REHABILITATION AND PHYTOREMEDIATION OF HEAVY METAL POLLUTED RIVERINE WETLANDS USING BAMBOO FOR PHYTOEXTRACTION IN KIBERA, KENYA

BY
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DECLARATIONS

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

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DEDICATION

In everything I do, how and when I do it; my greatest obligation has been and will be Praising You, Almighty God. I plan to achieve success as if I will never die, but as I pray I do and seek to do pray as if I will die the next minute. I humbly dedicate this script to You.

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ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
Ksp	Solubility Constant
MPA	Multi Purpose Analyzer
OSHA	Occupational Safety and Health Administration
USEPA	United States Environmental Protection Agency

ABSTRACT

Urban and peri urban unplanned settlements have mushroomed widely in Kenya today. Examples of settlements that are now congested with domestic wastes open draining into wetlands include Kibera (Africa's largest slum), Mathare and Dandora slums both, which drain their wastes into Nairobi River and its tributaries that pass through them. Nairobi River has through academic research, government and industrial partnerships attracted activities that seek to foster transformation from polluted to sustainable clean water. The river is a natural receptacle for possible heavy metal pollutants from domestic and industrial activities. Heavy metals are highly toxic when they fall beyond accepted standards. Their effects are evidenced as tetratogenic, mutagenic and others being carcinogenic. This is a great concern of whose subsequent research gives birth to bioremediation using plant species. Phytoremediation is a more cost-effective, more environmentally friendly, and more aesthetically pleasing method for polluted water purification and filtration. Plants also offer a permanent, *in situ*, no intrusive, self-sustaining method of soil and water contaminant removal. Potential danger might exist for animals and humans who live in the areas in which phytoremediators are grown, especially if they typically feed on the species of plant being used for phytoremediation. Rehabilitating the wetlands with an alternative that reinstates the purification and filtration capacity, and that is not consumed as food, is therefore urgently needed. A promising alternative is the bamboo that could serve to uptake heavy metals from polluted aquatic ecosystems. In this research, Zn Cu, Cd and Pb concentrations were investigated in soils, water and plants along Kibera riverine. Four species of bamboo and other plants investigated were grown on potted polluted soils from Motoine riverine, and the levels of Cd, Cu, Zn and Pb determined at regular intervals. Data was analysed by Genstat package for natural sciences for regression analysis and ANOVA. Soils were found to contain Pb in the range of 60.01±3.35 to 69.75±7.19 mg/kg, Cd in the range of 1.819±0.19 to 2.151±0.059 mg/kg, Zn from the range 34.76±6.27 to 38.47±9.4 mg/kg and Zn in the range of 875.84±215 to 1035.25±67.07 mg/kg. Results on soils further showed that the heavy metal levels reduced significantly after the growing plants on contaminated soils. For the vegetation comparative study herein, Bamboo plants showed trends that could serve to absorb Cd, Pb, Zn, and Cu. Bamboo absorption therefore translates to 6.66-16.65 kg of Zn, 7.29-18.225 kg of Cu, 1.08-2.7 kg of Pb, 39-97.5 g of Cd per ha/year. It will therefore be appropriate to argue that building up bamboo systems along Ngong/Motoine River in Kibera slums as a purifying model, substituting them for cultivated edible vegetation in slums currently around wetlands is highly encouraged.

INTRODUCTION

1 .1 Background

Sewage and domestic wastewater from open drains, use of fertilizers in agriculture, oil discarded from garages, as well as "jua kali" mechanics contribute to pollution of urban wetlands (UNEP, 2001). Examples of settlements that are now congested with domestic wastes draining into wetlands include Kibera slums, which drains its wastes to Motoine river. The Ngong/Motoine river is a tributary of the larger Nairobi river basin and joins it at Dandora slums. Dumping of solid waste is pronounced at bridges and crossing points (see Plate 1.1)

Plate 1.1 Dumping waste in parts of Ngong river tributary in Kibera slums which later joins Nairobi river at Dandora slums

Heavy metals may find their way into Motoine river through the above-mentioned drainage. When heavy metal polluted river water is directly taken in by fauna and flora (except for resistant and persistent ecotypes) and humans it causes adverse effects. Although toxicity of these heavy metals depends on their solubilities, stability and physico-

chemical form at the site of action, most of them are potentially toxic with some being carcinogenic, mutagenic while others are teratogenic (Istvan and Benton, 1997). The populations along the Motoine river have planted crops watered by this polluted water (Pascale, 1996). Among the foods grown are kale, sugarcane, arrowroots and napier grass for livestock as shown in plate1.2.

Plate 1.2 Napier grass, sugarcane and arrowroots grown and watered by a polluted river in Nairobi

These crops inevitably find their way into urban homes as the public generally buy the affordable and healthy looking produce (plate1.3) mostly from urban farmers, unaware of the potential dangers posed through the uptake of heavy metals. Therefore, there is need to assess the levels of the heavy metals in water, soils and crops grown in Motoine river system while at the same time looking for ways of rehabilitating this riverine wetland system.

Plate 1.3: Children tendering maize and sukumawiki (kale) along Motoine river

1 .2 Problem statement and justification of the study

Over the past two decades, Nairobi city has undergone rapid urbanization associated with industrial development and growth of human settlements and infrastructure. Examples of settlements that are now congested with domestic wastes in open drainage include Kibera (Africa's largest slum), Mathare and Dandora slums all, which drain their wastes into rivers that pass through them. A direct consequence of this is the heavy pollution of this river basin. This river is a source of water for domestic use by many city residents as well as the rural residents downstream. Domestic sewage systems, industrial effluents and agricultural activities are the main sources of water pollution. Wastes from city streets, motor vehicle garages and old rubber tyres near the river are other sources of pollution. All these and many other human activities along the river basin contribute to serious pollution of the

river. Many human diseases result from the build up of toxic pollutants in soil, wetlands and vegetation supported by the wetlands, making remediation of these areas crucial in the protection of human health (Istvan and Benton, 1997).

Rehabilitating these wetlands with an alternative plant species that reinstates the purification and filtration capacity and not consumed as food can be of economic importance to the farmers (UNIDO, 2004). Planted bamboo has shown potentials of serving as the crucial dual function of purification (through absorption of heavy metals, nitrogen and phosphorus) and filtration of polluted water and produce biomass suitable for a range of industrial, domestic and artisanal uses (UNIDO, 2004).

Bamboo is the strongest, versatile and fastest growing woody plant on earth, with some species achieving the phenomenal growth rate of one metre per day (Chin, 2005). For instance, *Arundinaria alpina*, the species native to Kenya, can yield up to 20,000 12 m-high culms per hectare per year. Its poles known as culms are the strongest, lightest natural material known to man. In South East Asia, is used to reinforce concrete and for scaffolding on skyscrapers. Bamboo performs remarkable environmental services (Chin, 2005). As an ecological plant, bamboo has several advantages. It prevents soil erosion and absorbs pollutants.

This study sought to investigate the levels of heavy metals along Kibera riverine tributary of Nairobi river and to establish the ability of bamboo to accumulate heavy metals.

1 .3 Research questions

This research therefore sought to answer the following questions.

i. Is Ngong/Motoine riverine system polluted by heavy metals?

ii. Do crops grown along the riverine accumulate heavy metals to unacceptable levels?

iii. Could bamboo serve to accumulate high levels and therefore act as an alternative to cleaning up these heavy metals?

1 .4 Hypotheses

i. Motoine river water and riverine soils are highly polluted by heavy metals

ii. Edible vegetation grown along and across wetland areas are highly polluted

iii. Growing of bamboo plants is effective for bioremediation

1 .5 Objectives

1.5.1 General objective

The main objective of this study was to quantify the levels of heavy metal pollution in riverine wetlands and assess bamboo potential for eco-sanitation of these riverine wetlands.

1.5.2 Specific objectives

The specific objectives were to:

i) Quantify the levels of Cd, Cu, Pb and Zn in water, plants and soils along the Ngong riverine

ii) Determine successive accumulation of Cd, Cu, Pb and Zn in the roots, stems and leaves of bamboo and other plants grown in pots containing contaminated soils.

iii) Determine the amount of Cd, Cu, Pb and Zn in the soils in pots after growing bamboo.

Chapter 2

LITERATURE REVIEW

2 .1 Introduction to heavy metals

Heavy metals are a group of elements between copper and lead on the periodic table of the elements, having relative atomic weights between 63.546 and 200.590 and specific gravities greater than 4.0 (Hawkes, 1997). Living organisms require trace amounts of some heavy metals, including cobalt, copper, manganese, molybdenum, vanadium, strontium, and zinc, but excessive levels can be detrimental to the organism (Istvan and Benton,1997). A good example is the 1998-2004 Southern Spain reserve episode where seven million tonnes of toxic sludge killed everything in the path of River Guadimar. Nothing survived in the mixture that contained lead, copper, zinc, cadmium and other metals (Short, 1999).

2 .2 Lead

This is a soft, weak, ductile, dull grey metal. It dissolves in nitric acid and tarnishes in moist air but stable to oxygen and water. It has 207.2 g atomic weight, exists commonly as Pb^{2+} and has lithosphere abundance of 14 mg/kg (Istvan and Benton, 1997). Lead in tetra methyl lead (IV) is used as a gasoline additive that increases octane rating. Another large application of lead is battery production and manufacture of lead–based paint. It is also used in mining and smelting operations and water pipe manufacture (ATDSR, 1999).

Industrial, domestic, transport, as well as natural sources are major means of lead entering into wetlands. It is one of the most difficult contaminants to remove from the soil and

water, as well as one of the most dangerous metal (Julia *et al.*, 2000). Use of leaded gasoline has contributed to high lead levels in waters (USEPA, 1999).

2.2.1 Lead exposure

According to the WHO air quality guidelines, the limit is given as 0.5 $\mu g/m^3$ of Pb (ATSDR, 1999). The Food and Drug Administration gives a guideline maximum as 0.005 mg Pb^{2+}/L for bottled drinking water (ATSDR, 1999). Uncontaminated soil contains lead concentrations of less than 50 ppm but in many urban areas in USA have soil lead levels generally exceeding 200 ppm (AAP, 1993). The EPA has gives 40 mg/kg for soil lead in residential soils as a guideline value that would be protective to public health (USEPA, 1999).

The presence of lead in the environment can have devastating effects on plant growth and can result in retarded growth (Lasat, 2000). Root vegetables uptake lead from the soil with additional atmospheric deposition into leafy vegetables (Mushak *et al.*, 1989). Both humans and livestock are exposed to toxic levels of lead through inhalation of particulate matter in the air as well as direct ingestion of contaminated food, water, or dust (Lasat, 2000). Almost all inhaled lead is absorbed into the body while 20-70% of ingested lead is absorbed. It is observed that children absorb more lead than adults do (ATDSR, 1999). It contaminates food and alcohol during production, processing or packaging (ATSDR, 1999). Thus, it is found in everyone's body; most people have lead levels that are orders of magnitude greater than that of ancient times (Flegal and Smith, 1992; 1995) and within an order of magnitude of levels that have resulted in adverse health effects (Budd *et al.*, 1998).

It is both carcinogenic and teratogenic. It adversely affects young children (2 to 3 years of age), affecting their intellectual development (Istvan and Benton, 1997). Lead is known to be a potent inhibitor of many enzymes working in the brain, thus possibly inducing functional problems in the brain under pathophysiological conditions (Garry ans Stephen, 2000). According to WHO, children with blood lead concentrations between 12 µg/dL and 120 µg/dL can suffer from lower IQ, shorter attention span, reading or learning disabilities, hyperactivity, impaired physical growth, learning and visual problems and impaired motor skills (Hwang *et al.*, 2002). At blood concentrations above 70 µg/dL, risk of encephalopathy, a neurological disorder, is higher and treatment is required (Blacksmith, 2004). It has recently been reported that high lead levels in blood may be associated with high blood pressure (USEPA, 2006). Inhibition of brain protein kinase C (PKC) subtypes by lead has been reported (Hwang *et al.*, 2002). The PKC is an important enzyme in mediating cellular signal transduction and neuronal plasticity (Hwang *et al.*, 2002).

2 .3 Cadmium

Cadmium is a silvery metal that tarnishes in air, is soluble in acids but not in bases, with 112.40 g atomic weight, and exists commonly as Cd^{2+}. It has lithosphere abundance of 0.18 mg/kg (Istvan and Benton, 1997). Cadmium hydroxide is a principal electrode in NiCd batteries that are frequently applied in railroad and aircraft industries (Morrow and Keating, 1997).

Cadmium sulphide and cadmium sulphoselenide are pigments used in plastics, ceramics, glasses and enamel. They withstand high temperature without chalking (Cook, 1994).

Cadmium coatings offer corrosion resistance especially in salt and alkaline media. They too exhibit excellent plating characteristics on varied substances, have good galvanic comparability with aluminium and are readily solderable (Morrow, 1996). Cadmium telluride and cadmium sulphide are applicable in photovoltaic cells (Cadmium Association and Council, 1991).

Naturally occurring cadmium concentration ranges from 0.1 ppm to 0.5 ppm (Cadmium Council, 1995). The levels may be higher in sedimentary rocks, marine phosphates and phosphorites; reported to be having levels as high as 500 ppm (Cook, 1994; WHO, 1992). Weathering, erosion and volcanic activity are other natural sources of cadmium emission to the atmosphere (WHO, 1992; OECD, 1994; Niragu, 1980; 1989). Anthropogenic sources of cadmium emissions, in an ascending order of importance, arise from Municipal solid waste incineration, non-ferrous metals production, iron and steel production and combustion of fossil fuel (Cook, 1994; Jackson and Macgillivray, 1993; Jones *et al.*, 1993;). Trace levels of cadmium may be available in naturally occurring wastes from grass, food and soil (Chandler, 1996). During the growth of grains such as wheat and rice, cadmium (from the soil) is concentrated in the core of the kernel (Whitaker, 2005).

2.3.3 Cadmium exposure

Ambient air is estimated to have cadmium at 0.1-5 ng/m^3 in rural areas, 2-15 ng/m^3 in industrialized areas (Elinder, 1985; WHO, 1992; OECD, 1994). Occupational cadmium exposure that assures no adverse human effects are now limited to 2-50 µg/m^3 (ILO, 1991; ACGIH, 1996; OSHA, 1992). Cadmium in major river systems has decreased significantly over the years especially since the 1960s and 1970s (Cook, 1994; Elgersma *et al.*, 1992;

Mukoniki and Fujimoto, 1996). River systems with excess cadmium can contaminate surrounding waters through flooding, irrigation and dumping of dredged sediments. Rivers can transport cadmium for up to 50 km from the source (WHO, 1992).

The major factors governing cadmium speciation, adsorption and distribution in soils are pH, soil organic matter and other metal ions (OECD, 1994). Cadmium solubility increases with decrease in soil pH. When exposed to high levels of cadmium in the rooting environment, some root crops (turnips) and leafy vegetables (for example, spinach) contain sufficient cadmium to pose a potential health hazard upon their consumption (Istvan and Benton, 1997). The effect of cadmium varies with species and other elements with both synergistic and antagonistic effects (Istvan and Benton, 1997).

Daily dietary intake of Cd is estimated at the range of between 0.007 to 30 mg, toxic intake at the range of between 30 to 330 mg and the lethal intake in the range of 1.5 to 9.0 g. The total mass of the element in an average person (70 kg) is 50 mg. Liquid intake of 16,000 µg/L can cause severe gastrointestinal symptoms (Istvan and Benton, 1997).

Though cadmium has no known useful biological functions in humans, it competes with zinc for binding sites and can therefore interfere with some of zinc's essential functions. In this way, it may inhibit enzyme reactions and utilization of nutrients. Cadmium may be a catalyst to oxidation reactions, which can generate free-radical tissue damage. Chronic cadmium toxicity to human sperm of heavy cigarette smokers has been reported (Zenzes, 2000).

Estimates have it that 98% of ingested cadmium comes from terrestrial foods, 1% from aquatic foods such as fish and shellfish, and 1% from cadmium in drinking water (Gleba *et al*, 1993). For exposure by ingestion, the principal effects are gastrointestinal disturbances such as nausea, vomiting, abdominal cramps and diarrhoea. Acute poisoning by inhalation may lead to respiratory manifestations such as severe bronchial and pulmonary irritation, sub-acute pneumonitis, lung emphysema and death from pulmonary oedema (Lauwerys, 1998). Chronic cadmium exposure affects mainly kidneys, lungs and bones. Kidney proteinuria, condition characterised by the presence of low molecular weight proteins in urine, has been associated with chronic cadmium exposure (WHO, 1992; OECD, 1994).

2 .4 Zinc

Zinc is a bluish-white metal, brittle when cast; tarnishes in air and reacts with acids and alkalis. It has 65.39 g atomic weight and exists commonly as Zn^{2+} (Istvan and Benton, 1997). A large proportion of all zinc, perhaps more than a third, galvanizes metals such as iron to prevent corrosion. Zinc metal finds use in dry batteries, roof cladding and protection of iron structures from corrosion by attaching zinc as sacrificial anodes. Zinc oxide is widely used in the manufacture of paints, rubber products, cosmetics, pharmaceuticals, floor coverings, plastics, printing inks, soap, textiles, electrical equipment and other products. Zinc sulphide has an application in making luminous dials, paints, X-ray and TV screens (http://www.webelements.com/Zn/uses, accessed 2006).

As an over-the-counter ointment, it is applied as a thin coating on the exposed skin of the face or nose to prevent dehydration of the area of skin. It can protect against sunburn in the

summer and windburn in the winter. Applied thinly to a baby's diaper area (perineum) with each diaper change, it can protect against rash. As determined in the age-related eye disease studies, it is part of an effective treatment for age-related muscular degeneration in some cases (http://fixedreference.org/2006-Wikipedia-CD-Selection/wp/z/Zinc.htm, accessed 2006).

Zinc on the roofs and gutters of buildings accounts for about 50% of the zinc emissions into the surface water in some areas including canals and rivers, which cause water and sediment pollution (Gouman, 2004). Zinc emissions to air mainly originate from industrial production activities (67.2 %) and traffic (25.2 %). Municipal waste (MW) incineration accounts for 2.4 % (Gouman, 2004). Total zinc emission contribution of incineration and landfill to the overall zinc emissions to the water compartment is only 0.2 % (Gouman, 2004).. Effluents from sewage treatment plants (41.3 %) are for this compartment the most important source (Gouman, 2004).

2.4.3 Zinc exposure and human health

Excessive absorption of zinc can suppress copper and iron absorption while on the other hand free zinc ion in solution is toxic to plants, invertebrates, and even vertebrate fish (Stowe *et al.*, 1978). Zinc toxicity, mostly in the form of the ingestion of US pennies minted after 1982, is commonly fatal in dogs where it causes a severe haemolytic anaemia (Stowe *et al.*, 1978). Zinc phosphide (Zn_3P_2) is a rodenticide used to control a variety of small mammal species (Robert *et al.*, 2005).

Daily dietary intake of Zn is between 5 to 40 mg, toxic intake is 150 to 600 mg, lethal intake is 6 g and total mass of the element in an average person (70 kg) is 2.3 g (Istvan and Benton, 1997). In many types of aquatic plants and animals, growth, survival, and reproduction can all be adversely affected by elevated zinc levels (Eisler, 1993). Zinc is toxic to plants at elevated levels, causing adverse effects on growth, survival, and reproduction (Eisler, 1993). Elevated zinc can cause a wide range of problems in mammals including cardiovascular, developmental, immunological, liver and kidney problems, neurological, haematological, pancreatic and reproductive disturbances (Eisler, 1993; Domingo, 1994).

2 .5 Copper

Copper is a brown metal, malleable and ductile with high electro thermal conductivities, fairly unreactive with air and water but slowly weathers to green form a carbonate (Istvan and Benton, 1997). It is a micronutrient and toxin. It strongly adsorbs to organic matter, carbonates and clay, which reduces its bioavailability. It is immobile in soils and can be precipitated easily and interacts readily with both organic and inorganic substances with widely varying solubilities depending on pH (Istvan and Benton, 1997).

Plant diseases amenable to control by copper fungicides include *Helminthosporiosis* in bananas, *Anthracnose* in French beans, avocado and pawpaw among others (http://www.copper.org, 2006). Pentahydrated copper sulphate is a mordant for dyeing and for electroplating, but today it is being employed in many industrial processes (http://www.copper.org, 2006). Copper is used for electrical equipment, in fashioning metal

products such as pipe, tubing, and other plumbing fixtures, hardware, and machine tool products. Copper use in the transportation industry, refrigeration equipment is reported (Encyclopaedia Britannica, 2006).

2.5.1 Copper exposure and animal health

Research findings suggest that chronic occupational exposure to manganese or copper, individually, or to dual combinations of lead, iron and copper, is associated with Parkinson's disease (Kumar, 2003). In man, toxic intake is $\geq$ 250 mg, and total mass of the element in an average (70 kg) person is 73 mg, while normal intake is 2 to 5 mg/day (Istvan and Benton, 1997). Toxic effects in birds include reduced growth rates, lowered egg production, and developmental abnormalities (Vymazal, 1995). While mammals are not as sensitive to copper toxicity as aquatic organisms, toxicity in mammals includes a wide range of effects such as liver cirrhosis, necrosis in kidneys and the brain, gastrointestinal distress, lesions, low blood pressure, and fetal mortality (Kabata-Pendias and Pendias, 1992; Vymazal, 1995).

2 .6 Urban agriculture in Kibera informal settlements

Kibera, with a population of about 800 000, is the largest informal settlement in Nairobi and is located not far from the city centre (UNEP, 2001). Most of the mud and wattle dwellings are located along a wide slope of the Motoine River and Nairobi Dam. Plate 2.5 shows aerial view of congestion of houses in Kibera slums (ICRAF-Nairobi, 2006).

Plate 2.5: Aerial photograph of Kibera slums, Nairobi

2 .7 Phytoremediation

The term Phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) refers to plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Cunningham *et al.*, 1997; Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Rai and Amit, 1999).Plants possess some characteristic features that enable them to

absorb, from soil and water, heavy metals that are essential for their growth and development. These metals include iron, manganese, copper, molybdenum and nickel. Plants also accumulate toxic metals that may not have any biological function; these include silver, cadmium, chromium, cobalt, mercury, lead, and selenium (Rai and Amit, 1999).

Worldwide, phytoremediation is being embraced as a noble way of heavy metal removal. Biochemists are working toward a day when plants and trees will replace earthmovers and landfills in cleaning contaminated industrial sites (Rai and Amit, 1999). Plants, especially, the grass family have been used to absorb heavy metals from contaminated soils (Melcer, 2004). In Zimbabwe, *Cynodon nlemfuensis* (star grass) was the main grass planted on the wastewater irrigated pasturelands (Madyiwa *et al.*, 2002). Most recently, researchers have used seaweeds to remove more than 95 per cent of metals such as zinc and cadmium from water coming out of redundant metal mines in Wales (RSC, 2006).

Phytoremediation essentially involves phytoextraction in which metal-accumulating plants are used to transport and concentrate metals from the soil into the harvestable roots and aboveground shoots and rhizofiltration in which plant roots absorb, precipitate and concentrate toxic metals from polluted effluents (Kumar *et al.*, 1995).

2.8 The bamboo

Bamboo is the strongest and fastest growing woody plant on earth, with some species achieving the phenomenal growth rate of one metre per day (Chin, 2005). Its poles known

as culms are the strongest, lightest natural material known to man. In South East Asia, bamboo is used to reinforce concrete and for scaffolding on skyscrapers. Bamboo performs remarkable environmental services. It prevents soil erosion and absorbs pollutants (Chin, 2005). With this in mind, the World Agro-forestry Centre, formerly the International Centre for Research in Agroforesry (ICRAF) researchers, in conjunction with Kenya Forestry Research Institute and Jomo Kenyatta University of Agriculture and Technology have joined forces in pursuit of unlocking bamboo potential available in Kenya (Kigomo, 1995), for native and exotic bamboo species. For effluent treatment work and value addition activities under this project, three species have been identified, namely, green bamboo (*Bambusa vulgaris*), black bamboo (*Dendrocalamus asper*) and giant bamboo (*Dendrocalamus gingateus*). Plate 2.8 shows bamboo fence in an outdoor setting.

Plate 2.8: Natural green bamboo fence in outdoor setting

2.9 Techniques of heavy metal analysis

Several methods have been used for heavy metal analysis. These methods include Atomic Absorption Spectrometry (Abulude, 2005; Sandra *et al.,* 2005), Inductively Coupled Plasma Mass Spectrometry and electron dispersive X-ray fluorescence spectrometry (Dean *et al.*,1986). In this study, the Atomic Absorption Spectrometry was used due to availability, convenience, and samples verified by the inductively coupled plasma mass spectrometry.

2.9.1 Atomic absorption spectrophotometer (AAS)

This is generally a mono element technique works as follows. The hollow cathode lamp emits radiation characteristic of the cathode material, usually single element (analyte). This beam, consisting largely of resonance radiation is electronically or mechanically pulsed. Analyte atoms are produced thermally in the atom reservoir. Ground state atoms, which predominate under experimental conditions, absorb resonance radiation from the lamp, reducing the intensity of incident beam. Monochromator isolates the desired resonance line and this radiation falls on the photomultiplier. Signals are processed hence electronic output proportional to the absorption by the analyte atoms (Dean *et al.*, 1986).

Chapter 3

3.0 MATERIALS AND METHODS

3 .1 Description of the study area

The Motoine river basin is located west of Nairobi city passes through Kibera, an informal human settlement. It extends from Dagoretti area in the west towards Nairobi Dam to the east. Small scale agriculture where the plants are cultivated along the riverbeds is a common feature. Kale, sugarcane, arrowroots are planted for human consumption while napier grass is grown for animal feed. Effluents from domestic waste, sewage sludge and small scale industries such as motor vehicle garages find their way into the river system. Figure 3.1 below shows the location of the study area.

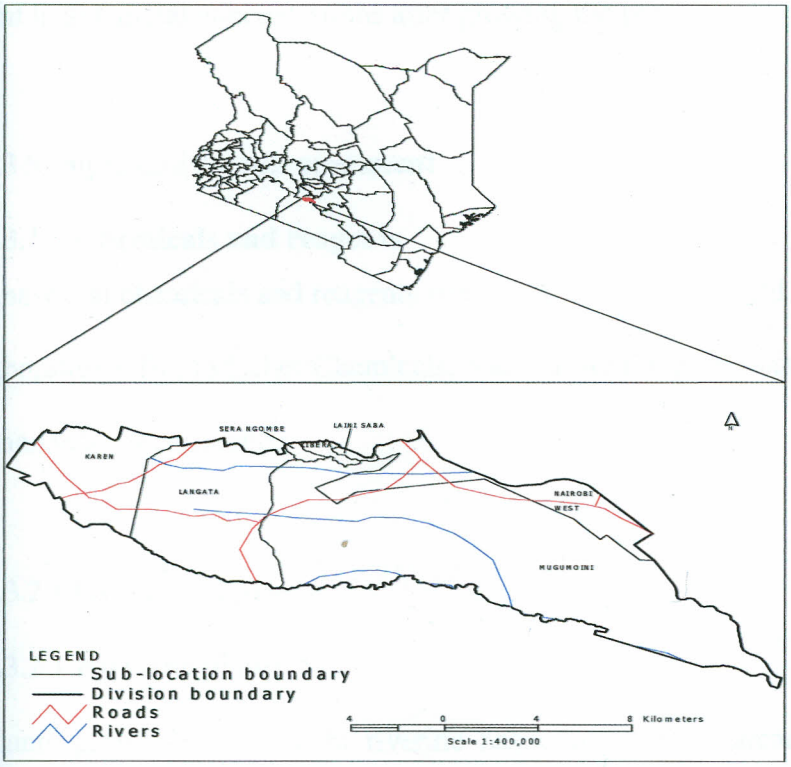

Fig 3.1: Location of Kibera riverine

3.2 Research design

A three-level design was adopted for analysis of samples for Zn, Cu, Cd and Pb.

Level 1: River water, soils and plants were sampled twice, during the dry and wet seasons. Sugarcane, napier grass, kale and arrowroots were all analysed for Zn, Cu, Pb and Cd.

Level 2: A controlled study involved the plant species above and four bamboo species. Sugarcane, napier grass, kale, arrowroots and 4 four bamboo specises namely,green bamboo, yellow bamboo, giant bamboo and water bamboo were partitioned and analysed for Zn, Cu, Pb and Cd.

Level 3: Quantify the heavy metals in water. Analyse, using two analytical techniques heavy metal contents in the soils. Quantify the levels of heavy metals in edible and non-edible parts of riverine vegetation. Check the absorption of heavy metals for the pot plants and the levels of soil heavy metal concentrations after growing the plants.

3.3 Sample and sample treatment

3.3.1 Chemicals and reagents

Analytical chemicals and reagents were obtained by the World Agro forestry Centre (ICRAF) laboratories from Fischer Chemicals, Nairobi. Analar grade salts were used to prepare standards for calibration.

3.3.2 Riverine samples

3.3.2.1 Riverine plants

Plants grown in the Kibera riverine basin included sugarcane, arrowroots, kale and napier grass. The four plants were harvested from five different sampling points at an approximate distance of 500 m apart along the Motoine tributary of Nairobi river, during both seasons; dry and rainy seasons. The plants were then packed in polythene bags and transported in cooler

boxes to the laboratory. In the laboratory, the plants were washed to remove any surface deposits. The plants were carefully separated into edible and non-edible parts and each part dried in an oven at 65°C for 4 days and ground to powder separately. The powdered samples were packed in well-labelled self-sealing PVC papers and then stored at 4°C.

3.3.2.2 Riverine water

Water samples were transferred into well cleaned and rinsed plastic bottles from the five sampling sites where the plants were grown at a random depth and acidified with 0.1 % HNO_3 acid to keep metal ions in solution. The samples were packed in clean plastic bottles then stored in laboratory at 4^0C.

3.3.2.3 Riverine soil samples

Soils were collected from the five different plant sampling points. A soil auger was used to scoop soil from a depth of 0-30 cm. The soil samples were transferred into cooler boxes and stored at 4°C overnight. The stored soil was dried at 160°C for four days to remove moisture and then ground to pass a 2 mm sieve. The ground soil was packed in well-labelled self -ealing PVC papers and then stored at 4°C.

3.3.3 Controlled study experiment

Soils from Kibera riverine farms were transferred into 46 plastic pots each measuring 40 cm in diameter and 60 cm high. The soil was loaded up to the 50 cm height of each pot, giving a soil volume of 78.6 litres. Four species of bamboo plant seedlings were obtained from Gigiri tree nurseries and planted in the pots. Napier grass and sugarcane from a farm in Kahawa were harvested by cutting them at a height of 5 cm from the ground and transported in wet bags then

planted in the pots. Arrowroot and kale were planted in pots from tubers and seeds, respectively; these were obtained from a seedling establishment in Nairobi. The plants were watered with equal volumes of tap water throughout their period of establishment.One sample of each was taken for analysis for Pb, Cu, Zn and Cd.

Bamboo plants, sugarcane, arrowroots, kale and napier grass grown, were harvested at three months, six months and seven months of their age. These pot plants were harvested at the aforementioned different stages and separated into various parts. The roots/ rhizome, stem/tuber and leaves were then cleaned by washing them under a tap followed by several distilled water rinses. The plant samples were oven dried at 67 ^{0}C for six days. The plant parts were ground to powder,passed through a 2 mm sieve and stored at 4^{0}C.

The soil samples were collected before growth of the plants at a depth of 0-30 cm in the various pots. Sampling using an auger was done before planting and after 8 months and packed in clean self-sealing polythene bags. The samples were then dried, ground and sieved through 2 mm sieve then stored.

3.4 Laboratory Procedures

3.4.1 Preparation of standards

Standards were prepared from commercially–supplied stock solutions of 1000 mg Cu, Cd, Pb or Zn/L. Intermediate stock standard solutions (100 mg Cu, Cd, Pb or Zn/L were prepared by pipetting 10 mL of the appropriate 1000 mg/L stock into labelled 100 mL flasks and make to volume with 0.5 M HCl. Mixed standards containing appropriate concentrations of all four metals were prepared in 250 mL volumetric flasks. An Eppendorf Multipette with appropriate

sizes of combitips was used to pipette the correct volumes of 100 mg/L intermediate stock standards.

3.4.2 Sample preparation

3.4.2.1 Plant sample digestion

One-gram portions of dry plant material was weighed in crucibles and covered. The plant materials were then ashed at 550°C for at least four hours or overnight in a muffle furnace. The ash was cooled to room temperature. The digestate was treated with 1 ml of de-ionised water. The digested wet ash was treated with 1 ml concentrated HNO_3 and heated to dryness at 80-100°C. The ash was then cooled and extracted using 5 ml of 1% nitric acid from each crucible and transferred into 60-ml plastic bottles. Five-fold dilutions were made and the resulting solution was ready for analysis.

One-gram portions of dried and ground plant material were wet digested in 10-ml portions of nitric acid according to procedures outlined by Jones *et al.* (1990). The mixtures were boiled on a hot plate at 140°C for 4 hours with occasional swirling. A reagent blank was run for each 10 samples. The mixtures were cooled, and carefully transferred to a 100-ml flasks. Two millilitres of H_2O_2 was added to the mixture and then homogenised. The digestate volume was adjusted to 50 ml with 0.1 % HNO_3 acid.

3.4.2.2 Soil sample digestion

One-gram samples of dried soil were placed in a digestion tube. Twenty millilitres of concentrated nitric acid was added to each sample and the mixture boiled to 140 °C. The mixture temperature was maintained at 140 °C for 2 hours to remove fumes of HNO_3. The mixture was cooled to room temperature and then 10 ml of hydrogen peroxide added. Again

the mixture was heated to 140 °C for one hour. The resulting mixture was cooled and then filtered through a quantitatively acid-washed filter paper into a 50-ml volumetric flask and the digestion tube rinsed with 0.1 % HNO_3 to volume.

3.4.3 Cleaning of apparatus

Glassware and porcelain crucibles were soaked in chromic acid overnight and then washed in dilute nitric acid. They were rinsed using de-ionised water followed by drying at 105^0C in an oven for 2 hours and then stored in clean dustproof drawers under lock and key.

3.5 Instrumentation

Table 3.1: The AAS measurement parameters

Metal	Zinc	Copper	Cadmium	Lead
Analytical line/wavelength	636.2 nm	324.75 nm	228.8 nm	217 nm
Support gas	Air- rate of flow 15 L/min	Air- rate of flow 15 L/min	Air- rate of flow 15 L/min	Air- rate of flow 15 L/min
Fuel gas	C_2H_2 - rate of flow 2.2 L/min	C_2H_2 flow rate 2.2 L/min	C_2H_2 - rate of flow 2.2 L/min	C_2H_2 - rate of flow 2.2 L/min
Flame type	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Lamp current	Low- 10mA High- 400 mA	Low- 10mA High- 500 mA	Low- 8 mA High- 100 mA	Low- 10 mA High- 300 mA
Slit width	0.7 nm	0.7 nm	0.7 nm	0.7 nm
Burner height	7 mm	7 mm	7 mm	7 mm

3.6 Sample analysis

The atomic absorption spectrophotometer was set up with working parameters as shown in Table 3.1. Warm-up time for the flame and lamp was at least 15 minutes, to allow lamp output and burner temperature to stabilise. For validation of results, some samples were sent to UK for analysis with ICP-MS. For calibration, standard solutions were prepared and run under the same conditions as the samples. The results of the standard solutions were used to prepare calibration curves, which was used to determine the concentrations of the digested samples. After each 10 samples, one of the preceding 10 samples was re-analyzed, and one standard solution analyzed. In this way, repeatability of the determination and stability of standard readings was assessed. The blank value was subtracted from instrument readings to obtain corrected solution concentration. The plant tissue metal (Pm) concentration (mg/kg) was given by:

$$\mathbf{Pm\ (mg/kg) = (Pm_C - Pm_B)\ (25)\ (D_F)/Pm_{wt}}$$

Where Pm_C = Sample concentration of metal (mg/L)

Pm_B = Blank concentration of metal (mg/L)

25 = Volume of dissolved ash solution

D_F = Dilution factor

Pm_{wt} = Weight of plant

3.7 Soil particle size analysis (SPSA) by hydrometer method

Fifty grams of air-dry soil, sieved to pass a 2 mm sieve, was weighed into a 400-ml beaker. With very sandy soils, 100 g was used. A 125 ml aliquot of double de-ionised water was added then placed in a hot water bath at 85 to 90 °C and stirred gently while adding 5 ml 30% hydrogen peroxide. The mixture was stirred gently and 1 or more drops of amyl alcohol was added to minimize foaming. A further 5-ml portion of hydrogen peroxide was added until frothing ceased, indicating complete destruction of organic matter. Exactly 10 ml of 10% sodium hexametaphosphate solution was added to each sample and the mixture was allowed to settle for 10 minutes. The resulting mixture in a mixer cup was stirred for two minutes with the high- speed stirrer. Quantitative transfer of the suspension into a 1000 ml measuring cylinder, using distilled water to wash all soil particles into the cylinder was done to the 1000-ml mark with distilled water. The sealed cylinder was inverted 10 times and 2 to 3 drops of amyl alcohol added to the cylinder. After 20 seconds, the hydrometer was gently placed into the suspension. At 40 seconds, and 2 hours hydrometer readings and the temperature readings of the suspension were taken. The corrected hydrometer readings were as given here under:

Corrected hydrometer reading at 40 seconds (PSH40COR) =

(PSH40SAM - PSH40BLK) + [(PST40 - 20) 0.36];

Corrected hydrometer reading at 2 hours (PSH2HCOR) =

(PSH2HS - PSH2HB) + [(PST2H - 20) 0.36];

Where PSH40S = Hydrometer reading at 40 seconds for sample

PSH40B = Hydrometer reading at 40 seconds for blank

PST40 = Temperature at 40 seconds

PSH2HS = Hydrometer reading at 2 hours for sample

PSH2HB = Hydrometer reading at 2 hours for blank

PST2H = Temperature at 2 hours

Percent clay in soil samples is given by:

Percent clay = (PSH2HCOR) 100/PSSLWT

Where PSSLWT = Weight of air-dry soil (g)

Percent sand in soil samples is given by:

Percent sand =100 - [(PSH40COR) 100]/PSSLWT

Percent silt in soil samples is given by:

Percent silt =100 - Sand – Clay

3.8 Soil pH in water

A pH meter calibrated with pH 4 buffer pH 7 buffer was used to read pH values. This method used a soil:water ratio of 1:2.5. Ten-mL soil portions were added into a 60 mL bottle. 25 mL distilled water was added into the bottle with dispenser, stirred for 10 minutes and let to stand for 20 minutes. The the electrode immersed into solution mixture ans readings taken.

3.9 Data analysis

The normality of the analyzed data was checked by statistical t-test, and mean scores using GENSTAT software checked the homogeneity of variances. Using the same software, differences between riverine plant and bamboo bioaccumulations with respect to mean concentrations of elements in soil and the different times were evaluated by analysis of

variance. Correlation coefficients were calculated to examine the relationships between the concentrations of elements in soil and bamboo plants.

4.0 RESULTS AND DISCUSSIONS

4.1 Introduction

Riverine water and wetland soil from Motoine river in Kibera were analysed using atomic absorption spectrometry for Pb, Cd, Zn and Cu. Riverine vegetation including kales, sugarcane and napier grass were also analysed for the same metals. The metal accumulations in bamboo species and other plants grown in pots with soils from Motoine rive were investigated. The results were validated using inductively coupled plasma mass spectrometry. This chapter contains the results together with discussions of the same.

4.2 The concentrations of heavy metals in Motoine river

4.2.1 Concentrations of Pb, Cd, Cu and Zn in Motoine river water

Motoine river water was mainly grey in colour with many floating waste particles. In general, the pH along the river was in the range of 8.10±0.5. Water from five different sites was analysed for Pb, Cd, Zn and Cu and the results obtained are presented in Table 4.1.

Table 4.1: Concentration of heavy metals at different sites along Motoine river (n=5)

Site	Heavy metal concentration in mg/L			
	Pb	Cd	Cu	Zn
Nairobi dam	*0.0870±0.005	0.009±.0.0005	0.0151±.0.002	0.0079±.0.002
Siranga	*0.06700±0.005	0.007±.0.0005	0.0151±.0.002	0.0075±.0.002
Soweto	0.06000±0.005	0.004±.0.0005	0.0149±.0.002	0.0074±.0.002
Lindi	0.06000±0.005	0.001±.0.0005	0.0145±.0.002	0.0074±.0.002
Gatwekera	0.0520±0.005	0.001±.0.0005	0.0133±.0.002	0.0062±.0.002

* Pb levels beyond internationally accepted standards

From table 4.1, the concentration of the metals increases downstream from Gatwekera to Nairobi dam. There were no significant differences in the metal concentrations in all the sites ($P>0.05$, DF 4, ANOVA). From table 4.1, it can also be observed that Pb concentrations in Nairobi dam and Siranga were above the maximum recommended levels for irrigation. This indicates that the water from the two sites is not suitable for agricultural use as per the Swiss Ordinance guidelines given in table 4.2.

Table 4.2: International tolerable standards of heavy metal concentrations for water

Guideline	Heavy metal concentration in mg/L			
	Pb	Cd	Cu	Zn
Swiss Ordinance on irrigation water	0.065	0.01	0.17	2
USEPA, 2001	0.2	0.03	0.7	0.5

Source: Swiss Ordinance on irrigation water, 2004

4.2.2 Lead, Cd, Cu and Zn contents of riverine soils

Mean total heavy metal levels and SD for soils from selected sites along Kibera riverine shows high levels of the heavy metals as shown in figure 4.1.

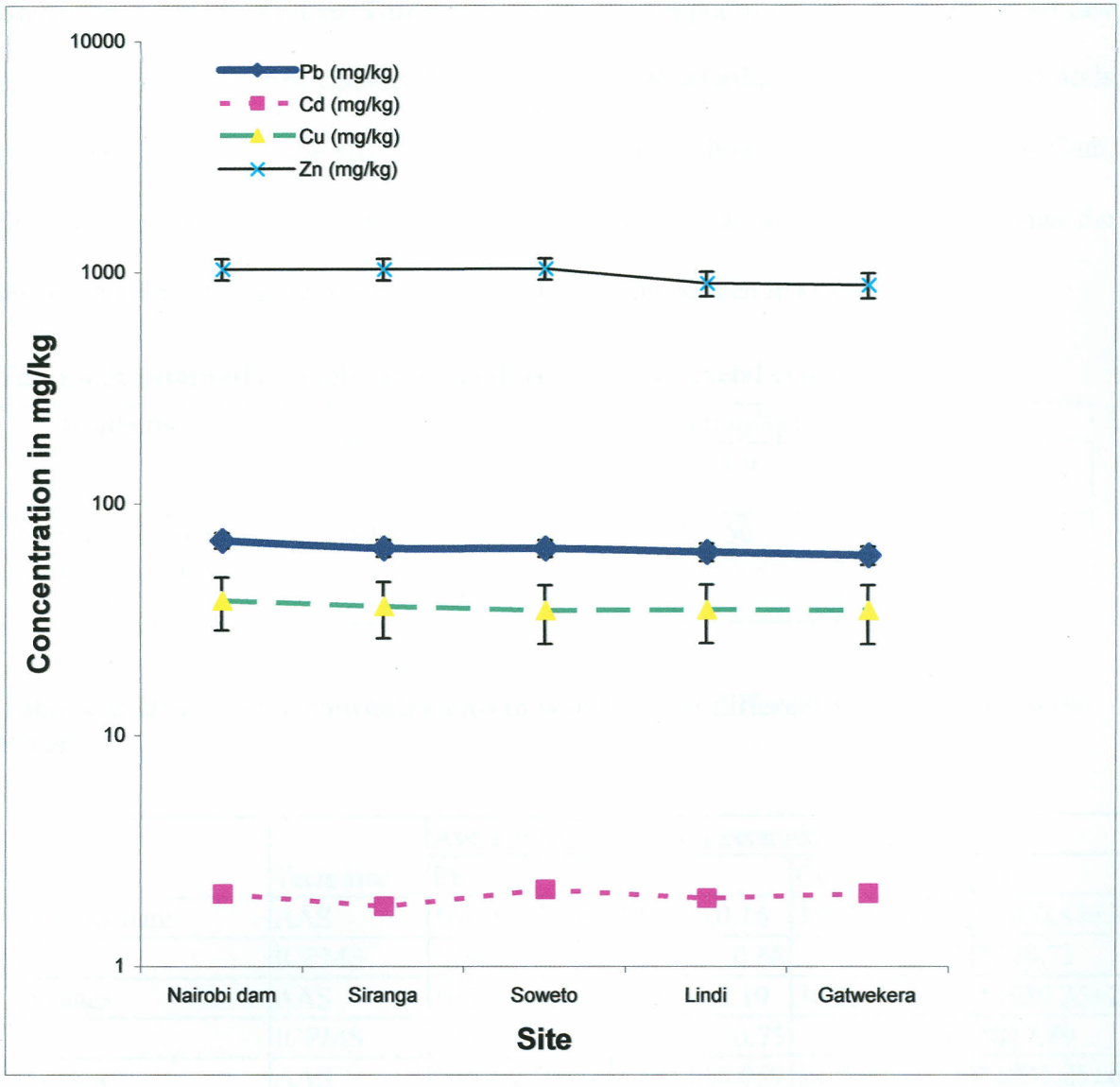

Fig. 4.1: Total heavy metal concentrations in soils at various sites along Motoine river

The total heavy metal contents in the riverine soils varied from one site to another as can be seen in Figure 4.1. The total Pb content ranged from 60.01±3.35 to 69.75±7.19 mg/kg, Zn ranged from 875.84±215 to 1032.48±3.8 mg/kg while Cu ranged from 34.78±10.07 mg/kg to 38.47±9.4 mg/kg at Gatwekera and Nairobi Dam respectively. Nairobi Dam had the highest concentration of Pb, Cd, Cu and Zn followed by Siranga. No significant differences were observed in heavy metal concentrations in the stated sites ($P>0.05$, DF 4, ANOVA). This study indicated an increasing accumulation of heavy metals from

Gatwekera and Nairobi dam. This is due to increased input of domestic discharge and raw sewage along the riverine channel. In line with the Swiss Ordinance on Contaminated Soils guidelines (table 4.3), the study found out that all the investigated sites; Nairobi Dam, Siranga, Lindi, Gatwekera and Soweto are not suitable for agricultural practice because the levels found exceeded the recommended values as can be seen in table 4.4.

Table 4.3: International tolerable standards of heavy metal contents in soils

Guideline	Heavy metal (mg/kg)			
	Pb	Cd	Cu	Zn
Swiss Ordinance on contaminated Soil	50	0.8	50	200

Table 4.4: Heavy metal concentrations in soils (n=3) at different sites along Motoine River

Site	Technique	Average heavy metal concentration in mg/kg			
		Pb	Cd	Cu	Zn
Nairobi dam	AAS	*69.75±7.19	*2.061±0.16	38.47±9.4	*1032.48±3.8
	ICPMS	*79.17	0.68	37.77	*828.73
Siranga	AAS	*64.56±4.29	*1.819±0.19	36.25±9.4	*1030.35±284.5
	ICPMS	*79.72	0.73	34.97	*817.89
Soweto	AAS	*64.48±2.95	*2.151±0.059	34.76±6.27	*1035.25±67.07
	ICPMS	*75.29	0.72	38.38	*779.89
Lindi	AAS	*62.03±2.84	*1.9665±0.29	34.95±11.09	*892.45±114.7
	ICPMS	*68.73	0.69	34.06	*858.8
Gatwekera	AAS	*60.01±3.35	*2.0645±0.14	34.78±10.07	*875.84±215
	ICPMS	*64.34	0.473	30.73	*649.98

Soils above the tolerable levels in agricultural soils (Swiss Ordinance on Contaminated Soils) represented by *

From table 4.4, the total Cu levels however, were within the recommended maximum concentrations. This study indicates the same levels of Pb, Zn, Cd and Cu contamination signifying the same sources of contamination.

4.2.3 Characteristics of riverine soils

Soil particle measurements and soil pH were analysed using Hydrometer and pH meter respectively. The results obtained are tabulated in table 4.5.

Table 4.5: Mean values (n=3) of soil characteristics for riverine soils

Site	Soil pH	Soil characteristics (%)		
		Sand	Silt	Clay
Nairobi Dam	8.41±0.1	65.00±5.00	20.00±5.00	15.00±5.00
Siranga	8.36±0.1	57.87±5.00	23.99±5.00	18.15±5.00
Soweto	8.31±0.1	61.86±5.00	19.99±5.00	18.16±5.00
Lindi	8.35±0.1	61.85±5.00	20.00±5.00	18.16±5.00
Gatwereka	8.37±0.1	57.85±5.00	25.99±5.00	18.15±5.00

From table 4.5, it is observed that the soils under this study were alkaline and mainly sandy Clay particles were the least.

4.2.4 Heavy metal levels in riverine vegetation

The concentrations of heavy metals in the plants examined were as shown in tables 4.6 and 4.7. Plant parts were divided into edible and non-edible parts. Any part directly consumed as animal feed and/or as human feed was considered edible. In particular, sugarcane roots, arrowroot roots and tuber peelings, napier grass roots and kale roots were considered non-edible whilst sugarcane stem and leaves, arrowroot tuber, napier grass leaves and stem and kale leaves were considered edible.

Table 4.6: Mean heavy metal contents (n=3) in Edible parts of the riverine plants

Plant type	Concentration of heavy metal in mg/kg			
	Pb	Cu	Cd	Zn
Sugarcane	8.19±0.67	144.49±24.7	0.04±0.005	41.19±13.76
Napier grass	3.13±0.09	64.32±10.87	0.31±0.002	34.44±7.8
Kale	14.18±2.54	40.16±3.678	0.19±0.01	105.35±21
Arrow root	20.63±4.87	105.00±21.23	0.23±0.024	121.00±22.85

Table: 4.7: Mean heavy metal contents (n=3) in non-edible parts of the riverine plants

Plant type	Concentration of heavy metal in mg/kg			
	Pb	Cu	Cd	Zn
Sugarcane	7.00±0.67	148.31±34.7	0.54±0.05	40.36±9.01
Napier grass	6.20±2.12	180.34±40.92	0.32±0.002	19.86±1.7
Kale	12.79±2.3	81.23±24	ND	97.41±3.5
Arrow root	9.56±0.5	122.94±35.84	0.10±0.000	43.11±5.89

Zinc contents in edible plant parts ranged from 41.19±13.76 mg/kg to 121.00±22.85 mg/kg in arrowroots and napier grass respectively whilst in non-edible plant parts it ranged 19.86±1.7 mg/kg to 97.41±3.5 mg/kg in napier grass and kale respectively. The highest accumulator of Zn is the kale and the least effective is napier grass. However, arrowroots accumulate most of the Zn in its edible parts. Generally, all species accumulate higher Zn contents in the edible parts, although the difference is not significant ($p>0.05$, Chi squared

test) for sugarcane, kale and napier grass. Arrowroots showed a significant difference in Zn accumulation between the edible and non-edible parts ($p<0.05$, t test).

Copper contents in edible plant parts ranged from 40.16±3.67 mg/kg to 144.49±24.7 mg/kg in kale and sugarcane respectively whilst in non-edible plant parts it ranged 81.23±24 mg/kg to 148.31±34.7 mg/kg in kale and sugarcane respectively. Total Cu accumulation followed the order sugarcane > napier grass > arrowroots> kale while the difference in partitions (edible and non-edible parts) was significant in napier grass and kale ($P<0.05$, ANOVA). Generally copper was higher in non-edible than in edible parts.

Lead contents in edible plant parts ranged from 3.13±0.09 mg/kg to 20.63±4.87 mg/kg in napier grass and arrowroots respectively whilst in non edible plant parts it ranged 6.20±2.12 mg/kg to 12.79±2.3 mg/kg in napier grass and kale respectively. Total Pb concentrations are higher in arrowroots than in sugarcane and kale and lowest in napier grass. Generally, Pb concentrations are higher in edible than in non-edible parts. Pennisetum accumulated most lead in the non-edible part. There is a significant difference in Pb contents between the edible and non-edible parts ($P<0.05$, DF 3, ANOVA).

Cadmium contents in edible plant parts ranged from 0.04±0.005 mg/kg to 0.31±0.002 mg/kg in sugarcane and napier grass respectively whilst in non edible plant parts it ranged 0.00 mg/kg to 0.54±0.05mg/kg in sugarcane and kale respectively. Total Cd was highest in napier grass followed by sugarcane. Cadmium levels in grasses are higher in non-edible than in edible partitions. Cd accumulation is ranked in the order napier grass > sugarcane >

arrowroots > kale and the difference in Cd between edible and non-edible parts was significant ($P<0.05$, DF 3, ANOVA). Significant differences were observed between plant heavy metal contents in both edible and non-edible parts and the soil heavy metal contents ($P<0.05$, DF 3, ANOVA).

This study on riverine plants established that different plant species accumulate different heavy metals in varying proportion. It also showed that heavy metal partitioning varied between species - for example, grasses showed a tendency to accumulate heavy metals in the roots and tubers while vegetables accumulated in the leaves. Specific grasses are known to be good accumulators of Cu; an example being *Cynodon dactylon* in river Guadimar (Madejon *et al.*, 2006). Nabulo *et al.* (2005) noted that heavy metal concentrations available to plants as trace elements vary with plant species. The edible parts of arrowroots are also the food storage organs forming an underground tuber with large biomass compared to other parts of the plant. This suggests the possible reason for the high zinc concentration at the roots. The effect of soil characteristics on bioavailability of these heavy metals is emphasized. According to Dean and Marisa (2006), the bioavailability of heavy metals to plants depends on a number of physical and chemical factors in the soil including soil pH.

4.3 Heavy metal concentrations in a controlled study

The Zn, Pb, Cu and Cd levels in plants grown in pots are shown in table 4.8. The separate plant parts were found to have different heavy metal contents likewise to the plant species. In this subsection four bamboo species were compared with the edible plants grown at the riverside and the results obtained were tabulated in 4.8

Table 4.8: Heavy metal concentrations in parts of potted plants at 8 mts of growth (n=3)

	Plant part	Heavy metal concentrations (mg/kg)			
		Zn	Pb	Cu	Cd
	Leaf	83.60±13.9	20.26±2.4	109.60±20.2	0.995±0.06
	Stem	135.30±23.2	17.67±2.9	180.50±17.9	0.890±0.04
	Root	83.60±6.3	34.05±2.2	143.10±23.5	0.730±0.01
	Leaf	190.90±25.0	12.93±0.3	124.30±23.9	1.140±0.15
	Stem	138.40±16.8	19.395±4.0	81.60±18.0	0.835±0.16
	Root	190.90±3.8	16.81±2.0	86.90±11.0	0.480±0.02
	Leaf	67.80±11.2	9.485±2.7	239.30±27.9	0.570±0.02
	Stem	234.00±30.9	17.67±3.7	30.80±1.6	1.025±0.05
	Root	67.80±4.7	14.655±1.8	176.50±18.9	1.010±0.10
	Leaf	42.80±11.0	16.38±5.9	184.50±27.9	0.715±0.01
	Stem	76.90±16.8	5.605±2.9	141.70±23.9	0.090±0.01
	Root	42.80±2.3	19.395±1.2	82.90±13.0	0.395±0.0
	Leaf	66.80±7.9	9.80±1.3	102.20±11.0	0.567±0.01
	Stem	211.40±21.0	15.085±3.6	38.80±5.9	0.935±0.23
	Root	66.80±2	41.81±0.8	183.20±12.3	1.065±0.01
	Leaf	63.40±2.3	5.605±0.7	128.40±12.8	0.790±0.03
	Stem	141.30±23.7	13.36±2.5	115.00±12.5	1.110±0.26
	Root	63.40±6.6	15.515±1.9	131.00±28.3	0.410±0.01
	Leaf	120.00±2.7	21.00±3.0	120.00±10.0	0.887±0.05
	Stem	115.30±23.8	7.90±1.3	134.00±12.7	0.879±0.20
	Root	120.00±7.3	12.24±1.5	84.00±10.0	0.991±0.02
	Leaf	89.40±11.0	15.95±1.0	157.80±19.0	1.040±0.03
	Stem	123.50±24.0	12.585±0.9	214.20±20.0	0.995±0.01
	Root	89.40±8.4	24.14±0.8	103.00±10.9	0.480±0.01

GB- giant bamboo, WB- water bamboo, YB-m yellow bamboo, GrB green bamboo, SC-sugarcane, NG- napier grass, Kl- kale and AR- arrow roots

The Zn concentrations in plant leaves ranged from 42.80±11.02 mg/kg in yellow bamboo to 190.90±25.00 mg/kg in water bamboo. Lead ranged from 5.605±0.71 mg/kg to 21.00±3.08 mg/kg in napier grass leaf and kale leaf respectively. Total Cu ranged from 102.20±11.05 mg/kg to 239.30±27.9 mg/kg in napier grass and green bamboo, respectively, and Cd ranged from 0.567±0.02 mg/kg to 1.140±0.15 mg/kg in sugarcane and water bamboo, respectively (table 4.8).

The Zn concentrations in plant stems ranged from 76.90±16.8 mg/kg in yellow bamboo to 234±30.90 mg/kg in green bamboo stem. Total Pb ranged from 5.605±2.98 mg/kg to 19.395±4.01 mg/kg in yellow bamboo stem and water bamboo stem respectively. Total Cu ranged from 30.80±1.67 mg/kg to 214.20±20.04 mg/kg in green bamboo stem and arrowroot respectively, and Cd ranged from 0.090±0.01 mg/kg to 1.110±0.267 mg/kg in yellow bamboo stem and napier grass respectively (table 4.8).

The Zn concentrations in plant roots ranged from 42.80±2.3 mg/kg in yellow bamboo to 190.90±3.81 mg/kg in water bamboo. Total Pb ranged from 12.24±1.53 mg/kg to 41.00±0.87 mg/kg in kale and sugarcane respectively. Total Cu ranged from 82.90±13.02 mg/kg to 183.20±12.32 mg/kg in yellow bamboo and sugarcane respectively, and Cd ranged from 0.395±0.009 mg/kg to 1.065±0.005 mg/kg in yellow bamboo and sugarcane roots respectively (table 4.8)

Zinc accumulation is in the order root > stem > leaves. Sugarcane was found to be a higher accumulator than napier grass, kale, arrowroots and bamboo. Higher Zn uptake was observed in water bamboo than in yellow bamboo. The same trend seems to be true for Pb. However, copper differs slightly from this trend. Different plants accumulate different amounts of copper in different parts, the highest accumulator being green bamboo with the highest concentrations of copper in root and leaves and least accumulated in the stem. Sugarcane accumulated most Cu in the root followed by the leaves and least in the stem. On the contrary, giant bamboo accumulated most in the stem and least in the root. The lowest Cu levels were observed in the leafy vegetable kale while yellow bamboo

accumulated more Cu than water bamboo. Cd accumulated most in stem, with almost equal contents partitioned to the root sand leaves. Yellow bamboo, however, accumulated least Cd in the stems and equal proportions in the root and leaves. Cd levels were higher in water bamboo than yellow bamboo.

No significant differences were observed in heavy metal concentration of the various plant species ($P>0.05$, DF 4, t-test). Significant differences were observed in Pb and Zn concentrations in leaf, stem and root parts of different species ($P<0.05$, DF 4, t-test). No significant differences were observed in Cd and Cu concentrations in leaf, stem and root parts of different species ($P>0.05$, DF 3, t-test).

4.3.1 Graphical summary of potted plants

A summarised version of this section is shown by two graphical presentations in figures 4.2 and 4.3.

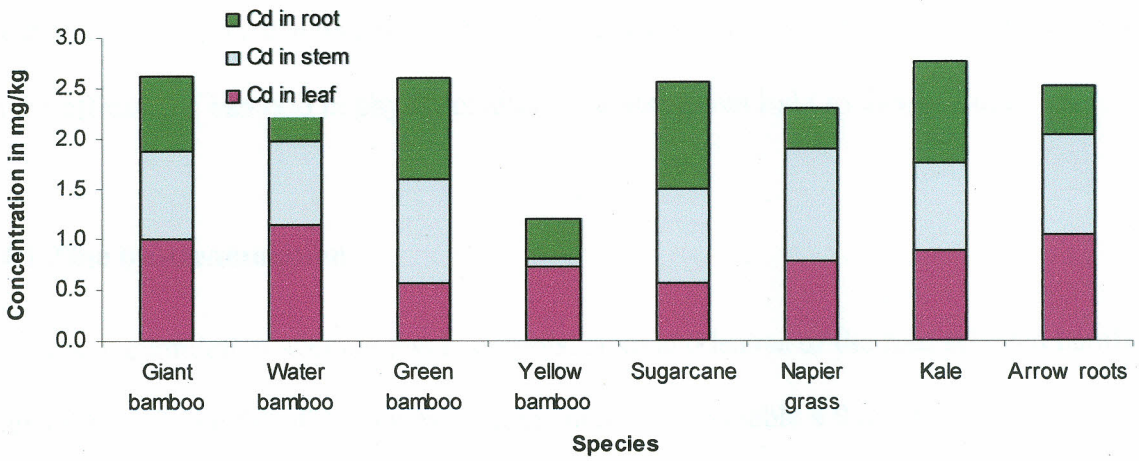

Fig 4.2: Cadmium concentrations in pot plants

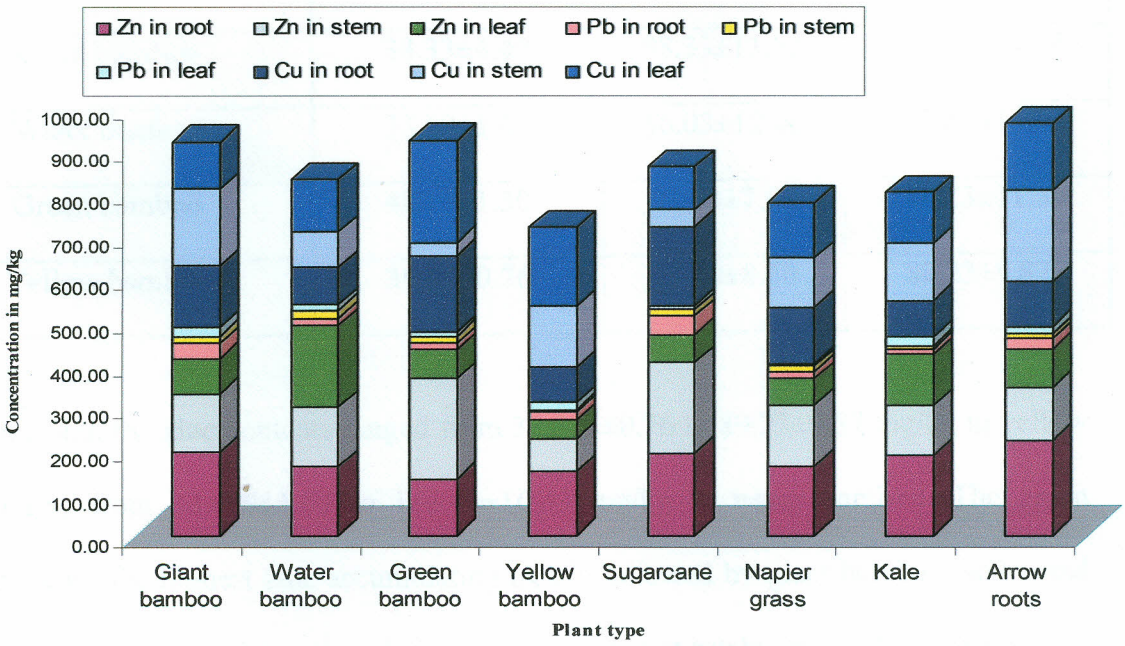

Fig 4.3: Zinc, Cu and Pb concentrations in potted plants

4.4 Heavy metal accumulations by selected bamboo species

Accumulation of Zn, Pb, Cu and Cd in giant bamboo, water bamboo, green bamboo and yellow bamboo, the four species of bamboo throughout the entire period of 8 months, is illustrated by graphical representations and tabulations in this subsection. The discussion on the efficacy of bamboo in phytoremediation is also given light in this section.

4.4.1 Zinc bioaccumulation

The zinc accumulation average levels in roots, stem and leaves of the four selected bamboo plants for the 8 months of the pot experiment are shown in table 4.9 and figure 4.4.

Table 4.9: Zinc average concentration in potted bamboo at various stages of growth (n=6)

Bamboo species	Zinc concentration in mg/kg		
	3 mts	6 mts	8 mts
Giant bamboo	38.33±4.40	88.33±11.35	137.53±21.34
Water bamboo	33.69±1.67	56.03±12.40	162.83±16.33
Green bamboo	48.10±1.30	91.43±7.90	144.23±11.50
Yellow bamboo	36.33±0.76	71.74±8.50	89.23±9.87

From table 4.9, the zinc contents ranged from 36.333±0.76 to 89.23±9.87 mg/kg in yellow bamboo and from 33.699±1.67 to 162.83±16.33 mg/kg in water bamboo. The green bamboo shows the highest zinc accumulating trends followed by giant bamboo, water and yellow bamboo in that order across all the months of the pot establishment (Figure 4.4).

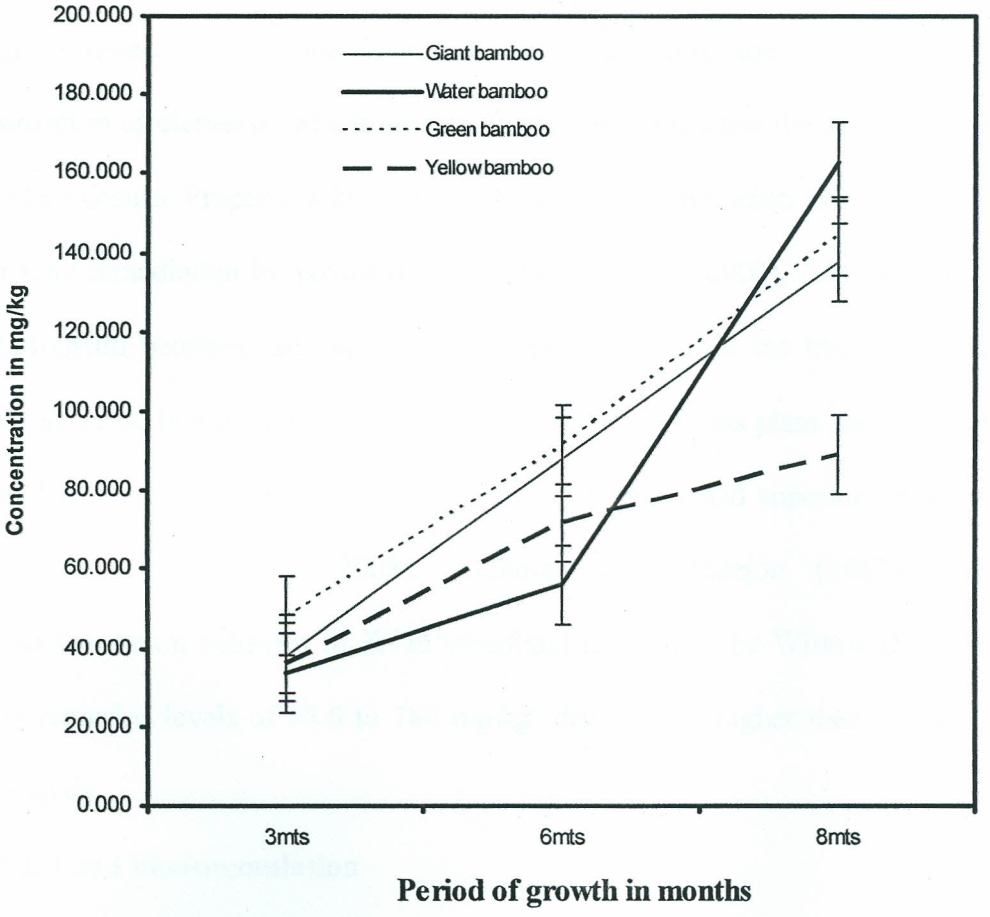

Fig. 4.4: Zinc accumulating trends in bamboo

Analysis of variance indicated a significant difference in the Zn contents after 3 months, 6 months and the last 8 months (P=0.05, DF 3, t-test). This indicates the rates of Zn absorption differing significantly in the initial stages and after 8 months. For the first three months the trends were green bamboo>giant bamboo>yellow bamboo>water bamboo. The same trend was observed after 6 months. A significant difference was observed between water bamboo and the other species after 6 months. However, at 8 months yellow bamboo was observed to have the lowest gradient a with the water bamboo having the steepest gradient. A significant difference was observed between yellow bamboo and the rest of the

species at the 8 month (P>0.05, DF 3, ANOVA). No significant differences were observed in zinc accumulations among all the bamboo species at 3 months (P>0.05, DF 3, ANOVA). Closely related research has found out that mineral nutrition influences plant growth and absorption of elements, which are two factors that influence the accumulation of elements in plant tissues. Proper nutrition may enhance Zn accumulation in plants and their potential for land remediation by phytoextraction (Hamlin *et al*, 2003). Another study analysed the relationship between zinc accumulation and tolerance in the hyperaccumulator, *Thlaspi caerulescens*, in which a cross between a nonmetallicolous plant and one from a calamine population suggested that the nonmetallicolous plants had superior Zn accumulation and tolerance (Ana *et al.*, 2003). Nicholas and Madejón (2007) investigated the bioaccumulation behavior of Zn in woodland dominated by Willow (*Salix*) species where they recorded levels of 83.0 to 784 mg kg^{-1}dry wt. Zn, higher than the levels reported in this study.

4.4.2. Lead bioaccumulation

The Pb accumulation average values in roots, stem and leaves of the four selected bamboo plants for the 8 months of the pot experiment are shown in table 4.10 and figure 4.5.

Table 4.10: Lead average concentration in potted bamboo at various stages of growth (n=6)

Bamboo species	Lead concentration in mg/kg		
	3 mts	6 mts	8 mts
Giant bamboo	11.36±1.33	17.70±1.26	23.99±4.30
Water bamboo	3.57±0.67	13.59±3.50	16.38±3.10
Green bamboo	13.94±2.21	14.04±2.34	20.71±1.70
Yellow bamboo	13.79±0.98	14.15±1.34	17.40±1.43

The Pb contents ranged from 3.57±0.67 to 16.38±3.1 mg/kg in water bamboo and from 11.36±1.33 to 23.99±4.3 mg/kg in giant bamboo (Table 4.11). The giant bamboo shows the highest Pb accumulating trends followed by green bamboo, yellow and water bamboo in that order across all the months of the pot establishment (Figure 4.5).

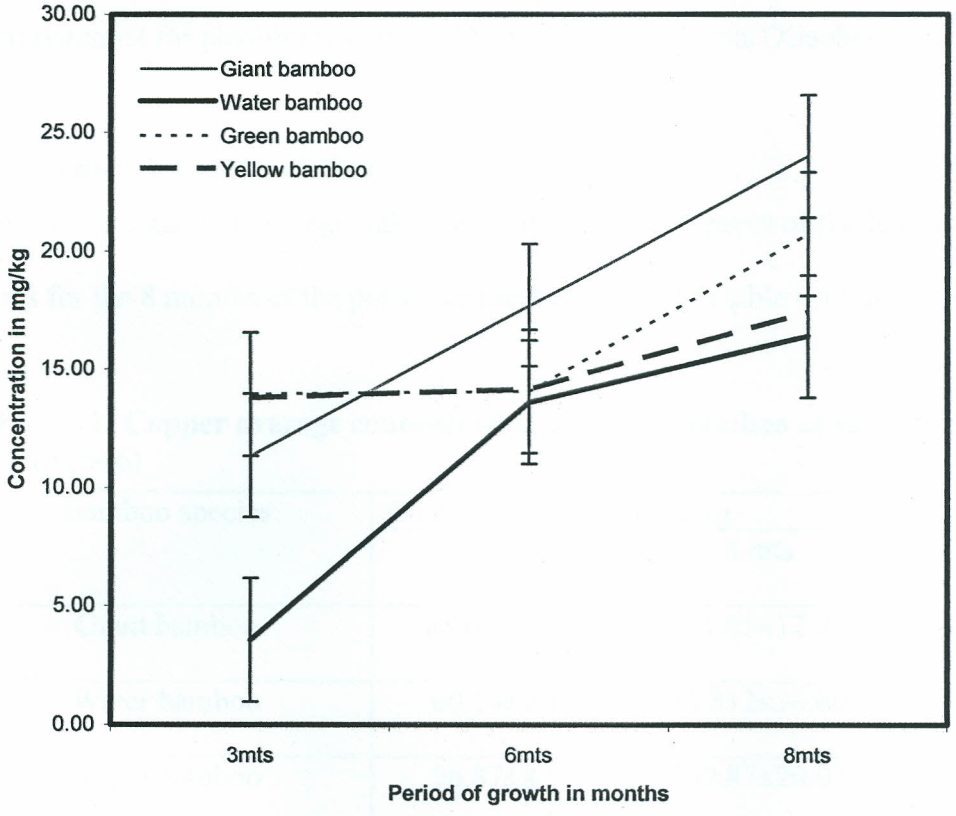

Fig. 4.5: Lead accumulating trends in bamboo

Significant Pb absorption differences were observed in accumulation levels of the first 3months, 6 months and the last 8 months ($P<0.05$, DF 3, ANOVA). This indicates the rates of Pb absorption differing significantly in the initial stages and last harvesting. Yellow and green bamboos have a similar Pb accumulating trend in the first and second periods of

plant harvests. The giant bamboo has the highest accumulation of Pb levels with the highest trend of accumulation. Significant Pb absorption differences were observed in accumulation between water bamboo and the rest of the species and giant bamboo and the rest of the species for the first three months and the last eight months respectively. No significant differences were observed in Pb accumulations among all the bamboo species in the second harvest ($P>0.05$, DF 3, ANOVA). Presently, it has been found out that *Ricinus communis* L. and two other species *Tephrosia candida* and *Debregeasia orientalis* have a great potential for phytoremediation of Pb contaminated soils (Xiaohai *et al.*, 2008).

4.4.3 Copper bioaccumulation

The Cu accumulation average values in roots, stem and leaves of the four selected bamboo plants for the 8 months of the pot experiment are shown in table 4.11 and figure 4.6.

Table 4.11: Copper average concentration in potted bamboo at various stages of growth (n=6)

Bamboo species	Cu concentration in mg/kg		
	3 mts	6 mts	8 mts
Giant bamboo	83.65±11.23	121.65±12.23	144.40±32.70
Water bamboo	60.14±7.10	84.812±14.60	97.60±18.00
Green bamboo	56.87±8.97	100.87±20.03	148.87±33.90
Yellow bamboo	81.66±4.80	103.40±23.07	136.37±14.70

The Cu contents ranged from 60.146±7.1 to 97.60±18 mg/kg in water bamboo and from 56.873±8.97 to 148.87±33.9 mg/kg in green bamboo (Table 4.11). The giant bamboo shows the highest Cu accumulation trends followed by green bamboo, yellow and water bamboo in that order across all the months of the pot establishment (Figure 4.6).

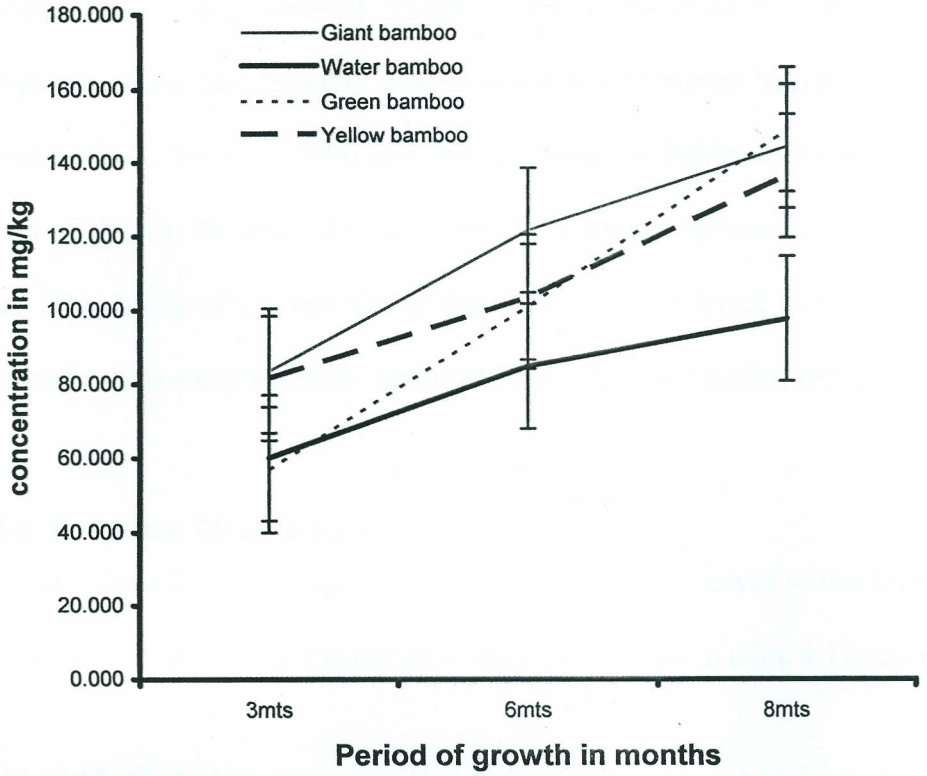

Fig. 4.6: Copper accumulation trends in bamboo

Analysis of variance indicated significant differences in the Cu accumulation levels of the first 3 months, 6 months and the last 8 months ($P<0.05$, DF 3, t-test). This indicates the rates of Cu absorption differed significantly in all stages of the pot establishment. Giant bamboo shows significant differences with water bamboo throughout the growth period. Water bamboo shows a significant difference with green and yellow bamboo at 8 months. ($P<0.05$, DF 3, ANOVA). Similar levels of accumulation were observed with giant and water bamboo at 3 months, green and water bamboo at 3 months, green and yellow bamboo at 6 months and green and giant bamboo at 8 months.

Under experimental conditions, *Groenlandia densa* (L.) proved to be a good accumulator of Cd and Cu (Yesim and Ali., 2007). Zhi-Ting and Hai (2005) reported that Chinese

cabbage *(Brassica pekinensis* Rupr) with an elevated Cu content, with no visible symptoms of damage, possibly could cause a risk to human health from the transfer of the metal in food. Between 2000 and 2001, a study in Pakistan revealed high accumulation levels of Zn, Cu Pb and Cd by over 40 plant samples grown on potentially contaminated sites. The levels of Zn and Cu in these plants were found to be lower than the levels obtained in this study while the levels of Pb and Cd were higher (Riaz *et al.*, 2005).

4.4.4: Cadmium bioaccumulation

The Cd accumulation average values in roots, stem and leaves of the four selected bamboo plants for the 8 months of the pot experiment are shown in table 4.12 and figure 4.7.

Table 4.12: Cadmium concentration in potted bamboo at various stages of growth (n=6)

Bamboo species	Cadmium content in mg/kg		
	3 mts	6 mts	8 mts
Giant bamboo	0.025±0.003	0.606±0.001	0.872±0.005
Water bamboo	ND	0.271±0.012	0.818±0.004
Green bamboo	0.047±0.005	0.629±0.002	0.868±0.009
Yellow bamboo	0.003±0.000	0.400±0.021	0.645±0.003

The Cd contents ranged from 0.003±0.000 to 0.645±0.003 mg/kg in yellow bamboo and from 0.025±0.003 to 0.872±0.005 mg/kg in giant bamboo (Table 4.13). The giant bamboo shows the highest Cd accumulation trends followed by green bamboo, water and yellow bamboo in that order across all the months of the pot establishment (Figure 4.7).

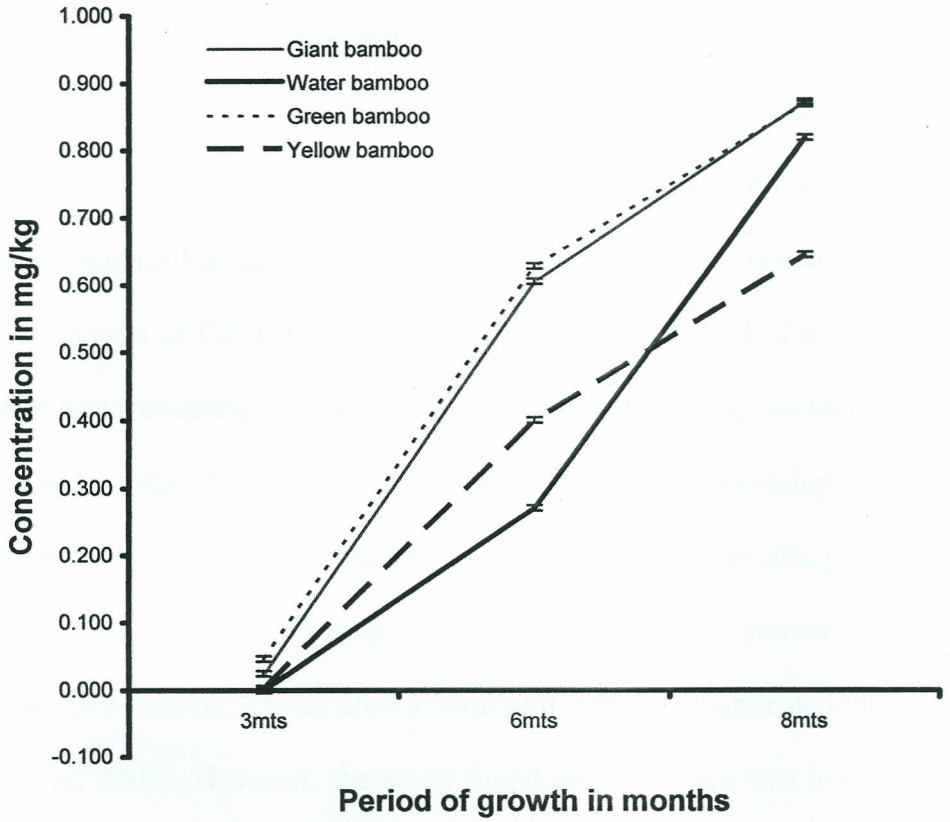

Fig 4.7: Cadmium accumulation trends in bamboo

Analysis of variance indicated significant differences in the Cd accumulation levels of the first 3 months, 6 months and the last 8 months ($P<0.05$, DF 3, t-test). This indicates the rates of Cd absorption differed significantly in all stages of the pot establishment. This trend is similar to Cu as shown in table 10 above. A significant difference was observed between water bamboo when compared with green and giant bamboo in which the latter species had accumulated the highest amount of cadmium. No significant differences were observed in Cd accumulation among all the bamboo species in the first 3 months and the last 8 months ($P>0.05$, DF 3, ANOVA). Cadmium uptake by *S. caprea* has elsewhere been correlated with differences in soil pH (Nicholas and Madejón, 2007).

Generally, this study shows that different bamboo species accumulate different metals at varied rates across the growth period. In particular, giant bamboo and green bamboo were found to bioaccumulate the highest levels of Cu, Cd and Pb among the bamboo species. Water bamboo had the best accumulation trend for zinc. Green bamboo had the highest accumulation of Cd and Cu while giant bamboo had the highest Pb accumulation. Total heavy metal concentration in bamboo species increased exponentially with increasing time in months. Figs; 4.4, 4.5, 4.6 and 4.7 illustrate these relationships for Zn, Pb, Cu and Cd respectively. Exceptionally, the white poplar plant (*Populus alba*), accumulated Cd and Zn in leaves up to 3 and 410 mg kg^{-1} respectively in a large phytomanaged site, the Guadiamar Valley (SW Spain), 7 years after a mine spill, which contaminated the area in 1998. (María T. *et al*, 2008). However, the study found out that there was low trace element transfer from contaminated soils to the aboveground parts of afforested woody plants under a semi-arid climate.

Bamboo production per year is estimated to be between 30-75 tons/ha per annum (Chin Ong, personal communication). From this study, it can be estimated that bamboo absorbs averagely 243 mg/kg of zinc, 1.3 mg/kg of Cd, 222 mg/kg of Cu and 36 mg/kg of lead per year. The total absorption therefore translates to 6.66-16.65 kg of Zn, 7.29-18.225 kg of Cu, 1.08-2.7 kg of Pb, 39-97.5 g of Cd per ha/year.

4.4.5 Heavy metal concentrations in soils after growing bamboo species

Increasing accumulation in bamboo plant is related to the decreasing contents in the soil in after the harvests. A study of the soil concentrations of these heavy metals showed a decrease of all heavy metal contents over a period of time (Table 4.13 and 4.14).

Table 4.13: Soil heavy-metal concentrations after first harvest of bamboo species (n=6)

Bamboo species' soils	Heavy metal concentration in mg/kg			
	Cu	Cd	Pb	Zn
Giant bamboo	25.27±1.98	1.84±0.01	64.56±7.90	756±40.20
Water bamboo	31.57±3.50	1.89±0.12	65.51±6.33	742±38.23
Green bamboo	27±3.94	1.78±0.04	62.04±2.09	742.55±56.93
Yellow bamboo	49±9.70	2.09±0.18	73.75±1.68	1017±45.34

Table 4.14: Soil heavy-metal concentrations after second harvest of bamboo species (n=6)

Bamboo species	Heavy metal concentration in mg/kg			
	Cu	Cd	Pb	Zn
Giant bamboo	26.41±34.4	1.84±0.10	62.09±4.56	678±45.23
Water bamboo	29.26±3.60	1.97±0.21	64.47±3.89	723±49.9
Green bamboo	23.75±2.34	1.98±0.02	58.9±6.70	685±51.3
Yellow bamboo	22.48±2.22	1.79±0.17	61.71±5.55	723±60.56

Significant correlations were observed between decreasing heavy metal concentrations in the soils after the harvests and the bioaccumulations in the bamboo species ($P>0.05$, DF 12, t-test). Cadmium had the highest correlation between the soil and an increasing bamboo

plant accumulation (r = -0.788). This suggests that in these soil types cadmium absorption by bamboo is highest among the heavy metals considered. No significant differences were observed in heavy metal reductions in soil used to grow the different bamboo species ($P>0.05$, DF 5, ANOVA)

5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Kibera settlements are congested with domestic wastes that drain into Motoine riverine wetland, which is a tributary of the larger Nairobi River basin. These domestic wastes are the main sources of heavy metal pollution into Motoine riverine and thus require attention in order to reduce this hazardous heavy metal pollution.

Motoine River has high levels of Pb that fall beyond the USEPA, 2001 maximum concentration standards for drinking water. It is therefore unsafe to use this water for domestic purposes and irrigation of crops. However, Cu, Zn and Cd levels in water are within the accepted standards.

Soils along Motoine riverine wetland have high levels of Pb and Zn that fall beyond the international set maximum concentration guidelines (Swiss Ordinance on Contaminated Soils). Copper and Cd levels in these soils are within the accepted standards. Growing crops on these soils is therefore a health hazard.

Most activities found in polluted places include agricultural practices. Arrowroots, sugarcane, kale and napier grass are the crops cultivated along the riverine. These crops have bioaccumulated toxic heavy metals in their tissues beyond the internationally accepted standards. The highest accumulator of Zn is the kale (*Brasicca oleraceae*) and the least effective is napier grass. Total Cu accumulation is ranked in the order sugarcane > napier

grass > arrowroots> kale. Total Pb concentrations are higher in arrowroots than in sugarcane and kale and lowest in napier grass. Cadmium accumulation is ranked in the order napier grass > sugarcane > arrowroots > kale.

Bamboo species can accumulate significant levels of heavy metals in their tissues. The green bamboo shows the highest zinc accumulating trends followed by giant bamboo, water and yellow bamboo in that order across all the months of the pot establishment. The giant bamboo shows the highest Pb accumulating trends followed by green bamboo, yellow and water bamboo in that order across all the months of the pot establishment. The giant bamboo shows the highest Cu accumulation trends followed by green bamboo, yellow and water bamboo in that order across all the months of the pot establishment. The giant bamboo shows the highest Cd accumulation trends followed by green bamboo, water and yellow bamboo in that order across all the months of the pot establishment

Due to fast growth rate and large biomass index, bamboo forms a good plant for biofiltration of these metals. Bamboo productions per year estimates are between 30-75 tons/ha per annum. From this study, bamboo absorbs averagely 243 mg/kg of zinc, 1.3 mg/kg of Cd, 222 mg/kg of Cu and 36 mg/kg of lead per year. The total absorption therefore translates to 6.66-16.65 kg of Zn, 7.29-18.225 kg of Cu, 1.08-2.7 kg of Pb, 39-97.5 g of Cd per ha/year. It will therefore be appropriate to argue that building up bamboo systems along Ngong/Motoine River in Kibera slums as a purifying model, substituting them for cultivated edible vegetation in slums currently around wetlands is highly encouraged.

5.2 RECOMMENDATIONS

There is need to establish improved clean water technologies through developing bamboo system. Building up bamboo-constructed wetlands along Ngong/Motoine river in Kibera slums as a purifying model, substituting them for cultivated edible vegetation in slums currently around wetlands can render positive ways of reduced heavy metal pollution.

Promote accelerated awareness and implementation of plant water ecosanitation technologies through motivation of members involved at community levels. This can be done through collaboration and networking with the main stakeholders in order to implement this pollution eradicating bamboo system

5.3 FURTHER RESEARCH

Other vegetation along the riverine should be investigated to give Metal speciation needs to be carried out to determine in which forms these metals are available to living organisms since toxicity of these metals is highly dependent on the forms in which they are available to them.

There is need to establish accumulation trends of lead, copper, cadmium and zinc by bamboo at later stages of its development. Further research and comparative studies between bamboo heavy metal absorption with other perennial plants such as mangrove and papyrus ought to be done.

Measurement of the investigated heavy metal levels absorbed under controlled conditions to distinguish atmospheric adsorption and absorption of heavy metals in air. Analysis of water sediments for heavy metals is necessary.

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APPENDICES

APPENDIX I

Bamboo vs soil correlation matrices

Correlation matrix

S_Zn	*1.000*	
Zn	*-0.586*	*1.000*
	S_Zn	*Zn*

52 CORRELATE [PRINT=correlations] Cd, S_Cd

Correlation matrix

Cd	*1.000*	
S_Cd	*-0.788*	*1.000*
	Cd	*S_Cd*

53 CORRELATE [PRINT=correlations] Cu, SCu

Correlation matrix

Cu	*1.000*	
SCu	*-0.312*	*1.000*
	Cu	*SCu*

54 CORRELATE [PRINT=correlations] Pb, S_Pb

Correlation matrix

Pb	*1.000*	
S_Pb	*-0.097*	*1.000*
	Pb	*S_Pb*

APPENDIX II

Table10: Mean total heavy metal concentrations and SD for leaves of potted plants

Plant	Zn (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Giant bamboo	83.60±13.98	20.26±2.43	109.60±20.2	0.995±0.065
Water bamboo	190.90±25.00	12.93±9.3	124.30±23.9	1.140±0.15
Green bamboo	67.80±11.2	9.485±2.7	239.30±27.9	0.570±0.02
Yellow bamboo	42.80±11.02	16.38±5.9	184.50±27.9	0.715±0.0123
Sugarcane	66.80±7.98	9.80±1.3	102.20±11.05	0.567±0.01
Napier grass	63.40±2.36	5.605±0.71	128.40±12.84	0.790±0.03
Kale	120.00±2.76	21.00±3.08	120.00±10.01	0.887±0.056
Arrow root	89.40±11.01	15.95±1.07	157.80±19.06	1.040±0.03

Table 11: Mean total heavy metal concentrations and SD for stems of potted plants

Plant	Zn (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Giant bamboo	135.30±23.23	17.67±2.99	180.50±17.9	0.890±0.046
Water bamboo	138.40±16.89	19.395±4.01	81.60±18.0	0.835±0.16
Green bamboo	234.00±30.90	17.67±3.75	30.80±1.67	1.025±0.05
Yellow bamboo	76.90±16.8	5.605±2.98	141.70±23.9	0.090±0.01
Sugarcane	211.40±21.0	15.085±3.67	38.80±5.9	0.935±0.23
Napier grass	141.30±23.7	13.36±2.55	115.00±12.54	1.110±0.267
Kale	115.30±23.87	7.90±1.33	134.00±12.78	0.879±0.2
Arrow root	123.50±24.02	12.585±0.98	214.20±20.04	0.995±0.016

APPENDIX III

Table 12: Mean total heavy metal concentrations and SD for roots of potted plants

Plant	Zn (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Giant bamboo	83.60±6.34	34.05±2.22	143.10±23.56	0.730±0.01
Water bamboo	190.90±3.81	16.81±2.01	86.90±11.01	0.480±0.023
Green bamboo	67.80±4.7	14.655±1.86	176.50±18.9	1.010±0.1
Yellow bamboo	42.80±2.3	19.395±1.2	82.90±13.02	0.395±0.009
Sugarcane	66.80±2.9	41.81±0.87	183.20±12.32	1.065±0.005
Napier grass	63.40±6.66	15.515±1.90	131.00±28.33	0.410±0.015
Kale	120.00±7.3	12.24±1.53	84.00±10.09	0.991±0.02
Arrow root	89.40±8.4	24.14±0.89	103.00±10.93	0.480±0.012

APPENDIX VI

4.3 TOTAL COPPER, IRON, MANGANESE AND ZINC IN PLANT TISSUE

Background

The wet digestion method (Kjeldahl) used for N, P and K analysis could be used also for analysis of micronutrients, but for two factors: first, copper concentration in the diluted Kjeldahl digest is too low to measure reliably, and second, the rubber stoppers used for sealing the digestion tubes contaminate the solutions with zinc. Therefore, for plant micronutrient analysis the organic matter is destroyed by high temperature dry ashing, and the ash is dissolved in acid. Elements are determined by atomic absorption spectrophotometry after appropriate dilution.

Equipment

1. Atomic absorption spectrophotometer
2. Bottle-top dispenser, 5 mL capacity
3. Balance, 0.001 g readability
4. Large hotplate
5. Fume cupboard
6. Muffle furnace

Supplies

1. Porcelain crucibles, about 15 mL
2. 60 mL plastic bottles and racks
3. Eppendorf Multipette pipettor, with combitips; 1.25, 2.5, 12.5, and 50 mL; and Oxford Macroset pipettor, 1-5 mL
4. Goggles
5. Gloves
6. Volumetric flasks (5000 mL, 1000 mL, 250 mL, 100 mL)
7. Volumetric pipettes, 10 mL
8. Pipette filler bulb
9. Heat resistant tongs
10. Heat resistant trays

Consumables

1. Acetylene gas, welding grade (at least 95% purity)

Chemicals

NOTE: All chemicals must be reagent grade unless otherwise noted.

1. Concentrated Hydrochloric acid (HCl, about 12 M, 36 to 38%) Nitric acid was used in case of Pb analysis

2. 1000 mg/L commercial stock standard solutions of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn)

Reagents

1. 0.5 M HCl: Add 208 mL of conc. HCl to a 5000 mL volumetric flask containing about 1000 mL of deionized water, cool and make to volume with deionized water.

Standards

1. Stock Solutions (1000 mg Cu, Fe, Mn and Zn/L):
Standards are prepared from commercially–supplied stock solutions of 1000 mg Cu, Fe, Mn or Zn/L. Stock solutions may also be prepared in the lab, but salts of these four elements are not available in high enough purity, so the standards should be prepared using the pure metals, which are available from various suppliers.

2. Intermediate stock standard solutions (100 mg Cu, Fe, Mn or Zn/L): Prepare separate stock solutions of each element by pipetting 10 mL of the appropriate 1000 mg/L stock into labelled 100 mL flasks. Make to volume with 0.5 M HCl and mix well.

3. Working standards in 0.5 M HCl: Mixed standards containing appropriate concentrations of all four metals are prepared in 250 mL volumetric flasks. The following table gives the final concentrations of the elements, and the appropriate volumes to be pipetted for each element. Use an Eppendorf Multipette with appropriate sizes of combitips to pipette the correct volumes of 100 mg/L intermediate stock standards. When all elements have been added, make the flasks to volume with 0.5 M HCl and mix well.

Procedure

1. Ashing

Ashing is carried out in batches of 40, including 33 plant samples, 4 standard plant samples and 3 blanks.

a) Weigh to 3 decimal places about 1.000 g plant material into numbered crucibles. If samples are not to be ashed immediately, cover the crucibles.

b) Place the crucibles in a cool muffle furnace. Slowly (over a time of about 1 hour), raise the temperature from room temperature to 500°C, to prevent sudden, explosive combustion of the dry plant sample. Hold at 500°C for at least 4 hours or overnight. Overnight ashing is more convenient to avoid staff contact with the choking smoke developed during initial heating.

c) The next morning, turn off the muffle furnace. Open the door just a crack, and very slowly, to avoid blowing of the ash by escaping hot air. Leave the samples to cool inside the furnace for about 30 minutes.

d) Slowly open the muffle furnace some more, and allow to cool until samples and the furnace are cool enough.

e) Using gloves and tongs, transfer the crucibles containing the ashed samples onto heat resistant trays and allow to completely cool. Ensure that the crucibles are covered, to prevent any currents from blowing away or contaminating the dry ash.

2. Preparation and dilution of samples

a) Transfer the crucibles to the fume cupboard.

b) Wet the ash by adding 1 mL of deionised water.

c) Add 1 mL of conc. HCl to each sample, very slowly to avoid splattering, and heat slowly to dryness on the hotplate, set at about 80 to 100°C. Remove from the hotplate and allow to cool.

NOTE: The acid hydrolysis and drying step serves to dehydrate silica, and also to help solubilise difficultly-soluble Mn compounds.

d) Using an Eppendorf Multipette or bottle-top dispenser, add 5 mL of 0.5 M HCl to each crucible. Warm briefly on the hotplate, and then transfer samples to 60 mL plastic bottles.

e) Wash the crucibles with 4 more 5-mL portions of 0.5 M HCl, and add the washings to the plastic bottles, for a total volume of 25 mL.

f) If appropriate dilutions are known, prepare these using suitable pipetters and dispensers. Cap the samples tightly until analysed.

NOTE: If dilutions are necessary, it is important that the standards and samples are "matrix-matched", that is that the solutions are matched with respect to acid or salt concentration. A set of standards should be prepared which is more concentrated than the usual standards by a factor equal to the desired dilution factor. Then the same dilution step may be performed on both samples and standards. This assures proper matrix matching, and also eliminates any bias from the dilution step, since the samples and standards have been handled in exactly the same way.

3. Analysis

a) Set-up of the atomic absorption spectrophotometer: refer to Section 3.3: EXCHANGEABLE CALCIUM, MAGNESIUM AND SODIUM for instructions on the set-up of the AAS. Set wavelengths and lamp currents according to recommendations on the lamp and the AAS operating manual.

b) Warm-up time for the flame and lamp should be at least 15 minutes, to allow lamp output and burner temperature to stabilise.

c) Read standards in absorbance mode to check for proper set-up and linearity of the standard curve. Then set the standard curve in concentration mode. Read standards and samples in concentration mode.

NOTE: Quality control samples are included in the following manner: After each 10 samples, one of the preceding 10 samples is re-analyzed, and one standard solution is analyzed. In this way repeatability of the determination and stability of standard readings can be assessed.

NOTE: For some elements, an alternate and less sensitive absorption wavelength may be used in lieu of dilutions, thus saving time. This is possible for the elements Cu, Fe and Mn, though for Cu it is rarely necessary due to low plant Cu concentration. This procedure effectively expands the linear range for analysis about 4-fold for Fe (0 to 20 mg/L) and about 10-fold for Mn (0 to 30 mg/L). Refer to operating manual for alternate wavelengths.

Calculations

The blank value must be subtracted from instrument readings to obtain corrected solution concentrations.

Plant tissue Cu, Fe, Mn or Zn concentration (mg/kg) (PCU, PFE, PMN or PZN):

$$\frac{(\text{PCUCONC} - \text{PCUBLNK})\ (25)\ (\text{DF})}{\text{PCUWT}}$$

where PCUCONC = Sample concentration of Cu (mg/L)
PCUBLNK = Blank concentration of Cu (mg/L)
25 = Volume of dissolved ash solution
DF = Dilution factor, if any
PCUWT = Weight of plant

3.6 SOIL PARTICLE SIZE ANALYSIS BY HYDROMETER METHOD

Background

The particle size analysis of soil estimates the percentage of sand, silt and clay particles comprising the soil. Based on the proportions of different particle sizes, a soil textural category may be assigned to the sample.

The hydrometer method of silt and clay measurement relies on the effects of particle size on the differential vertical velocities of the particles through a water column, i.e. the

sedimentation rate. Sedimentation rate is dependent upon liquid temperature, viscosity, and the diameter and specific gravity of the falling soil particles.

Soil is dispersed into individual particles after pretreatment with hydrogen peroxide to destroy organic matter, and addition of sodium hexametaphosphate to aid dispersion, then dispersed throughout a water column and allowed to settle. Hydrometer measurements quantify the amount of material remaining in suspension at specific time intervals, which in turn can be related to the amounts of sand, silt and clay in the soil.

Equipment

1. High speed stirrer with cup receptacle ("milk-shake mixer")
2. Balance, 0.01 g readability
3. Mechanical shaker (if stirrer is not available)
4. Hot water bath

Supplies

1. Bouyoucos hydrometer, graduated in g/L
2. Measuring cylinders, 1000 mL, one per soil sample
3. Plastic beakers, 400 mL, one per soil sample
4. Wash bottle
5. Thermometer, 0 to 110°C
6. Watch glasses to fit 400 mL beakers
7. Stop watch
8. Glass or plastic stirring rods fitted with rubber tips, one per soil sample

9. Rubber stoppers to fit measuring cylinders, or plunger and rod to fit cylinders, for mixing soil suspensions.
10. Volumetric flasks, 1000 mL
11. Stopwatch, or clock with sweep second hand

Chemicals

1. Hydrogen peroxide, 30% solution, GPR grade
2. Amyl alcohol
3. Sodium hexametaphosphate, technical grade

Reagents

1. Sodium hexametaphosphate, 10% solution: Dissolve 100 g of sodium hexametaphosphate in 1 litre of distilled water. This solution should not be stored over one month.

Procedure

1. Weigh 50 ± 0.5 g of air-dry soil, sieved to pass a 2 mm sieve, into a 400 mL beaker. If soil is very sandy, use 100 g of soil. In each day's analysis, include one standard soil sample and one blank.

2. Add 125 mL of distilled water and stir the mixture to wet the soil thoroughly.

3. Place beakers with soil into a hot water bath at 85 to 90°C.

4. Add 5 mL 30% hydrogen peroxide and stir gently with a stirring rod. If necessary, add 1 or more drops of amyl alcohol to minimize foaming. Cover with a watch glass. Add further 5-mL portions of hydrogen peroxide until reaction (frothing) ceases, indicating complete destruction of organic matter. Unless soil is high in organic matter, about 20 mL total of hydrogen peroxide is usually sufficient.

5. Heat the beakers for a short while longer, until no more bubbles appear.

NOTE: Ensure that the hydrogen peroxide is fully destroyed, as bubbles from residual hydrogen peroxide will cause erroneous hydrometer readings.

6. Remove the beakers from the water bath and allow to cool.

7. Add 10 mL of 10% sodium hexametaphosphate solution to each sample. Allow to stand for 10 minutes.

8. Transfer the sample to the mixer cup, and mix for two minutes with the high-speed stirrer. NOTE: If high-speed stirrer is not available, transfer samples to leakproof bottles and shake overnight on a flat-bed or end-over-end shaker.

9. Quantitatively transfer the suspension into a 1000 mL measuring cylinder, using distilled water to wash all soil particles into the cylinder. Fill to the 1000 mL mark with distilled water.

10. Prepare a blank cylinder containing 10 mL of 10% sodium hexametaphosphate solution, and fill to 1000 mL with distilled water.

11. Thoroughly mix the cylinders by fitting with a rubber bung and inverting the cylinder 10 times. Alternatively, the cylinders may be mixed with a circular plunger attached to a metal or wooden rod. Start the stopwatch immediately when mixing is complete.

12. After mixing, quickly add 2 to 3 drops of amyl alcohol to the cylinder, and after 20 seconds place the hydrometer gently into the suspension.

13. At 40 seconds, take a hydrometer reading and measure the temperature of the suspension. Also take a hydrometer reading in the blank cylinder.

14. Allow the cylinders to stand undisturbed for two hours. Avoid locations which are windy or in direct sun.

15. After two hours, take hydrometer and temperature readings in both sample and blank cylinders.

Calculations

1. Corrected hydrometer readings

a) Corrected hydrometer reading at 40 seconds (PSH40COR):

(PSH40SAM - PSH40BLK) + [(PST40 - 20) 0.36]

b) Corrected hydrometer reading at 2 hours (PSH2HCOR):

(PSH2HSAM - PSH2HBLK) + [(PST2H - 20) 0.36]

where PSH40SAM = Hydrometer reading at 40 seconds for sample
PSH40BLK = Hydrometer reading at 40 seconds for blank
PST40 = Temperature at 40 seconds
PSH2HSAM = Hydrometer reading at 2 hours for sample
PSH2HBLK = Hydrometer reading at 2 hours for blank
PST2H = Temperature at 2 hours

2. Percent clay (CLAY)

$$\frac{(\text{PSH2HCOR})\ 100}{\text{PSSLWT}}$$

where PSSLWT = Weight of air dry soil (g)

3. Percent sand (SAND)

$$100 - \left[\frac{(\text{PSH40COR})\ 100}{\text{PSSLWT}}\right]$$

4. Percent silt (SILT)

100 - SAND – CLAY

Section 3. DRY SOILS--ROUTINE ANALYSES

3.1 SOIL PH IN WATER

Background

This standard method uses a soil:water ratio of 1:2.5.

Equipment

1. pH meter
2. Multiple dispenser, 25 mL (Custom Laboratory Equipment)
3. Stirrer, 33 place (Custom Laboratory Equipment)

Supplies

1. Combination electrode for pH meter
2. Calibrated spoon, 10 mL (Custom Laboratory Equipment)
3. Plastic bottles, 60 mL, with holders (Custom Laboratory Equipment)

Consumables

1. pH 4 buffer
2. pH 7 buffer

NOTE: The pH of buffer solutions should bracket the expected pH values of soil samples.

Procedure

1. Extraction

Analyses are conducted in batches of 33 with 30 soil samples, 2 repeated samples and 1 standard soil sample.

a) Scoop 10 mL of soil and add to 60 mL bottle.
b) Add 25 mL distilled water to bottle with dispenser.
c) Stir for 10 minutes on the 33-place stirrer.
d) Let stand for 20 minutes.
e) Stir again for 2 minutes.

2. Calibration of pH meter

a) Immerse the electrode into pH 7 buffer.
b) After the reading stabilizes (about 1 minute), adjust the buffer knob on the meter to read 7.00.
c) Remove electrode, rinse with distilled water, and touch off the remaining drop of water with tissue paper. NOTE: Do not wipe the electrode tip with the tissue, as this can create static charge and cause unstable readings.
d) Immerse the electrode into pH 4 buffer. After 1 minute, adjust the slope (or sensitivity) knob of the pH meter to read 4.00.
e) Repeat the calibration until the values obtained for pH buffers agree within ± 0.02 pH unit of the theoretical values.

3. Determination of soil pH

a) Immediately before pH measurement of each sample, stir the sample 5 seconds with a glass or plastic stirring rod. Allow the soil to settle 30 seconds before proceeding. Do not continue stirring during pH measurement.
b) Immerse electrode into 60 mL bottle with soil. Always immerse the electrode to the same depth in the bottles, because repeatability of readings depends upon the procedure being exactly the same each time. Take care not to strike the bottom of the sample bottle with the electrode tip.
c) Record pH reading after reading stabilizes. About 30 seconds to 1 minute is usually sufficient. If pH reading is very slow to stabilize, it is probably due to malfunction of the combination electrode. Follow manufacturer's instruction for maintenance of electrodes before proceeding.
c) Remove electrode from bottle, rinse with distilled water, and continue with samples. Soil pH for the standard soil should be repeatable to about ± 0.1 pH unit.
d) After each 11 samples, re-check one of the buffer solutions to ensure instrument and electrode stability. After each tray of 33 samples, check and record pH values for both buffer solutions. If values are more than ± 0.02 from theoretical, reset the correct values before continuing with samples.