UNIVERSITY OF NAIROBI

DEPARTMENT OF CHEMISTRY

HEAVY METALS IN SUGARCANE JUICE AND SOILS: A COMPARATIVE STUDY OF PART OF NAIROBI RIVER BASIN AND NYAHURURU FARMS

BY:

SOLOMON KAMAU

I56/79623/2012

A Thesis Submitted for Examination in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Analytical Chemistry of the University of Nairobi.

2016

DECLARATION

I declare that, this is my original work and has not been submitted elsewhere for examination, award of a degree or publication.

Signature…………………………Date……………………

Solomon N. Kamau

I56/79623/2012

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

	Signature	Date
Prof. Lydia W. Njenga	……………………	……………………
Department of Chemistry		
University of Nairobi		
Mr David M. Maina	……………………	……………………
Institute of Nuclear Science and Technology		
University of Nairobi		

DEDICATION

To my beloved parents, Dad Sammy B. Kamau and Mum Grace N. Kamau for their enduring support.

ACKNOWLEDGMENT

ABSTRACT

The contamination of food is increasingly becoming a concern in developing countries due to the mounting amounts of heavy metals in urban environments. Juices are common beverages in the usual diet and contribute significantly to the nutritional intake of essential and harmful trace elements. Currently, there is inadequate information regarding the amounts of heavy metals in juice from sugarcane produced along rivers in Nairobi city. In the present study, the levels of heavy metals in sugarcane juice and soils from Nairobi and Nyahururu are determined. This study quantitatively determined the levels of heavy metals such as Fe, Cu, Zn and Mn in 32 juice samples and 16 soil samples. The analysis of the juice and soils were done using the Total Reflection X-Ray Fluorescence (TXRF) and Energy Dispersive X-Ray Fluorescence (EDXRF) techniques respectively. The general results of soil samples varied from 39958-126667, 2196-8683, 141-834 and 28.9 – 123 mg/kg for Fe, Mn, Zn and Cu respectively. The amounts of Fe, Mn, Zn and Cu in the juice varied from 4.63- 49.07, 1.82-31.38, 0.37-12.32 and 0.14-0.92 mg/l respectively. The transfer factors of the heavy metals in the sugarcane juice indicated a trend in the order: Fe < Mn < Cu < Zn. Significant differences in the trace element concentration in samples from Nairobi and Nyahururu were observed. Greater levels of Mn, Zn and Cu were observed in juice samples from Nairobi. The Zn levels in juices from Kibera and Tasia samples exceeded the Food and Agriculture Organization/World Health Organization maximum permissible limits for the metal (5mg/kg). The levels of Fe and Mn in the soils were noticeably higher than amounts observed for other heavy metals. Unlike Nairobi samples, the amounts of metals in Nyahururu samples were found to have close relationships between the various samples. It was observed that levels of Zn and Cu in Nairobi soils were higher than those from Nyahururu. The Korogosho, Dandora Mile 7, Mathare and Kibera soil samples were found to have higher concentrations of heavy metals than the rest. Some of the heavy metal levels in the soil were beyond the permitted levels for agricultural soil. Since the impact on health of human beings regarding heavy metals is usually from consumption of plants grown on contaminated soils, the data and information obtained from this study can be used to curb this problem.

Keywords: accumulation, heavy metals, Saccharum officinarum and transfer factors

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION ACRONYMS AND SYMBOLS

AAS   Atomic Absorption Spectroscopy

EDXRF   Energy Dispersive X-ray Fluorescence

TXRF   Total reflection X-ray Fluorescence

XRF   X-ray Fluorescence

WHO   World Health Organisation

FAO   Food and Agriculture Organization

US   United States of America

EU   European Union

GPS   Global Positioning System

GLONASS   Global Navigation Satellite System

UNEP   United Nations Environmental Programme

NEMA   National Environment Management Authority

CCME   Canadian Council of Ministers of the Environment

CHAPTER ONE

INTRODUCTION

1.1 Background

The interaction of heavy metals with the environment is an important issue in science. These elements are a collection of metals or metalloids that have large specific density and are usual constituents of the earth's crust (Nogueira *et al*., 2013). Even though heavy metals are abundant in the earth crust they are usually in trace quantities in plants and in a human body (Alissa & Ferns, 2011). Some of these metals are important for metabolic processes and, in fact, organisms may show nutrient deficiency when these elements are at relatively low avialabilty. On the other hand, heavy metals can be toxic if they are in excess amounts in the body while some are poisonous even in trace amounts.

The source of heavy metals in the human body is primary food (Mudgal *et al*., 2010). Human beings are exposed to heavy metals by inhalation, ingestion and dermal contact absorption (Wei & Yang, 2010). However, it is the ingestion of food contaminated with heavy metals that contributes upto 90% of exposure to the toxic elements (Khan *et al*., 2013). This is because there is a constant need to consume food crops since they are the source essential nutrients. Some of the plants have an ability to absorb and retain heavy metals using parts that are exposed to air in a polluted environment or from contaminated soils (Inoti *et al*., 2012). Therefore, it has generated numerous studies on the harmful food contaminants such as heavy metals (D'mello, 2003).

Soil is considered to be an important resource that provides a habitable environment and plays a major role in food production and hence important in sustaining essential human needs. Consequently, any heavy metal contamination of this resource will result in the possible contamination of food crops. Indeed, high amounts of heavy metals in soils are resulting to increased concentrations of these elements in food crops. Elevated concentrations of heavy metals in soil can be attributed to both natural and anthropogenic causes (Park *et al*., 2011). However, it is the rapidly rising anthropogenic activities that are predominantly leading to heavy metal enrichment in soils. For example, metal mining and smelting contributes more than half of

the estimated annual worldwide emmision of Cd (22 10^6 kg), Cu (954 10^6 kg), Pb (796 10^6 kg) and Zn (1372 10^6 kg) into soils (Jung, 2008).

The heavy metal contamination of food is a vital environmental problem that is increasingly becoming severe all over the world and particularly, in the developing nations (Akinyele & Shokunbi, 2015). Due to increased population and high levels of poverty, developing countries are under pressure to industrialize. This has led to rapid urbanization with increased growth in the number of industries. Consequently, industrial waste with elevated amounts of heavy metals is mounting. Most developing countries have poor methods of waste disposal and therefore industrial effluent rich in toxic metals is dumped into rivers and landfills. Moreover, urbanization has influenced production of food crops on the contaminated land and using waste water for irrigation. As a result, crops cultivated in urban areas are often found to contain heavy metals levels that are beyond the permitted amounts (Kapungwe, 2013; Kihampa, *et al*., 2010; Inoti *et al*., 2012; Nabulo *et al*., 2010).

It is the properties of heavy metals in the environment and their interaction with plants that make these elements interesting. These metals are non-biodegradable and tend to persist in the environment, and form soluble compounds. In addition plants are inclined to bioaccumulate heavy metals whereby plants absorb and retain these elements over time (Du *et al*., 2013). The concentrations of elements tend to increase through the food chain in a process known as bio-magnification. Human beings are usually at the tail end of the food chain and therefore at great risk of having unhealthy concentrations of heavy metals.

Urban agriculture is one of the activities that contribute significantly to heavy metal bioaccumulation and consequent biomagnification. It involves farming of food crops, horticulture, poultry and even livestock in cities and towns (UNEP, 2001). It is often done in waste dump sites as a result of their organic matter deposits and plant nutrients. However, waste dump sites are poorly managed and are used to discard sewage and industrial wastes which have heavy metals, dangerous organic chemicals, salt and high pH. In fact research has shown that there has been a rise in amounts of heavy metals in the urban environments of emerging nations such as Kenya due to rapid and disorderly urban expansions (Inoti *et a*l., 2012). Plants cultivated under these environments could absorb and retain high amounts of these metals. When the crops are consumed over a period they pose health risk to human beings.

2

It has been reported the urban agriculture in contaminated environments is extensive in Nairobi city (UNEP, 2001). A report by United Nations Environment Programme (2001) reported of intensive farming of sugarcane, arrowroots and vegetables along rivers along the Nairobi city. It also noted that the rivers and banks in the Nairobi river basin were likely to be polluted due to the numerous industrial activities and large human settlements in the areas where the rivers pass. It reported that pollution of the river basin begins from Dagoretti, the point where the rivers enters the city and increases as it flows through Riruta, Kileleswa, Chiromo, Kijabe Street, Kirinyaga road, Gikomba Market, Bahati, Eastleigh, Mathare, Korogocho, Kariobangi and Dandora before joining Athi River near Ruai.

Sugarcane is associated to the Graminae family and its genus is Saccharum. It exists three species: *Saccharum officinarum, Saccharum spontaneum* and *Saccharum. robustum* (Kent, 2007). Its origin can be linked to New Guinea island approximately 8000 B.C. The plant spread to other areas of Asia and later to the rest of world. The farming of the plant was introduced in Kenya in 1902, largely in the western part of the country. Within Nairobi County, it is commonly cultivated along rivers in the Nairobi river basin. It grows to a height of 2-6 m and contains glucose, fructose, sucrose, and other nutrients for good human health (Tzeng *et al*., 2010). It is therefore a vital plant to human beings and in fact is the top ranking crop worldwide with a production of more than 1.59 billion tonnes annually. However, the plant has been shown to accumulate heavy metals in concentrations that are beyond permitted levels by the World Health Organization (Abdus-salam *et al*., 2008). This implies that prolonged consumption of contaminated sugarcane could be detrimental to human health.

1.2 Statement of the problem

There has been an increased public awareness and concern on the safety of food sourced from urban and peri- urban farms in developing countries. It has been prompted by the ever increasing pollution of the urban environments. Cities in developing countries have experienced unplanned massive expansions accompanied with the disregard to the environment and hence, leading to the dumping of waste into rivers and on land. In addition, as a result of the large population and a high cost of living there has been extensive farming within these cities (Shackleton *et al*., 2009.) Crops farmed in these urban environments are often found to have large concentrations harmful substances.

Kenya has experienced increased pollution of her urban environments and heavy metal contamination of crops farmed in these locations. For example Inoti *et al*., (2012) reported on heavy metal contamination of soils and plants in Thika town. However the main degradation of Kenyan urban environment is happening along rivers of the Nairobi river basin. In fact recent studies have reported on the degradation of the environment and contamination of food crops along these rivers (Mutune *et al*., 2014; Ndeda & Manohar, 2014; Orwa, 2015, Sirengo, 2001). Nevertheless, most studies have focused on vegetables and have ignored sugarcane which is consumed raw.

Since the impact on human health from heavy metals is usually from crops grown on contaminated soils, sugarcane cultivated along rivers in Nairobi County are of interest. A report by UNEP, (2001) warned on the pontential contamination of plants that are cultivated along the rivers in Nairobi County. Among the widely grown crops in the river basin is sugarcane. Several studies have shown that sugarcane is capable of absorbing and retaining heavy metals in significant amounts (Abdus-salam *et al*., 2008; Sereno *et al.,* 2007; Zhang *et al.,* 2014; Nogueira *et al*., 2013). Therefore there was a need to study the heavy metal content in soils and sugarcane along the river and compare with other samples from a non-urban area. This is because there was no adequate information on the heavy metals levels in sugar cane grown along the Nairobi Rivers. There is also insufficient data on the effect of farming sugarcane on Nairobi soils as compared to those cultivated in a rural area.

1.3 Objectives

1.3.1   Overall Objective

The overall objective was to investigate the heavy metal levels in sugarcane juice and soils in part of Nairobi River Basin and compare with samples from Nyahururu farms.

1.3.2   Specific Objectives

1. To investigate the heavy metal levels in sugarcane juice from plants grown along Nairobi, Ngong and Mathare rivers of the Nairobi River Basin, and in farms from Igwamiti, Subukia and Mairu Inya places in Nyahururu.

2. To assess the concentrations of heavy metals in the soils affiliated to the sugarcane of interest.

3. To carry out a comparison of the amounts of heavy metals in sugarcane juice and soils from Nairobi and those from Nyahururu farms.

1.4 Justification and significance of the study

There is a great concern arising from food contamination from heavy metals. This is because of the fact that more than 90% of human exposure to harmful heavy metals is through ingestion of contaminated food (Khan *et al*., 2013). Foods are primarily contaminated by the cultivation of plants on polluted soil.

Urban agriculture has posed a risk to heavy metal accumulation of food crops. Rapid and unorganized industrialization coupled with poor management of waste have resulted to large amounts of heavy metals in environments of urban areas in developing countries, particularly in soils (Kihampa, *et al*., 2010). There has also been an increased agricultural activity in these urban areas with sugarcane cultivation being common. Plants grown in these environments could absorb and retain high amounts of heavy metals.

It is important to provide facts of sugarcane cultivated in urban areas since information relating to heavy metal accumulation in the plant is inadequate. Sugarcane is among the most important plant grown in the world and therefore a concern that very few studies have paid attention on the amount of heavy metals in sugarcane (Collin & Doelsch, 2010). Furthermore, consumption of sugarcane grown in potentially polluted soil could lead to adverse health effects to human beings.

This research intends to provide accurate and reliable information on sugarcane grown in an urban area and gives a comparison with those grown in rural farms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

There are certain class of metal elements whose interactions with living system are considered either essential or toxic. These elements have specific density of more than $5g/cm^3$ and are referred to as heavy metals. Common examples include Pb, Cu, Zn, Cd, Fe and Hg. Some of the metals, in trace concentrations, are crucial for survival of plants and animals. However at certain concentrations the essential elements become harmful to the organism. In addition some elements are harmful even in trace amounts. The difference from an element to be considered essential to toxic depends on the metal itself and its quantity in an organism. For example zinc is micronutrient for growth of animals when present in trace amounts while lead is poisonous even in trace quantities. The impact on human health from heavy metals has been from occupational exposure, environmental contamination and accumulation in foods. They are known to have poisonous effects that lead to chronic degenerative changes nerves system, organs, and are also known to be carcinogens and teratogens (Karalliedde & Brooke, 2012).

Plants form an important part of diet for both human beings and animals. As plants extract essential nutrients from soil, they also uptake non-essential and toxic elements to their tissues. These elements build up amounts over a period and they could reach toxic levels at the edible parts (Wang *et al.,* 2012). Heavy metal accumulation and bio-magnification across the food chain pose a health risk to both plants and animals. Therefore, importance in determining heavy metals in foods and other food contaminants have been increasing. On the other hand, the characteristic tendency of some plants to accumulate relatively large concentrations of heavy metals has resulted to an interest in using these plants for environmental treatment of soil contaminated by heavy metals.

Plant uptake of heavy metals and resultant accumulation is determined by several factors. Heavy metals are both essential and toxic to plants. Moderate concentrations of some of these elements in the soil are a requirement for plant growth. However, soils with relatively large heavy metals levels hinder plant growth. Other factors that influence heavy metal uptake by plants are organic

content, soil pH, cation exchange capacity, metal content and solubility sequence, and plant species (Abdus-salam *et al.,* 2008; Chandra *et al.,* 2008).

Though heavy metals are natural components of the earth's crust, anthropogenic activities progressively contribute to the elevated concentrations in the soil. Heavy metal pollutants have chief distinction with organic pollutants; they are not subject to chemical change or bio-degradation (Zhang *et al*., 2008). Therefore continuous addition of heavy metals into the environment has resulted to accumulation of these elements. For example heavy metal pollution of agricultural land has been due to activities such as mining, agronomics and irrigation with water from industrial waste. Due to the increasing economic development and human activities, this problem is expected to intensify (Zhao *et al.,* 2012).

2.2 Heavy metal in soils

Heavy metals origins in soil are both natural (geogenic or pedogenic) and anthropogenic (Khan *et al.,* 2013; Zhao *et al.,* 2012). They are constituents of the earth's crust and therefore it is expected that soil will have a substantial amount of these elements. However human activities have also resulted to additional amounts of the elements in the environment. The fact that the metals in the environments could attributed to these two sources has been illustrated by Jirsa *et al*., (2013) findings on the high levels of Zn and Fe in Lakes Nakuru and Bogoria sediment. They concluded that an elevated Fe level was because of the volcanic activities in the area. Fe is the most plentiful metal in the earth crust and so that observation was expected. On the other hand the unanticipated high amount of Zn in Lake Nakuru sediment could only be attributed to the anthropogenic activities in Nakuru City.

Heavy metals from geogenic sources are usually present in forms that have low availability to plants. The processes of sorption and desorption determine the availability of specific chemical species and their content in the soil (Minkina *et al.*, 2012). This is determined by the interaction between metals and soil's solid phase, water content and constituent air which is comprised of four physico-chemical processes: cation exchange, co-precipitation, specific adsorption and organic–ligand complexation (Omwoma *et al*., 2010). These process are dependent on covalent bonding with the matrix of the soil, metal-anion exchange at the surface of the soil and creation of water insoluble precipitates. The metals availability plant uptake is highly influenced by the elements' solubility and mobility. It has been found that heavy metals in soils resulting from

natural sources have relatively low mobility and hence low phyto-availability. Collin & Doelsch, (2010) found out that despite the high levels of heavy metals in soil from natural sources, the elements had low mobility and a phyto-availability that was below the threshold. They found, for instance, that the mobile faction of Cr, Cu and Zn was very small. The measured mobile Zn concentration was 0.81% of the total amount of the metal in the soil. This phenomenon was confirmed by the concentration of these metals in the juice which had no significant correlations with the total content of heavy metals in the soil. In fact the highest correlation coefficient (r^2) was a mere 0.19 for Cu.

It is anthropogenic activities that are the main cause of increase metal levels in soils and accumulation in plants (Park *et al.,* 2011). Accumulation of heavy metals in plant is influenced largely by bioavailability of the elements in soil. The dynamic process of heavy metal bioavailability in soil is dependent on combination of biological, chemical and environmental parameters (Hajar *et al*., 2014). Metals resulting from anthropogenic sources have speciation that influences positively their bioavailability in soil. Hu *et al*., (2014) compared the total heavy metal concentrations and the bioavailable (Diethylenetriaminepentaacetic acid - extracted) in paddy fields and deduced a strong positive correlation ($r_{Zn} = 0.435$, $r_{Cd} = 0.855$, $r_{Mn} = 0.735$, $r_{Cu} = 0.798$, $r_{Ni}=0.812$, $r_{Co} = 0.580$; $p<0.01$). They concluded that the heavy metal bio-availability of was strongly influenced by the total levels of the metals in the soil which was as a result of pollution of paddy fields.

Soils plays an important role in our society as it is the basis on which food crops are grown. However it is also the sink for most heavy metals. Heavy metals are not subject to chemical or biological degradation and therefore heavy metal pollution of soil poses long-term environmental problem with serous health implications on human beings and animals. Moreover, contaminated soil is the primary route of heavy metal exposure to human beings (Mahmood & Malik, 2014; Wei & Yang, 2010). In fact it has been suggested that soil ingestion is the main source of Pb in children's blood (Florido *et al*., 2011). In addition, Oyoo-Okoth *et al*., (2013) linked the heavy metal concentration in children's hair to those detected in the soil. For example the geometric mean of Cr levels in children hair varied from 0.4 to 1.6 µg/g at exposed locations as compared at the reference site which had four times less (0.1–0.4 µg/g). The Cr amounts in soil from the

8

reference site were 6.7 µg/g while the levels in the exposed areas were 11.8 µg/g. The exposure was attributed to dust inhalation, water, soil ingestion or through food crops.

Although heavy metals do not degrade, the chemical conditions in the soil determine the fate and transport of the metals from the source (Omwoma *et al.,* 2010). The chemical form of a metal element in soil controls its availability, non-availability and bioaccumulation in soils and plants. There are several factors that influence the chemical form of an element in soil: parent materials, soil organic matter, crop residues, soil pH, solubility, presence of other metals, fertilizers and the type and amount of clay (Barzegar *et al.,* 2005; Nayak *et al*., 2014). These factors create chemical characteristics of the soil that determines the solubility and mobility of the metals. For instance, pH of soil and organic constituent influences significantly mobility of metals in soils (Yang *et al.*, 2010). Kamau *et al*., (2014) reported a positive correlation value ($r = 0.67$) from a comparison between sediment pH and dissolved Zn concentration which was meant that an increase in pH corresponded to a bigger amount of Zn in solution. This was attributed to chemisorption of oxides and aluminosilicates at higher pH values. They also reported positive correlation ($r = 0.50$) between dissolved Zn and organic matter, which was due to the release of organic bound Zn. Harguinteguy *et al*., (2014) also reported significant correlations ($r_{Cu}=0.58_{p<0.001}$, $r_{Pb}=0.32_{p<0.05}$, $r_{Zn}=0.46_{p<0.001}$) between heavy metal concentration and the organic content in sediment from Suquía River in Argentina.

Activities such as industrialization, urbanization, agriculture and mining are among the main causes of elevated amounts of heavy metals. Mining and application of waste water or sludge have been singled out as the leading sources of heavy metal pollution of soil (Jung, 2008; Li *et al.,* 2006). It is projected that approximately 240, 0.5, 310 and 250 million tonnes of Pb, Cd, Cu and Zn respectively, have ended up in soil as a result of mining activities (Yun-Guo *et al*., 2006). Muohi *et al*., (2003) used the Turkey honest test to reveal that there was a substantial difference ($p = 0.00012$) for the average amounts of Cd, Pb, Zn and Cu between Makupa Creek and Port Reitz Creek. The notably elevated values were attributed to the increasing anthropogenic activities. Kelepertzis, (2014) also used the Turkey honest test to detect significant statistical differences between the levels of Pb ($p = 0.044$), Cd ($p = 0.013$), Zn ($p= 0.001$) and Cu ($p = 0.000$) in agricultural soil and those from reference samples. Therefore, it was an indication that agriculture was contributing to the increase of heavy metals levels in soils. A study by Ngure *et*

al., (2014) reported that concentration of As, Pb, Hg and Cd in the Lake Victoria region had exceeded the maximum allowable concentration standards by both World Health Organization (WHO) and Food and Agriculture Organization (FAO). For example they reported that the concentration ranges of Cd and Pb in soil were 4.5-570 and 5.5-619 mg/kg in comparison to WHO/FAO limits 0.1-10 and 10-70 mg/kg respectively (Ngure *et al*., 2014).

Several works have shown that the use of sludge for agricultural activities has resulted to the significant increase of heavy metals levels in the environment and their availability (Jamali *et al*., 2009). Latare *et al*., (2014) detailed that application of sludge could lead to an increase of extractable Zn and Fe content in post-harvest rice soils by 68% and 49% and in post-harvest wheat soil by 87% and 36% respectively. Another study by Florido *et al*., (2011) reported statistical correlation between the total concentration of Zn (r = 0.97), Cu (r = 0.72) and Pb (r = 0.6) in soil and the available amounts of the metals after the soil had been amended with bio-solid from waste water treatment. Similarly, there was an increase of Mn, Cu, Cd and Zn by 40% and 20% for Pb, Ni and Cr concentrations in sludge amended soil in comparison to the unamended (Singh & Agrawal, 2007). Moreover, Nogueira *et al*., (2013) reported that sludge application in soil led an increase of extractable Cd (p<0.01) and total recoverable Cd (p<0.01). The authors also reported significant corelation between extractable Cd and total recoverable Cd (r^2=0.91)in soil which was a comfirmation that sludge was the source of the element.

The heavy metals sources in soil can be indicated by the use of correlation studies. The ratios of specific metal element to other metals in a soil can indicate the parent material from which the soil originated from. The elements concentrations in the soil are usually closely correlated. However, an external source can alter the proportions of the metals in the soil. Therefore the initial association between the amounts of metal in the soil could be eliminated and new ones formed depending on the external source. For example a study by Nabulo *et al*., (2010) reported a close correlation (r^2 = 0.90) between the concentrations of Zn and Cd in soil. It was an indication that the two elements had shared industrial and geochemical source. The deficiency of covariance for the other metal concentration showed that they originated from different sources rather than a common one.

2.3 Heavy metals in plants

The tendency of plants to accumulate heavy metals in substantial amounts has ramifications on human and animal health. The existence of plants is heavily reliant on the water and nutrients in soils. Plants absorb trace metal elements from the soil and retain them in their tissues. Most edible crops do not discriminate in the extraction of nutrients from the soil and therefore uptake unwanted heavy metals as well as required vital nutrients (Oyedele *et al*., 2008). Heavy metal uptake by plant and resultant bio-magnification across the food chain and bioaccumulation in human and animal tissues is important to both the environment and human health (Singh & Kalamdhad, 2013). Nakayama *et al*., (2012) compared the amount of heavy metals in plants and that in *hippopotamus amphibius* liver and reported a bio-accumulation factor value 5.0 for Hg an indication of bioaccumulation of the metal from plant to the animal. Several factors influence the heavy metals concentration within and on the plants. They include atmospheric deposition, climate, type of soil where a plant is growing and plant's maturity at the time it is harvested.

Plants uptake of trace elements is distinctive for each element and varies with species or varieties. The interaction of individual elements with plants is relative to factors specific to that element. For example, uptake of Cu is depended on total amount of Cu in the soil and plants ability to transfer the metal across the soil–root interface (Hajar *et al*., 2014). Rahman *et al*., (2014) reported that rice samples Bangladesh, Vietnam, India and Pakistan had substantial differences in the heavy metal concentrations in comparison with Australian grown rice. For example the amount of Cu, Mn, Pb and Zn in Bangladesh rice was 1.6 µg kg^{-1}, 14.7 µg kg^{-1}, 19.0 µg kg^{-1} and 13.4 µg kg^{-1} as compared to those in Australian rice which were 2.9 µg kg^{-1}, 24.4 µg kg^{-1}, 375 µg kg^{-1} and 17.1 µg kg^{-1} respectively. It was also observed that the levels of heavy metals in different Australian grown rice varieties differed significantly. It reported that the concentrations of Cu, Mn, Ni and Zn in brown rice varieties were higher than those in white rice varieties, demonstrating that Australian brown rice could be a potential contribute to dietary heavy metals. Another study by Li *et al*., (2007) reported great differences in metal concentrations in plants species with Mn ranging from 16-3280 mgkg^{-1}, Cd 0.06-2.27 mgkg^{-1}, Cu 2.9-27.2 mgkg^{-1}, Fe 88-9311 mgkg^{-1} and Pb 0.2-10.6 mgkg^{-1}.

The connection between plants and soil, in relation to heavy metals, is classified into three types namely excluders, indicators and accumulators. Excluders are plants that limit metal transport to

the shoot and retain low amounts of the metals in the shoot in comparison to the levelsof the metals in the soil. Indicators are plants that show a response to metal levels in the soil. Accumulators are plants that tend to translocate and retain relatively large amounts of heavy metals in sections of the plant that are above ground. A group of the accumulators called hyper-accumulators have the ability of retaining more than 1000 ug/g. For instance, Ghaderian & Ravandi, (2012) found out plants such as *Polypogon fugax* and *Epilobium hirsutum* Cu concentrations were 4000 and 1500 µg/g respectively and therefore could be considered as Cu hyper-accumulators.

For plant species that can accumulate a certain heavy metal species, it must able to resist the harmful effects of that element. Since heavy metals can be toxic, plants are required to have a certain level of tolerance to the elements in order to accumulate these elements in their tissues. The heavy metal tolerance level of a certain plant to is associated to the balance between the rate of metal uptake and their detoxification effectiveness within that specific plant (Hajar *et al*., 2014). Some plants species have developed strategies to resist accumulation of high amounts heavy metals. Li *et al*., (2007) reported that *Melastoma dodecandrum* had surprisingly normal levels of Fe, Mn, Pb and Cu which was an indication of the plant's ability to avoid metal accumulation.

Plant tolerance to heavy metals is evaluated using different methods. Bio-concentration factor describes the accumulation level of chemical elements in plants and is derived by the ratio of the amount of metal in the plant and the total amount in the soil. Another index is the translocation factor which is the ratio between the metal concentration in the plant and the amount of the metal in the soil that is mobile (Minkina *et al*., 2012). Transfer factor is an important parameter that describes the bioavailability of an element at a specific part of a plant species. It is the ratio of the metal concentration in the edible part and the total metal levels in the soil (Gebrekidan *et al*., 2013). The effectiveness of diverse plants species in absorbing the heavy metals can be assessed by either soil to plant transfer factors or plant uptake of the heavy metals (Wang *et al.,* 2012).

Several studies have also shown that crops accumulate trace elements varyingly within the different plant parts. Hajar *et al*., (2014) investigated the amounts of metal in the leaves, flowers and stems of *Stevia rebaudiana* and reported that the metals were concentrated differently in the different parts of plants. For instance the amount of As and Cd was in the order leaves < flowers

< stems, while the amount Cu were in the order flowers < leaves < stems. Amount of Fe was in the order stems < flowers < leaves. Another study by Li *et al*., (2006) reported that a general trend where the roots accumulated slightly larger Cd, Pb and Cu than the leaves. Minkina, *et al*., (2012) also reported different amounts of Zn, Pb and Cu in the various parts of barley. They found out that Zn amounts were higher roots, Cu in the grains while Pb in the straw.

Waste water irrigation is a practice that has resulted to an increase in levels of heavy metals in soils and plants. In order to sustain farming of crops throughout the year, irrigation of plants is usually done substitute dry seasons. However water is a scarce resource and hence the use of waste water has increased. The waste water, especially in urban environments, usually has high levels of heavy metals. The practice of waste water irrigation has therefore resulted to an increase of amounts heavy metals in soil and plants (Muchuweti *et al*., 2006; Zhao *et al*., 2012). An investigation on heavy metals in vegetables by Inoti *et al.,* (2012) attributed elevation of these elements in the plants to irrigation with waste water. Furthermore, the amounts of Fe, Cd, Cr and Pb in soils from the vegetable farms varied from 16225-11803, 9.43-21.4, 1806-2020 and 57.7-693 mgkg^{-1} respectively. These amounts were above the permitted levels for agriculture of 425, 0.1, 100 and 0.3 mgkg^{-1} for Fe, Cd, Cr and Pb respectively.

Another activity that contributes to increased amounts the metal in plants is the use of sewage sludge for agriculture purpose. Sludge is used in agriculture because of its large content of plants nutrients. However, it is often that sewage sludge is contributor of heavy metals in soils and also changes chemical conditions of soil. Singh & Agrawal, (2007) reported that the amounts of Zn, Pb, Cd, Cu and Cr were higher for plants grown in soils amended with sewage sludge than amounts in plants from unamended soils. The amount of Cd, Ni and Zn surpassed the Indian allowable limits for edible parts of *Beta vulgaris*. The metal uptake increased with the increased sludge amendment with the exception of Pb and Mn. This was indicative that uptake of metals by *Beta vulgaris* was influenced by increasing availability of heavy metals in the soil. However, the discrepancies in the trend for Pb and Mn were because the plants did not absorb all metals uniformly and metal uptake is not concentration dependent for all elements. Another study by Chandra *et al*., (2008) reported that wheat and mustard plants accumulated heavy metals in when irrigated with effluents. The plants retained metals to amounts that were beyond permissible limit and therefore the plants were not suitable for consumption by both human and cattle. Latare *et*

al., (2014) showed that application of sludge could lead to rise in the amounts Zn, Mn, Cu, and Fe in wheat and rice grains. The amounts of Cu, Zn, Fe and Mn increased by 29%, 43%, 23% and 54% in rice and 64%, 40%, 40%, and 38% in wheat respectively. They attributed the increased uptake to the rise of extractable heavy metals content with the use of larger doses of sludge.

Soil pH is singled out as among the important influences that determine heavy metals content in food crops. Soil pH influences the solubility and mobility of heavy metals by altering their forms in soil. For example, a change in pH would lead to chemical changes in the soil which could convert naturally occurring metals and those introduced by anthropogenic means bioavailable by changing their speciation and influencing chemisorption (Omwoma *et al.,* 2010; Zhao *et al.,* 2011). Zeng *et al*., (2011) reported significant negative correlation coefficients between the soil pH with concentrations of Zn (r = -0.652, P < 0.001), Mn (r = -680, P < 0.001), Cu (r = -0.593, P < 0.001) and Fe (r = -0.501, P < 0.001) in the straw, suggesting that amounts of Zn, Cu, Mn and Fe were lower with relatively higher pH and vice versa.

Another factor that influences the heavy metal levels in plant is the total levels of heavy metals in soil. Soil is an important resource from which plants absorb essential and non-essential nutrients. Even though plants absorb heavy metal from deposits on parts that are exposed to air and soils, the latter is the main origin of these elements in plants. Minkina *et al*., (2012) reported that an increase of Zn, Cu and Pb amountss in soil would result to more accumulation of the metals in the plant. They showed addition of Zn in concentrations of 100mg/kg or more led to concentration in grain that in the amount exceeding the maximum allowable concentration of Zn (50 mg/kg). Khan *et al.,* (2013) reported significant correlation between levels of heavy metals in soil with those in plants. For example Cr and Ni levels in *Zea mays* were significantly and positively correlated (*r* = 0.886 and *r* = 0.975) with amounts of these metals in the soil. There was also a positive correlation (*r* = 0.879) between the levels of Mn in *Benincasa hispada* and in the soil. However, Zn soil concentration revealed a negative significant correlation (*r* = -0.909) with the amount of the element in *Allium cepa*. This denoted that not all heavy metals in the soil were present in extractable forms for plant uptake.

Several studies have reported that a surge in the amounts of heavy metals in soils would result to the increase in the amounts of the metals in plants. This is an indiation of the effect of the

pollution of soil relative to bioaccumulation of heavy metals in plants. Nayak *et al*., (2014) found out that amounts of Fe, Cu, Mn, Zn, Cd, Cr and Pb in rice grain and straw increased with increasing metal concentrations in soil. The metal amounts in straw were greater than those in grain for all metal elements studied with the exception of Cu, which was higher in the grain. This was attributed to the different cellular bioaccumulation mechanisms that influence the translocation of metals in plant systems. Similarly, Hu *et al*., (2014) found out rice grown in polluted soils in paddy fields accumulated heavy metals with Cd being singled out because 90% of the samples exceeding the Chinese maximum allowable amounts (0.2 $\mu g\ g^{-1}$) and 50% had amounts that made the rice toxic (1.0$\mu g\ g^{-1}$). Grytsyuk *et al*., (2006) three years work reported a high positive correlation between the metal concentration and accumulation of the elements in clover and perennial cereal grasses (in some cases $R^2 > 0.70$). It showed increase in concentration of metals in the soil corresponded to an increase in the amounts of the metals in the plant. Harguinteguy *et al*., (2014) compared levels of Cu, Fe, Pb and Zn in *Stuckenia filiformis* for a period of three years and reported a relative increase in the amounts of the metals. They reported a positive significant correlation between amounts of the metals in sediments and the amount in leaves of *Stuckenia filiformis* ($r_{Cu} = 0.55$, $r_{Mn} = 0.53$, $r_{Pb} = 0.62$ and $r_{Zn} = 0.76$, p<0.001).

Plants have been used to remediate contaminated soils in a cost effective and environmental friendly method referred to as phytoremediation (Chen *et al*., 2014). Phytoremediation involves a process of extraction of metals from soils using plants by uptake and accumulation of the elements into biomass (Abreu *et al.,* 2012). The process of removal and retention in the plant's biomass is referred to as phytoextraction. Abreu *et al.,* (2012) reported that the castor oil plant could be used as a viable alternative to decontamination of soil. Another study by Li *et al.,* (2007) suggested that a variety of plants could be used to remedy a restored Mn mine land. They suggested the use of indigenous species *Phytolacca acinosa*, *Castanea henri* and *Pinus massonian* to remedy the mine. Yun-Guo *et al*., (2006) work on several plants showed that *Poa pratensis, Gnaphalium afine, Phytolacca acinosa, Coynza canadensis* and *Phragmites communis* were hyper-tolerant to Mn and had a high tolerance for Pb and Cd. Therefore these plants could be considered for phytoremediation since they had a relatively high ability to accumulate these metals though not as hyper-accumulators. For instance the reported concentrations of Pb, Cd and Mn in *Phragmites communis* were 549.50, 39.65 and 146500.00 $\mu g\ g^{-1}$, respectively.

2.3.1 Heavy metals in vegetables

There have been extensive attentions in the investigation of heavy metal accumulation in vegetables. Vegetables are important as part of the human diet since they comprise carbohydrates, proteins, vitamin, minerals and trace elements (Chen, *et al*., 2013). These plants are also the main crops cultivated in urban zones due to an increase in vegetables consumption because of their food value. These plants also farmed as a survival approach for the urban poor to manage with the declining living standards in developing countries. Increased anthropogenic activities in these urban areas have resulted to an increase of heavy metal pollution of environment. Moreover waste water irrigated agriculture is among the common practice in the urban areas of these emerging countries (Kihampa *et al*., 2010). Vegetables have the ability to absorb and retain heavy metals in large amounts capable of causing harm to consumers. Hence, there have been increased interests to determine the potential risk posed by heavy metal accumulation in vegetables.

Some of the studies on vegetables have reported that these plants are able to retain heavy metals within their biomass to levels that are beyond the allowable limits. Vegetables are known to uptake large amounts essential nutrients along with other harmful elements in relatively short time (Wang *et al.,* 2012). A study by Rahman *et al.,* (2014) reported that leafy crops cultivated in polluted soils had higher levels of heavy metals than those cultivated in uncontaminated soils. 14 species of Australian and Bangladeshi grown vegetables were found to have Cd amounts which were beyond the maximum permitted levels for Australia (100 mg/kg). They also reported that the metal concentrations in vegetables were considerably different as compared to other plant species. After a comparison with rice, vegetables were observed to have exceedingly higher amounts of heavy metals and therefore posed risk as sources of dietary heavy metals. For instance, Cd concentration in rice was 7.5 μgkg^{-1} while in vegetables was 1285 μgkg^{-1}. Similarly, Muchuweti *et al.,* (2006) found out that a leafy vegetable (*Brassica juncea*) easily accumulated Cd levels that were eighteen times the more than the permissible levels. These findings show the tendency of vegetables to accumulate heavy metals in large amounts while maintaining growth and appearing healthy.

It has been observed that soil characteristics influence the amount of trace elements in vegetables. Oyedele *et al.,* (2008) reported a significant correlation between plant uptake of the

metal and heavy metal content in the soil. They also reported that the transfer factor from control site (0.05 for Pb and 0.8 for Cd) varied significantly to a dump site which range from 0.29 for Zn to 4.05 for Pb. This difference was because of changes in phyisco-chemical characteristics at damp locations due to interactions with various soil properties such as pH. Another work by Yang *et al.,* (2014) reported that reductions in soil parameters such as pH and soil organic matter led to phytoavailability of Cd, Zn and Cu and hence higher accumulation in leafy vegetables. The average pH values of the soil in green vegetable production ranged from 5.0 - 8.1. Another study by Li *et al.,* (2012) also found out that the accumulation factor for Cd was greater than for Pb, Cr, Zn, Cu and Ni because of the salty saline soil which improves Cd mobility and hence its accumulation. The average accumulation factors were 0.535, 0.019, 0.016, 0.116, 0.176, and 0.074 for Cd, Pb, Cr, Ni, Zn and Cu respectively. Heavy metals accumulation factor are generally dependent on chemical speciation of heavy metals in soil, crop genetic features as well as other properties, such as pH and salinity. For instance, Li *et al.,* (2012) reported a negative correlation between the AFs of Cd, Cr and Cu with soil pH which was an indication that dissolution of the heavy metals and their respective phyto-availability was enhanced by acidity. Soil acidification enhances bioavailability of state bound cations by inducing chemical processes that make them mobile (Wang *et al*., 2000).

Irrigation of vegetables with polluted water and cultivation in contaminated soils has been contributing to the elevated amounts of trace elements in the plants. Mahmood & Malik, (2014) reported that soil irrigated with wastewater had significantly higher metal amounts in comparison to soil watered with tube well water. The study also revealed that vegetables grown with wastewater had greater heavy metal concentration those farmed with the ground water ($p \geq 0.05$). Moreover, transfer factors of Cu^{2+}, Pb^{2+}, Mn^{2+} and Zn^{2+} for vegetables farmed with waste water irrigated were higher than those from tube. The differences were attributed to increased bioavailability of metals in waste water irrigated soils due to changes in oxidation states of the metals. Another study by Gebrekidan *et al.,* (2013) linked concentrations of heavy metals in vegetables with amounts of the metals in irrigation water and soils. The study was carried out in two areas (Laelay Wukro and Tahtay Wukro) with one being a reference sampling location as it was relatively less polluted; concentrations of Cu, Zn, Fe and Mn in Laelay Wukro were lower as compared to those observed for Tahtay Wukro. Consequently, it was observed mean levels of heavy metals in vegetables planted in irrigated soil samples were greater. For example levels of

Cu, Zn, Fe and Mn in tomato were 8.0, 22.2, 1.1 and 200 mg/kg in Laelay Wukro as compared as compared to 26.7, 46.0, 2.4 and 789.2 respectively, in Tahtay Wukro. A similar study was carried out by Kapungwe (2013) with the main distinction being that there was no reference sampling site. The amounts of Cu (0.58 mg/l) and Cr (0.18 mg/l) in New Farm irrigation water, and Ni (0.12 mg/l), Co (0.09 mg/l), Cr (0.33 mg/l) and Cu (0.23 mg/l) in Chilumba gardens water were found to be above the permitted levels. As a result the amounts of Cu, Pb, Cr and Ni in food crops were all above allowed levels.

The consumption of vegetables sourced from peri-urban farming has been considered as health hazard (Lagerkvist *et al.*, 2013). One of the main aspects in peri-urban farming is the continuous irrigation with wastewater. These actions influence the soil physicochemical properties which increases plant uptake of heavy metals which have adverse effects to human health. Koumolou *et al.,* (2013) investigated on the risk posed by urban farmed vegetables and reported bioaccumulation of Cd and As in the crops; the plant levels of these toxic metals were larger than those in the soil. However, Chen *et al.,*(2013) suggested that well managed farming methods could result reduced accumulation of the metals in vegetables. A harmless greenhouse vegetable system of farming led to more heavy metals in soil than in organic greenhouse vegetable system because of its limited use of irrigation water.

Varieties of vegetables have different capabilities to accumulate heavy metals and each metal element is accumulated distinctly. It takes longer time to transfer heavy metals from the root to the stem and finally into the fruit. This means it's more likely that fruits have lesser amounts of trace elements than other vegetables grown in the same environment. In fact the transfer factors of many elements are influenced heavily by the vegetable species (Wang *et al.,* 2012). Moreover, metal elements have individual factors that influence their accumulation. Wang *et al.,* (2012) reported that concentrations in vegetables were in general order of Cd < As < Pb < Cr < Cu < Zn. For example Pb uptake was dependent on pH, cation exchange capacity and particle size of the soil as well on root exudation and other physio-chemical factors. On the other hand, Cd was uniformly distributed in plant because is a relatively mobile element which makes it easily absorbed by roots and transported to the shoots with ease (Wang *et al.,* 2012). Huang *et al.,* (2014) found that accumulation of heavy metals was different for each vegetable species and metal element. Rape had the highest mean levels of As and Cd (0.022 and 0.046 mg/kg

respectively) while parsley had the highest mean level of Hg (0.029mg/kg) and the highest mean level of Pb (0.0056mg/kg) was observed in spinach. Kihampa *et al.,* (2010) also reported that the concentrations of heavy metals varied in different species. The following trend *Solanum nigram < Amaranthus blitum < Ipomea batata < Cucurbita maxima < Vigna unguiculata* was observed for metal accumulation in the vegetables. This was attributed to characteristics of soil such as pH and organic matter and also the ability of the plant's roots type to penetrate where the heavy metals were concentrated.

Other works have reported that amounts of heavy metals vegetables cultivated in a same setting are relative to each variety. The ability of a metal element to be transported from the soil into different parts of the plants can be expressed by transfer factors. Gebrekidan *et al.*, (2013) reported tranfer factors values for Cu, Mn, Fe, Pb, Zn and Cd were different for each vegetables species. The general transfer pattern for the metals was was Co = Cr < Zn < Ni < Zn < Cu < Mn < Cd < Pb < Fe. A study by Tsafe *et al.,* (2012) revealed transfer factors varied with metal elements (Pb < Fe < Mn < Zn < Ni < Cu) and vegetables species, which could have been due to variances in metal amounts in the soil and differing element uptake by the diverse plants. In addition, Nabulo *et al*., (2010) work on Cd concentration in 15 types of vegetables from Kampala revealed that *Gynandropsis gynandra* had highest ability to accumulate Cd. On the other hand *Cucurbita maxima* had the least ability to accumulate the metal; *Gynandropsis gynandra* Cd amount was 6 times the concentration found in *Cucurbita maxima*.

Leafy vegetables have been found to have conspicuous greater levels of heavy metals when compared with other varieties. Leafy vegetables usually have larger moisture content and higher transpiration rates than other crops. They also open to atmospheric deposition on their leaves which makes leafy vegetables likely to have higher concentrations of heavy metals. Mahmood & Malik, (2014) reported that leafy vegetables such as *Spinacia oleracea*, *Coriandrum sativum*, *Brassica oleracea capitata*, *Brassica campestris* had higher heavy metal concentrations than those found in tuber and bulb type vegetables. Those findings were similar to Muchuweti *et al.,* (2006) who found out that a leafy vegetable (*Brassica juncea*) accumulated higher than other plants in the study. In fact the concentration of Cd in the plant was 18 times more than the maximum permissible level of 0.2 mg kg^{-1}. Findings by Wang *et al.,* (2012) were also in agreement that leafy vegetables tend to accumulate larger amounts of heavy metals in their

edible parts than in roots and fruits. For example the cauliflower transfer factor for Pb ranged from 0.00 - 0.19 as compared those observed for cabbage which varied from 0.07 - 0.29. Another study by Inoti *et al.,* (2012 reported levels of Pb and Cd in spinach (13.3 - 29.5 and 0.41 - 0.70 mg/kg respectively) and tomatoes (3.5 - 20.5 and 0.7 - 0.95 mg/kg respectively) that were beyond the permitted value by World Health Organization (0.1 mg/kg). The reason for noticeable difference in the amount of Pb in leafy vegetable was attributed to depositions from industrial and vehicles emissions.

2.3.2 Accumulation of heavy metals in sugarcane

The importance of sugarcane (*Saccharum officinarum*) cannot be underestimated. It is the highest ranking crop in worldwide with a yearly production in excess of 1.59 billion tonnes (Collin & Doelsch, 2010). It is main raw material for production of sucrose, molasses and bioethanol. Despite it obvious usefulness, it is therefore a concern that very few studies have concentrated on the amount heavy metals in sugarcane. The plant and its products are consumed by billions of people and hence the lack of enough information on the heavy metal accumulation by the plant could adversely affect human health.

A number of studies have found out that sugarcane has the ability to uptake and retain heavy metals in significant amounts. For instance, a study by Abdus-salam *et al.,* (2008) in Nigeria reported sugarcane had the ability to bioaccumulate heavy metals and therefore posed health risk to humans by biomagnification of the elements through the food chain. The study found out that even though the concentrations of Pb and Cd were not detected (below the detection limit of AAS) in irrigation water, the elements were detected in sugarcane juice extracted from plants farmed in the same area (0.16 - 0.42 mg/l for Pb and 0.01 - 0.02 mg/l). These amounts were beyond the permitted levels of World Health Organization standards for drinking water (0.05 mg/l for Pb and 0.01 mg/l for Cd). They also found significant correlation (95% confidence limit) for the two elements which suggested of common source of the pollutants. Muchuweti, *et al.*, (2006) work in Zimbabwe also reported that amounts of Zn and Cu in sugarcane stalks were beyond the permitted levels by European Union (50 mg/kg for Zn) and United Kingdom (20 mg/kg for Cu) guidelines.

It has been reported that amounts of heavy metals in soil influence the amounts of these elements in sugarcane. A study by Kapungwe, (2013) reported that the concentration of Cu (29.45 ± 2.03

mg/kg), Pb (12.09 ± 1.27 mg/kg), Cr (4.21 ± 1.20 mg/kg) and Ni (6.19 ± 1.42 mg/kg) in sugarcane stems were above the permitted limits by Food and Agriculture Organisation/World Health Organisation. They singled out Cu contamination and attributed it to the large amounts of Cu in the soils (219 ± 25.34 mg/kg) from copper mines. Li *et al.,* (2006) found out that sugarcane cultivated in a reclaimed Mn mine had Pb (1.3 mg/kg) and Cd (0.19 mg/kg) levels in their edible parts that were beyond the safety limits and thus not safe for human consumption. This was attributed to large amounts of the two elements in the soil. Nogueira *et al.,* (2013) showed increase of the concentrations of Cu ($p<0.05$), Ni ($p<0.05$) and Zn ($p<0.01$) in soil with the amount in the sugarcane juice. They however could not find correlations for Se and Cr which was an indication that heavy metal amounts in the soil was not the only factor determining levels in the plant. The other factors include type of soil, plant species, and other elements in the soil. For example Barzegar *et al.,* (2005) reported high Cd amounts in sugarcane (15.8mg/kg). This was due to presence of Cl in the soil which is known to increase the bioavailability of Cd. Another work by Adekola & Akinpelu, (2002) indicated that the irrigation water quality also influenced the levels of trace elements in the cane juice. There was a strong correlation between the metal levels in irrigation water and the concentrations of heavy metals in two varieties of sugarcane juice ($r = 0.9848$ and 0.7570). There was also a strong association of the metal concentrations in juice with those in top soil ($r = 0.5677$). The study concluded that indiscriminate farming of sugarcane along riverbeds on which untreated industrial effluents and heavy domestic waste was dumped, was a serious risk on health.

Some sugarcane varieties have been observed to have relatively lower abilities to uptake and retain heavy metal in their biomass. Wang *et al.*, (2012) work on a selection of cane varieties reported that a sugarcane variety (Guiyin9) had comparatively lower heavy metals accumulation in contrast to six other varieties in the study. They also found out that there was no significant correlation between concentration of heavy metal in sugar cane juice and soil's heavy metal concentration. They suggested that the level of heavy metals in the cane's juice is influenced relative ability of the cane to enrich the metals. This finding was similar to Collin & Doelsch, (2010) who reported that there was correlation between total heavy metal concentration in soil and subsequent heavy metal levels in juice. In fact, the highest correlation coefficient (r^2) observed was for Cu (0.19). However, Sirengo (2001) reported that Zn levels sugarcane roots and leaves positively correlated ($r = 0.75$) with the Zn levels in the soil.

On the other hand, sugarcane has been thought as an alternative plant for phytoremediation, a plant based method to remove heavy metals from polluted soils. A suitable plant species suited for phytoremediation should be an efficient accumulator of the metal, produce high biomass and is tolerant to heavy metal concentration in soil. Sugarcane is an outstanding biomass producer and is known to be tolerant to heavy metals. In an investigation by Zhang *et al.,* (2014), soil was induced with Cu (60–125 mg/kg), Zn (150–300 mg/kg) and Pb (500–1000 mg/kg). Despite very high extractable metal concentrations and heavy metal contents greater than the concentration threshold, sugarcane root biomass increased ($p < 0.01$). Sereno *et al.,* (2007) also showed that sugarcane is an outstanding Cd accumulator and also tolerant to high amounts of Cd. They demonstrated that the sugarcane could tolerate 500 µM Cd whereas most convection plants are affected by amounts below 20 µM. The plant also showed Cd accumulation in its shoot tissues to 451 mg/kg. They also reported that sugarcane could accumulate significant amount of Cu (45 mg/kg) even though it is not highly tolerant to the metal.

Studies have shown uneven distributions of various metal elements between the various parts of the sugarcane. Zhang *et al.,* (2014) found out that the distribution of heavy metal in the sugarcane stem was irregular. The top part of the cane had higher Zn concentration ($p < 0.001$) in the juice while higher amounts Pb, Sn and Cu were found to be in the juice from the bottom parts. The retention of most metals in the lower parts is due to the because of heavy metal bindings to negatively charged walls and extracellular precipitation. Furthermore, Segura-Mun˜oz *et al*., (2006) used transfer factors to show most heavy metal were retained mainly in the roots. The soils to root transfer factors for Cu, Mn, Pb and Zn were 0.25, 1.28, 0.93 and 0.67 respectively as compared to soil to stem transfer factors which were 0.23, 1.03, 0.79 and 0.64 respectively. They further noted that amounts of these elements in comestible parts of sugar cane were about 80% to 90% of the amounts detected in the root. This was in agreement with Sirengo (2001) who reported that Zinc levels in sugarcane roots were larger than in leaves and stems.

2.4 Heavy metals and their harmful nature to human health

Heavy metals have developed an interest to the scientific community as a result of their toxic effects to human beings. Heavy metals are those elements whose interactions with biological systems are determined by their Lewis acid and ionic indices properties. This narrows down to most of the transitional elements, rare earth elements and some p-group elements that are either

metals or metalloids (Duffus, 2002; Nogueira *et al.,*2013). Plants constantly uptake heavy metals and concentrations within the organisms could build-up to levels larger than those in the environment (Gebrekidan *et al.,* 2013; Nogueira *et al.,* 2013). Since the toxicity of any material to a living system is dependent on the amount of that substance in cells of the organism, these metals are not always considered as toxic (Du *et al.,* 2013). In fact some heavy metals, at trace levels, are essential. However the metals are non-biodegradable and tend have an extended biological half-lives and hence prolonged consumption of food crops with risky levels of heavy metals can result to a chronic accumulation of these elements in the kidney and liver of human beings, thereby triggering disruptions of many biochemical processes (Inoti *et al.,*2012; Mahmood & Malik, 2014; Tsafe *et al.,* 2012). Therefore numerous studies have been done on the heavy metals and their accumulation in food crops.

Some heavy metals species have been singled out because of their toxicity even in trace amounts. Among the most common heavy metals are Pb, Cr, Ni and Cd. Pb and Cd have ability to accumulate in crucial organs of humans and their half-life could reach 30 years (Hussein, *et al*., 2008). Excessive exposure to Pb is associated with the damage of the skeletal, enzymatic, circulatory, endocrine, nervous and immune systems. Cd has been linked with lung cancer, hypertension, kidney dysfunction and bone fractures among other complications (Li *et al.,* 2014). Pb and Cd are also known to cause teratogenesis and mutageneis (Alissa & Ferns, 2011; Gebrekidan *et al.,* 2013). Furthermore, hexavalent chromium is regarded to be carcinogenic to human beings by the International Agency for Research on Cancer and the United States Environment Protection Agency while Ni is associated with emphysema, asthma, chronic bronchitis and cancer of the lungs (Zhao *et al.,* 2011).

Even though some metal elements are considered essential, they are harmful to human health when present at elevated amounts. Zn and Cu are needed for biochemical and physiological functions to maintain health. Their deficiencies lead to immunological defects and various abnormalities. Cu is a constituent of several enzymes and hence contributes to collagen synthesis and is also important to the formation of nerves, connective tissues and the immune system. Zn plays a vital role in the well-functioning of biological systems such as transport of proteins, enzymes structure and functions, hormonal activities and specific receptor locations (Oyoo-Okoth *et al*., 2013). However, the excessive concentration of Cu and Zn in food and feedstuff is

known to have toxic effects to human beings and animals (Wang, *et al*., 2012). Cu is known to cause irritation to the mouth, nose and eyes and also dizziness, headaches, stomach aches and severe gastrointestinal problems (Rahman *et al.,* 2014). It is also associated with liver damage when in excess, while high amounts of Zn can lead to reduction of immune faction and levels of high density lipoproteins. Other essential elements, Iron and Manganese, have been associated with Parkinson's disease, malfunction of the immune system and infertility in mammals (Gebrekidan *et al.,*2013; Li *et al.,*2007; Wang *et al.,*2012).

2.5 X-Ray Spectrometry

Spectrophotometric methods such as atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) are the most used methods for environmental analysis of toxic elements (Marguí *et al*, 2010). This techiniques have low detection limit and provide rapid and highly reproducible results. However these techniques are hampered by several challenges in analysis of samples with high organic load, suspended solids and high viscosity. These methods may require digestion which uses corrosive reagents and could be a source of contamination of the sample. Therefor a method of analysis that can analyse sample in variuos forms including liquids, suspensions and solids.

X-ray fluorescence (XRF) spectrometry has become one of the most used techniques for analysis of trace elements. X-ray spectrometry is efficient, reproducible and accurate for the determination of elements with Z >13 (Jbrgensen et al 2005). It is considered to have low detectability limit (ppm-ppb) and quality selectivity (Szyczewski *et al*., 2009). This technique is non-consumptive and is applied for multi-element determination and quantification of samples with a wide range of matrix forms (Towett *et al*., 2013). For example total refelective x-ray fluorescence spectrometry (TXRF) has been utilized as an alternative method for analysis of liquids and powdered samples. This technique has several advantages which include simultaneous multielement analysis, ability to analyse micro-samples (ug or mg), low detection limits, simplicity of operation, short analysis time (100-1000 s), doesn't use cooling agents, easy calibration with an internal standard and reduced matrix effects and background noise (De La Calle *et al*., 2013). Marguí *et al*., (2010) used TXRF for analysis of effuents and compared the results with those obtained from other techniques. The results compared favourably with those obtained by ICP-OES or ICP-MS for the anlysis of Zn, Cu, Ni, Cr and Sn and thus considered

this technique accurate. Another study by Towett *et al*., (2013) colaborated this findings. They compared values obtained from TXRF and with those from ICP-MS reported that Mn, V, Ti, , , Fe, Ni, Cu, Al, Ga, K and Zn were determined with accuracy (R^2 > 0.80). Nevertheless, it was realized that values for Ca, Rb, As, Y,Ta and Pb were under- or overestimated though with relatively good accuracy (R^2 0.60 to > 0.80). Furthermore, Muohi *et al*., (2003) compared the results obtained from Atomic Absoprtion Spectroscopy (AAS) and those from energy despersive x-ray fluorescence spectroscopy with certified values for the elements Cu, Zn and Pb . They reported a conformity between the experimental and certified values. For example, the values for Zn were 92.2 mg/kg from AAS while those from EDXRF were 99.0 mg/kg with certified value ranging from 101-113 mg/kg.

X-ray spectrometry has been used widely to study substances that would be difficult to analyse with other conventional methods because of sample matrixes. X-ray spectroscopy can be used in non-destructive analysis which means that the sample matrix is preserved. Therefore it has been utilized for determination of trace elements in various types of samples with an advantage of avoiding digestion with corrosive acids. For example Parth *et al*., (2011) used EDXRF technique for analysis of heavy metals in soils. X-RF is also the method preferred for the analysis of samples containing high sugar content and other soluble solid materials. While AAS and ICP-AES are more suitable for the analysis of solutions, they are however have limited for the analysis of solutions containing significant amount of soluble solid substances which causes difficulties in the sample injection of their instrumentation (Bao *et al*., 1999). This is because of the solution's high viscosity and solid content. Examples of these solutions are sugar cane juice and fruit juice. The method was also employed in the determination of Sn in canned food by Mino, (2006) and also in the analysis of As and Hg in Chinese traditional medicine (Mino & Yamada, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The study was focused along three rivers in Nairobi river basin and eight farms in Nyahururu region. The Nairobi, Ngong and Mathare rivers pass through industries and heavily inhabited settlements and industries. The rivers are polluted by industrial waste and raw sewage. Just like most developing nations experiencing rapid development, urban farming along these rivers is intensive. One of the crops widely cultivated along these rivers in this area is sugarcane. The other sugarcane samples were obtained from farms in the Nyahururu region which is a rural area and is considered unpolluted. These samples were compared to the ones from Nairobi County. In this chapter, the sampling area, sampling procedures, sample preparation, quality control and sample analysis procedures are presented.

3.2 Study Area

The study area includes three rivers of the Nairobi River Basin, and also farms in Igwamiti, Mairo Inya and Subukia areas in the Nyahururu region. Mathare, Ngong' and Nairobi rivers flow through the capital of Kenya, join up in the east of Nairobi and near Ruai, drain into the Athi river which flows into the Indian Ocean. The sampling along the rivers was done south of the equator at latitudes 01 ^{o}S, longitudes 36 ^{o}E and altitude above 1539 m. The farm samples were collected in the rural areas located in the north of the equator at latitudes 00 ^{o}N, longitude 36 ^{o}E and altitude above 2028 m. The specific GPS points for the sampling locations are shown in table 3.1. The sampling locations are illustrated in figure 3.1 and figure 3.2.

Table 3.1: Sampling points and their respective GPS coordinates

Nairobi				Nyahururu			
Sampling Point	Elevation (M)	Longitude (-)	Latitude	Sampling Point	Elevation (M)	Longitude (-)	Latitude
Tasia	1612	01.30248°	036.89180°	SBK1	2058	00.00806°	036.25048°
Kayore Soweto	1602	01.29069°	036.90738°	SBK2	2028	00.00734°	036.24853°
Dandora Mile Saba	1569	01.24355°	036.91402°	SBK3	2045	00.00886°	036.24946°
Dandora Nairobi River	1539	01.23866°	036.92179°	IGW1	2423	00.03921°	036.29092°
Huruma Kia Maiko	1607	01.25075°	036.87376°	IGW2	2433	00.02819°	036.29506°
Korogosho	1590	01.25015°	036.88999°	IGW3	2457	00.03615°	036.29497°
Nairobi Dam	1695	01.31832°	036.79692°	MRI1	2380	00.02512°	036.41033°
Kibera Lindi	1698	01.31556°	036.79169°	MRI2	2348	00.01713°	036.40806°

NAIROBI RIVER BASIN

Dandora Nairobi River
Dandora Mile Saba
Huruma Kia Maiko Korogocho
Nairobi CBD
Kayole Soweto
Tasia
Kibera Lindi
Nairobi Dam

MAP OF KENYA

Legend

Sampled Points   Railway Line

CBD Nairobi   Road

River

1:115,901

0 1.25 2.5 5 7.5 10
Kilometers

Geographic Coordinate System Arc_1960
Datum: D_Arc_1960
Prime Meridian: Greenwich (0.0)

Date: October, 2015
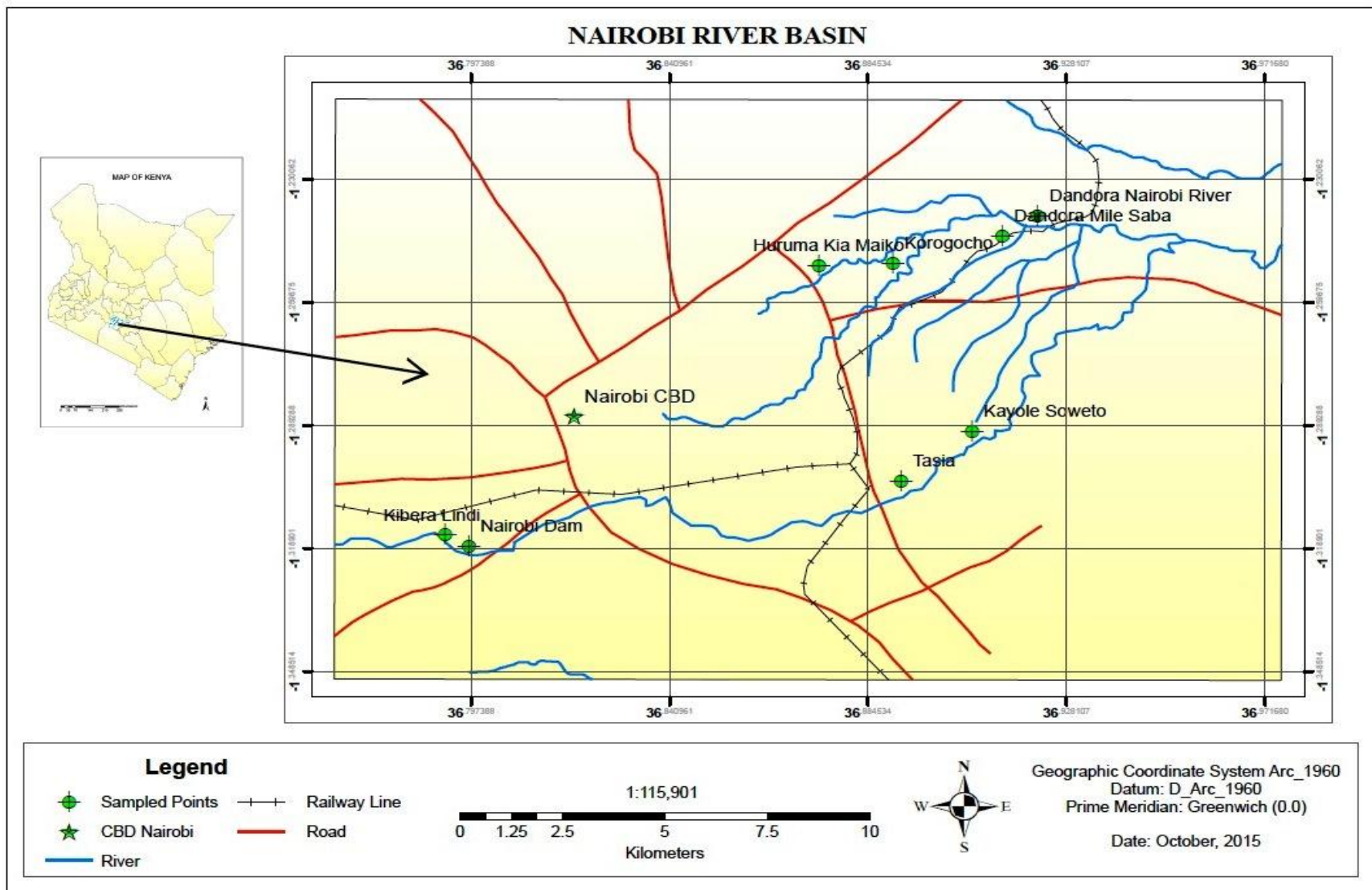
Figure 3.1. Map showing sampling points in Nairobi County (Survey of Kenya)
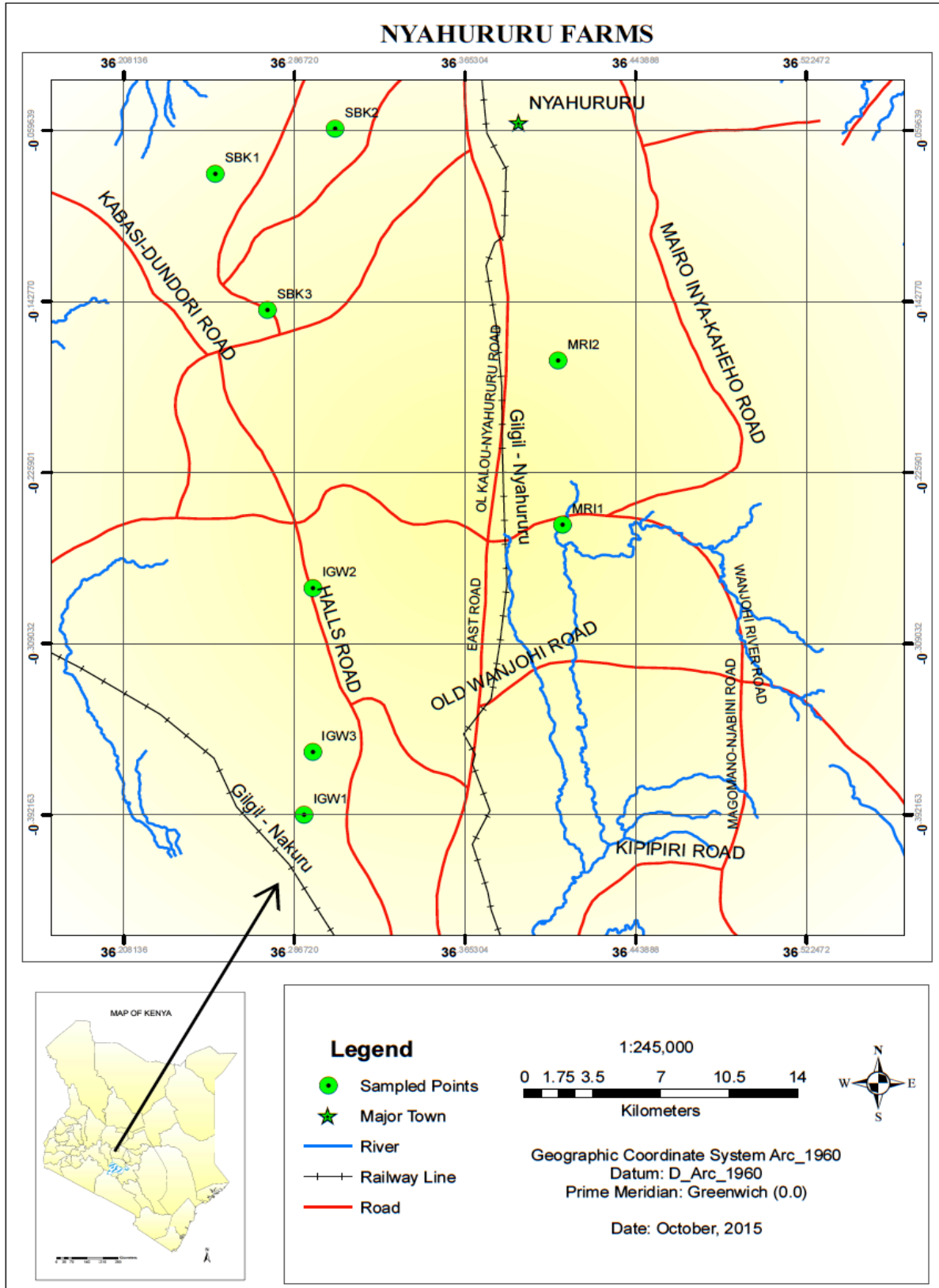
Figure 3.2. Map showing sampling points in Nyahururu Farms (Survey of Kenya)

3.3 Sampling Procedures

Sugarcane stems and soil samples were collected at every sampling point concurrently. The coordinates of the sampling site were obtained by the aid of Garmin e Trex GPS instrument that uses GPS and GLONASS (samples were only sampled when the instrument indicated that the point could be mapped with an accuracy of 3m). The soil was collected at the place where the sugarcane was sampled. The samples were put in plastic bags, labelled, sealed and taken to the laboratory for analysis.

3.3.1   Sampling of the sugarcane

In October 2013, a reconnaissance survey was conducted on in order to identify sugarcane farms in Nyahururu and Nairobi. Eight sampling points were established along three rivers in Nairobi and another eight sampling locations were selected in Nyahururu. Three sugarcane plants were sampled from mature plants (12-15 months) using a stainless steel knife. The shoots were removed and the stems were cut into two parts (top and bottom). The bottom parts were bagged separately from the top parts in plastic bags and properly labelled to differentiate. Hence, thirty two sugarcane samples were obtained for this study; sixteen top and sixteen bottom parts of sugarcane plants. The samples were taken to the laboratory for preparation and analysis.

3.3.2   Sampling of soil

Sixteen soil samples were collected concurrently with the sugarcane plants. The soil was sampled adjacent to the sampled sugarcane so as to ensure the relevance of the soil to the cane. The surface soil was first removed by scooping it until 15 cm depth was achieved. The selected depth for sampling was 15 cm below the surface in order to avoid arbitrary surface conditions and also consider the reach of the sugarcane roots. The soil was collected with an aid of plastic scoop from three points (300 g from each site) in the sampling location and thoroughly mixed to make a merged soil sample. The three points were 30 cm from the stump of the sampled sugarcane and 120° from each other. The samples were put in zip locking plastic bags, labelled and sealed to avoid contamination and loss of sample.

3.4 Development of sugarcane juice extractor

A sugarcane juice extractor was developed in science workshop to enable the juice extraction. The extractor consisted of stainless steel perforated container, plates and piston. First, the container was prepared by welding a stainless steel plate on the base of a stainless steel pipe. The resultant container had a diameter of 8.5 cm and a height of 12.5 cm. The wall and base of the container were perforated with small holes in order to allow juice to pass through (Figure 3.2). A stainless steel piston was developed to fit in the container in order to provide means of exerting pressure from a hydraulic press. A stainless steel circular plate which could fit in the container (8cm diameter) was developed to prevent clogging of bagasse on the bottom of the container. Lastly, a stainless plate was prepared on which squeezed juice could flow to the collecting container. The use of stainless steel was to reduce contamination from the container.

Figure 3.3. Sugarcane juice extractor; piston, circular plate and perforated container

3.5 Cleaning of apparatus

Using clean apparatus is important to minimize sample contamination during preparation and analysis. The glassware and plastic containers were soaked overnight in water with detergent. They were then cleaned thoroughly with warm water and soap. They were then rinsed with tap water and finally with double distilled water. They were finally put in a rack to dry.

3.6 Quality control

3.6.1 Routine quality control procedures for TXRF

Resolution, sensitivity and intensity of the TXRF instrument were determined before any analysis of samples was commenced. Resolution was found by analysing 1ng of Mn standard while sensitivity was determined by analysing 1ng of Ni standard. The intensity was checked by analysis of 1μg of As standard.

3.6.2 Accuracy of the measurement instrument

10 μl of a multi element standard from Bernd Kraft was spiked on a sample carrier and dried on a hot plate. The carrier was transferred to the TXRF instrument and measurement was done.

3.6.3 Accuracy of the analytical procedure

The accuracy of the TXRF procedure was assessed by carrying out analysis of Bowen Kale standard reference material, while for the EDXRF method a river clay standard reference material was analysed. 0.3 g of Bowen kale was put through the same digestion procedure used for the juice and then analysed using the TXRF procedure. River clay standard pellets were prepared and analysed using the EDXRF procedure.

3.6.4 Reliability Analysis / Recovery

Recovery was determined by adding pre-digestion spikes of standard solutions (5mg/l Fe, 2mg/l Zn and 0.5mg/l Cu) to some of the juice samples before going through the digestion and analysis process. Firstly, Fe, Cu and Zn standards were commercially acquired with each containing a concentration of 1000 mg/l of the particular element. From a single juice sample, two 50 ml sub-samples were pipetted into separate vials. To one sub-sample, 250μl of Fe, 100μl of Zn and 25μl of Cu standards were added in order to effectively add 5 ppm Fe, 2 ppm Zn and 0.5 ppm Cu to the juice. Finally, the two sub-samples were digested and analysed using the TXRF spectrometer.

3.6.5 Assessment of the juice extractor

The extractor consisted of a container, piston and several plates which were all made of stainless steel. Where welding was needed, it was done using a stainless steel rod. In order to determine whether the extractor was releasing metal contaminants into the juice, it was tested in distilled water for different time periods. The tests involved immersion of the extractor in 1 litre double distilled water for 30 minutes, 60 minutes and 120 minutes periods. When a time period had elapsed the extractor was removed from the water and 10 ml of the water was measured. 10 µl of 10 ppm Ga internal standard was added to 10 ml of the above solution and resultant solution was analysed using TXRF technique.

3.7 Analysis of sugarcane juice

3.7.1 Juice extraction and digestion

The sugarcane was washed with tap water, rinsed with double distilled water and cut to smaller sizes using a stainless knife. The circular plate was placed in the container and the sugarcane pieces were added. The container was placed on a steel plate, the piston was placed on top and the cane was crushed using a hydraulic press. The juice was collected in a plastic container and refrigerated (4°C) awaiting analysis. The process is illustrated in figure 3.4
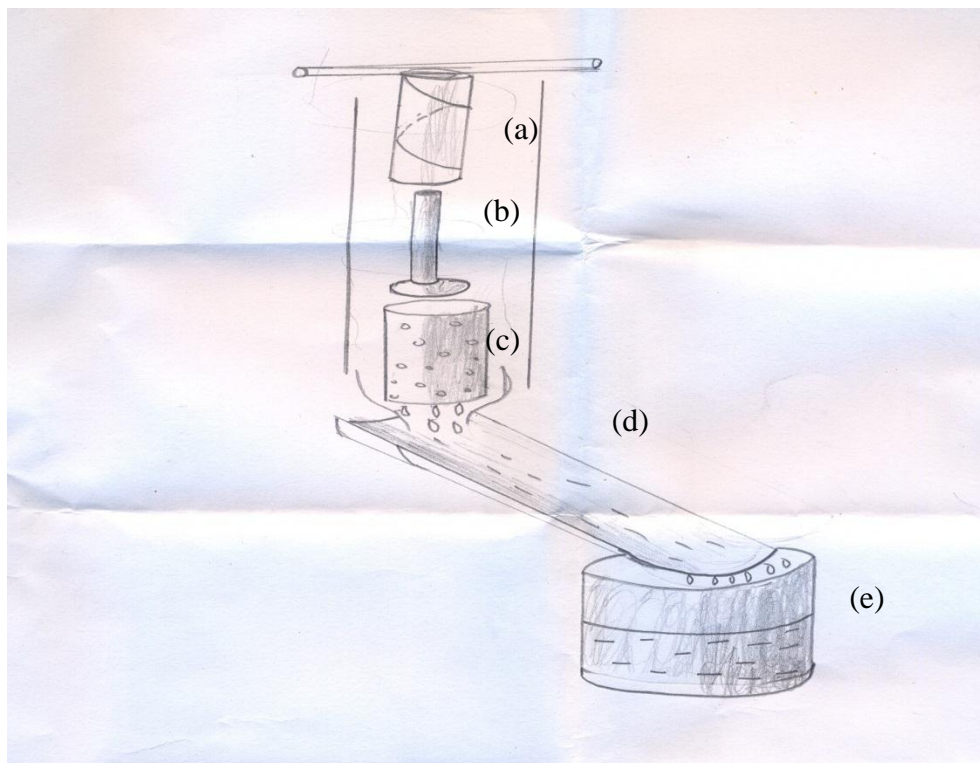
Figure 3.4. Illustrating the sugarcane extraction process

Where, (a) is the hydraulic press, (b) is the stainless steel piston, (c) is the perforated stainless steel container, (d) is the stainless steel plate and (e) is a plastic container.

Since juice analysis was to be analysed in solution form, there was need to carry out wet digestion of the juice. Microwave assisted digestion technique was applied because of its ability to achieve high temperature and pressure and therefore attaining quick complete matrix destruction. The conditions for each digestion procedure were monitored and saved by the machine in order to ensure that the process is analytically reproducible. A temperature program was developed for the preparation of the juice as shown in table 3.2. 5 ml of the juice was pipetted directly into polytetrafluoroethylne (PTFE) digestion vessel and 10 ml of analytical grade concentrated HNO_3 was added. PTFE polymer digestion prevents loss of volatile elements and minimises contamination. This was done in triplicates for each sample. Reagent blanks were prepared by putting only the acid in the vessels. The vessels containing the samples were closed tightly, positioned into the rotor and digestion was initiated. The samples were digested for 40 minutes. After digestion, the digestates were transferred to a 50 ml volumetric flask and topped

to the mark with distilled water. The resultant solution was transferred into a plastic container and was ready for TXRF analysis.

Table 3.2. Microwave digestion program for sugarcane juice (programmed in microwave digester)

Step	Temperature($^{\circ}$C)	Ramp(min)	Hold(min)	Fan Speed
1	180	15	0	1
2	150	0	15	2
3	70	0	10	2

3.7.2 Washing sample carriers

The carriers contribute to the achievement of the optical analytical results in TXRF analysis (Marguí *et al.*, 2010). They could influence the concentration of the sample since they are the only link of the sample to the measuring instrument and therefore carriers must be clean before applying the sample. Initially, the carriers were pre-cleaned with a fluff-free cleaning tissue. They were then mounted on washing cassette and transferred in a glass beaker filled with a cleaning solution (5% RBS solution) and heated without boiling for five minutes on a hot plate. After that, the carriers were rinsed with distilled water and the cassette was immersed in 10% nitric acid and heated again for two hours on a heating plate. In next step, the cassette was transferred to a beaker filled with distilled water, was heated for five minutes and then rinsed thoroughly with double distilled water, followed by wiping with acetone. Then, 10 µl of silicon solution was applied at the centre of the carriers and the carriers were then dried on a hot plate at 80 $^{\circ}$C for 30 minutes. The carriers were assessed for cleanliness using the TXRF technique.

3.7.3 Juice analysis using TXRF

10 ml of digested juice sample was pipetted into a clean vial and 10 µl of 1000 ppm Gallium internal standard was added. The mixture was then shaken vigorously to homogenize and 10µl of the resultant sample was pipetted onto a clean carrier. The carrier was then dried and gently inserted in the TXRF spectrometer (Bruker S2 Picofox Benchtop TXRF Spectrometer) and the sample was irradiated for 500 seconds using a 50kVand a current of 1000 µA. A software

provided by the manufacturer (S2 Picofox) was used for quantification using the following formula:

$$C_x = \frac{N_x/S_x}{N_{is}/S_{is}} \times C_{is}$$

Where, C_x is the analyte concentration, C_{is} is the internal standard concentration, N_x is the analyte net intensity, N_{is} is the internal standard net intensity, S_x is the analyte relative sensitivity and S_{is} is the internal standard's relative sensitivity.

3.8 Analysis of Soil

The soil analysis involved pH determination and trace elemental analysis.

3.8.1   Soil Preparation

Pellets were prepared for the determination of trace elements in soil using EDXRF spectrometry technique. Initially, soil samples were dried in the sun and heated in an oven at 60 ^{o}C for a period of 48 hours. The soil samples were now crushed using a mortar and pestle. The soil was then homogenized by crushing with mechanical crusher and moved through a mesh of 75 µm mesh size. 1.6 g of the sieved sample and 0.40 g (20%) starch binder were weighed, followed by a through mixing. The intention of adding the binder was to make the pellet structurally strong. Finally, a 0.40 g sample was pelletized using a hydraulic press. For each sample three pellets were prepared.

3.8.2   Determination of pH

The pH of the soil was determined using the 1:2.5 soil to water ratio procedure. A 20 g of pulverized soil sample was weighed and put in a beaker. 50 mL of de-ionized water was then added to the soil sample and the mixture was stirred thoroughly for 10 minutes. The mixture was then allowed to stand for 30 minutes and finally stirred again for 2 minutes. The pH reading was taken with the aid of a pH meter (HANNA pH p11).

3.8.3   Analysis using EDXRF

The pellet was inserted into the EDXRF spectrometer (Shimanzu 800 HS) and irradiated for 50 seconds. The spectrum was developed and quantification of the elements was done using the Quantitative X-ray Analysis System software (QXAS 3.6) which is developed and provided by

the International Atomic Energy Agency. It uses fundamental parameters (FP) as the basis for quantification.

3.8.4 Limit of detection of the EDXRF spectrometer

The limit of detection of the system is determined by the following formula:

$$D = (3C\sqrt{N_B})/N_P$$

Where, D is the detection limit, C is the element concentration (µg/g), N_p is the element's net peak area and N_B is the net background area under peak of the element

3.9 Statistical analysis

The data was subjected to Pearson correlation with 2-tailed significance to determine the relationship between two data sets while the means were analysed using Tukey's range test to determine the significant difference between two data sets. These statistical assessments were done using SPSS Version 17.0 software (SPSS-Inc., 2002) and as suggested by Miller & Miller (2010).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Quality assurance

The accuracy of any measurement technique is very important and is described as the degree to which a measured value conforms to a standard or a true value (Muohi *et al*., 2003). To assess the accuracy and reliability of TXRF and EDXRF methods of analysis and microwave assisted digestion method for sample preparation, the results from analysis of Bernd Kraft multi element standard, PTXRFIAEA09 river clay certified reference material and Bowen Kale standard reference material were compared with the certified values. Recoveries of Fe, Zn, and Cu were also compared with the expected values. It was noted that the means of the experimental and certified values did not have statistical differences (p<0.01)

4.1.1   Bowen kale certified reference material

Analysis of Bowen Kale standard reference material from the University of Reading was done using TXRF spectrometry technique. The results obtained were used to assess the dependability of the digestion and measurement method. The experimental concentrations of Zn, Cu and Fe were within the recommended range of the certified amounts as shown in table 4.1. In addition, a significant test for mean values for each element was performed according to the method described by Miller & Miller (2010). It was revealed that the experimental values and certified values did not have a statistical difference (p< 0.01). Furthermore recoveries were calculated with a method described by Zhao *et al.,* (2012). The recoveries for Cu and Zn (100% and 107%) for the current work compared favourably with those reported by Zhao *et al.,* (2012) for rice (104% and 95%) and cabbage (127% and 89%) standard reference materials.

Table 4.1. Comparison of the experimental values of Bowen Kale SRM (mg/l) to certified values (mg/l)

	Certified values	Experimental values	|t|
Mn	14.8±1.68	17.20±1.20	2.44
Fe	119±14.80	121± 2.10	0.23
Cu	4.89±0.63	4.90±0.7	0.03
Zn	32.30±2.80	34.60±1.00	1.41

ǀtǀ is the calculated t value without regard of sign for significance test.

4.1.2   Standard Reference Material (SRM)

PTXRFIAEA09 river clay standard reference material from the international atomic energy agency (IAEA) laboratories, Seibersdorf was analysed using EDXRF spectrometry technique to test its accuracy. The recommended range for most elements was achieved as shown in table 4.2. The comparison of the experimental mean with the certified means was done with the aid of Turkey test as shown by Miller & Miller, (2010). It was noted that there was no significant difference ($p < 0.01$) between experimental and certified values.

Table 4.2. Comparison of experimental values (µg/g) of PTXRFIAEA09 (river clay) sample with the certified values (µg/g)

	Certified Values	Experimental Values	|t|
Mn	1000±60	1012±85	0.32
Fe	29700±1000	30700±1820	1.56
Cu	20.10±2.05	18.10±3.70	1.48
Zn	96.10±7.73	88.90±12.00	1.48

ǀtǀ is the calculated t value without regard of sign for significance test.

4.1.3   Multi Element Standard

The accuracy of the total reflection x-ray fluorescence spectrometer was assessed by analysing a multi element standard. The multi element standard from Bernd Kraft GmbH was a mixture comprising diverse metals with equal concentration of 10ppm each in the mixture. Three metals were used as internal standard separately and experimental values obtained were compared with

expected values. When Cu was used as the internal standard, the concentrations of elements ranged from 8.80 to 10.76 mg/l, while for Se and Ti values ranged from 9.27 to 11.33 and 9.47 to 11.59 respectively as shown in table 4.3. A comparison between the mean experimental values and the certified was done to determine whether there was evidence of systematic errors (Miller & Miller, 2010). The |t| values observed in this test were less than the critical value and hence there were no evidences of systematic errors (p< 0.05).

Table 4.3. Comparison of experimental values of multi element standard and expected values

Element	Cu	Se	Ti
Ti	9.29	9.78	10.00
V	9.63	10.14	10.37
Cr	9.31	9.80	10.02
Mn	10.76	11.33	11.59
Fe	10.12	10.66	10.90
Ni	10.64	11.20	11.45
Cu	10.00	10.53	10.77
Zn	10.28	10.82	11.07
As	8.80	9.27	9.47
Se	9.50	10.00	10.23
|t|	0.26	0.53	0.86

4.1.4   Recovery analysis

It was important to assure that the method of analysis did not result to significant loss of analytes or contamination of the samples. Therefore, the reliability of the procedure for determination of metal elements in the sample was assessed by spiking selected samples with metal standards and measuring their respective recoveries. Juice from the Dandora mile 7 and Kibera sugarcane juice were used for reliability analysis. For each sample, two sub-samples were taken and spiked with standards at concentrations relative to those in the juice (5 ppm Fe, 0.5 ppm Cu and 2 ppm Zn). The recoveries were calculated and the recoveries obtained for Fe, Cu and Zn are shown in table 4.4. The recoveries achieved in the present work compared well with those reported by Akinyele & Shokunbi, (2015); the recovery rates for Fe, Zn and Cu were 90.3%, 90.4% and 97.0% respectively.

Table 4.4. Percentage recovery of Fe Zn and Cu from digested spiked juice from bottom and top parts of sugarcane samples

	Kibera		Dandora Mile 7	
	Bottom	Top	Bottom	Top
Fe	90	92	93	91
Zn	92	90	91	92
Cu	98	98	95	97

4.1.5   Evaluation of the cane juice extractor

The possible sources of errors from the equipment used for the juice extraction were assessed and results are given in table 4.5. The Cu and Zn concentrations released by the apparatus were constant at the three time phases. This observation suggested that release of Zn and Cu by the apparatus was not affected by the amount of time the squeezer was being used.

Table 4.5. Amount of metals (mg/l) released by the squeezer based on the time

Element	30 mins	60 mins	120 mins
Ti	BDL	BDL	BDL
Mn	0.011	0.015	0.018
Fe	0.179	0.161	0.206
Cu	0.005	0.005	0.006
Zn	0.013	0.014	0.012
Pb	BDL	BDL	BDL

BDL represents concentration that is below the detection of TXRF spectrometer

It was noted that there were marginal increases in the amount of Mn and Fe released as time progressed. These increases were observed when the squeezer was soaked for 120 minutes which were not representative of the actual contribution to the juice since it took an average of 30 for the squeezer to extract a juice sample. The amounts of Zn and Cu released were constant in all time periods. The amounts of Zn, Fe, Cu and Mn detected in the assessment of the squeezer were averaged for all time periods and deducted from those detected in the juice.

4.2 Heavy metal content in sugarcane juice and soil samples

The research involved the determination of heavy metals in soils and sugarcane cane juice from: a) rivers in Nairobi County and b) Nyahururu farms.

4.2.1 Heavy metals in Nairobi Soils

The presence of heavy metals in soils can be attributed to either natural or anthropogenic origins (Khan *et al.,* 2013; Zhao *et al.,* 2011). It was noted that the levels of Fe and Mn in the soils were higher than amounts observed for other heavy metals which could have been due to the fact that they are the most plentiful elements in the lithosphere (Zeng *et al*., 2011).

The association between heavy metal content in soil and its pH is very important when considering trace metals in plants. The availability of these elements in soils for plant uptake is determined by their solubility and mobility. The speciation of a metal element in soil and chemisorption process, which controls solubility and mobility, are highly influenced by the soil pH. The pH values for Nairobi County were found to range from 4.75 to 7.75 with an average of 6.75 (table 4.6). Six of the eight samples had pH values that indicate that the soils were neutral. However, soils from Dandora Mile 7 and Nairobi River were found to have acidic pH values. Wang *et al*., (2000) studied the influence of pH on metal uptake by soybean and tomato plants with regard to acidic rain. Soybean uptake of Sr, Ru, Rh, Zr, Rb, Zn, Tc, Co and Y increased with acidification of soil. However soybean uptake of Se and absorption of the trace elements by tomato plants was lowered by acidification of soil. In addition, Segura-Mun˜oz, *et al*., (2006) reported no association ($p<0.05$) between soil pH and metal concentration in roots, stems or leaves of sugarcane. Thus the influence of pH can be considered to be specific to a metal element and plant species. Even so, lower pH elevates the bioavailability of most elements in soil and hence risk of increased uptake and retention of harmful elements by increased ionization, water solubility and mobility of metal species (Zhao *et al.,* 2011). Soil with acidic properties would not be adequate for farming and in fact the Grade II Chinese environmental quality standard for soils requires a pH <6.5. It is clear that the Dandora Mile 7 and Nairobi rivers do not meet this requirement. They also did not meet the Canadian requirements agricultural soils which only permit a pH range of 6-8 (CCME, 1999).

Table 4.6. pH and Metal concentrations (mg kg⁻¹) of soils sampled along three rivers of Nairobi River Basin

	pH	Mn	Fe	Cu	Zn	Pb
Kayole	7.24±0.04	6370±435	52133±3390	93.7±13.9	343±32	34.8±5.0
Mathare	7.18±0.01	6293±432	58333±3793	110±15	746±58	<15.0
NB Dam	7.75±0.08	4637±316	50667±3293	43.7±3.4	155±17	<15.0
Tasia	7.10±0.04	4357±304	43267±2817	37.9±9.7	327±29	37.1±7.7
Korogosho	6.99±0.01	7290±493	58800±3820	114±15	834±62	57.1±10.9
Kibera	7.07±0.02	7130±484	77567±5047	123±17	527±45	<15.0
D.N/River	4.75±0.07	3773±267	57800±3760	57.1±12.5	219±25	<15.0
D.Ml 7	5.94±0.01	8683±580	61433±3990	61.1±11.2	580±44	31.7±7.4

NB Dam represents Nairobi Dam

D.N/River represents Dandora Nairobi River

D.Ml 7 represents Dandora Mile 7

The total element content in soil also influences the amount of trace elements in plants since the soil is the main source of metals for plant uptake. The heavy metal concentrations in the soil were found to be in the ascending order Pb<Cu<Zn<Mn<Fe. Fe was the most abundant heavy metal ranging 43.3-77.8 mg/g while the concentration of Pb was the least (ranging from below detection limit to 57.1 mg/kg) as shown in table 4.6. The levels of Mn, Zn and Cu were found to range from 3773 -8683 mg/kg, 155- 834 mg/kg and 37.9 - 110 mg/kg respectively. The levels of Zn for this study were higher than those reported by Sirengo (2001) for a study along Nairobi river. A study by Parth *et al*., (2011) soils in a dumpsite in Hyderabad, India, reported amounts of Zn (26.9 - 882.1 mg/kg) and Cu (11.0 - 186 mg/kg) which were close to those reported in this study. Moreover, some samples in the current study had levels of Zn and Cu that were beyond maximum permissible standards (63 - 100 mg/kg for Cu and 200 - 300 mg/kg for Zn) (CCME, 1999; Ewers, 1991; Pendias & Pendias, 1992). However, Li *et al*., (2014) work on soils from mines in China reported concentrations of Pb (641.3 mg/kg), Zn (1163 mg/kg) and Cu (211.9 mg/kg) which were higher than those observed in this study. In comparison to sources of heavy metals pollution in the environment, mining is considered to be among the highest contributors. On the other hand, the amounts of Fe (43267 - 77567 mg/kg) in the current study were significantly higher than those reported by Inoti *et al*., (2012) for soils in Thika town (11803.0 -

16224.8 mg/kg). They also stated that these amounts were beyond the accepted limits and attributed this observation to contamination by effluents from major industries. Hu *et al*., (2014) reported average amounts of Cu (73.7 mg/kg), Zn (239.5 mg/kg), Pb (40.4 mg/kg), Mn (224.5 mg/kg) and Fe (27.0 mg/g) in soils from paddy fields. The concentration of Cu and Zn was considered to be have exceeded the limits for Chinese soil quality. Notably, most samples from Nairobi County were found to be have more amounts of Cu and Zn. Moreover, all samples from Nairobi county were found to have Fe and Mn levels that were higher than those reported in Hu *et al*., (2014). However, only the Korogosho sample had higher Pb levels (57.1 as compared to 40.4 mg/kg).

It was noted that the distribution of heavy metals in the different sampling points was unique for each elements. However, some samples were observed to have higher levels of the metals than the rest. It was an indication that pollution along the rivers was not uniform, in that some areas were observed to be more polluted than others. Mn concentrations were found to be in the increasing order of Dandora Nairobi river < Tasia < Nairobi Dam < Mathare < Kayole < Kibera < Korogosho < Dandora mile 7. The Fe amounts on the other hand followed the order Tasia < Nairobi Dam < Kayole < Dandora Nairobi river < Mathare < Korogosho < Dandora mile 7 < Kibera. The Cu levels in the soil were in ascending order Tasia < Nairobi Dam < Dandora Nairobi river < Dandora mile 7 < Kayole < Mathare <Korogosho < Kibera, while Zn amounts followed the order Nairobi Dam < Dandora Nairobi river < Tasia< Kayole < Kibera < Dandora mile 7<Mathare<Korogosho. The samples in which Pb was detected had concentrations in the order Dandora mile 7<Kayole<Tasia<Korogosho. Among the eight samples, Mathare Korogosho and Dandora mile 7 were consistently found to have elevated amounts of heavy metals. For instance, amounts of Zn in Korogosho and Dandora mile 7 were higher than that those reported by Jirsa *et al*., (2013) study of Lake Nakuru sediment. The elevated Zn levels in that sediment were attributed to pollution arising from increased anthropogenic activities around the lake such as industries in Nakuru City.

The concentrations of Zn were found to have significant ($p<0.05$) correlations with Mn, and Cu as shown in table 4.7. It was also noted that the Fe amounts in soil correlated with the amount of Cu and Mn. Nabulo *et al*., (2010) reported a close correlation ($r = 0.90$) between the

concentrations of Zn and Cd in soil which suggested that the two elements had a common source such as the recycling of scrap metals.

Table 4.7. Pearson correlation values for associations between heavy metals in Nairobi soils

	Mn	Fe	Cu	Zn
Mn	1	0.55	0.56	0.72*
Fe	0.55	1	0.68	0.41
Cu	0.56	0.68	1	0.73*
Zn	0.72*	0.41	0.73*	1

*. Correlation is significant at the 0.05 level (2-tailed).

4.2.2   Heavy metals in sugarcane juices from Nairobi County

It was observed that concentrations of metals in juice varied in the different parts of the sugarcane. It was also observed that the amounts metals in the juice varied widely relative to the sample and with a specific element. However, some elements were below the detection limit. After analysis of the juice with the TXRF technique, Cr (<0.20), Ni (<0.05), As (<0.02) and Pb (<0.01) were not detected in the juice. The levels of heavy metals in sugarcane juice are presented in table 4.8.

It was observed that the trends in the distribution of Fe and Cu in the parts of cane were different to those observed for Zn and Mn. The Fe concentrations were the highest among the heavy metals while the lowest concentration detected was that of Cu. However the metals had a similar trend in their distribution between the top and bottom parts of the cane. The amounts of Fe and Cu in the bottom were marginally higher than the top part of sugarcane. The amounts of Fe and Cu in the bottom varied from 5.95-10.03 mg/l and 0.28-0.92 mg/l respectively while the top concentration ranges were 3.77-9.21 mg/l and 0.22-0.84 mg/l. The trend for Cu was in agreement with a study by Zhang *et al.,* (2014) also reported that Cu concentrations were higher in the bottom part of sugarcane. The study found out that most of Cu was retained in the roots. They attributed this observation to either the poor ability of sugarcane to transport Cu or the counter flow of Cu back towards the roots. On the contrary, Mn and Zn concentrations (mg/l) in the top (2.41-12.12 for Mn and 1.67-12.32 for Zn) were higher than in the bottom (1.67-10.14 for Mn

and 1.31-9.78 for Zn). This was also in agreement with Zhang *et al.,* (2014) who found out that these elements were relatively concentrated in the top part of the sugarcane (p<0.001).

It was observed that the trend for the distribution of trace elements between the bottom and top of juice samples was relative to each element. The amounts of Mn and Zn were higher in the juice from the top part while, on the contrary, Fe and Cu levels were higher in the bottom part. However, the amounts of heavy metals in the top were higher as compared to the bottom.

Table 4.8. Heavy metal content (mg/l) in juice from bottom and top parts of sugarcane from Nairobi County

	Mn		Fe		Cu		Zn	
	Bottom	**Top**	**Bottom**	**Top**	**Bottom**	**Top**	**Bottom**	**Top**
D Ml 7	2.56±0.13	3.58±0.22	10.03±0.27	9.21±0.30	0.28±0.02	0.22±0.01	4.34±0.13	5.43±0.11
D N/River	10.14±0.26	12.12±0.53	9.11±0.34	8.31±0.36	0.39±0.02	0.31±0.02	1.43±0.05	2.28±0.13
Kibera	2.78±0.11	3.29±0.21	8.34±0.18	7.53±0.15	0.54±0.08	0.41±0.03	5.14±0.17	6.01 ± 0.13
Korogosho	4.99±0.17	5.84± 0.26	17.52±0.41	14.43±0.34	0.53±0.04	0.38±0.04	2.46±0.05	3.34±0.17
Kayole	1.93±0.13	3.61±0.26	5.95±0.28	3.77±0.31	0.31±0.02	0.27±0.04	1.42±0.04	2.04±0.12
Mathare	2.79±0.24	4.10±0.20	6.93±0.23	4.17±0.19	0.92±0.03	0.84±0.06	1.99±0.06	2.12±0.01
NB Dam	3.12±0.15	4.64±0.07	10.15±0.11	8.65±0.21	0.41±0.04	0.32±0.04	1.31±0.08	1.67±0.05
Tasia	1.67±0.07	2.41±0.15	8.97±0.16	6.79±0.20	0.56±0.02	0.43±0.01	9.78±0.07	12.32±0.31

NB Dam represents Nairobi Dam

D.N/River represents Dandora Nairobi River

D.Ml 7 represents Dandora Mile 7

Some samples were observed to have high amounts a particular element while others had low amounts. Dandora Nairobi river samples had the highest amount of Mn while Tasia samples had the lowest (10 mg/l less). On the hand, the Korogosho samples had the highest amount of Fe while Kayole had 10 mg/l less of the metal. The highest levels of Cu were found in Mathare while the least amounts were in Dandora Mile 7. Additionally, Zn levels in Tasia were large as compared to the amounts in Kayole or Dandora Nairobi river.

The amounts of Zn, Mn and Fe in the juice were higher than those allowed by World Health Organization guidelines and United States of America standards for water (UNEPGEMS, 2006).

In addtion, some juice samples had higher Zn levels and a bigger sum of Zn, Cu and Fe than the permitted limits by FAO/WHO for juice (FAO/WHO, 2001). The amounts of Zn in Kibera, Tasia, and Dandora Mile 7 were above the recommended amounts (3-5 mg/l). The amounts of Fe and Mn in the juice were all above permited levels (0.3 mg/l and 0.5 mg/l for Fe and Mn respectively). Excess amount of Zn can lead to reduction of immune faction and levels of high density lipoproteins (Rahman *et al.,* 2014) while Fe and Mn have been associated with Parkinson's disease, malfunction of the immune system and infertility in mammals (Gebrekidan *et al.,*2013; Li *et al.,*2006; Wang *et al.,*2012). However Cu levels in the juice were below the recommended limits.

The concentrations of a select group of heavy metals in the juice were in increased in the order $Cu < Zn < Mn < Fe$. Collin & Doelsch, (2010) work on sugarcane juice from the island of Reunion also reported that the concentration of the trace elements they investigated to be in the order $Cd < Pb \approx Cr < Ni < Cu << Zn$ which was in agreement for Zn, and Cu for the current work research. In addition Nayak *et al*., (2014) also reported metal concentartions in plants were in the order $Cd < Cr < Pb < Cu < Zn < Mn < Fe$. This variations in metal concentrations suggested that the translocation of metals in plant systems was controlled by different cellular mechanisms of bioaccumulation of the elements. In addition, availability of the element in the soil also influences the uptake of metals. Heavy metals in fly ash (which was used to amend soil) followed the order $Cd < Cr < Pb < Cu < Zn < Mn < Fe$ in comparison to the soil's order which was $Cd < Cr < Cu < Pb < Zn < Mn < Fe$. The amounts of the metals in plants were in the same order as that of fly ash which suggested it influenced the availability of the metals.

The concentrations of Fe, Mn and Zn in the current study were either higher or lower than those detected in sugarcane juices in other studies. The amounts of Zn, Fe and Mn (mg/l) detected in the juice for current work was higher than those reported by Abdus-salam *et al*., (2008). The concentrations (mg/l) of Fe, Mn and Zn in sugarcane juice from Nigeria varied from 0.17 - 0.61, 0.11 - 0.69 and 0.15 - 1.54 respectively which is significantly lower than those observed for the present work. The presence of these elements in the juice was attributed to the irrigation with polluted water. However the amounts of Cu reported for this work was less than those reported by other works. For instance Kapungwe, (2013) reported unusually high amount of Cu (29.45 ± 2.03 mg/kg) in sugarcane from Zambia which was attributed to the total concentration of Cu in

the soils (219 ± 25.34 mg/kg). Another work by Sereno *et al.,* (2007) reported concentrations of Cu in sugarcane of up to 45 mg/kg.

The variations in the levels of metals in the various sugarcane samples could be due to either different cane varieties (Wang *et al*., 2012), increases in the amount of metals in soil (Nogueira *et al.,* 2013) or irrigation water (Adekola & Akinpelu, 2002). For example, Nogueira *et al.,* (2013) reported an increase in the concentrations of Zn ($p<0.01$) in sugarcane juice as a result of an increase of elevation of metal levels in the soil. Another factor that could determine plant uptake of Zn is its availability in under influence of soil pH. Kamau *et al*., (2014) found out that an increase in pH corresponded with a larger amount of dissolved of Zn. Though higher pH instigates the chemisorption of oxides and aluminosilicates and hence reduction of Zn^{2+} activity, it increases mobility and solubility of Zn-organic complexes.

The uptake and distribution of trace elements between the bottom and top of juice samples suggested individual uptake mechanisms for each element. This was supported by an observation of a few significant correlations between the concentrations of trace elements as seen in table 4.9. The finding for the lack of relationships between the amounts of Fe, Zn and Mn in the juice is in agreement with the findings by Abdus-salam *et al*., (2008). They attributed this observation to natural physiological processes of mineral uptake and bio-regulatory mechanisms of the sugarcane. On the other hand, it could indicate differing availability of metals which would suggest that the amounts of the elements in soil could be from different sources rather than a common one.

Table 4.9. Pearson Correlation values for associations between the concentrations of Mn, Fe, Cu and Zn in Nairobi juice samples

	Mn	Fe	Cu	Zn
		Bottom		
Mn	1	0.29	-0.12	-0.42
Fe	0.29	1	0.05	0.03
Cu	-0.12	0.05	1	0.12
Zn	-0.42	0.03	0.12	1
		Top		
Mn	1	0.25	-0.18	-0.45
Fe	0.25	1	-0.49	-0.06
Cu	-0.18	-0.49	1	-0.06
Zn	-0.45	-0.06	-0.06	1

4.2.3 Heavy metals in Nyahururu Soils

The pH for Nyahururu soils were found to vary from 4.94 to 7.49 as presented in table 4.10. It was observed that IGW3, MRI2 and SBK2 soils were acidic while the rest were neutral. Low soil pH is usually associated with H^+ and Al^{3+} ions. The two ions in soil solution and surface exchange sites establish the active acidity in soils. pH is a measure of H^+ ions which reduces with an increase of the ions. Al^{3+} undergoes solvolysis forming aqua acids. It is also known that even though some soils may have mineral oxides as pH buffer, large amounts of rain would surpass the buffering capacity of these soils (Wang *et al*., 2000). Increased levels of organic matter and leaching of nitrogen beyond plant root are among the factors that lead to lower pH (Omwoma *et al*., 2010). Omwoma *et al*., (2010) work in Western Kenya reported a soil pH range of 5.10 - 6.16 during the wet season. The samples for this study were collected in Nyahururu farms during the rainy season which may be the reason of some having relatively low pH.

The amounts of metals in Nyahururu samples revealed close relationship between some samples. For example, the concentration trend of Zn was SBK2<SBK3<SBK1. It was also observed that IG2, IG3 and SBK1 were found to have the highest amount of Fe and Pb. On the other hand, MRI2 had the lowest levels of Fe, Mn, Zn and Pb. The occurrence of metals in soil is associated with the soil type, location, clay content, drainage waters, oxidation-reduction potential, cation

exchange capacity and plant species growing in that soil (Segura-Mun˜oz *et al*., 2006). The amounts of Fe, Cu, Zn and Mn were found to have the following concentrations ranges: 40.0 - 127 mg/g, 28.9 - 58.6 mg/kg, 141 - 708 mg/kg and 2481 - 7687mg/kg respectively. Pb was detected in seven of the eight samples and varied from 22.1 - 43.3 mg/kg. The concentration trend of Mn was MRI2 <IGW3 <MRI1 <SBK2 <IGW1 <IGW2 <SBK3 <SBK1, while the levels of Fe followed in the order MRI2 <SBK3 <SBK2 <MRI1 <IGW1 <IGW3 <SBK1 <IGW2. On the other hand, the Cu concentration trend was SBK2 <SBK3 <MRI2 <IGW3 <MRI <IGW2 <IGW1 <SBK1 while the trend of Zn levels was MRI2 <IGW3 <MRI1 <IGW1 <IGW2 < SBK2 <SBK3. Furthermore, the levels trend for Pb amounts was in the order MRI2 <IGW1 <SBK2 <SBK3 <MRI1 <SBK1 <IGW2 <IGW3.

Table 4.10. pH and metal concentrations (mg kg^{-1}) of soils from farms in the Nyahururu region

	pH	Mn	Fe	Cu	Zn	Pb
IG1	7.49±0.06	4038±275	101750±6600	58.6±10.6	298±26	22.1±7.0
IG2	6.58±0.04	4058±276	126667±8213	53.9±10.8	318±27	43.3±7.4
IG3	4.94±0.07	2481±184	116750±7563	49.6±13.5	214±26	41.7±6.7
MRI1	6.78±0.04	3821±272	96375±6263	53.5±13.3	251±28	29.0±6.4
MRI2	5.55±0.02	2196±178	39958±2613	40.2±13.8	141±23	<15.0
SBK1	7.02±0.06	7687±515	118333±7667	74.7±13.1	780±58	36.3±7.1
SBK2	6.31±0.01	4006±273	94938±6163	28.9±8.7	325±28	24.3±4.6
SBK3	6.96±0.04	6325±428	76292±4950	33.3±8.9	402±34	25.2±4.5

The Zn and Mn trends were observed to be similar and this observation was confirmed by a high correlation between the amounts of the two metals as seen in table 4.11. It was also observed that the soils with relatively low pH had conspicuously lower amounts of Zn and Mn. For example the Zn and Mn concentrations in MRI2 (pH=5.55) were 141 and 2196 mg/kg while those detected in MRI1 (pH=6.78) were 251 and 3821 mg/kg respectively. This could be as a result of higher mobility and leaching of the two elements in the soil with lower acidity. The behaviour of heavy metals is highly influenced by pH; in acidic conditions the metals are more mobile (Oyedele *et al*., 2008). For example cation exchange capacity (CEC) reduces with lowering pH which results to more free ions at the soil solution.

Table 4.11. Pearson correlation values for associations between heavy metals in Nyahururu soil samples

	Mn	Fe	Cu	Zn
Mn	1	0.30	0.38	0.93**
Fe	0.30	1	0.56	0.43
Cu	0.38	0.56	1	0.57
Zn	0.93**	0.43	0.57	1

**. Correlation is significant $p < 0.01$ (2-tailed).

4.2.4 Heavy metals content in sugar juices from Nyahururu Region

Sugarcane from this region was grown in farms unlike those from Nairobi which were from along rivers. A comparison on the distribution of trace elements in the samples from this region showed similar as well as different trends.

The amounts of Cu, Fe and Zn in the sugarcane juice from Nyahururu farms (table 4.12) were different to those reported in other studies. Collin & Doelsch, (2010) reported a Zn concentration range 4.2-31.8 mg/kg in sugarcane juice which is relatively higher than detected in the present work. The difference could have been as a result of variations in metal content in the soil, availability and varieties. Another work by Muchuweti *et al*., (2006) reported amounts of Zn in sugarcane stems from Zimbabwe that were beyond the permited levels by Britain (50mg/kg). They atrributed these findings to contamination of soil by the use sewage slugde and waste water for agricultural purposes. On the other hand, the amounts of Fe and Cu in the current study were higher than those reported by Adekola & Akinpelu, (2002) for sugarcane from Nigeria Bacinta Estate. They reported concentrations of Fe and Cu in sugarcane juice were 0.266 mg/l and 0.04 mg/l respectively. In additon, it was noted that Cu concentrations in the juice was comparative to each sampling area. For example Cu amounts in IG1, IG2 and IG3 was nearly the same which could be due to common soil type of these samples. Availability and accumulation of a metal element is dependent on environmental factors such as soil mineralogy, texture and pH (Nayak, *et al*., 2014).

Table 4.12. Heavy metal content (mg/l) in juice from bottom and top parts of sugarcane from Nyahururu farms

	Mn		Fe		Cu		Zn	
	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top
IGW1	6.52±0.24	7.23±0.73	28.70±0.61	16.42±0.89	0.31±0.02	0.28±0.09	2.83±0.10	3.14±0.21
IGW2	10.07±0.43	11.17±0.32	14.16±0.47	11.94±0.34	0.30±0.02	0.29±0.02	2.31±0.12	4.37±0.14
IGW3	12.53±0.35	12.87±0.21	6.36±0.21	4.63±0.24	0.30±0.03	0.27±0.01	1.98±0.08	2.39±0.11
SBK1	1.82±0.16	2.03±0.18	10.27±0.56	8.50±0.43	0.18±0.01	0.15±0.01	0.79±0.06	1.38±0.13
SBK2	3.57±0.28	4.13±0.20	7.38±0.57	5.92±0.23	0.17±0.01	0.14±0.01	0.37±0.02	0.48±0.04
SBK3	1.89±0.13	2.30±0.17	7.12±0.41	5.74±0.25	0.31±0.02	0.22±0.01	0.57±0.05	0.99±0.07
MRI1	23.69±0.77	31.38±1.17	49.07±1.58	32.69±0.73	0.22±0.01	0.15±0.01	1.06±0.08	2.60±0.17
MRI2	18.34±0.98	20.33±1.53	25.54±0.70	23.12±0.78	0.30±0.02	0.24±0.01	0.95±0.06	1.45±0.16

The amounts (mg/l) of Mn in MRI1 and MRI2 were considerably higher (20.33-31.38 in top and 18.34-23.69 in bottom) than those found in IGW1, IGW2 and IGW3 (7.23-12.87 in top and 6.52-12.53 in bottom). The variation in the amounts of Mn could be as a result of the differences on the phytoavailabilities of the metal from two areas. Soil pH and total elemental content of the soil are the factors that determine Mn uptake and retention in sugarcane. Segura-Munoz *et al*., (2006) work on the metal levels in sugarcane reported an elevated amount of Mn in the cane tissues associated with lower soil pH (5.3-6.5). Acidification influences speciation changes of Mn to Mn^{2+} form which has higher solubility and thus resultant higher bioavailability. Moreover, presence of hydroxonium ions (associated with acidity) results to a reduction of metal adsoprtion through cation exchange and organic complexation since they displace other cations at the negative surface charges (Omwoma *et al.,* 2010). Furthermore, the efficiency of trace element extraction could be influenced by variation in varieties of the sugarcane. Wang *et al*., (2012) reported that a sugarcane variety, 'Guiyin9', had lesser amounts of heavy metals in comparison to other six varieties.

It was also noted that there were some large, positive and significant relationships between the concentrations of trace elements in the juice as shown in table 4.13. For instance the amount of Fe in the juice significantly and positively correlated with Mn concentrations. The ratio of these

two elements in plant tissues is crucial for metabolism in plants (Hajar *et al*., 2014). In addition the two metals are the most abudant in natural environments and hence the relative concentrations of the elements could be affected by the nature of the soil.

Table 4.13. Pearson Correlation values for associations between the concentrations of Mn, Fe, Cu and Zn in Nyahururu juice samples

	Mn	Fe	Cu	Zn
		Bottom		
Mn	1	0.74*	0.17	0.15
Fe	0.74*	1	-0.02	0.18
Cu	0.17	-0.02	1	0.60
Zn	0.15	0.18	0.60	1
		Top		
Mn	1	0.87**	-0.04	0.31
Fe	0.87**	1	-0.11	0.25
Cu	-0.04	-0.11	1	0.66
Zn	0.31	0.25	0.66	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

There was also a positive correlation between the concentrations of Zn and Cu in the juice. According to Collin & Doelsch, (2010) the amounts of Cu and Zn in sugarcane would be more dependent to soil environment rather than on the differing varieties. It was therefore an indication of a common source of these elements in soils from Nyahururu.

4.3 Comparison between the distributions of trace elements in Nairobi and Nyahururu sugarcane juice samples and respective soils

Trace elements in sugarcane showed distinct trends in their distribution between bottom and top. For both Nairobi and Nyahururu Zn and Mn concentrations were higher in the top section while Cu and Fe concentrations were higher in the bottom section. This tendency of different distribution of individual elements in different parts of plants was also reported by Hajar *et al*., (2014). They found out that metal concentrations differed in the different parts of *Stevia rebaudiana* and was relative to each element. For instance, the levels of Fe were in the order stems<flowers<leaves while Cu levels were in the order flowers<stems<leaves. Another study by Minkina, *et al*., (2012) reported that the accumulation of Zn, Pb and Cu in barley was relative

to the parts of the plant. They reported that Zn was likely to be accumulated in the roots, Cu in the grains while Pb in the straw. It was also observed that the concentration of the heavy metals in the soil were in the order Pb<Cu<Zn<Mn<Fe which was identical to the order observed for the amounts of the metals in the juice.

The concentrations of Fe, Zn, Cu and Mn in Nyahururu juice were different from those observed in Nairobi juice. It was observed that Mn and Fe in Nyahururu samples were generally higher than those detected in Nairobi as shown in figure 4.1. Five Nyahururu samples had Mn concentration of more than 5 mg/l while only two from Nairobi had amounts that exceeded 5 mg/l. On the other hand, four Nyahururu samples had Fe concentrations exceeding 10 mg/l while only one sample from Nairobi had more than 10mg/l. On the contrary, Cu and Zn concentrations in Nairobi were higher than those in Nyahururu. For instance, six Nairobi samples had Cu amounts higher than 0.3mg/l while only one sample from Nyahururu exceeded those amounts. These disparities could be due to the differences in concentrations of the respective metals in the various soils. Gebrekidan *et al.,* (2013) compared the accumulation of Cu, Zn, Fe and Mn in plants from two sites in Northern Ethiopia. They reported greater concentrations metals in plants from the site whose soil had higher amounts of heavy metals
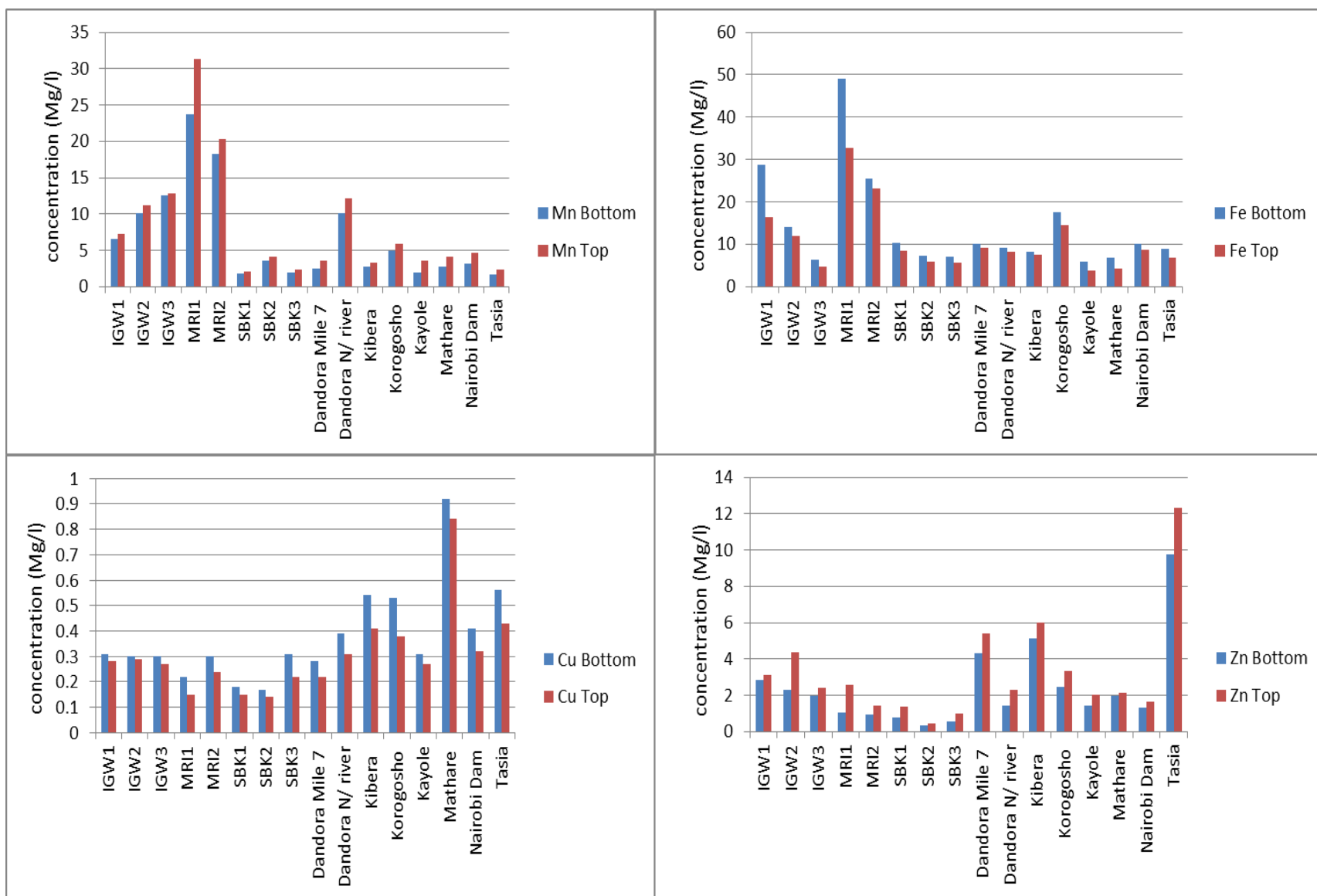
Figure 4.1. Comparative illustrations of the amounts of Cu, Fe, Zn and Mn for both Nyahururu and Nairobi juice samples

55

Just like the findings for the juice, the amounts of Fe levels in Nyahururu were generally higher than those from Nairobi as illustrated in figure 4.2. It suggested that the total amounts of Fe in the soil had a significant influence in the levels of the element in the juice. The amounts of Zn and Cu in Nairobi soil were higher, a similar trend to the one in juice. However, the Mn levels in Nairobi soils were higher than those from Nyahururu which was contradictory to what was observed for the juice. In addition, it was realized that Mn amount in SBK1 juice is disproportionate to that in the soil. Furthermore, it was noted that the amount of Zn in Tasia juice was the highest but the level in the soil was moderate. It suggested that the amounts of Mn and Zn were not only reliant on total metal content in the soil but also on the other factors. One of the most important factors that influence metal for plant uptake is speciation of trace elements in the soil. Heavy metal mobility and bioavailability for plant uptake is strongly influenced by their specific chemical forms in the soil (Singh & Kalamdhad, 2013). The most available chemical forms for plant uptake are those that are water soluble. The pH and anthropogenic activities influence highly the speciation of metals in soil. According to Chandra et al., (2008) the availability of Zn, Mn and Cu is highly regulated by pH.
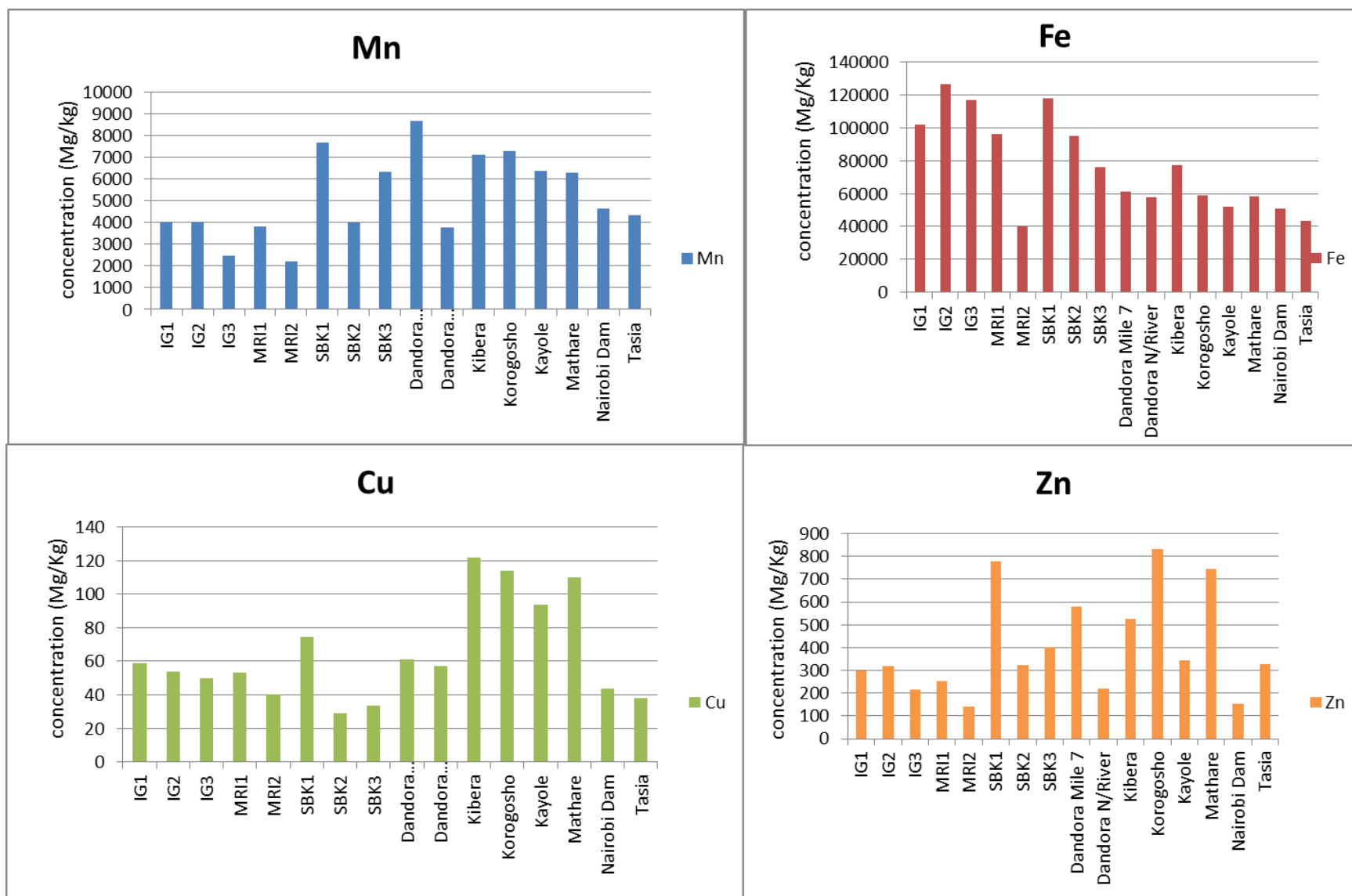
Figure 4.2. Comparative illustrations of the amounts of Cu, Fe, Zn and Mn for both Nyahururu and Nairobi soil samples

4.3.1 Transfer factors (TF) of trace element in sugarcane stem

Since it was observed that there were differences in amounts of elements in the bottom and top, it was important to show the availability of each element in specific part of the sugarcane. Transfer factor is the parameter that describes the bioavailability of an element at a specific part of a plant (Tsafe *et al*., 2012). It is the ratio of the concentration of the metal in the edible part and the total metal concentration in the soil (Gebrekidan *et al*., 2013). It is the relative tendency of a particular species of plant to accumulate a metal element. Thus soil-to-plant transfer factors were used to evaluate the efficiency of the plant in absorbing trace elements from the soil. They were obtained by dividing the amount of an element in the plant with the corresponding amount in the soil.

The heavy metals transfer factors for both top and bottom (table 4.16) were found to increase in the trend Fe<Mn<Cu<Zn. This order was reverse to the one observed for the same elements in soil and juice (Cu<Zn<Mn<Fe). The trend for transfer factors and their relation to the trend in plants and soils in the current work is in agreement to a study from Nigeria by Tsafe *et al*., (2012). They reported that the amount trace metals in the vegetables and soil were in increasing the order Cu<Pb<Zn<Mn<Fe while the transfer pattern for the same elements was in ascending order Pb<Fe<Mn<Zn<Cu.

Table 4.14. Mn, Fe, Zn and Cu transfer factors (10^{-5}) of sugarcane.

		Mn	Cu	Fe	Zn			Mn	Cu	Fe	Zn
D.N/River	Bottom	268.8	683.0	15.8	653.0	IGW1	Bottom	161.5	529.0	28.2	949.7
	Top	321.2	542.9	14.4	1041		Top	179.1	477.8	16.1	1054
Kibera	Bottom	39.0	439.0	10.8	975.3	IGW2	Bottom	272.8	556.6	11.2	726.4
	Top	46.1	33.3	9.7	1140		Top	275.3	518.7	9.4	1374
N.Dam	Bottom	67.3	938.2	20.0	845.2	IGW3	Bottom	505.0	604.8	5.5	925.2
	Top	100.1	732.3	17.1	1077		Top	518.7	518.7	4.0	1117
Tasia	Bottom	38.3	1478	20.7	2991	MRI1	Bottom	620.0	411.2	50.9	422.3
	Top	55.3	1135	15.7	3768		Top	821.3	280.4	33.9	1036
D.Mile 7	Bottom	29.5	458.3	16.3	748.3	MRI2	Bottom	835.2	746.3	63.9	673.8
	Top	41.2	360.1	15.0	936.2		Top	925.8	597.0	57.9	1028
Mathare	Bottom	44.3	836.4	11.9	266.8	SBK1	Bottom	23.7	241.0	8.7	101.3
	Top	65.2	763.6	7.2	284.2		Top	26.4	200.8	7.2	176.9
Kayole	Bottom	30.3	330.8	7.2	414.0	SBK2	Bottom	89.1	588.2	7.8	113.9
	Top	56.7	288.2	7.2	594.8		Top	103.1	484.4	6.2	147.7
Korogosho	Bottom	68.5	464.9	29.8	295.0	SBK3	Bottom	29.9	930.9	9.3	141.8
	Top	80.1	333.3	24.5	400.5		Top	36.4	660.7	7.5	246.3

4.3.2 Comparison of manganese amounts in soil and juice

Using the Pearson correlation tool to assess relationships, it was found that the levels of Mn in the top and bottom of sugarcane had significant correlation (r = 0.987, p<0.01). Further subjecting the data to t-test, it was revealed that the amounts in the top and bottom did not have statistical difference (p<0.05). It is indicative that similar factors influenced the relative Mn concentrations in Nyahururu in both bottom and top. This observation was supported the findings in the Mn transfer factors whereby there was a strong significant correlation (r = 0.990, p<0.01) between the top and bottom parts of Nyahururu sugarcane. Additionally, transfer factors of the bottom and top did not have a statistical difference (p<0.05). Furthermore, it was noted there was strong correlations between the Mn amounts in the top (r = 0.921, p<0.01) and bottom (r = 0.919, p<0.01) with their respective transfer factors.

The findings for Nyahururu juice were similar to those observed for Nairobi samples but with one distinction. There was strong relationship (r = 0.96, p<0.01) between the Mn concentration in the top and the bottom was obtained. There were also significant correlations between the Mn amounts in the juice and their respective transfer factors. Furthermore, a t-test assessment on the amounts of Mn in both Nyahururu and Nairobi samples revealed that there were no statistical differences between the two sample data sets. However, it was noted that there was a difference between Mn levels of the bottom and top parts of Nairobi samples. The Mn concentration in the top was significantly higher (p<0.05) than the amount detected in the bottom.

An evaluation on the relationships between the amounts of the Mn in the soil and that in sugarcane revealed negative correlations. The correlation coefficients for Nyahururu samples were large but not significant. The relationships between the Mn amounts in soil and juice from Nairobi were both large and significant. A t-test assessment of soil Mn concentration from both sampling areas shows that the two data sets do not have a significant statistical difference (p<0.05). This was in agreement to the findings by Khan, *et al*., (2013) on the relationship between the amounts of Mn in *Zea Mays* and the soil (r = -0.965). However they also reported a positive correlation coefficient (*r* = 0.879) for the amount of Mn in *Benincasa hispada*. This suggests that the ability of the plant to extract the Mn was relative to each plant species which would imply that sugarcane, in this case, is a poor accumulator of Mn. Additionally, Singh & Agrawal, (2007) showed that plants had a reduced Mn uptake despite the increase of the metal

levels in the soil due to soil amendments. Plants are known to develop strategies to resist heavy metal toxicity and as much as Mn is an essential element, elevated amounts of the element hinders growth (Hajar *et al*., 2014).

It was further observed that relationship between soil pH and Mn levels in the juice was negatively correlated. The Nyahururu soil pH had moderate association with Mn concentration (r = 0.387). On the other hand, Nairobi soils pH had large significant negative correlations with Mn juice concentration (r = - 0.770_{top}, p<0.05) and transfer factors (r = -0.781_{bottom}, p< 0.05). Zeng *et al*., (2011) reported a significant negative correlation coefficient between the soil pH with concentrations of Mn (r = -0.680, P< 0.001), in rice straw. It implies that the higher the pH of the soil led to reduced plant uptake of Mn. According to Wang *et al*., (2000) a reduction of pH in soil could lead to processes that would increase mobility bound cations and thus make them available for plant uptake. This observation was supported by the large correlation coefficients between Mn transfer factors and respective concentrations in Nairobi juice (r = 0.92_{bottom} and 0.96_{top} p<0.01).

4.3.3   Comparison of iron amounts in soil and juice

The Fe trend in Nyahururu and Nairobi samples showed few relationships between the soil and juice. In fact the highest relationship was between the Fe levels in the top part of Nyahururu cane and in the soil (r = -0.415). This finding was backed by the significant correlation (p<0.05) between the Nyahururu top transfer factors and Fe concentration in the soil. There was also a large negative relationship between the bottom transfer and Fe amounts in the soil. These observations suggested that the higher the amount of Fe in Nyahururu soils resulted to a lower availability of the metal for plant uptake. It was noted that the Nyahururu soils had a significant higher amount of Fe (p<0.05) than in Nairobi soil. According to Hajar *et al*., (2014) high concentrations of Mn, Ni and Co could reduce uptake and translocation of Fe. Moreover, higher amounts of Fe compounds in the soil negatively influences the uptake of other trace elements. The relationships between Fe levels in Nairobi soils and respective transfer factors and Fe concentrations in the juice were relatively small.

The relationships between soil pH and Fe amounts in the juice and transfer factors were small. It was also noted that even though some correlation coefficients observed for the relationship with the Fe levels in the juice were positive, negative values were observed for the transfer factors.

For example correlation coefficient for the relationship between soil pH and Fe levels in top Nyahururu juice was 0.291 while for the transfer factor was -0.039. These observations suggested that pH had an insignificant influence on the plant uptake and transport of Fe. This was in disagreement to the findings by Zeng *et al*., (2011) who reported a significant negative correlation coefficent (r = -0.501, p<0.001) for the relationship between the amount of Fe concentration in plant and soil pH. They also noted a significant positive correlation between soil organic matter and Fe concentrations in rice straw and grains. Organic matter is among the factors that determine the availability of metals for plant uptake. It increases the solubilty of metals in soil solution by forming complexes with the metals (chelates) and increasing cation exchange capacity in soils. Thus the transfer of Fe from the soil into the sugarcane could have been influenced by organic matter rather than soil pH.

Even though soil Fe concentrations were statistically different (p< 0.05), the mean amount of Fe in Nyahururu juice samples was not statistically different (p< 0.05) from that found in Nairobi juice samples. The levels of Fe in the top and bottom were not stastically different from each other for both data sets. This observation was supported by the same findings on the respective transfer factors of both the bottom and top. Transfer factors can be used to to show the the ability of a metal species to transfer from soil to various parts of plants (Gebrekidan *et al*., 2013). The ability of the sugarcane to translocate Fe in this case seems to be moderate and intended to achive certain amount of Fe in the cane. Plant metabolism is highly influenced by the ratio of Mn and Fe in the plant tissue and medium (Hajar *et al*., 2014) rather than the total concentration of the elements. It had been observed that there was no statistical difference between the Mn Nyahururu and Nairobi juice samples.

4.3.4   Comparison of copper amounts in soil and juice

It was observed that the relationships between the Cu concentrations in juice and those in the soil were irregular. It was seen that there was a medium positive correlation between the Nairobi soil Cu amount and the element's level in the juice. However a small negative correlation was observed for the relationship between the Cu concentration in Nyahururu top juice and respective amount of the metal in the soil. Nogueira *et al*., (2013) reported a positive correlation (r = 0.68) between the soil Cu concentration with those in sugarcane juice. They also reported a negative correlation (r = -0.34) between Cu concentration in cane plant juice and that in the soil. On ther

hand, Collin & Doelsch, (2010) reported a small positive correlation between the total heavy metal concentration in soil and subsequent Cu levels in sugarcane juice. This was attributed to a low Cu phytoavailability which was not proportional to the metal levels in the soil.

However the transfer factors revealed a clearer picture to the relationship with the Cu amounts in the soil. The Cu transfer factors were negatively correlated with the respective Cu levels in the soils, with the relationships observed for Nyahururu samples being significant. These findings suggested that there was indication of the plant inability or resistance to extract and transport Cu into the stem. Zhang *et al*., (2014) reported that majority of Cu in the cane (78% - 93%) was detected in the root of the cane. This was attributed to either binding of Cu to the roots, poor transport of the element or counter of the metal back to the roots. It was also observed that the amounts of the Cu in the bottom parts of the cane was statistically higher ($p < 0.05$) than those in the top which was also in agreement with findings by Zhang *et al*., (2014). They also reported that the juice Cu amounts increased in different stem parts with the increase of the metal amounts in the growth medium ($p < 0.001$). The Cu concentrations in juice from Nairobi were higher ($p < 0.05$) than those detected in Nyahururu juice which was the same observation for the Cu levels in the particular soils. The plant uptake of Cu is influenced by soil depends on the total amount of Cu in the soil and plants ability to transfer the metal across the soil–root interface (Hajar *et al*., 2014).

The difference in the trend seen in Nyahururu sample from that observed in Nairobi samples was in the relationships between soil pH and Cu concentration in the juice and transfer factors. Nyahururu soil pH was slightly negatively correlated to both Cu levels in the juice and transfer factors. This was in agreement with work by Zeng *et al*., (2011) that there was negative and significant correlation Cu ($r = -0.593$, $P < 0.001$) between soil pH and Cu amount in straw. Yang *et al.,* (2014) also reported decrease on soil pH led to the elevated availability of Cu and increase in the metal level in green vegetables. However the correlations observed in the current work were relatively small and insignificant. Segura-Mun˜oz *et al*., (2006) also reported no significant correlations ($P > 0.05$) between soil pH and the levels of metal in sugarcane stems or leaves. On the contrary, the Nairobi soil pH was moderately and positively correlated to the amount of Cu in the juice. There were also a slight positive relationship between Nairobi soil pH and Cu transfer factors. Zhao *et al.,* (2011) found out that some metal elements uptake of plant increased with

higher pH value.   They reported was positive correlation coefficients for Cu and Pb   an indication that there higher uptake of the elements at relatively higher pH values.

4.3.5   Comparison of zinc amounts in soil and juice

There was a general negative relationship between the juice Zn concentration and transfer factors with the amount of the metal in the soil. There were small correlations between Nyahururu juice Zn levels and those in the soil. The correlation coefficients ($r = -0.235_{top}$ and $r = -0.277_{bottom}$) were in agreement to those found by Khan *et al.,* (2013) in work on heavy metals in agricultural soils and crops. They reported a negative correlation between Zn levels in soils with those in *Juglans regia* ($r = -0.264$). The relationships between the Zn concentrations in Nairobi juice with soils were negligible. The correlation coefficient for the relationship between Zn levels in Nyahururu top and soil ($r = -0.041$) was similar to that reported by Khan *et al.,* (2013) for the relationship between *Mormordica charantia* and Zn concentration in the soil ($r = -0.039$). This observation was in agreement with Collin & Doelsch, (2010) who reported no correlation between Zn total soil concentration and the element concentration in juice. This was atrributed to the natural origin of the element in the soil and hence Zn phytoavailability in this case was low. According to Walker *et al*., (2004) the availability and uptake of Zn is mainly influenced by the effect of compost and manure on soil pH. However Adekola & Akinpelu, (2002) work on sugarcane in Nigeria reported positive correlation values ($r = 0.302$) between the Zn concentrations in soil and sugarcane juice.

There was a small relationship between the amounts of Zn in the juice and the soil pH for both data sets. However it was noted that Nyahururu transfer factors were moderately negatively correlated to the pH. It was an indication that the availabilty and uptake of the metal to the different parts increased with reduction of pH. Yang *et al.,* (2014) reported that reductions in soil pH led to an increased Zn phytoavailability in the soil and hence higher concentration of the metal in leafy vegetables.

It was also noted that the Zinc concentration in top section was statistically higher than the levels in the bottom section ($p<0.05$) in both Nyahururu and Nairobi juices. This observation was in agreement with Zhang *et al.,* (2014) who reported an irregular distribution of Zn in the sugarcane stem with the top part of the cane having higher Zn concentration ($p <0.001$). Sirengo (2001) also reported that zinc is accumulated more in roots than in leaves and stems.

CHAPTER FIVE

CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

The sugarcane juice contained substantial amounts of Fe, Zn, Cu and Mn. The heavy metal concentrations in sugarcane juice followed the trend Cu < Zn < Mn < Fe. The concentrations of metals in juice, however, varied in the different parts of the sugarcane. Fe and Cu concentrations in bottom sections were higher while Mn and Zn levels in top sections of the cane were larger. In addition, the metal concentrations in sugarcane juice varied widely depending on each sample and specific elements. For Nairobi samples, the uptake and distribution of metals between the bottom and top section of the sugarcane was dependent on individual uptake mechanisms for each element. On the other hand, there were positive and significant correlation between the concentrations of trace elements in Nyahururu juice which was an indication of common factors determining the uptake and distribution of metals in the plant. The Kibera, Mathare and Tasia samples, from Nairobi, were found to have Zn concentrations that were beyond the permitted level.

It was noted that the levels of Fe and Mn in the soils were higher than amounts observed for other heavy metals. The heavy metal concentrations in the soil were found to be in the trend order Pb < Cu < Zn < Mn < Fe. Nairobi soils were observed to have unique distribution of heavy metals for each sampling point. It was noted that the Mathare, Korogosho, Kibera and Dandora mile 7, all from Nairobi, had large concentrations of heavy metals that were also above the levels prescribed for agricultural soils. On the other hand, concentration and distribution of heavy metals between the Nyahururu soil samples had common trends. Zn and Mn concentrations in Nyahururu samples were significantly correlated and were affected by the pH value of the soil. Nonetheless, it was observed that most of soil samples, both from Nyahururu and Nairobi, had neutral pHs.

It was observed that Mn and Fe concentrations in Nyahururu juice samples were generally higher than that those detected in Nairobi samples. On the contrary, Cu and Zn concentrations in Nairobi juice samples were higher than those in the Nyahururu samples. Just like in the

observations for the juice, the levels of Fe in Nyahururu soil samples were generally higher than those from Nairobi. However, the Mn levels in Nairobi soils were higher than those from Nyahururu which was contradictory to what was observed for the juice. The heavy metals transfer factors for both top and bottom were found to increase in the trend Fe<Mn<Cu<Zn which was reverse of the concentration trend in soils and juice. It was also observed that the soil pH was negatively correlated to the Mn concentration in the juice. Even though, Fe concentrations in Nyahururu soils were higher ($p< 0.05$), the mean amount of Fe in Nyahururu juice was not statistically different ($p < 0.05$) from of Nairobi juice samples. Nyahururu soil pH was slightly negatively correlated to both Cu levels in the juice and transfer factors while Nairobi soil pH was moderately and positively correlated to the concentrations of Cu in the juice. There was a negative correlation for the relationships between the juice Zn concentration and transfer factors with the amount of the metal in the soil.

Therefore data and information obtained from this study is of importance in addressing contamination of food consumed by vulnerable populations in Nairobi. For instance, some areas were found to have high levels of heavy metals in soil and in juice. Thus this information could be used by the ministry of health in the national government of Kenya or the health department of the county government of Nairobi in developing policies in relation to consumption of sugarcane and other crops grown along rivers of Nairobi river basin. The data could also be used by organizations such as NEMA and UNEP to address the heavy metal pollution of Nairobi soils.

5.2 Recommendations

The following recommendations were made:

1) Since some sugarcane samples from the three rivers in Nairobi were found to have high levels of heavy metals, consumption of sugarcane juice from these areas should be discouraged because it may pose increased health risks to the local population in the long term.
2) Some of Nairobi soil samples were not suitable for agriculture and hence a program should be initiated to remediate soils from these areas.
3) In addition, more studies should be done on heavy metal contamination of other crops grown in Nairobi river basin and also on other contaminants such as pesticides.

REFERENCES

Abdus-salam, N., Adekola, F. A., & Bolorunduro, O. J. (2008). Environmental Assessment of the Impact of Feed Water on the Quality of Sugarcane Juice. *International Jour. Chem. 18(3)*, 129-135.

Abreu, C. A., Coscione, A. R., Pires, A. M., & Paz-Ferreiro, J. (2012). Phytoremediation of a soil contaminated by heavy metals and boron using castor oil plants and organic matter amendments. *Journal of Geochemical Exploration 123*, 3-7.

Adekola, F. A., & Akinpelu, A. A. (2002). Some trace elements in juice and bagasse of two varieties of sugarcane, location soil and irrigation water from Bacita Sugar Estate, Nigeria. *Bioscience Research Communications 14(2)*, 17 -22.

Akinyele, I. O., & Shokunbi, O. S. (2015). Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. *Food Chemistry 173* , 682–684.

Alissa, E., & Ferns, G. (2011). Heavy Metal Poisoning and Cardiovascular Disease. *Journal of Toxicology 2011*, 1-22.

Bao, S.-X., Wang, Z.-H., & Liu, J.-S. (1999). X-Ray Fruorescence Analysis of Trace Elements in Fruit Juice. *Spectrochimica Acta Part B54* , 1893-1897.

Barzegar, A. R., Koochekzadeh, A., Xing, B., & Herbert, S. J. (2005). Concentration Changes of Cd, Ni and Zn in Surgarcane Cultivated Soils. *Water, Air, and Soil Pollution 161*, 97-112.

CCME. (1999). Canadian Council of Ministers of the Environment updated 2001. Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health. Canadian Environmental Quality Guidelines, No.1299. Winnipeg: CCME.

Chandra, R., Bharagava, R., Yadav, S., & Mohan, D. (2008). Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents. *Journal of Hazardous Materials 162*, 1514-1521.

Chen, L., Luo, S., Li, X., Wan, Y., Chen, J., & Liu, C. (2014). Interaction of Cd-hyperaccumulator Solanum nigrum L. and functional endophyte Pseudomonas sp. Lk9 on soil heavy metals uptake. *Soil Biology & Biochemistry 68*, 300-308.

Chen, Y., Hu, W., Huang, B., Weindorf, D. C., Rajan, N., Liu, X., et al. (2013). Accumulation and health risk of heavy metals in vegetables from harmless and organic vegetable production systems of China. *Ecotoxicology and Environmental Safety 98*, 324-330.

Collin, B., & Doelsch, E. (2010). Impact of high natural soilborne heavy metal concentrations on the mobility and phytoavailability of these elements for sugarcane. *Geoderma 159*, 452-458.

D'mello, J. P. (2003). *Food safety: Contaminants and toxins.* London: Oxford University Press.

De La Calle, I., Cabaleiro, N., Romero, V., Lavilla, I., & Bendicho, C. (2013). Sample pretreatment strategies for total reflection X-ray fluorescence analysis: A tutorial review. *Spectrochimica Acta Part B 90* , 23–54.

Du, Y., Gao, B., Zhou, H., Ju, X., Hao, H., & Yin, S. (2013). Health risk assessment of heavy metals in road dusts in urban parks of Beijing, China. *Procedia Environmental Sciences*, 299-309.

Duffus, J. (2002). "Heavy Metal" - a meaningless term? *Pure Appl Chem*, 793-807.

Ewers, U. (1991). Standards, guidelines and legislative regulations concerning metals and their compounds. In Merian, E. *Metals and their Compounds in the Environment: Occurrence, Analysis and Biological Relevance* (458–468). Weinheim: VCH.

FAO/WHO. (2001). Food Additives And Contaminants. *Codex Alimentarius commission: Joint FAO/WHO Food Standards Program, 01/12a, pp.*, 1-289.

Florido, M. C., Madrid, F., & Madrid, L. (2011). Effect of an organic amendment on availability and bio-accessibility of some metals in soils of urban recreational areas. *Environmental Pollution 159*, 383-390.

Gebrekidan, A., Weldegebriel, Y., Hadera, A., & Bruggen, B. (2013). Toxicological assessment of heavy metals accumulated in vegetables and fruits grown in Ginfel river near Sheba Tannery, Tigray, Northern Ethiopia. *Ecotoxicology and Environmental Safety 95*, 171-178.

Ghaderian, S. N., & Ravandi, A. G. (2012). Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh. *Journal of Geochemical Exploration 123*, 25-32.

Grytsyuk, N., Arapis, G., Perepelyatnikova, L., Ivanova, T., & Vynograds'ka, V. (2006). Heavy metals effects on forage crops yields and estimation of elements accumulation in plants as affected by soil. *Science of the Total Environment 354*, 224-231.

Hajar, E. W., Sulaiman, A. Z., & Sakinah, A. M. (2014). Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of Stevia rebaudiana Plant. *Procedia Environmental Sciences 20*, 386 – 393.

Harguinteguy, C. A., Cirelli, A. F., & Pignata, M. L. (2014). Heavy metal accumulation in leaves of aquatic plant Stuckenia filiformis and its relationship with sediment and water in the Suquía river (Argentina). *Microchemical Journal 114*, 111-118.

Hu, X.-F., Jiang, Y., Shu, Y., Hu, X., Liu, L., & Luo, F. (2014). Effects of mining wastewater discharges on heavy metal pollution and soil enzyme activity of the paddy fields. *Journal of Geochemical Exploration147* , 139–150.

Huang, H., Pan, X.-D., Wu, P.-G., Han, J.-L., & Chen, Q. (2014). Heavy metals in vegetables and the health risk to population in Zhejiang, China. *Food Control 36*, 248-252.

Hussein, F., Njue, W., Murungi, J., & Wanjau, R. (2008). Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya. *Science of the T otal Environment 393*, 376–384.

Inoti, J., Kawaka, F., Orinda, G., & Okemo, P. (2012). Assessment of heavy metal concentrations in urban grown vegetables in Thika Town, Kenya. *African Journal of Food Science 6(3)*, 41-46.

Jamali, M. K., Kazi, T. G., Arain, M. B., Afridi, H. I., Jalbani, N., Kandhro, G. A., et al. (2009). Heavy metal accumulation in different varieties of wheat (Triticum aestivum L.) grown in soil amended with domestic sewage sludge. *Journal of Hazardous Materials 164*, 1386-1391.

Jbrgensen, N., Laursen, J., Viksna, A., Pind, N., & Holm, P. E. (2005). Multi-elemental EDXRF mapping of polluted soil from former horticultural land. *Environment International 31* , 43– 52.

Jirsa, F., Gruber, M., Stojanovic, A., Omondi, S. O., Mader, D., Körner, W., et al. (2013). Major and trace element geochemistry of Lake Bogoria and Lake Nakuru, Kenya, during extreme draught. *Chemie der Erde 73*, 275– 282.

Jung, M. (2008). Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine. *Sensors 8*, 2413-2423.

Kamau, J. N., Gachanja, A., Ngila, C., Kazungu, J. M., & Zhai, M. (2014). The seasonal influence on the spatial distribution of dissolved selected metals in Lake Naivasha, Kenya. *Physics and Chemistry of the Earth 67–69* , 111–116.

Kapungwe, E. M. (2013). Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. *Journal of Geography and Geology 5(2)*, 1-18.

Karalliedde, L., & Brooke, N. (2012). *Toxicity of heavy metals and trace elements. In essentials of toxicology for health protection: A handbook for field professionals.* Oxford: Oxford University Press.

Kelepertzis, E. (2014). Accumulation of heavy metals in agricultural soils of Mediterranean: Insights from Argolida basin, Peloponnese, Greece. *Geoderma 221–222*, 82-90.

Kent, A., & Riegel, R. E. (2007). *Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology: Vol. 1*. New York: Springer- Verlag.

Khan, K., Lu, Y., Khan, H., Ishtiaq, M., Khan, S., Waqas, M., et al. (2013). Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. *Food and Chemical Toxicology 58*, 449-458.

Khan, M. U., Malik, R. N., & Muhammad, S. (2013). Human health risk from Heavy metal via food crops consumption with wastewater irrigation practices in Pakistan. *Chemosphere 93*, 2230-2238.

Kihampa, C., Mwegoha, W., & Shemdoe, R. (2010). Heavy metals concentrations in vegetables grown in the vacinity of the closed dumpsite. *International Journal of Environmental Sciences 2(2)*, 1-7.

Koumolou, L., Edorh, P., Montcho, S., Aklikokou, K., Loko, F., Boko, M., et al. (2013). Health-risk market garden production linked to heavy metals in irrigation water in Benin. *Comptes Rendus Biologies 336*, 278-283.

Lagerkvist, C. J., Hess, S., Okello, J., Hansson, H., & Karanja, N. (2013). Food health risk perceptions among consumers, farmers, and traders of leafy vegetables in Nairobi. *Food Policy 38* , 92-104.

Latare, A. M., Kumar, O., Singh, S. K., & Gupta, A. (2014). Direct and residual effect of sewage sludge on yield, heavy metals content and soil fertility under rice–wheat system. *Ecological Engineering 69*, 17-24.

Li, M. S., Luo, Y. P., & Su, Z. Y. (2007). Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. *Environmental Pollution 147*, 168-175.

Li, Q., Chen, Y., Fu, H., Cui, Z., Shi, L., Wang, L., et al. (2012). Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary, China. *Journal of Hazardous Materials 227– 228*, 148-154.

Mahmood, A., & Malik, R. N. (2014). Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry 7(1)*, 91-99.

Marguí, E., Tapias, J. C., Casas, A., Hidalgo, M., & Queralt, I. (2010). Analysis of inlet and outlet industrial wastewater effluents by means of benchtop total reflection X-ray fluorescence spectrometry. *Chemosphere 80*, 263-270.

Miller, J. N., & Miller, J. C. (2010). *Statistics and Chemometrics for Analytical Chemistry.* Harlow: Pearson Education Limited.

Minkina, T. M., Motuzova, G. V., Mandzhieva, S. S., & Nazarenko, O. G. (2012). Ecological resistance of the soil–plant system to contamination by heavy metals. *Journal of Geochemical Exploration 123*, 33-40.

Mino, Y. (2006). Determination of Tin in Canned Foods by X-Ray Fluorescence Spectrometry. *Journal of Health Science 52(1)*, 67-72.

Mino, Y., & Yamada, Y. (2005). Detection of High Levels of Arsenic and Mercury in Some Chinese Traditional Medicines Using X-Ray fluorescece Spectrometry. *Journal of Health Science 51(5)*, 607-613.

Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M., & Lester, J. N. (2006). Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agriculture, Ecosystems and Environment 112* , 41-48.

Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B., & Mishra, S. (2010). Effect of Toxic Metals on Human Health. *The Open Nutraceuticals Journal 3*, 94-99.

Muohi, A. W., Onyari, J. M., Omondi, J. G., & Mavuti, K. M. (2003). Heavy metals in sediments from Makupa and Port–Reitz Creek systems: Kenyan Coast. *Environment International 28*, 639–647.

Mutune, A. N., Makobe, M. A., & Abukutsa-Onyango, M. O. (2014). Heavy metal content of selected African leafy vegetables planted in urban and peri-urban Nairobi, Kenya. *African Journal of Environmental Science and Technology 8(1)*, 66-74.

Nabulo, G., Young, S. D., & Black, C. R. (2010). Assessing risk to human health from tropical leafy vegetables grown on contaminated urban soils. *Science of the Total Environment 408*, 5338-5351.

Nakayama, S. M., Ikenaka, Y., Muzandu, K., Choongo, K., M'kandawire, E., Yasuda, J., et al. (2012). Metal and metalloid levels and bio-accumulation characteristics in soil, sediment, land

plants and hippopotami (Hippopotamus amphibius L) from the South Luangwa National Park, Zambia. *Ecotoxicology and Environmental Safety 80*, 333–338.

Nayak, A. K., Raja, R., Rao, K. S., Shukla, A. K., Mohanty, S., Shahid, M., et al. (2015). Effect of fly ash application on soil microbial response and heavy metal accumulation in soil and rice plant. *Ecotoxicology and Environmental Safety114*, 257–262.

Ndeda, L. A., & Manohar, S. (2014). Determination of Heavy Metals in Nairobi Dam Water, (Kenya). *Journal of Environmental Science, Toxicology and Food Technology 8(5)* , 68-73.

Ngure, V., Davies, T., Kinuthia, G., Sitati, N., Shisia, S., & Oyoo-Okoth, E. (2014). Concentration levels of potentially harmful elements from gold mining in Lake Victoria Region, Kenya: Environmental and health implications. *Journal of Geochemical Exploration 144* , 511–516.

Nogueira, T. A., Franco, A., He, Z., Santoro, B. V., Firme, L. P., & Abreu-Junior, C. H. (2013). Short-term usage of sewage sludge as organic fertilizer to sugarcane in a tropical soil bears little threat of heavy metal contamination. *Journal of Environmental Management 114*, 168-177.

Omwoma, S., Lalah, J., Ongeri, D., & Wanyonyi, M. (2010). Impact of Fertilizers on Heavy Metal Loads in Surface Soils in Nzoia Nucleus Estate Sugarcane Farms in Western kenya. *Bulletin of Environmental Contamination and Toxicology 85(6)*, 602-8.

Orwa, T. O. (2015). *Assessment of selected plants growing along Nairobi River for uptake of copper, zinc and cadmium, Nairobi County, Kenya.* Masters Thesis: Jomo Kenyatta University of Agriculture and Technology.

Oyedele, D., Gasu, M., & Awotoye, O. (2008). Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump sites in IIe-Ife, Nigeria. *African Journal of Environmental Science and Technology 3 (5)*, 107-115.

Oyoo-Okoth, E., Admiraal, W., Osano, O., Manguya-Lusega, D., Ngure, V., Kraak, M. H., et al. (2013). Contribution of soil, water and food consumption to metal exposure of children from geological enriched environments in the coastal zone of Lake Victoria, Kenya. *International Journal of Hygiene and Environmental Health 216* , 8– 16.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., & Jae-Woo, C. (2011). Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. *Journal of Hazardous Materials 185*, 549–574.

Parth, V., Murthy, N., & Saxena, P. (2011). Assessment of heavy metal contamination in soil around hazardous waste disposal sites in Hyderabad city (India): natural and anthropogenic implications. *Journal of Environmental Research and Management 2(2)*, 027-034.

Pendias, A. K., & Pendias, H. (1992). *Elements of group VIII, Trace Elements in Soils and Plants.* Boca Raton: CRC Press.

Rahman, M. A., Rahman, M. M., Reichman, S. M., Lim, R. P., & Naidu, R. (2014). Heavy metals in Australian grown and imported rice and vegetableson sale in Australia: Health hazard. *Ecotoxicology and Environmental Safety 100*, 53-60.

Segura-Mun˜oz, S. I., Oliveira, A. d., Nikaido, M., Trevilato, T. M., Bocio, A., Takayanagui, A. M., et al. (2006). Metal levels in sugar cane (Saccharum spp.) samples from an area under the influence of a municipal landfill and a medical waste treatment system in Brazil. *Environment International 32* , 52 – 57.

Sereno, M. L., Almeida, R. S., Nishimura, D. S., & Figueira, A. (2007). Response of sugarcane to increasing concentrations of copper and cadmium and expression of metallothionein genes. *Journal of Plant Physiology 164*, 1499-1515.

Shackleton, C. M., Pasquini, M. W., & Drescher, A. W. (2009.). *African indigenous vegetables in urban agriculture.* London: Earthscan.

Singh, J., & Kalamdhad, A. S. (2013). Effect of Eisenia fetida on speciation of heavy metals during vermicomposting of water hyacinth. *Ecological Engineering 60*, 214-223.

Singh, R. P., & Agrawal, M. (2007). Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. *Chemosphere 67*, 2229–2240.

Sirengo, J. S. (2001). *Uptake Characteristics of Trace elements: Lead, Zinc, Cadmium and Mercury by Selected Food Crops Grown Along Nairobi River.* Masters Thesis: University of Nairobi.

SPSS-Inc. (2002). *SPSS Base 11.0 for Windows User's Guide.* Chicago: Prentice-Hall.

Szyczewski, P., Siepak, J., Niedzielski, P., & Sobczyński, T. (2009). Research on Heavy Metals in Poland. *Polish Jnalour of Environmental Studies 18(5)*, 755-768.

Towett, E. K., Shepherd, K. D., & Cadisch, G. (2013). Quantification of total element concentrations in soils using total X-ray fluorescence spectroscopy (TXRF). *Science of the Total Environment 463–464*, 374–388.

Tsafe, A., Hassan, L., Sahabi, D., Alhassan, Y., & Bala, B. (2012). Evaluation of Heavy Metals Uptake and Risk Assessment of Vegetables Grown in argalma of Northern Nigeria. *Journal of Basic and Applied Scientific Research 2(7)*, 6708-6714.

Tzeng, D., Chia, Y., Tai, C., & Ou, A. (2010). Investigation of Chemical Quality of Sugarcane (Saccharum Officinarum L.) Wine During Fermentation by Saccharomyces Cerevisiae. *Journal of Food Quality 33*, 248-267.

UNEP. (2001). *Nairobi river Basin Project.* Nairobi: Regional Office for Africa, UNEP.

UNEPGEMS. (2006). *Water Quality for Ecosystem and Human Health.* Burlington: United Nations Environment Programme Global Environment Monitoring System.

Walker, D. J., Clemente, R., & Bernal, M. P. (2004). Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of Chenopodium album L. in a soil contaminated by pyritic mine waste. *Chemosphere 57* , 215–224.

Wang, H. F., Takematsu, N., & Ambe, S. (2000). Effects of soil acidity on the uptake of trace elements in soybean and tomato plants. *Applied Radiation and Isotopes 52*, 803-811.

Wang, X., Chen, T., Lei, M., Song, B., Wan, X., & Li, Y. (2012). Selection of Sugar Cane Varieties with a Low Heavy Metal Accumulation Abilty for the Ecological Remediation of Contaminated Farmland. *Journal of Resources and Ecology 3(4)*, 373-378.

Wang, Y., Qiao, M., Liu, Y., & Zhu, Y. (2012). Health risk assessment of heavy metals in soils and vegetables from wastewater irrigated area, Beijing-Tianjin city cluster, China. *Journal of Environmental Sciences 24(4)*, 690-698.

Wei, B., & Yang, L. (2010). A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchemical Journal 94*, 99-107.

Yang, L., Huang, B., Hu, W., Chen, Y., Mao, M., & Yao, L. (2014). The impact of greenhouse vegetable farming duration and soil types on phytoavailability of heavy metals and their health risk in eastern China. *Chemosphere 103*, 121-130.

Yang, S.-X., Liao, B., Li, J.-t., Guo, T., & Shu, W.-S. (2010). Acidification, heavy metal mobility and nutrient accumulation in the soil–plant system of a revegetated acid mine wasteland. *Chemosphere 80*, 852-859.

Yun-Guo, L., Hui-Zhi, Z., Guang-Ming, Z., Bao-Rong, H., & Xin, L. (2006). Heavy Metal Accumulation in Plants on Mn Mine Tailings. *Pedosphere 16(1)*, 131-136.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., et al. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution 159* , 84-91.

Zhang, X., Zhu, Y., Zhang, Y., Liu, Y., Liu, S., Guo, J., et al. (2014). Growth and metal uptake of energy sugarcane (Saccharum spp.) in different metal mine tailings with soil amendments. *Journal of Environmental Sciences 26*, 1080-1089.

Zhang, Y., Zhao, L., Wang, Y., Yang, B., & Chen, S. (2008). Enhancement of heavy metal accumulation by tissue specific co-expression of iaaM and ACC deaminase genes in plants. *Chemosphere 72*, 564-571.

Zhao, H., Xia, B., Chen, F., Zhao, P., & Shen, S. (2012). Human health risk from soil heavy metal contamination under different land uses near Dabaoshan mine, Southern China. *Science of the Total Environment 417-418*, 45-54.