of bio-accumulated contaminants within the tissues /components of organisms, which indicate the net amount of pollutants integrated over a period of time (Lovett-Doust *et al*., 1994).

Bio-monitoring of pollutants using plants as indicator species accumulate relatively large amounts of certain pollutants, even from much diluted solutions without obvious noxious effects (Ravera *et al*., 2003). It may be performed in two ways, based on the kind of sampled organisms, that is, ‘endemic’, or native, organisms (passive biomonitoring) and introduced organisms (active biomonitoring) (Chaphekar, 1991).

The metal pollution load (Siegel *et al*., 1994) and its biomonitoring in aquatic plants (Ramdan, 2003) were intensively investigated in Manzala lake of Nile delta, Egypt. Ravera *et al*., 2003 studied trace element concentration in freshwater macrophytes. Moreover, macro and microelement accumulation in *Typha angustata* and *Phragmites australis* was assessed in relation to spatial gradients of lake by Baldantoni *et al.*, 2004; 2005.

Studies carried out in Kenya include: phytoremediation of heavy metals in mimics of chemically polluted soils and waste water and phytoremediation of polychlorobiphenyls (PCBs) in landfill E-waste lecheate in water hyacinth (Kimenyu 2014; Auma, 2014). Therefore, it is necessary to carry out phytoremediation of heavy metal contaminated water and sediments by selected aquatic macrophytes viz. *P. senegalensis, A. hybridus* and *E. crassipes* in Nairobi River, Nairobi, Kenya.

1.4 Problem statement

Human settlements along the Nairobi River have increased dramatically due to the growth of the city and population increase. This has raised serious concern for the environmentalists on the state of Nairobi River for several decades. The River has seen a massive deterioration in quality with the increase in population of the city.

This increase in the city’s population coupled with a sluggish economy has led to the mushrooming of slums, which tend to be next to the riverbanks of which sanitation facilities are non-existent. Since the communities are not served by amenities and public utilities, they discharge their raw sewage into the streams next to them. This has led to the well- being of communities living downstream of the Nairobi River being adversely affected. Also lack of environmental awareness and law enforcement capacity has left Nairobi residents with a deplorable situation impacting adversely on all who live in, or indeed visit the city.

Nairobi River is currently faced with high turbidity, presence of solid wastes, absence of aquatic life and effluvia from raw sewage (UNEP, 2008). The problem of water contamination in Nairobi River is constantly growing due to the fact that there are limited financial and technological resources to remediate polluted soils and water.

This study attempts to bridge the existing gap of knowledge on phytoremediation, by reporting major research findings elsewhere and highlighting the potential applicability of those findings to a developing country in tropical environments like Kenya.

1.5 Justification

Kenya is among developing African countries with high economic growth potential. Nairobi city is traversed by three rivers and many tributaries whose potential in economic promotion is yet to be unlocked. As a water scarce country, Kenya needs to conserve and manage its water resources more effectively and efficiently now than ever before.

Most of the conventional methods for environmental remediation of polluted sites are expensive and often poorly implemented or not implemented at all. Some methods, such as soil washing, have an adverse effect on biological activity, soil structure and fertility, and some require engineering costs. Consequently, the low technology, in situ approach of phytoremediation is attractive as it offers site restoration, partial decontamination maintenance of the biological activity and physical structure of soils, and is potentially cheap, visually unobtrusive, and there is possibility of bio-recovery of metals (Baker, 1994).

Thus there is a clear need to develop new cost effective and environmentally friendly clean-up methods to remediate the contaminated areas in Kenya. This approach could help the country to achieve its Millennium Development Goals principles guiding the restoration and rehabilitation of the Nairobi Rivers, as well as Vision 2030 which proposes that the Kenyan population by 2030 will be living in a clean, secure and sustainable environment. The constitution of Kenya 2010 also states that every citizen has a right to access clean and safe water.

1.7 Hypothesis

The concentration of Cu, Zn and Cd in water and soils along Nairobi River are not significantly different from those found in plants (*P. senegalensis*, *A. hybridus* and *E. crassipes*)

1.8 Objectives

1.8.1 General Objective

To determine heavy metals uptake capability of *P. senegalensis*, *A. hybridus* and *E. crassipes* growing along Nairobi River

1.8.2 Specific Objectives

1. To determine the concentrations of Cu, Zn and Cd in water samples from the water column of Nairobi River.
2. To determine the concentrations of Cu, Zn and Cd in soil samples collected along Nairobi River
3. To determine the concentrations of Cu, Zn and Cd in *P. senegalensis, A. hybridus, E. crassipes* growing along Nairobi River

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Water is a precious natural resource on which we rely the sustainability of agriculture and the civilization of mankind. Unfortunately, it has been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and non-point sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers (McGrath *et al*., 2001).

Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001; Gisbert *et al*., 2003). Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

In developing countries, rivers and streams that flow through cities are badly kept with their banks as disposal sites for municipal wastes and with public sewage system draining into the streams. They are heavily polluted and even those in the industrial areas are no exception. However, most of the available land by the side of the streams is used for irrigation of vegetables, fruits and other high valued crops for the consumption of city dwellers (Binns *et al*., 2003). The contamination of urban and peri-urban surface water through such process are due to inadequate sewage facilities, landfills with wastes, agro – chemicals, domestic sewage and industrial effluents.

In order to maintain good quality of soils and waters and keep them free from contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. The use of plant species for cleaning polluted soils and waters named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology (Ramdan, 2003).

2.2 Heavy metals

Heavy metals refer to any metallic chemical element that has a density greater than 5g/cm^3. These include lead, mercury and cadmium among others. Many other definitions of heavy metals have been proposed based on density, atomic number and atomic weight.

Depending on the context, the term can include elements lighter than carbon and can exclude some of the heaviest metals (Duffus, 2002). Any element that exhibits metallic properties, and belongs to the transition metals, metalloids or lanthanides and actinides can pass as a heavy metal. At one time, International Union of Pure and Applied Chemists' (IUPAC) technical report described the term heavy metal as a "meaningless and misleading term" due to its contradictory definitions, lack of a "coherent scientific basis" and unclear boundaries (Duffus, 2002). Recently, the definition has been based on chemical properties particularly toxicity.

Heavy metals have thus been defined collectively as metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms (Harrison and Waites, 1998).

2.3 Classifications of heavy metals

Heavy metals can be broadly classified into three groups; those that are essential for certain bio-chemical processes, but are toxic when their concentration exceeds certain thresholds. These include copper, zinc, cobalt, selenium and iron. The second group consists of metals with no known biological function and toxic if present in concentrations above trace amounts. These include arsenic, bismuth, indium, antimony and thallium. The last and evidently the most dangerous group includes lead, cadmium and mercury which serve no known biological function and are toxic at all concentrations (Fernandez *et al.*,1992).

Some elements are necessary for human health in trace amounts (Co, Cu, Cr, Ni) while others are carcinogenic or toxic, mainly affecting the central nervous system

(Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu), skin, bones or teeth (Ni, Cd, Cu, Cr), (Zevenhoven and Kilpinen, 2001).

2.4 Sources of heavy metal pollution

Land and water pollution by heavy metals is a worldwide issue. All countries have been affected, though the area and severity of pollution vary enormously. In Western Europe, 1,400,000 sites were affected by heavy metals (McGrath *et al*., 2001), of which, over 300,000 were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and Eastern European countries (Gade, 2000).

In USA, there are 600,000 brown fields which are contaminated with heavy metals and need reclamation (McKeehan, 2000). According to government statistics, coal mine has contaminated more than 19000 km of US streams and rivers from heavy metals, acid mine drainage and polluted sediments. More than 100,000 ha of cropland, 55000 ha of pasture and 50 000 ha of forest have been lost (Ragnarsdottir and Hawkins, 2005).

Soil and water pollution is also severe in India, Pakistan and Bangladesh, where small industrial units are pouring their untreated effluents in the surface drains, which spread over near agricultural fields. In these countries raw sewage is often used for producing vegetables near big cities.

The problem of land pollution is also a great challenge in Kenya, where one-sixth of total arable land has been polluted by heavy metals, and more than 40% has been degraded to varying degree due to erosion and desertification (Liu, 2006). Heavy metals that have been identified in the polluted environment include As, Cu, Cd, Pb, Cr, Ni, Hg and Zn. The sources of various heavy metals are listed in Table 2-1.

Table 2-1: Sources of heavy metals

Heavy metal	**Sources**
As	Semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, herbicides, volcanoes, mining and smelting (Nriagu, 1994)
Cu	Electroplating industry, smelting and refining, mining, bio-solids (Liu *et al*., 2005)
Cd	Geogenic sources (Baize, 1997), anthropogenic activities, metal smelting and refining, fossil fuel burning, application of phosphate fertilizers, sewage sludge (Alloway, 1995; Kabata-Pendias, 2001)
Cr	Electroplating industry, sludge, solid waste, tanneries (Knox *et al*., 1999)
Pb	Mining and smelting of metalliferous ores, burning of leaded gasoline, municipal sewage, industrial wastes enriched in Pb, paints (Gisbert *et al*., 2003)
Hg	Volcano eruptions, forest fire, emissions from industries producing caustic soda, coal, peat and wood burning (Lindqvist, 1991)
Se	Coal mining, oil refining, combustion of fossil fuels, glass manufacturing industry, chemical synthesis (e.g., varnish, pigment formulation) (Knox *et al* 1999)
Ni	Volcanic eruptions, land fill, forest fire, bubble bursting and gas exchange in ocean, weathering of soils and geological materials (Knox *et al*., 1999)
Zn	Electroplating industry, smelting and refining, mining, bio-solids (Liu *et al*., 2005)

The presence of any metal may vary from site to site, depending upon the source of individual pollutant. Excessive uptake of metals by plants may produce toxicity in human nutrition, and cause acute and chronic diseases. For instance, Cd and Zn can lead to acute gastrointestinal and respiratory damages and acute heart, brain and kidney damages.

High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Garbisu and Alkorta, 2001; Schmidt, 2003; Schwartz *et al*., 2003). Soil contamination with heavy metals may also cause changes in the composition of soil microbial community, adversely affecting soil characteristics (Giller *et al*., 1998; Kozdrój and Elsas, 2001; Kurek and Bollag, 2004).

2.5 Toxicity of heavy metals

The toxicity of trace metals arises from their interference with an organisms' uptake of essential metal ions such as sodium and calcium. For instance, cadmium and zinc block the uptake of calcium ions which is essential for bone and teeth development. The interaction of some heavy metals with enzymes and their tendency to bind to protein and other biological tissues also cause trace metal poisoning in organisms (Campbell, 1995).

The common results of trace metal toxicity to living organisms include brain disorder, gross deformities in development, carcinogenic effects and generally, disruption of biological processes. In most cases these elements find their way into the environment through human industrial processes such as mining, electroplating, battery manufacture, leather tanning, and manufacture of printing pigments and paints, among others.

A high concentration of heavy metals in the environment is of great health concern because they are non-biodegradable and end up accumulating in food chains in various forms such as organic, inorganic or organo-metallic species (Cordero *et al*., 2004) with disastrous consequences. Due to the toxicity of trace metals, it is important to remove them from the environment.

2.6 Remediation of polluted sites

A two-tiered remediation strategy has been adopted for remedying water and soil contaminated by heavy metal. The first tier aimed at in situ increasing the stabilization of metals on sediment particles (e.g. immobilization) and the second tier aimed at ex situ extracting or separating metals from sediment (e.g. washing, flotation) (Iskandar and Andriano, 1997, Susan *et al*., 2005).

The first strategy focuses on improving metal stabilization, by enhancing metal sorption, precipitation and complexion capacity on sediment, the potential mobility or bioavailability of the toxic metals to environment are lowered. Due to these stabilization techniques usually being carried out “in situ”, their remediation cost is relatively low (Enzo *et al*., 2002, Raicevic *et al*., 2006). This technique improves the immobility of heavy metal on sediment but does not decrease their total content. Therefore, in some special conditions, part of these immobilized metals will be released into the water again.

The other strategy is extraction, in which polluted water or soil is dredged from the river bed and heavy metal is extracted through a series of chemical, physical, biological methods in a specially designed reactor. This kind of remediation techniques is usually carried out “ex situ”, and can remove almost all mobile metal. However, it also implies the sediment structure deterioration and high costs, which limit their popularization on vast contaminated areas (Alfredo *et al*., 2005, Rafael *et al*., 2006).

Compared with ex situ remediation techniques, the key advantage of in situ stabilization approach is that it only needs a simple mixture of amendments with sediment. And the major disadvantage of this approach is that the final product of remediation, although existing in inactive form, still remains in sediment.

2.6.1 In situ remediation technology

Due to low cost and non-disruptive to natural hydrological conditions than conventional ex situ extraction technologies, in situ metal immobilization technologies are developing rapidly.

2.6.1.1 Amendments

Amendment, usually possessing high cation exchange capacity, can lower metal mobility and bioavailability in sediment by precipitation or sorption, thereby decreasing their solubility. The in situ immobilization of metals, usually using inexpensive amendments such as minerals (e.g. apatite, zeolites, steel shot, or beringite), is considered as a promising alternative to the currently available remediation methods.

In sediment remediation process, metal firstly incorporates into the apatite lattice through ion exchange with Ca^{2+}. This reaction can stimulate apatite dissolution and phosphate is released correspondingly. Due to the lower solubility between metal ions and phosphate, a new metal-phosphate solid phase would form (Shrestha *et al.*, 2003). Through these fixing process, apatite minerals can effectively immobilize almost all Pb, Mn, Co, Cu, Cd, Zn, Mg, Ba, U, and Th in sediment (Raicevic *et al.*, 2005, Raicevic *et al.*, 2006).

2.6.1.2. Sand cap application

Normally, decreasing the direct contact area between water and the contaminated sediment is a good choice for lowering the release content of heavy metal. Therefore, capping the contaminated sediment with sandy materials, such as clean sediment, sand, or gravel becomes an effective remediation technique.

Through physical isolation, chemical isolation or sediment stabilization, the mobile and the exchangeable metals are transformed from the contaminated sediment into the clean cap and combined with particles in more stable forms. When properly designed, the placement of a relatively coarse-grained cap does not disturb or mix with underlying very soft fine-grained sediments. Some researchers also showed that a good cap thickness was approximately 50 cm; and through capping the sediment by sands materials, the heavy metal concentration in water could reduce to 80% (Theofanis *et al.*, 2001).

Additionally, compared with other in situ remediation methods, this approach has a lower cost. However, this remediation technique can only reduce the transfer rate of metal in sediment, while their immobilization effect for heavy metal is small.

Therefore, for enhancing their immobilization capacity, some amendments (such as apatite, rock phosphate, lime or zeolite) can also be added into the sand cap. These reactive materials would demobilize heavy metals from solution and enhance the cap quality.

2.6.1.3 Phytoremediation and phytorestoration

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants (including grasses, forbs, and woody species) to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water (Lovett-Doust *et al*., 1994).

This definition includes all plant-influenced biological, chemical, and physical processes that aid in the uptake, sequestration, degradation, and metabolism of contaminants, either by plants or by the free-living organisms that constitute the plant's rhizosphere. It takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body.

Several comprehensive reviews have been written on this subject, summarizing many important aspects of this novel plant-based technology. The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source. However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective, and environmentally friendly technology. It can be applied to both organic and inorganic pollutants, present in solid substrates (for example soil), liquid substrates (such as water), and the air (Lone *et al*., 2008).

Simply, phytoremediation is the use of plants to extract, sequester, or detoxify pollutants. This technology is widely viewed as an ecologically responsible alternative to the environmentally destructive chemical remediation methods currently practiced (Meagher, 2000). This technology is popularly applied in soil remediation, and also shows some excellent remediation effects in some shallow rivers, lakes and wetlands. At present, this technology presents good immobilization effects for Zn, Fe, Mn and Cd in sediment.

Phytoremediation is comprised of two tiers, one by plants themselves and the other by the root colonizing microbes, which degrades the toxic compounds to further non-toxic metabolites. Normally, hydrophytes have the ability to uptake and accumulate various heavy metals by the action of phytochelatins and metal lothioneins (Suresh and Ravishankar, 2004).

However, mass balances experiments show that metal uptake by hydrophytes were not high enough for phytoextraction.This indicates that in phydroremediation, the direct uptake of hydrophytes is small, and the indirect reactions, such as stimulation of microbial activity, redox reactions/formation and precipitation of insoluble metal compounds in the rhizosphere, may play a relative important role (Clemente *et al*., 2005).

Therefore, the direct uptake capacity of phytoremediation for heavy metal can be achieved by most plant species able to grow in contaminated sediment. Correspondingly, the choice of hydrophytes would not depend on their apparent uptake capacity, but on their practical immobility capacity for metal.

2.6.2 Ex-situ remediation technology

In situ remediation techniques are usually applied for the sediment slightly polluted by heavy metal. However, for the sediments heavily polluted, their remediation effects can be ignored to a large extent. Under such conditions, ex situ sediment remediation becomes the first choice (Catherine *et al*., 2001). Most ex situ remediation technologies for soil or mineral ores can be used for dredged sediment. However, due to higher workload and different environmental characteristics in sediment, some technologies have higher costs and more complex when used in sediment remediation.

2.6.2.1 Soil/sediment washing

Sediment washing is a relatively simple and useful ex situ remediation technology, by adding washing water, heavy metal can be transferred from the dredged sediment to wash solution. To enhance the performance of sediment washing, various additives can be employed, such as acid (H_2SO_4 and HNO_3) washing, chelating agents (such as ethylenediamine-N,N'-EDTA) or surfactants (like rhamnolipid). These additives can assist in the solubilization, dispersal and desorption of metal from dredged sediments.

This technology is most appropriate for the weaker bound metals in the form of exchangeable, hydroxides, carbonates and reducible oxides fraction. Residual fractions, the most difficult ones to remove, are not affected during the washing process (Catherine *et al*., 2001, Ortega *et al*., 2008).

Additionally, fine grain sediments are difficult to decontaminate through washing solutions, therefore washing is most applicable to sands and gravels. Optimization of chelating agents should be the research direction in future. Surfactants, especially bio surfactants, usually possess excellent surface active properties, anionic nature and low toxicity.

2.6.2.2. Electrochemical remediation

Electrochemical remediation involves applying low direct current or a low potential gradient to electrodes that are inserted into the sediment and encompass the contaminated zone (Tsai *et al*., 2003). When DC electric fields are applied to the contaminated sediment, migration of charged ions occurs. Positive ions are attracted to the negatively charged cathode, and negative ions move to the positively charged anode.

For example, under an induced electric potential, the anionic Cr(VI) migrated towards the anode, while the cationic Cr(III), Ni(II) and Cd(II) migrated towards the cathode. The contaminants, which are accumulated at the electrodes, are extracted by electroplating, precipitation/co-precipitation, pumping water near the electrodes, or complexing with ion-exchange resins (Krishna *et al*., 2001).

2.6.2.3. Flotation

Flotation, using gas bubbles attachment to dispersed phase, is a separation method of hetero-phase systems. The formed aggregates are floated and separated from the dispersing medium.Flotation is widely used in mining industries to separate valuable mineral ores (Matis, 1995).

Presently, flotation technology, as a possible treatment procedure for metal sulfides, shows some advantages in the remediation of anaerobic sediment, especially fine-textured substrates (20–50m) of sediment. It is expected that various metal ions (e.g. Ca, Cu, Pb and Zn) would be present as sulfides in dredged anaerobic sediments. The surface of these metal sulfides is hydrophobic in nature, and then can be selectively separated from suspensions by means of collector less flotation.

For most heavy metals in sediment, up to 80% of removal efficiencies can be achieved in flotation process. In flotation process, some metal sulfides are oxidized, released and subsequently redistributed in other fractions, such as freshly precipitated iron oxides, which would lower the efficiency of flotation. Consequently, in general, the froth fractions have a lower extractability than other remediation technology (Cauwenberg *et al*., 1998).

2.6.2.4 Ultrasonic-assisted extraction

One of the key limitations of traditional heavy metal remediation technologies is that they are extremely time consuming. However, the use of ultrasound coupled with vacuum pressure can effectively improve the extraction efficiencies of heavy metal from dredged sediment (Jay and Rusini, 2001). Ultrasound can cause high-energy acoustic cavitations: the formation, growth and implosive collapse of bubbles in liquid.

During cavitational collapse, intense heating of the bubble occur. These localized hot spots roughly have the temperatures of 5000 °C, the pressures of 500 atmospheres, and a lifetime of a few microseconds, the impact of which is sufficient to melt most metals. These bubbles collapse, create very minute, but high-energy movements of the solvent that results in localized high shear forces, which can remove the material adhering to particles surface.

Additionally, these “cavities” or areas of low pressure provide a sink of the metal into which adsorbed material will be desorbed. Depending on particles sizes, the removal efficiencies for heavy metal change correspondingly. When ultrasound is applied to treat coarse grains, almost all metal can be separated from sediment and 92% can be removed in the whole remediation process; when used for silt (>2_m), separation efficiency still can reach 100%, but only 82% of the removal efficiency can realize; when used for clay (<2_m), no significant removal can be found (Jay and Rusini, 2001).

Further research shows that the metal associated with clay is too stable to be removed inmost remediation processes. Therefore, the ultrasound technology is an effective and economical remediation process, especially for those sediments with lower clay contents (Hanna *et al*., 2004).

2.6.2.5 Heavy metal immobilization

Most amendments used for sediment remediation are also used for heavy metal immobilization in dredged sediment. Though these immobilization methods cannot remove metal from sediment, due to their low cost and fast remediation effect, they are still popularly applied.

2.7 Accumulation, Distribution and Chemical status of heavy metals in plants

Phytoremediation processes involve several biochemical and biophysico processes. These include accumulation, distribution and purification.

2.7.1 Accumulation and Distribution

The accumulation ratio usually indicates the accumulation capacity of plants, is the ratio of the contents of the metal in the plant or organ to the content of this element in the cultivated solution and/or soil. At individual sites, plants absorb elements selectively. In copper (Cu) polluted soil, the content of Cu in above ground parts of

Eriachne pallescens R. Br. was 132 mg/kg DW, 20 fold higher than that seen in other plants.

Grown in acid soil, *Lycopodiaceae* and *Melastomaceate* species accumulated large amounts of Aluminium (Al), so that the contents even reach 1% in dry weight, although it was seen to be lower in other species (Liao, 1993). Woody plants accumulate a high amount of Cd, but the capacities are different in varying species. Zhuang and Wang, 2000 studied the heavy metal accumulation in plant leaves at a single site and found that they were different. Plants absorb heavy metals from soil and they predominantly accumulate in the roots, then some portions are transported to other parts of the plant.

Generally, the contents of heavy metals in underground parts are higher than that found in those parts above the ground (Liao, 1993) and follows a pattern that root>leaf>shoot (stem)>fruit and lateral root>main root, old leaf>young leaf (Cheng *et al.*, 2002). For vegetables, the content of heavy metals in organs is: leaf>stem>root>fruit for cucumber and tomatoes, root>above ground part of cabbage, and above ground part> root for carrot.

Vegetable leaves> root and vegetable rhizomes> fruit in waste water irrigation farmland (Wang and Bai, 1994). On the other hand, plants transport large portions of heavy metals from the root to the stem, while the accumulation in the stem and leaf are even higher than that in the root. The accumulation of heavy metals in plants is related to the elements and the chemical status of the heavy metals. Song *et al.*, 1996 reported that the accumulation of heavy metals in the edible parts of spinach depended on the doses, status of the elements in the soil and interaction with an accumulation rate order: Cd>Zn>Pb.

Therefore, the accumulation and distribution of heavy metals in plants depends on the environmental factors, such as, plant species, element species, chemical and bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Xu and Yang, 1995, Wu *et al.*, 1998a, Bi *et al.*, 2000, Yu *et al.*, 2000, Su *et al.*, 2000).

2.7.2 Chemical status

The transportation of heavy metals in plants is related to the chemical status in plants Xu *et al*., 1999a reported that the tolerance of plants to heavy metals was also related to the chemical form of heavy metals in plants, and that a higher portion of sodium chloride extractive metals showed a lower tolerance to Cd. They found out that the different distribution of Cd and Pb in cells may be the reason that Cd is much more toxic to plants than Pb. Cu is mainly in a water-soluble and ethanol-soluble form, which can easily be trans-located in plants.

The total content of chemically bound forms of copper was higher in the above ground parts than in the underground parts in wheat, and their content was decreased in the order of water soluble form>ethanol soluble form> residual form> acid soluble form (Wang *et al.*, 2000). Zinc is mainly in the form of acid soluble, which is hard to be transferred in crops. The total content of various chemically bound forms of Zn was lower in the above ground parts of wheat than in the roots (Wang *et al.*, 2000a).

In spite of the chemical reaction forms, heavy metals also combine with inorganic substances e.g. sulphides, small molecular organic substances e.g. glutathione, oxalic acid and metal binding proteins in the plant (Zhang *et a*l., 1999). The contributions of metallothionein and phytochelatin in the heavy metal transport in the plants, however, have not been studied.

2.8 Purification of heavy metal polluted water by plants

Since plants have a capacity to uptake heavy metals from the environment, plants are used to purify heavy metal polluted water, to keep the water clean and/or to be used for some utilization, as well as to reclaim metals. Macrophytes and the eco-system are commonly used to purify mine tailing water (Tang, 1993). The purification capacity of *Typha latifolia* Linn and *Cyperus alternifolius* in the ecosystem are listed in Table 2-2.

Macrophytes show a high performance in the removal of individual heavy metals. *Erichhornia crassipes* and *Leminar minor* show a high purification capacity for removing silver, while *Erichhornia crassipes* also has the potential for reclaiming gold from waste water (Fan and Jai, 1999). Economy aquatic plants are potential plants for removing heavy metals. You *et al*., 2000 investigated the contents of heavy metals (Cu, Cd, Pb and Zn) in *Ipomuea aquatic* and *O. javanica* for purification of eutrophic water and revealed that heavy metals mainly accumulated in the roots.

The purification capacities of plants to heavy metals form waste water are affected by several factors, such as concentrations of heavy metals, species of elements, plant species, exposure durations, temperature and pH (Gu *et al*., 2000)

Table 2-1: Removal performance of heavy metals by certain plants (%)

Species	Al	Cd	Cu	Fe	Hg	Mn	Pb	Zn	Ref.
Typha latifolia	-	60	-	41.2	-	-	97.1	84.8	a
Cyperus alternifolius	100	100	100	100	-	42.2	100	100	b
a) Zheng and Li, 1996 b) Cheng *et al*., 2002									

2.9 Heavy metal phytoremediation techniques

There are several ways to remediate heavy metal pollution in soil: to abate the toxicity of heavy metals in soil by using bacteria, to add bonds in soil to solidify and stabilize heavy metals, to remove heavy metals from soil by utilization of electricity dynamics, by thermic absorption, extraction and washing and phytoremediation (Wu *et al*., 2000).

Metal is different from organic substances, it cannot be decomposed by bacteria, and can only be absorbed and removed from the environment by organisms. To use bacteria for heavy metal remediation in large-scale sites has limitations: the biomass is small and the absorption quantities of heavy metals will be small and disposal will be more difficult because of the micro-bodies.

The advantage of a big biomass an easy disposal makes it possible for plants to remove heavy metals on-site, and phytoremediation is a potential choice for solving the heavy metal pollution. Phytoremediation in-situ is based on four points: phyto-stabilization, phyto-volatilization, phyto-extraction and phyto-filtration.

2.9.1 Phytostabilisation of heavy metals

It is used by plants and some additive substances to decrease the mobility, bioavailability and toxicity of persistent pollutants. Although the heavy metals ions are stabilized temporarily and are not removed from the environment by phytostabilization, the bioavailability of those heavy metals will be changed when the condition alters. Thus, phytostabilization is not an optimistic way to remediate heavy metal pollution.

2.9.2 Phytovolatilisation of heavy metals

It uses plants to remove volatile pollutants from the environment. Plants absorb the pollutants and transfer them into gaseous substances, which are then released into the atmosphere. Therefore, using plants to remove mercury and selenium from the soil is possible. Since it is only used for volatile pollutants, limitations are revealed for its further utilization. Moreover, the pollutants are transported into the atmosphere and threaten the health of humans and wildlife. As a consequence, they should be used carefully with equipments that can collect the volatile metals before it is released to the atmosphere.

2.9.3 Phytoextraction of heavy metals

Phytoextraction uses plants to accumulate and transport heavy metal ions and store them in above ground part organs, it has more potential to remove heavy metals from the environment. To study the accumulation discipline of heavy metals by plants, and to scan and select the hyper-accumulator are key points for phytoextraction techniques. A perfect plant species for phytoextraction: reveals a higher accumulation efficiency of heavy metals in lower concentrations, accumulates higher contents of heavy metals in organs which are easy to harvest, accumulates several kinds of heavy metals, grows rapidly and has a large biomass, resists diseases and pests and demonstrates some environment-friendly economic utilization.

2.9.4 Phytofiltration of heavy metals

Phytofiltration and rhizofiltration uses the uptake capacity and large surface area, or the whole plant, to remove heavy metals from the waste water. The mechanism is similar to phytoextraction. Aquatic plants, wetland plants and terrestrial plants are suitable plants for phytofiltration. Constructed wetland is one of the potential methods to use phytofiltration to remove heavy metals from waste water (Cheng *et al.*, 2002).

2.10 Selected plants growing along Nairobi River

Introduction of invasive species for phytoremediation purposes in Kenya may affect the local biodiversity. Therefore, identification and selection of locally available plant species for phytoremediation research and implementation is one of the challenges that need to be met and a pre-requisite for successful phytoremediation research.

Careful selection of plant and plant variety is critical, first, to ensure that the plant is appropriate for the climatic and soil conditions at the site, and second, for effectiveness of the phytoremediation of the pollutant at hand. Plant species that are long-term competitors and survivors under adverse changing conditions normally have an advantage.

Phytoremediation research studies have examined numerous plants, but interest has focused on a smaller group for reasons such as widespread distribution, ready availability, ease of growth, an existing large knowledge base, or even the plant's commodity value (Pivertz, 2001; Schnoor, 2002). Further studies in tropical countries like Kenya, will undoubtedly add many more candidates, as there is little information on potential locally available plant species that may be used for phytoremediation in Kenya.

2.10.1 *Amaranthus hybridus*

Amaranthus hybridus (*A. hybridus)* (Figure 2-1), commonly called smooth amaranth, smooth pigweed, red amaranth, or slim amaranth, is a species of annual flowering plant. Local name: “terere”. *A. hybridus* grows to 2m (6ft 7in). It is frost tender (Wikipedea). It is in flower from July to September. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind, self. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils. It prefers moist soil.

No members of this genus are known to be poisonous, but when grown on nitrogen-rich soils they are known to concentrate nitrates in the leaves. This is especially noticeable on land where chemical fertilizers are used. Nitrites are implicated in stomach cancers, blue babies and some other health problems. It is inadvisable, therefore, to eat this plant if it is grown inorganically.

Figure 2-1: ***Amaranthus hybridus***

2.10.2 *Eicchornia Crassipes*

Eicchornia Crassipes (Figure 2-2) is an erect, free-floating, stoloniferous, perennial herb that has proven to be a formidable weed in fresh water bodies’ through-out the world. Originating from the Amazon of South America, it has since been spread to lakes and rivers throughout the tropics and sub-tropics globally – even California and the South Eastern states of USA have proven susceptible to infestations by the weed (Center *et al*., 2002).

Initially, it was spread by unwitting floriculturists who sought to grow the weed in their domestic ponds because of the unique beauty of the hyacinth's flower. However, failure to strictly confine the weed to their ponds, coupled with the weed's uncanny ability to adapt in many aquatic environments, led to the endemic spread of the weed.

It is commonly known as water hyacinth. It is a fast growing, floating plant with a developed fibrous root system and large biomass. It adapts easily to various aquatic conditions and plays an important role in extracting and accumulating metals from water. Hence, water hyacinth is considered to be an ideal candidate for use in the rhizofiltration of toxic trace elements from a variety of water bodies.

Figure 2-2: ***Eicchornia Crassipes***

2.10.3 *Polygonum senegalensis*

Polygonum senegalensis (Figure 2-3) is a unique plant. There isn't much information available on this plant only that it belongs to the polygnum family.

Figure 2-3: ***Polygonum senegalensis***

2.11 Previous studies on phytoremediation

Several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water. These include sharp dock (*Polygonum amphibium* L.), duck weed (*Lemna minor* L.), water hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort (*Oenathe javanica* (BL) DC), calamus (*Lepironia articulate*) and pennywort (*Hydrocotyle umbellate* L.) (Prasad and Freitas, 2003).

Dos Santos and Lenzi, 2000 tested aquatic macrophyte (*Eiochhornia crassipes*) in the elimination of Pb from industrial effluents in a green-house study and found it useful for Pb removal. Wang *et al*., 2002 conducted a pot experiment to test five wetland plant species, that is, sharp dock, duckweed, water hyacinth, water dropwort and calamus for their possible use in remedying the polluted waters.

The results show that sharp dock was a good accumulator of N and P. Water hyacinth and duckweed strongly accumulated Cd with a concentration of 462 and 1420 mg/Kg, respectively. Water dropwort achieved the highest concentration of Hg, whereas the calamus accumulated Pb (512 mg/Kg) substantially in its roots. Other studies show that microorganisms, genetically modified organisms, bacteria and algae can also be used to remediate polluted sites.

2.12 Atomic Absorption Spectroscopy (AAS) instrumentation technique

This technique was introduced for analytical purpose by Walsh and Alleemade, under the designation Atomic absorption spectroscopy. Atomic absorption spectroscopy is a quantitative method of analysis. It is a technique for determining the concentration of a particular metal element in a sample. It is found to be superior to other technique as it can be used to determine 50-60 elements from trace to large quantities. These may include metals and non-metals.

2.12.1 Principle of AAS

Atomic absorption spectroscopy (AAS) occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analytes present in known standards can be used to determine unknown sample concentrations by measuring the amount of light they absorb.

The sample is first converted into an atomic vapor and then the absorption of atomic vapor is measured at a selected wavelength characteristic of atoms of each element. The amount of light absorbed is determined because the absorption is proportional to the concentration of the element.

2.12.2 AAS instrumentation

Performing atomic absorption spectroscopy requires a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be measured, a detector to measure the light accurately, and electronics to process the data signal and a data display or reporting system to show the results (Figure 2-4). The light source normally used is a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL).

In general, a different lamp is used for each element to be determined, although in some cases, a few elements may be combined in a multi-element lamp. In the past, photomultiplier tubes have been used as the detector. However, in most modern instruments, solid-state detectors are now used. Flow Injection Mercury Systems (FIMS) are specialized, easy-to-operate atomic absorption spectrometers for the determination of mercury. These instruments use a high-performance single-beam optical system with a low-pressure mercury lamp and solar-blind detector for maximum performance.

Whatever the system, the atom source used must produce free analyte atoms from the sample. The source of energy for free atom production is heat, most commonly in the form of an air/ acetylene or nitrous-oxide/acetylene flame. The sample is introduced as an aerosol into the flame by the sample-introduction system consisting of a nebulizer and spray chamber. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed.

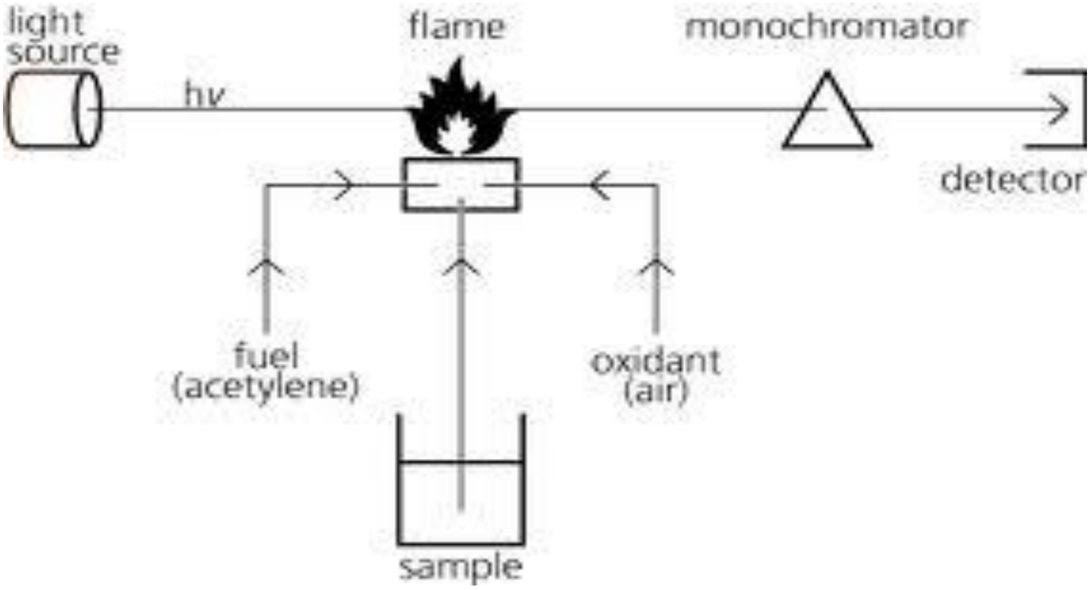

Figure 2-4: Components of AAS

2.13 Sample preparation procedures

Many analytical methods including atomic absorption spectrometry for trace element analysis requires decomposition of the sample (Roychowdhury *et al*., 2005). The two techniques used in this study are dry ashing at a defined temperature and wet digestion with mineral acid.

2.13.1 Dry Ashing

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600^oC. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates.

Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, for example, iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures.

There are a number of different types of crucible available for ashing food samples, including quartz, Pyrex, porcelain, steel and platinum. Selection of an appropriate crucible depends on the sample being analyzed and the furnace temperature used. The most widely used crucibles are made from porcelain because it is relatively inexpensive to purchase, can be used up to high temperatures (< 1200^oC) and are easy to clean.

Porcelain crucibles are resistant to acids but can be corroded by alkaline samples, and therefore different types of crucible should be used to analyze this type of sample. In addition, porcelain crucibles are prone to cracking if they experience rapid temperature changes. A number of dry ashing methods have been officially recognized for the determination of the ash content of various foods (Official Methods of Analysis AOAC). Typically, a sample is held at 500-600^oC for 24 hours.

2.13.2 Wet digestion

Wet digestion is primarily used in the preparation of samples for subsequent analysis of specific metals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents such as nitric, per-chloric and/or sulfuric acids, and then heated.

Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350°C. The resulting solution can then be analyzed for specific metals.

Advantages: Little loss of volatile minerals occurs because of the lower temperatures used, more rapid than dry ashing. Disadvantages: Labor intensive, requires a special fume-cupboard if per-chloric acid is used because of its hazardous nature, low sample throughput.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study area

Six sampling sites along the pollution gradient of Nairobi River were selected for this study based on the physical appearance of the water, land use patterns and economic activities. The sites were Kikuyu (site 1), Kawangware (site 2), Chiromo (site 3), Gikomba (site 4), Njiru (site 5) and Fourteen Falls (site 6) as shown in Figure 3-1.

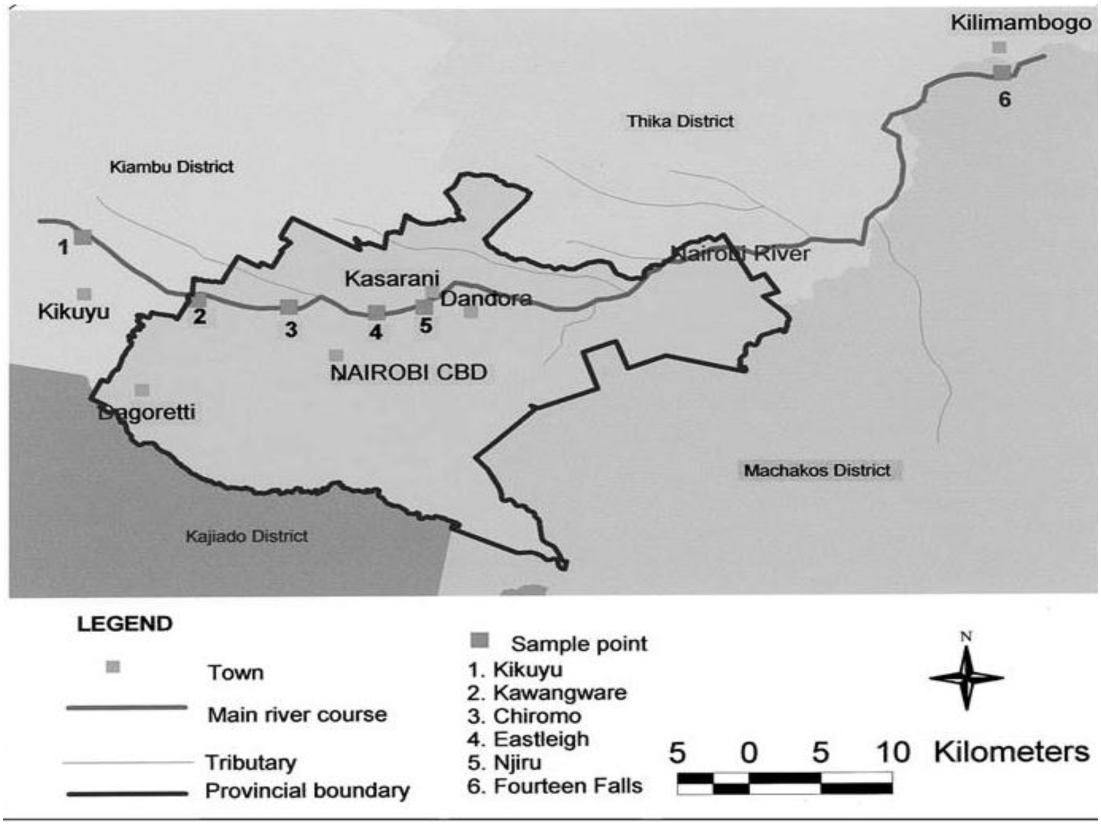

Figure 3-1: Sampling sites on map of Nairobi County

3.2 Sample collection

The sample collection design chosen was purpose sampling. Samples were collected in triplicate from six sampling sites along Nairobi River (Figure 3-1). The sample size, n, was 15.

The sites were purposely chosen considering relevance as point sources of pollution, this included sites at mid-point of the river, along feeder river discharge canals and the source to serve as a control. Samples were collected during the months of February and March 2012, the dry season only. Plant samples were sent to the National Museum of Kenya for identification in April, 2012.

3.2.1 Collection of plant species

Three native aquatic plants growing along the river were selected for estimating the concentration status induced by three heavy metals (Cu, Zn and Cd). The plant species were identified (Botany department, National Museum of Kenya) as *P. senegalensis*, *A. hybridus* and *E. crassipes* (Figure 2-3, 2-1 and 2-2 respectively).

The aquatic plants were collected by hand, washed with river water to remove sediment particles. The collected plant species were placed in plastic bags, labeled carefully and brought to the laboratory. Polythene tools were used in sampling and storing the collected matrices to avoid metal contamination.

3.2.2 Soil and water collection

Surface water and soil samples were collected, in triplicate, from six sites (Figure 3-1). Water samples were collected as grab samples in pre-cleaned containers in triplicates from all sites and treated with nitric acid (2%); these were stored in a cool box and transported to the laboratory. Soil samples, approximately 500g were scooped with a shovel at a maximum depth of 10 cm and homogenized before a laboratory sample was drawn. Samples were stored in polythene bags.

3.3 Sample preparation

3.3.1 Water Samples

Water samples were thoroughly mixed and aliquots of 50 ml taken in triplicates. These were acid digested with nitric acid until clear solutions were obtained; digests were filtered with Whatman No. 41 and stored in bottles.

3.3.2 Soil Samples

Soil samples were air dried, then crushed in a mortar and pestle and sieved through 2 mm governorates sieve to remove plant parts and debris. Well mixed samples of approximately 2g each were weighed using a digital analytical balance (model: Mettler Toledo) with an accuracy of 0.001g, were placed in 250 ml glass beakers and wet digested with nitric acid until clear solutions were realized.

The digests were filtered using Whatman filter paper no. 41 and then diluted to 100 ml with distilled water. The digests were stored in the refrigerator in pre-cleaned containers and analysis carried out within one week.

3.3.3 Plant Samples

Plant samples were dried in an oven (WTB Binder) at 105°C. Samples was divided into two. The first halves of aquatic plants species were crushed using pestle and motor. Second half aquatic plant species was sorted into different parts: roots, stems and leaves. The different parts were then crushed using pestle and mortar for further analysis. 2g each of the crushed samples were wet digested with aqua regia (HCl: HNO_3 - 3:1) and filtered through Whatman filter paper no. 41. The digests were then diluted to 100 ml with distilled water. The digests were stored in the refrigerator in pre-cleaned containers and analysis carried out within one week.

3.4 Materials

3.4.1 Apparatus

(a) Atomic absorption spectrophotometer - with an air-acetylene burner for flame determinations with deuterium lamp for background correction, Table 3-1, was used in this study.

Table 3-1: Instrumental parameters for flame determination

Element	**Flame**	**Wavelength, nm**
Cu	Air-acetylene, oxidising	324.8
Zn	Air-acetylene, oxidising	213.9
Cd	Air-acetylene, oxidising	217

(b) Hollow cathode lamp for Cu, Zn and Cd determination

(c) Hot plate– with heating control, to heat up to about 300°C.

(d) Furnace – to ash at 500°C

(e) Beakers– Pyrex, 250 ml

(f) Volumetric flasks– Pyrex, 25, 50 and 100 ml

All glassware were washed and rinsed with 10% HCl followed by distilled water to avoid metal contamination.

3.4.2 Reagents

All reagents were of analytical grade unless stated otherwise.

(a) Water- distilled with resistivity $\geq 18 M\Omega.cm$.

(b) Hydrochloric acid– 6M, diluted 500 ml HCl (37% w/w) with distilled water to 1L.

(c) Nitric acid – 65% w/w

(d) 0.1M nitric acid – Dilute 7 ml HNO_3, (c), with water, (a), to 1 L.

(e) Cadmium, zinc and copper standard solutions – 1000 mg/l stock solution for each element supplied by Sigma and Aldrich (USA).

(f) Working standard solutions – standards, (e), were diluted with 0.1M HNO_3, (d), to a range of standards that covers the linear range of the elements to be determined (Table 3-2).

3.5 Sample Analysis

Flame atomic absorption spectrophotometer (AAS - Shimadzu AA 7000) was used in the analysis. The concentrations of the metals were determined in triplicates. The accuracy and precision of the analytical procedure were determined. A series of standards were prepared for instrumental calibration by serial dilution of working solutions (100 mg/L) prepared from analytical grade stock solutions (1000 mg/L) from Sigma and Aldrich INC., USA.

For the elements (Cu, Zn, Cd) six standard solutions of different concentrations were prepared in 0.1M HNO_3 within linear concentration range (Table 3-2). The calibration curves were prepared for each of the metals investigated by least square fitting. Quality assurance was guaranteed through triple determinations and use of blanks for correction of background and other sources of error.

Table 3-2: Concentration range of calibrating standards

Metal	**Concentration of calibrating standards, mg/L**
Cu	0, 0.2, 0.4, 0.6, 0.8 and 1.0.
Zn	0, 0.2, 0.4, 0.6, 0.8 and 1.0.
Cd	0, 0.02, 0.04, 0.06, 0.08 and 0.1

3.6 Data Analysis

The concentrations of heavy metals in various matrices were presented as arithmetic mean with standard deviation (mean ± standard deviation). Statistical analyses were done at $p = 0.05$ (Miller and Miller, 1998). The results are presented in tables and graphs. Significant differences in heavy metal in water, sediment and plants were tested using the student t-test.

Pearson correlation coefficient analysis was done between metal-pairs in plants to check if differences exist between different metal combinations in either root, stem or leaf system. The products of the correlation coefficient (r) were evaluated (Norusis, 1993) as follows: 0–0.3: No correlation; 0.3–0.5: Low correlation; 0.5–0.7: Medium correlation; 0.7–0.9: High correlation; 0.9–1.0: Very high correlation. In all cases, the level of significance was set at $p<0.05$.

The comparison of the concentration of an element in an aquatic organism with that of the same element in the water in which the organism lives is the ratio between the concentration of the element in the organism and that in the soil, which is known as Bio-concentration factor (BCF) (Zayed *et al.*, 1998) and was evaluated as:

$$\mathrm{BCF} = \left(\frac{\mathrm{P}}{\mathrm{E}}\right)\mathrm{i} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Equation 1)}$$

Where i denote the heavy metal and BCF is the bio-concentration factor and is dimensionless. P represents the trace element concentration in plant tissues (μg/Kg dry weight); E represents the trace element concentration in the water (μg/L). A larger ratio implies better phyto-accumulation capability. When the BCF value is at equilibrium, the release rate of the element from the organism is equivalent to its intake rate, so that the element concentration in the organism is fairly constant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

Method validation was determined by replicate analysis, use of spikes and calibration curves. The difference between replicates was acceptable i.e. A-B ≤ 2 at 95% confidence level. Recovery obtained for spikes at 50ppb for Cu, Zn and Cd was 98%, 97% and 95%. The calibration curves were obtained as below:

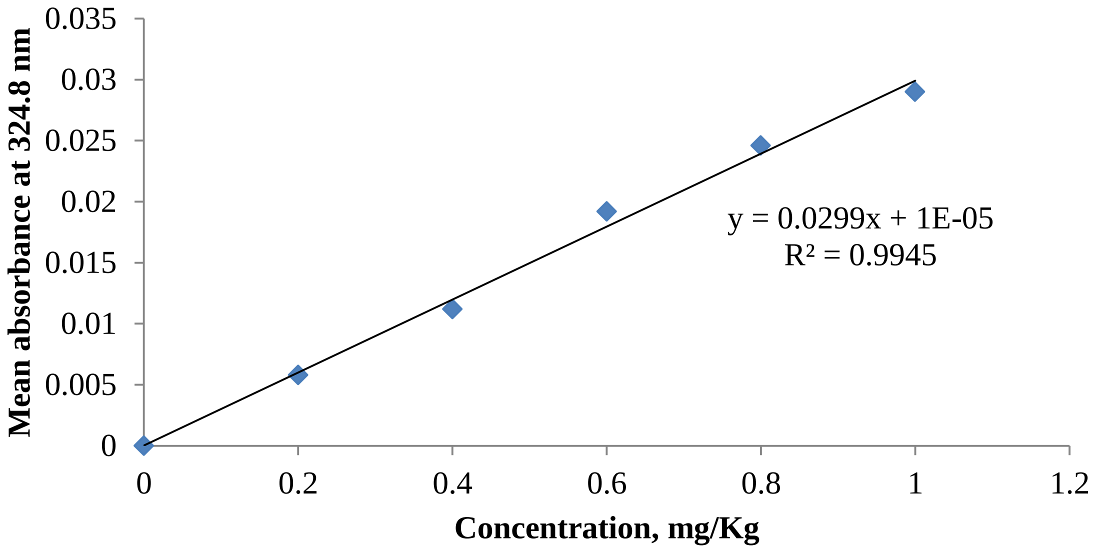

Figure 4-1: Copper calibration curve

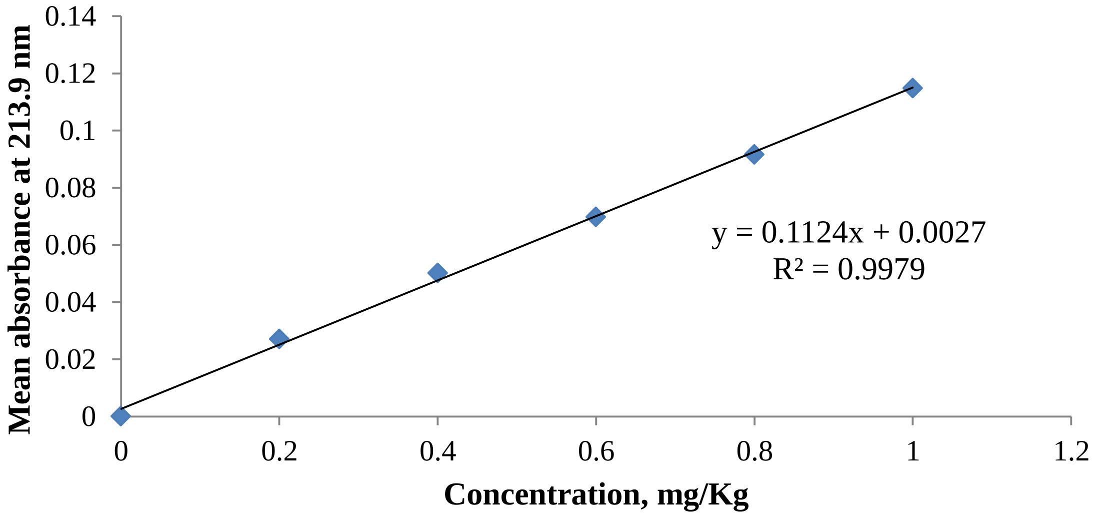

Figure 4-2: Zinc calibration curve

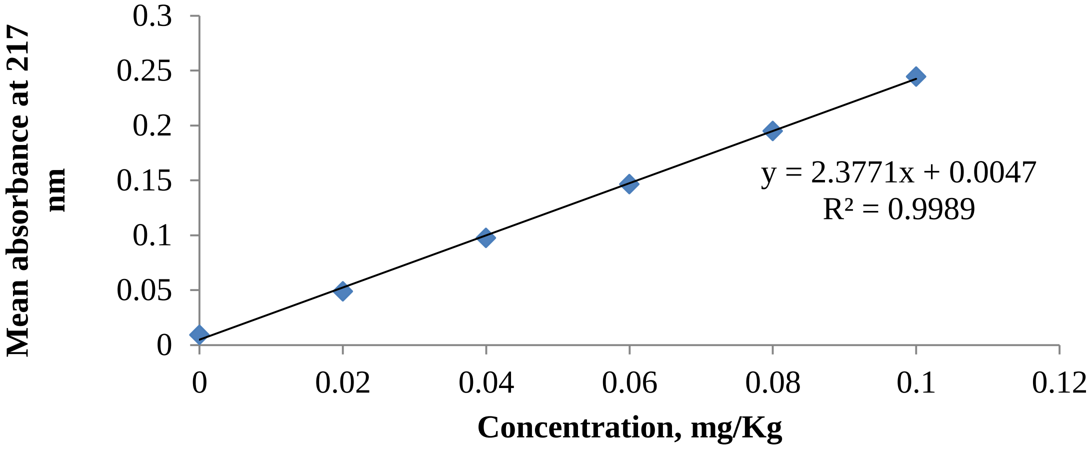

Figure 4-3: Cd calibration curve

4.1 Heavy metal concentration in water samples

The six sampling sites recorded varying concentration levels of the metals investigated (Table 4-1).

Table 4-1: Heavy metal concentration in water samples (µg/L)

Site	Cu	Zn	Cd
1	17.15 ± 0.5	30.58 ± 2.6	21.58 ± 0.8
2	18.35 ± 0.2	40.38 ± 3.8	25.78 ± 1.8
3	23.25 ± 0.8	39.54 ± 4.5	37.85 ± 4.5
4	28.65 ± 1.2	85.64 ± 5.6	40.35 ± 5.6
5	25.65 ± 0.3	80.54 ± 6.2	36.28 ± 2.5
6	25.65 ± 1.5	70.95 ± 4.8	28.69 ± 1.2
Mean±standard deviation, n=15 key: Cu- copper, Zn- zinc, Cd- cadmium			

Zinc was the most predominant followed by cadmium then copper. The mean concentration of Zn in water ranged from 85.64 ± 5.6µg/L in site 4 to 30.58 ± 2.6µg/L in site 1. This difference is statistically significant ($p<0.05$) and could be as a result of traffic pollution, effluent discharge or informal settlements along the two sites.

The highest concentration of copper was found in site 4 at 28.65 ± 1.2µg/L while the lowest concentration was found in site 1 at 17.15 ± 0.5µg/L. This difference is not significant ($p > 0.05$). The highest concentration of cadmium was found in site 4 at 40.35 ± 5.6µg/L while the least amount in site 1 (21.58 ± 0.8µg/L). This difference is statistically significant ($p < 0.05$).

The results show that site 1 is also polluted with Cu, Zn and Cd despite being source of the river, which was used as a control. This could be attributed to the presence of flower farms in the area. The pollution may be due to the intensive use of agrochemicals containing heavy metals as active ingredients or contaminants.

The three investigated heavy metals (Cu, Zn and Cd) were detected in measurable quantities in Nairobi River. The concentrations of these heavy metals recorded in the water column in this study are higher than those obtained from previous studies of Nairobi River. Budambula and Mwachiro, 2005 reported mean concentration of Cu, Zn and Fe as all below detectable limits. Kithia and Ongwenyi, 1997 reported mean concentration Cu and Zn as 0.1µg/L and 0.2µg/L respectively. This increase in heavy metal content (Cu, Zn, and Cd) may be due to increased vehicular traffic, industries close to Nairobi River some of which may empty their effluents into the river and informal settlements along the river bank.

The range of values of these metals (Cu = 17.15 ± 2.5 - 28.65 ± 4.3, Zn = 30.58 ± 5.5 - 85.64 ± 8.2, Cd = 21.58 ± 4.6 - 40.35 ± 3.2µg/L) in water from the six sites are lower than the World Health Organization (WHO) limits (Cu = 1, Zn = 5, Cd = 0.05 mg/L) for drinking water (WHO 2010).

4.2 Metal concentration in soil samples

The heavy metal contents in soil is presented in Table 4-2, indicating wide variations of heavy metals along Nairobi River. Similar to the water samples, zinc was found to be the most predominant of the heavy metals studied, followed by cadmium then copper. The highest amount of zinc was observed in site 4 (101.7 ± 6.2 µg/Kg), while the least amount was observed in site 1 (35.25 ± 2.6 µg/Kg). The high Zn content could be attributed to the sewage treatment plant or quarry activity in the area. Copper and cadmium levels were high in site 6 (32.01 ± 1.5 μg/Kg) and 4 (49.7 ± 2.5 μg/Kg) respectively.

Table 4-2: Heavy metal concentration in soil (μg/Kg)

Site	**Cu**	**Zn**	**Cd**
1	18.77 ± 0.5	35.25 ± 2.6	22.71 ± 0.8
2	20.35 ± 0.2	42.1 ± 3.8	28.33 ± 1.8
3	25.5 ± 0.8	44.5 ± 4.5	45.87 ± 4.5
4	31 ± 1.2	92.5 ± 5.6	48.6 ± 5.6
5	31.7 ± 0.3	101.7 ± 6.2	49.7 ± 2.5
6	32.01 ± 1.5	72.1 ± 4.8	31.02 ± 1.2
Mean ± Standard deviation, n = 15. Key: Cu- copper, Zn- zinc, Cd- cadmium			

The concentrations of heavy metals were higher in the soil samples than those calculated for the same heavy metals in the river water. Of the analyzed heavy metals, Zn was the most abundant in soil (101.7 ± 6.2 μg/Kg) and water (85.64 ± 5.6μg/L), followed by Cd with a concentration of 49.7 ± 2.5 μg/Kg in the soil and 40.35 ± 5.6μg/L in the water. Cu exhibited the receding trend in both soil and water. Cu varied significantly ($p<0.05$) between the following sites: site 2 and 5, site 1 and 2 and site 1 and 6, Cd: site 1 and 2 and site 4 and 1. This trend provides an understanding that the soil gets the heavy metals mainly from the water. The values of the ratio between element concentrations in the soil and those in the water were low (0.82-1.19, 0.99-1.39, 0.65-1.65) for Zn, Cd and Cu respectively (Table 4-3).

The mean concentrations in soil (Cu = 26.55 ± 0.6, Zn = 64.77 ± 1.5, Cd = 37.71 ± 0.8 μg/Kg) and water (Cu = 23.12 ± 0.4, Zn = 57.94 ± 0.9, Cd = 31.76 ± 0.5 μg/L) represent approximately 1.15 fold increase. This may be due to pre-concentration of the heavy metals in the soil and dilution effect in water due to water flow. This observation also agrees with the study of Oyewo, 1998 on Lagos Lagoon in Nigeria.

Table 4-3: Heavy metal concentration in soil, water and soil to water ratio

Metal	**Soil, µg/Kg**	**Water, µg/L**	**Soil/water**
Cu	26.55 ± 0.6	23.12 ± 0.4	0.65 – 1.65
Zn	64.77 ± 1.5	57.94 ± 0.9	0.82 – 1.42
Cd	37.71 ± 0.8	31.76 ± 0.5	0.99 – 1.39
Mean ± Standard deviation, n = 15. Key: Cu- copper, Zn- zinc, Cd- cadmium **Overall mean of all sites was used*			

Oyewo, 1998 reported that this observation is due to the fact that sediments acts as sinks of heavy metals derived from weathering as well as those from anthropogenic inputs. The biological significance of this observation is that flora and fauna especially benthic organisms which live on and forage on bottom soil will be exposed to greater risks of damage and or bioaccumulation.

4.3 Heavy Metal Concentration in *P. senegalensis*, *A. hybridus* and *E. crassipes*

Table 4-4 shows the mean values of Cu, Zn and Cd concentration in *A. hybridus, P. senegalensis*,and *E. Crassipes*. The mean concentration values of the elements in the plants decrease according to this sequence: Zn > Cu > Cd. The highest mean concentration value was recorded in *P. senegalensis* (11.14±0.48 µg/Kg), followed by *A. hybridus* (9.6 ± 0.32 µg/Kg), and *E. crassipes* (8.6 ± 0.012 µg/Kg). Among all studied metals, the lowest Cd content was recorded in water hyacinth (2.15 ± 0.01 µg/Kg), while the greatest amount of Zn was observed in *P. senegalensis* (21.79 ± 0.4 µg/Kg).

Table 4-4: Heavy metal concentration in *P. senegalensis, A, hybridus* and *E crassipes* (µg/Kg)

Element/Taxon	**Cu**	**Zn**	**Cd**
P. senegalensis	8.73 ± 0.08	21.79 ± 0.4	2.91 ± 0.01
A. hybridus	7.27 ± 0.05	17.42 ± 0.2	4.11 ± 0.03
E. crassipes	7.23 ± 0.05	16.32 ± 0.1	2.15 ± 0.01
Mean ± Standard deviation, n = 15. Key: Cu- copper, Zn- zinc, Cd- cadmium			

The concentration of individual metal varies from species to species. The content of Zn were 21.79 ± 0.4 µg/Kgin *P. senegalensis* to 17.42 ± 0.2 µg/Kg in *A. hybridus* and 16.32 ± 0.1 µg/Kg in *E. crassipes*. The Cd content was found lowest in *E. crassipes* (2.15 µg/Kg), followed by 2.91 µg/Kg in *P. senegalensis* and highest in *A. hybridus* (4.11 ± 0.03 µg/Kg). On the other hand, *P. senegalensis* showed higher concentrations of Cu (8.73 ± 0.08 µg/Kg), while equal concentration of the same was recorded in *A. hybridus* (7.27 ± 0.05 µg/Kg) and *E. crassipes* (7.23 ± 0.05 µg/Kg).

Table 4-5 gives the toxicity status of *A. hybridus*, *P. senegalenisis* and *E. crassipes*. The concentration of Cu, Zn and Cd are within the normal range found in plants (Kabata-Pendias and Pendias, 1992).

Table 4-5: Toxicity status of measured plants

Metal	**Mean range in tested plants (µg/Kg)**	**Normal range in plants (mg/Kg)***	**Critical range in plants (mg/Kg)***	**Toxicity status**
Cu	7.59-8.73	7.53-8.44	25-90	Normal
Zn	16.32-21.79	1-4	100-400	Normal
Cd	2.15-4.11	0.1-2.4	10-30	Normal

*Data from Kabata-Pendias and Pendias, 1992

4.4 Heavy metal concentration in roots, stem and leaves of *P. senegalensis,E. crassipes* and *A.hybridus.*

The heavy metal content in roots, stems and leaves of *P. senegalensis, A. hybridus* and *E. crassipes* is presented in table 4-6. All the species studied had high amounts of Zn in the roots; 36.3±3.2, 32±2.8 and 27.6±2.5 µg/Kg in *P. senegalensis, A. hybridus* and *E. crassipes* respectively.

Roots of aquatic plants absorb heavy metals from the sediments and accumulate high concentrations (Baldantoni *et al.*, 2004). Similarly, the findings in this study revealed the high accumulation of heavy metals registered in roots of *P. senegalensis* and *A. hybridus*.

The mean concentration of heavy metal of stems of three aquatic plants varies from species to species. The higher values of Zn and Cu were observed in *P. senegalensis* and *A. hybridus*, respectively, compared to Cd. On the other hand, stems of *E. crassipes* showed high amount of Zn, Cu and Cd in a recessive manner. The accumulation of heavy metals in leaves of three native aquatic plants is exhibited by high accumulation of Zn, and Cu in all the plants, while a high accumulation of Cd in *A. hybridus* (1.41 ± 0.03 mg/Kg).

Table 4-6: Heavy metal concentration in different plant part (µg/Kg)

Plant	**Element**	**Roots**	**Stems**	**leaves**
1	Cu	14.5±2.1	7±0.6	4.7±0.4
	Zn	36.3±3.2	19.8±1.7	9.3±0.4
	Cd	5.4±05	2.1±0.1	1.2±0.1
2	Cu	12.7±1.5	6.7±0.5	6.8±0.5
	Zn	32±2.8	14±2	6.3±03
	Cd	5.6±0.6	5.3±0.4	1.6±0.1
3	Cu	12.3±1.5	5.4±04	3.9±0.2
	Zn	27.6±2.5	14.3±2	7.2±03
	Cd	5.2±0.3	0.7±0.05	0.5±0.02

Mean ± Standard deviation, n = 15. Key: Cu- copper, Zn- zinc, Cd- cadmium
1-P. senegalensis, 2- *A. hybridus,* 3- *E. crassipes*

The order of the accumulation of heavy metals in various parts of aquatic species:

1. Root heavy metal accumulators: *P. senegalensis, A. hybridus,* and *E. crassipes*.
2. Stem heavy metal accumulators: *P. senegalensis, A. hybridus,* and *E. crassipes*.
3. Leaves heavy metal accumulators: *P. senegalensis, E. crassipes, and A. hybridus.*

The stems and/or leaves of submerged plants accumulated lower concentrations of trace elements than roots, which is well substantiated with the findings of Baldantoni *et al.*, 2005. Thus, among the selected plant species, *P. senegalensis* and *A. hybridus* appear to be good monitoring species due to their bio-accumulation capabilities and availability along Nairobi River.

4.5 Bio-concentration of heavy metals (Cu, Zn and Cd) in the native plants

The ability of the plants to take up heavy metals (BCF) was evaluated from the ratio of metal concentration in the plants and water (Equation 1). Table 4-7 gives mean values of Bio Concentration Factor (BCF.) for each species and element.

The mean BCF value of the elements in the plants decreases according to this sequence: Zn > Cu > Cd. This sequence (which is rather different from that of the mean concentrations of elements in the plants) reflects the capacity of the plant species to accumulate elements independently from their concentration in the water, that is, the regulation capacity of the plants.

Mean concentration factors for the various elements calculated are higher for *P. senegalensis* (0.28 ± 0.01), followed by *A. hybridus* (0.25 ± 0.01), then *E. crassipes* (0.22 ± 0.01).

Table 4-7: Bio-concentration factor (BCF)

Matrix	**Plant**	**Cu**	**Zn**	**Cd**	**Mean**
Water(µg/L)	Mean	23.12±2.5	57.94±5.1	31.76±3.0	
Plant (µg/Kg)	1	8.73 ± 0.08	21.79 ± 0.4	2.91 ± 0.01	
	2	7.27 ± 0.05	17.42 ± 0.2	4.11 ± 0.03	
	3	7.23 ± 0.05	16.32 ± 0.1	2.15 ± 0.01	
BCF	1	038±0.02	0.38±0.02	0.09±0.01	0.28±0.01
	2	0.31±0.01	0.30±0.01	0.12±0.01	0.25±0.01
	3	0.31±0.01	0.28±0.02	0.07±0.005	0.22±0.01
1-*P. senegalensis* 2- *A. hybridus* 3- *E.crassipes* , mean of the six sites					

Of all metals, the lowest BCF value of Cd was observed in *E. crassipes* (0.07 ± 0.005), while the highest value of Zn was recorded in *P. senegalensis* (0.38 ± 0.02). Low BCF of Cd was recorded in water hyacinth (0.07 ± 0.005), while highest concentration of the same were registered in *A. hybridus* (0.12± 0.01), no significant difference (p>0.05).

On the other hand, minimum BCF of Cu was registered in *E. crassipes* (0.31 ± 0.01) and *A. hybridus* (0.31±0.01), while high amount of the same was observed in *P. senegalensis* (0.38 ± 0.02), no significant difference (p>0.05).

The BCF of Zn is 0.38 ± 0.02, 0.30±0.01 and 0.28±0.02 in *P. senegalensis*, *A. hybridus* and *E. crassipes* respectively.

The accumulation of heavy metals by various species in descending order is as follows:

Zn – *P.* senegalensis >*E. crassipes* >*A. hybridus*.
Cu – *P. senegalensis* > *E. crassipes* > *A. hybridus*.
Cd – *P. senegalensis* > *A. hybridus* >*E. crassipes*.

4.6 Gradient of Heavy Metals in Root, Stem and Leaf System

The output of Pearson correlation coefficient (r) analysis on combinations of different metal-pairs which are present together in either roots, stems or leaves of the tested plant species showed high correlation (r = 0.7-0.9) between only a single metal pair (Cu and Zn) and medium correlation (r = 0.5-0.7) between Cd and Zn, Cu and Cd metal pairs (Table 4-8).

Table 4-8: Correlation coefficients

Plant species	**Analysis metal pair**	**Correlation coefficient, r**		
		Root system	**Stem system**	**Leaf system**
1	Cu x Zn**	0.546	0.918	0.015
	Cu x Cd*	0.627	0.418	0.598
	Zn x Cd*	0.619	0.684	0.04
2	Cu x Zn**	-0.312	0.838	-0.312
	Cu x Cd	0.114	0.352	0.211
	Zn x Cd	-0.531	0.049	-0.479
3	Cu x Zn**	-0.189	0.832	-0.275
	Cu x Cd	0.168	-0.511	-0.575

	Zn x Cd	-0.187	-0.411	0.057

*medium correlation (0.5-0.7), **high correlation (0.7-0.9) 1- *P. senegalensis*, 2-*A. hybridus* 3- *E. crassipes*

Thus, results indicate that both roots and stem systems may have a kind of natural controlling mechanism regarding the quantity of specific metals taken from the ambient environment, but they don't have controlling mechanism to suppress the combination between specific metal pairs in their tissues/components (Ravera *et al.*, 2003).

Study revealed the transport mechanism of metals from abiotic environment (soil) to biotic environment (macrophytes) and their accumulation in various parts. The translocation mechanism and accumulation pattern of heavy metals can be elaborated as follows: Soil > Root system >Stem system > Leaf system (Figure 4-4).

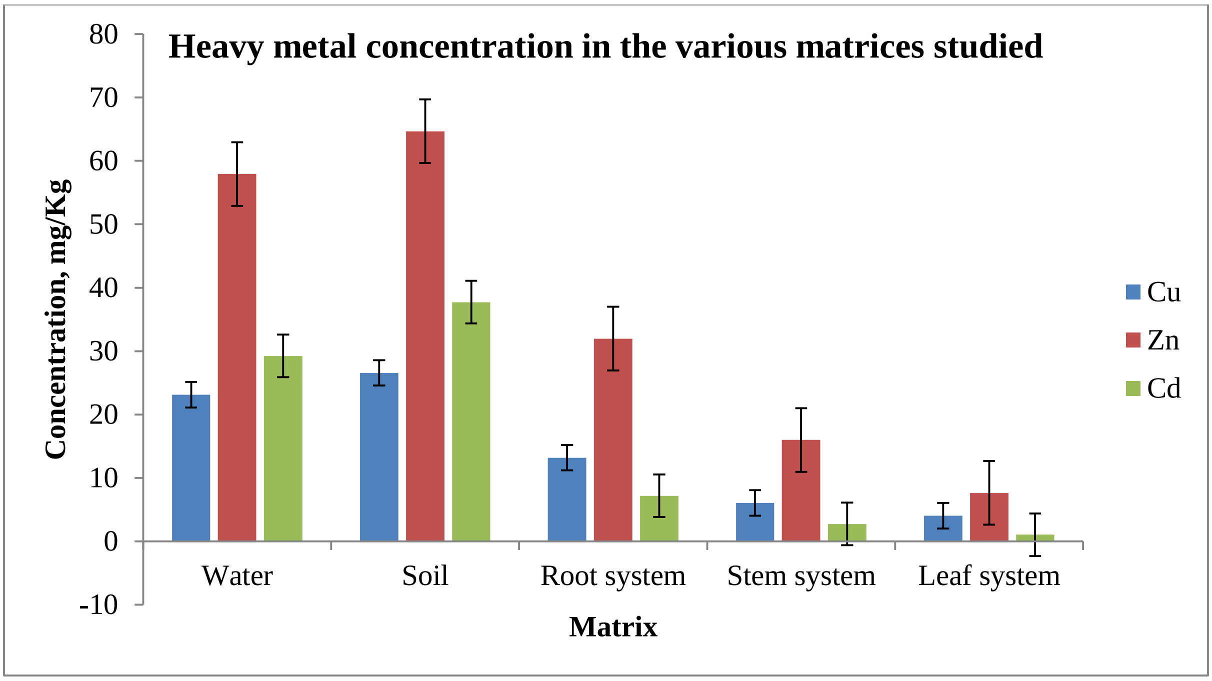

Figure 4-4: Mean concentration of heavy metals in the various matrices studied

The accumulation of heavy metals (in sediments and plant components) could be arranged in descending order as follows: Zn > Cu > Cd, which was based on the degree of toxicity status induced heavy metals in plants as well as sediments: Zn > Cu > Cd. The above sequence agrees with the findings of Ramdan, 2003 and Siegel *et al.*, 1994 in Lake Manzala, Nile Delta, Egypt.

The ratio of heavy metals was two times higher in the sediments than in selected plants. This also agrees with findings of Ramdan, 2003. Accordingly, the component systems of aquatic plants in Nairobi River could be arranged in the following order based on their accumulation capacity of heavy metals as: Soil > Root system > Stem system > Leaf system. Similarly, Lovett-Doust *et al.*, 1994 reported that the accumulation levels of pollutants in aquatic ecosystems might be higher in soil than in plants.

The high level of Zn and Cu, an important source of contamination in the river, might be due to agricultural run-off on sediments in the reservoir, carrying various Zn and Cu-based pesticides used in agricultural practices. This largely agrees with findings of Jones *et al.*, 1991 in Lake Averno, and Siegel *et al.*,1994 in Ginka sub-basin, south of Lake Manzala.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECCOMENATIONS

5.1 Conclusions

This study investigated the potential of *P. senegalensis*, *A. hybridus* and *E. crassipes* to accumulate Cu, Zn and Cd. The uptake and accumulation varied among the selected plants. The three species studied accumulated Cu, Zn and Cd to a certain degree; BCF was found to be 0.28±0.01 for *P. senegalensis*, 0.25±0.01and 0.22±0.01 for *A. hybridus* and *E. crassipes* respectively.

The metals concentration in soil and water of the measured area (Nairobi River) did not exceed the limited values by World Health Organization for agricultural soils and drinking water respectively. The mean heavy metal concentration in water samples collected from the six sites was 23.1±2.5, 57.9±5.1 and 31.76±3.0 µg/L for Cu, Zn and Cd respectively. In the digested soil samples metals concentration was 26.6±2.6, 64.7±5.8 and 37.7±3.1 µg/Kg for Cu, Zn and Cd respectively.

Mean contents of metals (Cu, Zn and Cd) in the studied plants 11.1±1.2, 9.6±0.8 and 8.6±0.5 µg/Kg for *P. senegalensis*, *A. hybridus* and *E. crassipes* respectively. The results show that the plants removed some of the metals from the water and soil. The results also confirm findings of work cited in literature that pants have the ability to uptake heavy metals from contaminated sites. This could be as a result of pre-concentration of the metals.

The research hypothesis "The concentration of Cu, Zn and Cd in water and soils are not significantly different from those found in *P. senegalensis*, *A. hybridus* and *E. crassipes*" is accepted. *P. senegalensis*, *A. hybridus* and *E. crassipes* have the capability to uptake Cu, Zn and Cd from contaminated water, such as, Nairobi River.

5.2 Recommendations

This research indicates that there is considerable uptake of Cu, Zn and Cd by *P. senegalensis*, *A. hybridus* and *E. crassipes*. The recommendations of this study are:

1. More metals should be studied, as this research focused on three (Cu, Zn and Cd) only.
2. Factors affecting plant growth and metal uptake should be considered before introducing plants for phytoremediation
3. Studies in controlled conditions, such as, pot experiments which focuses on optimizing agronomic management practices
4. Carry out genetic engineering and mutation breeding to modify plants' characteristics. This can enhance utilization of plants for phytoremediation, hence environmental sustainability.
5. Laboratory experiments should be carried out to check the performance of plants in a controlled environment.

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