DETERMINATION OF THE LEVELS OF SELECTED HEAVY METALS IN SOIL AND IN KHAT (*Catha edulis* Forsk) GROWN IN KENYA

Sailus Mugendi Ireri (B.Sc., PGDE)
Chemistry department

A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science (Analytical Chemistry) in the School of Pure and Applied Sciences of Kenyatta University.

June, 2014
DECLARATION

I declare that this thesis is my original work and it has not been presented to any other university.

Sailus M. Ireri........................................Date………………………...

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

Signature: ______________________ Date: __________________________

Prof. Jane Murungi
Department of chemistry
Kenyatta University

Signature: ______________________ Date: __________________________

Dr. Harun mbuvi
Department of chemistry
Kenyatta University
DEDICATION

This work is dedicated to my family.
ACKNOWLEDGEMENTS

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ACRONYMS AND ABBREVIATIONS

AAS         Atomic Absorption Spectroscopy
AOAC        Association of Official Analytical Chemists
ATSDR       Agency for Toxic Substances and Disease Registry
CAC         Codex Alimentarius Commission
CCME        Canadian Council of Environmental Ministers
CNS         Central Nervous System
DNA         Deoxyribonucleic Acid
DW          Dry Weight
EDXRF       Energy Dispersive X-ray Fluorescence
ECDD        Expert Committee on Drug Dependence
EPA         Environmental Protection Agency
GI          Gastrointestinal
ABSTRACT

Heavy metal pollution is among the leading health concerns all over the world because of their long-term cumulative effects. Consumption of food crops contaminated with heavy metals is a major food chain route for human exposure. Khat (Catha edulis forsk), a plant used as a stimulant is grown in certain areas of East Africa and the Arab Peninsula. In Kenya, it is cultivated in Embu and Meru counties and distributed to consumers in other counties who include young children. Due to increased demand and value, many farmers have not only begun growing it but have also adopted modern farming methods which include application of fertilizers, pesticides, compost manure, and irrigation. Yet some of these agricultural practices such as application of fertilizers and pesticides are known to increase the concentration of heavy metals such as Cd, Pb, Zn and Cu in the soil. Some of these heavy metals such as Pb and Cd are toxic even at low concentrations while Zn, Cu, Fe and Cr though essential in the body, are toxic at high levels. This call for monitoring to make sure that the levels of heavy metals in khat do not exceed the threshold limits recommended by WHO due to their adverse health effects to man. This study therefore determined the levels of Zn, Cu, Pb, Cd and Cr in khat and in the soils where the plant is grown. Soil and khat samples were collected from the three main khat growing regions namely Embu, Mbeere and Nyambene. Known weights of oven
dried khat and soil samples were digested using nitric and perchloric acids. The digests were analysed for selected heavy metals using flame atomic absorption spectrophotometer. The following concentration ranges in dry weight (µg/g) were obtained in khat: Zn(25.15-73.95), Cu(0.10-41.80), Cr(ND-39.50), Cd(ND-0.90) and Pb(0.50-13.00). Cd was only detected in khat samples from Embu. In soil, the concentrations of selected metals in dry weight (µg/g) were as follows: Zn(25.50-440.30), Cu(0.65-54.40), Cr(7.50-170.50) Cd(0.05-1.40) and Pb(5.00-119.00). Levels of these metals in soil were below the permissible limits for agricultural land use set by FAO/WHO. Correlation study revealed positive correlation between metals in soil and khat except for Zn, Cu and Pb in Embu. The ANOVA results suggested that there was significant different (p’0.05) in the levels of heavy metals between khat from various regions. Levels of studied heavy metals in khat were below the maximum limits recommended by WHO except for Pb and Cr. Therefore children should be discouraged from chewing khat since they are more susceptible to adverse effects of Pb than adults.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Khat (*Catha edulis* Forsk) is an evergreen perennial shrub plant that belongs to the Celastraceae family. It is widely cultivated in East Africa and Arabian Peninsula more specifically in Yemen (Tilahun, 2009). Its young leaves and stem tips contain higher proportions of cathinone, which is responsible for much of the stimulant effect of khat (Graziani *et al.*, 2008). Khat chewing is highly prevalent in East African and some Middle Eastern countries. Its use is both a social and a culture-based activity and it is said to enhance social interaction. It is chewed by both the young and old people in Kenya. On average, almost 70% of households in Yemen and 50% in Djibouti use khat (Milanovic, 2008), and more than 30% of Ethiopians have been reported to use khat (Belew *et al.*, 2000). In Kenya the total amount of khat consumed is estimated as 12,000 kg daily (Maitai, 2013).

Khat farming in Kenya is legal and it is popular in Meru and Embu counties. It is a major source of income besides being chewed for its stimulating effect. The employment created through khat farming is very high as a large number of people are involved in growing, harvesting, parking, transporting and selling. The varieties of khat depend upon the geographical area of cultivation (Tilahun, 2009). They differ in color, size and height of the leaves and size and height of the plant as a whole (Atlabachew *et al.*, 2011).
The soil on which khat grows is a good source of essential elements for human beings. However the presence of non-essential elements such as lead and cadmium in the soil leads to their uptake by khat. These metals end up in the soil from different sources which include agricultural practices such application of phosphatic fertilizers, pesticides and refuse derived composts (Alloway, 1995). Nartey et al. (2012) reported that heavy metals in fertilizer amended soils were higher when compared to those in natural soils due to soils retaining heavy metals sourced from the applied fertilizers. Additionally, foliar uptake of atmospheric heavy metals emissions have been identified as an important pathway of heavy metal contamination in crops (Salim et al., 1992). Some livestock manures such as pig and poultry manures also contain elevated concentrations of copper and zinc which come from animal feeds with added minerals to improve food conversions efficiency (Alloway, 1995).

The input of heavy metals to soil from various sources may prove detrimental to plant through its uptake to toxic limit, thereby facilitating its entry into food the chain. These metals have the capacity to affect adversely the activity of a living organism, its growth, health, life span and reproduction performance (ATSDR, 1998; Ferner, 2001). They are extremely persistent in the environment, non-biodegradable and thus readily accumulate to toxic levels (Sharma et al., 2007) and therefore pose a
significant health risk to human particularly in elevated concentrations (Gupta and Gupta, 1998).

Lead and cadmium are among the most abundant trace heavy metals and are particularly toxic (Radwan and Salama, 2006). Excessive content of these metals in food is associated with a number of diseases, especially those of the cardiovascular, renal, nervous and skeletal systems (WHO, 1992, 1995; Steenland and Boffetta, 2000; Jarup, 2003). These heavy metals are also implicated in carcinogenesis, mutagenesis and teratogenesis (Radwan and Salama, 2006). Other metals, such as copper, chromium and zinc are essential for important biochemical and physiological functions and necessary for maintaining health throughout life (Prentice, 1993; ATSDR, 1994; Linder and Azam, 1996). However at elevated levels these metals can also lead to metal poisoning. For example zinc accumulation causes vomiting and renal damage while copper toxicity may induce hypertension, coma and sporadic fever whereas hexavalent chromium may induce gastrointestinal ulceration and cancer (Prabu, 2009).

The accumulation of high quantities of metals in plants parts that are consumed is an important environmental problem. There is also a paucity of information on the levels of heavy metals in khat. Therefore this study was carried out to determine the levels of selected heavy metals in soil and khat grown in Kenya.
1.2 Problem statement and justification

In Kenya, khat is sold commercially in many parts of the country and where it is easily accessible to even primary school children. Chewing khat is a common recreation activity among many individuals of all age groups. The total amount of khat consumed in Kenyan market is estimated as 12,000 kg daily (Maitai, 2013). Despite objection of khat by National Agency for the Campaign against Drug Abuse, (NACADA), its cultivation is fast expanding in different parts of the country. As a result wide areas of fertile land are now being used to grow khat. However, agricultural officers do not include khat in the list of scheduled crops and therefore no technical advice on how to grow and apply agrochemicals to khat is provided to the farmers.

In order to meet its ever increasing demand for both domestic consumption and for the export market, farmers are now employing different methods of farming such as application of fertilizers, pesticides, sewage sludge, compost materials, manure and irrigation to improve and protect the khat. However, agricultural practices such application of phosphatic fertilizers, pesticides and refuse derived composts can be important source of heavy metals in the soil (Alloway, 1995). Lidia et al. (1997) reported that continuous application of fertilizers to the soil may increase the heavy metal contents making it exceed the natural abundances in soils, and transfer of these metals into the human food chain despite the fact that these heavy metals may be present in minute quantities in fertilizers. Some of these metals such as Pb and Cd have no known use in the body and are toxic even at low levels. Excessive content
of these metals in food is associated with a number of diseases, especially those of the cardiovascular, renal, nervous and skeletal systems (WHO, 1992, 1995; Steenland and Boffetta, 2000; Jarup, 2003).

During the last two decades, important progress has been made in understanding the pharmacological and social effects of khat in Kenya, but less attention has been paid to concentration of heavy metals in khat. Yet studies done in other countries such as Yemen by Matloob (2003), and in Ethiopia by Atlabachew et al. (2011), have reported presences of heavy metals in khat. The presence of high levels of heavy metals such as Pb and Cd in khat could have serious health effects especially to the young users.

Therefore this study aimed at assessing the levels of selected heavy metals in khat and soil that support its growth in order to establish whether the users are exposed to high levels besides creating awareness. Analyzing the soil where khat is grown was important in correlating accumulation of particular metal in the plant and in the soil.

1.3 Objectives

1.3.1 Main objective

To determine the levels of selected heavy metals in soil and in khat grown in Kenya.
1.3.2 Specific objectives

(i) To determine the levels of Zn, Cu, Pb, Cd and Cr in soils from Mbeere, Embu and Nyambene where khat is grown.

(ii) To determine the levels of Zn, Cu, Pb, Cd and Cr in khat from Mbeere, Embu and Nyambene districts.

1.4 Hypotheses

(i) The levels of heavy metals in soils from different khat growing regions do not have different levels of heavy metals.

(ii) Khat plants grown in different regions do not have different levels of heavy metals.

1.5 Significance of the study

The analytical results from this study provided important baseline data on the levels of selected heavy metals in khat besides being an important assessment of environmental pollution in rural areas where khat farming is predominant. The study also provided awareness of the health effects of khat to the public.

1.6 Scope and limitations of the study

This study determined the levels of Pb, Cd, Zn, Cu and Cr in soil and khat samples though there are many other heavy metals. Two of these metals, Pb
and Cd were selected since they are non essential while the others are essential elements. The roots and the stems of the khat plant were not studied. This is because the consumption of khat only involves chewing of young leaves and stem tips. Only the khat varieties grown in the studied region was considered since khat variety depends on the geographical location.
CHAPTER TWO
LITERATURE REVIEW

2.1 The khat plant (Catha edulis)

Khat (Catha edulis Forsk) is an evergreen perennial shrub that belongs to the
celastraceae family. It is a mild stimulant herb that contains the alkaloids
cathine and cathinone which have amphetamine like properties and produces
a variety of pleasurable effects (ECDD, 2006). The plant is known with
different vernacular names such as miraa and veve in Kenya, qat in Yemen
and eschat in Ethiopia. In Africa it is well distributed in Ethiopia, Eriteria,
Somalia, Kenya, Zambia, Rwanda and South Africa. Outside Africa, it is
planted in Arabian Penesula, Afghanistan, Yemen and Sir Lanka (Lemessa,
2001). Fresh leaves from khat trees are chewed daily by over 20 million
people on the Arabian Peninsula and East Africa (Al-Motarreb, 2002; Saha
and Dollery, 2006).

The khat plant grows to a height of between one and six metres (Al-Hebshi
and Skaug, 2005), however, what grows in plantations is normally pruned to
a height that is manageable for harvesting. The time between planting a
cutting and producing a viable plant that can be harvested is about two years
(Anderson et al., 2007). It can be rain fed or irrigated especially during the
dry season. The crop can be grown in home gardens or in plantations. The
shapes of the leaves are elliptical and have serrated edges. Old leaves are
leathery in texture, highly polished on their upper surface and deep green in
colour (NACADA, 2011).
The most favored part of the plant is leaves, particularly the young shoots near the top of the plant. The twigs are normally tender and users chew the outer cover or the back of the twigs. The fresh leaves, twigs or shoots of the khat plant are chewed and then retained in the cheek and chewed intermittently to release the active drug. Sometimes chewers take tea or soft drinks (soda) while chewing khat. Its use has traditionally been confined to the regions where khat is grown, because only the fresh leaves have the desirable stimulating effects. In recent years improved roads, off-road motor vehicles and air transport have increased the global distribution of this perishable commodity (NACADA, 2011).

2.1.1 The chemical profile of khat

The environment and climate condition determine the chemical profile of khat leaves. Many different compounds are found in khat including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino-acids, vitamins and minerals (Kalix and Braenden, 1985; Nencini and Ahmed, 1989; Cox and Rampes, 2003). The phenylalkylamines and cathedulins are the major alkaloids. The khat phenylalkylamines comprises of cathinone and the two diastereoisomers cathine. Cathinone is unstable and undergoes decomposition reactions after harvesting and during drying. As cathinone is presumably the main psychoactive component of khat, this explains why fresh leaves are preferred and why khat is wrapped up in banana leaves to preserve freshness (ECDD, 2006).
2.1.2 Adverse effects of khat in man

Khat contains many different compounds and therefore chewing may have different effects. As cathinone and to a lesser extent cathine are held responsible for the effects of khat on the nervous system, the effects of many other constituents of the khat are frequently overlooked (ECDD, 2006). Khat use affects cardiovascular, digestive, respiratory, endocrine and genitourinary system. In addition, it affects the nervous system and can induce paranoid psychosis and hypomanic illness with grandiose delusions (Kalix, 1988). Adverse effects of khat may be summarized according to the system involved as shown in table 2.1.
Table 2.1: Reported and suggested adverse effects of khat in man.

<table>
<thead>
<tr>
<th>System</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td>tachycardia, palpitations, hypertension, vasoconstriction, myocard infarction,</td>
</tr>
<tr>
<td></td>
<td>cerebral hemorrhage, pulmonary edema</td>
</tr>
<tr>
<td>Gastro-intestinal system</td>
<td>dry mouth, polydipsia, dental caries, periodontal disease, chronic gastritis,</td>
</tr>
<tr>
<td></td>
<td>constipation, paralytic ileus, weight loss, duodenal ulcer, upper gastro-</td>
</tr>
<tr>
<td></td>
<td>intestinal malignancy</td>
</tr>
<tr>
<td>Genito-urinary system</td>
<td>urinary retention, spermatorrhoea, spermatozoa</td>
</tr>
<tr>
<td>Obstetric effects</td>
<td>malformations, impotence, libido change</td>
</tr>
<tr>
<td>Metabolic and endocrine</td>
<td>low birth weight, stillbirths, impaired lactation</td>
</tr>
<tr>
<td>effects</td>
<td>hyperthermia, perspiration, hyperglycaemia</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>dizziness, impaired cognitive functioning, fine tremor, insomnia, headaches</td>
</tr>
<tr>
<td>Psychiatric effects</td>
<td>lethargy, irritability, anorexia, psychotic reactions, depressive reactions,</td>
</tr>
<tr>
<td></td>
<td>hypnagogic hallucinations</td>
</tr>
</tbody>
</table>


2.2 Heavy metal toxicity

The toxicity of heavy metals is attributed basically to the role they play in biological processes as well as the way they interact with essential elements both at intestinal and organ level once absorbed in the body (WHO, 1993). They are able to remove electrons from the amino-acids or bases of
DNA that cause a reaction which disrupts the ability of the cell to carry out their biological functions (Jamnicka et al., 2007). These modified biological molecules lose their ability to function properly and result in malfunction or death of the affected cells (Hoekman, 2011). Nowak and Chmielnicka (2000), observed that in some cases toxic metals displace chemically related metal ions that are required for important biological functions. The alteration of these structures leads to toxic consequences that have a array of disorders.

Further, heavy metals are non biodegradable, thermostable and persistent (Sharma et al., 2007) and undergo bioaccumulation and biomagnification up the food chain when ingested. The cumulative effects occur after along exposure to low levels of heavy metals. Exposure of consumers to health risks are usually expressed as provisional maximum tolerable daily intake (PMTDI) or provisional maximum tolerable weekly intake (PMTWI) reference values established by the FAO/WHO (FAO/WHO, 1999). The symptoms are periodically progressive resulting into gradual and severe problems.

### 2.2.1 Biochemistry of toxicity

The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted to their stable oxidation states and combine with the body’s bio-molecules such as
proteins and enzymes to form very stable biotoxic compounds, which become difficult to be dissociated, due to their bio-stabilities, during extraction from the body by medical detoxification therapy (Ogwuegbu and Ijioma, 2003). The equations below show their reactions during bond formation with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH$_3$) (Ogwuegbu and Ijioma, 2003).

Where: (A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal

The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme (Duruibe et al., 2007).
2.2.2 Heavy metals in the soil

Soil is a crucial component of rural and urban environment. It is a valuable and indispensable natural resource. It is in the soil that most of the foods we eat grow. The water we drink seeps through the soil in to the ground reservoirs or flows over it to the rivers. Mining, manufacturing and use of synthetic products such as pesticides, paints, industrial wastes, fertilizers and land application of industrial or domestic sludge result in heavy metal contamination of urban and agricultural soil. Although fertilizers are soil nutrient enhancements applied to the soil to promote plant growth they are however found to contain in minutes quantities other elements mostly heavy metals which either have no known use or may be toxic to man and plants are, most of which are responsible for many adverse health effects (Alloway, 1995).

Bon fires and burial of metal containing house hold wastes in domestic gardens can also lead to significant metal accumulation in soils used for growing food crops. Besides, temporary waste stockpiles can cause significant soil contamination. Foliar uptake of atmospheric heavy metals emissions have been identified as an important pathway of heavy metal contamination in crops (Salim et al., 1992). Land filling of municipal solid waste can lead to several metals including Cd, Cu, Pb, Sn, and Zn being dispersed in to the soil (Alloway, 1995). Prabu (2009), observed the concentration of heavy metals in soil is dependent on clay content because
clay size particles have a large number of ionic binding sites due to the higher amount of surface area. This results in the immobilization of heavy metals and there is little leaching through the soil profile. Immobilization can increase the concentration of heavy metals in soil and ultimately lead to toxicity of contaminated soil.

Higher soil heavy metal content can result in higher levels of uptake by plants. Soil acts as a long term sink for heavy metals such as Zn, Cu, Pb, Ni and Cd which have residence times that range from hundreds to thousands of years depending on the element and soil properties (Kaara, 1992). Heavy metals, also occur naturally but rarely at toxic levels. Excess heavy metal accumulation in soils is toxic to human and other animals. Exposure to heavy metals is normally chronic due to food chain transfer. Metal contamination in agricultural soil is of increasing concern due to food safety issues and potential health risk associated with intake of contaminated plants.

2.2.3 Uptake of heavy metals by plants

Plants grow by absorbing nutrients from the soil. Their ability to do this depends on the nature of the soil. Soil contains some combination of sand, silt, clay and organic matter. The makeup of a soil texture and its acidity or pH determines the extent to which nutrients are available to plants. The path taken by metal to transport into the plant is: soil → roots → stem → leaves. The minerals, dissolved in the water, get transported often accompanied by various organic molecules supplied by root cells. Once in the xylem, water
with the minerals that have been deposited in it move up in the vessels and tracheids (Kirmani et al., 2011). Minerals enter the root by active transport into the symplast of epidermal cells and move toward and into the stele through the plasmodesmata connecting the cells. Usually metals preferentially concentrate in the roots, followed by the twigs and leaves. Large number of factors control metal accumulation and bioavailability associated with soil and climatic conditions, plant genotype and agronomic management (Kirmani et al., 2011). Additional sources of these metals for plants are rainfall, atmospheric dusts and plants protection agents, which could be adsorbed through the leaf blades. An important source of contamination, in vegetable crops, is considered to be foliar uptake of atmospheric heavy metals emissions (Salim et al., 1993).

Studies conducted by Kisku et al. (2000), in Kalipur, Bangladesh, on the uptake of Cu, Pb, Ni and Cd by Brassica oleracea from fields irrigated with industrial effluent indicated widespread contamination from heavy metals despite showing a healthy and gigantic external morphology. High levels of accumulation of heavy metals from soil by common garden vegetables have been reported by many environmental researchers (Boon and Soltanpour, 1992; De Pieri et al., 1997; Xiong, 1998). Therefore, heavy metal contamination of plants widely consumed cannot be underestimated.

Plant uptake is a critical pathway by which biosolids-borne, potentially toxic trace metals can enter the food chain
Plant availability of trace metals differs widely among plant species and organs. Hooda et al. (1997), measured variability in the accumulation of Cd, Cu, Ni, Pb, and Zn in wheat, carrots, and spinach grown on bio-solids amended soils. Cd, Ni, and Zn increased in plants to a greater extent than Cu and Pb compared to their background levels suggesting that Cd, Ni, and Zn might pose the greatest hazard among the trace metals studied. Therefore the importance of heavy metals in health and nutrition of both human and animal requires continuous monitoring of their levels in the environment. The next section discusses the sources and health effects of the selected heavy metals.

2.3 Sources and health effects of selected heavy metals

2.3.1 Zinc

Zinc occurs in small amounts in all indigenous rocks. In nature Zn occurs only rarely in its metallic state and the vast majority of environmental samples contain the element only in the form of zinc compounds. Zinc is widely used as a protective coating of other metals, in dye casting and the construction industry, and for alloys. Inorganic Zn compounds have various applications, for example, for automotive equipment, storage and dry cell batteries, and dental, medical and household applications. Organo-zinc compounds are used as fungicides, topical antibiotics and lubricants (IPCS, 2001).
The main pollutant sources of Zn in the soils are metalliferous mining activities, agricultural use of sewage sludge and composed materials and the use of agrochemicals such as fertilizers and pesticides (Alloway, 1995). The mobility of zinc in soils is dependent on its speciation, the soil pH, and content of organic matter (IPCS, 2001). Asada et al., (2010) reported that application of compost manure not only results in Zn accumulation in soil but also causes an increase in zinc mobility and enhances Zn leaching. Natural background total Zn concentrations are usually < 0.1–50 µg/litre in fresh water and 10–300 mg/kg dry weight (DW) in soils (IPCS, 2001). Mitei (1996) reported 106.9 µg/g of Zn levels in garden soil at Kiserian and 243.4 µg/g at Limuru while Oyaro (2002) reported 270.0 µg/g at Ngara roundabout and 87.8 µg/g at Ndarugu river. The mean Zn levels along Thika-Nairobi highway for top soil range between 94.4 to 267.2 µg/g during the dry season (Magothe, 2009) while in Ethiopia, levels of Zn in khat farms range from 49.9 to 131.3 µg/g (Atlabachew et al., 2011).

Environmental Zn pollution greatly influences the concentration of this metal in plants. Some plants are zinc accumulators, but the extent of the accumulation in plant tissues varies with soil and plant properties. High amounts of Zn in soil may lead to high uptake, and on contaminated sites the concentration can exceed 500 µg/g plant dry matter, a level considered to be generally toxic (Wild, 1993). Normal levels of Zn in most crops and pastures are in the range 10–100 mg/kg DW (IPCS, 2001). Zinc plays essential metabolic roles in the plant, of which the most significant is its activity as a
component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phosphohydrolases (Yap et al., 2010). Mitei (1996), reported mean Zn levels of 101.08 µg/g in tobacco leaves and 67.63 µg/g in pumpkin leaves from Runyenjes while at Limuru, the mean level of Zn in spinach was reported as 30.38 µg/g (Kaara, 1992). Levels of Zn ranging from 24.1 to 46.9 µg/g have been reported in khat from Ethiopia (Tilahun, 2009).

For humans, the most important route of exposure to Zn is through ingestion of food (IPCS, 2001). Zinc is an essential mineral that is naturally present in food, added to others and available as a dietary supplement. It is involved in numerous aspects of cellular metabolism. It is required for catalytic activity of approximately 100 enzymes (Sandstead, 1994; IMFNB, 2001) and it plays roles in immune function (Prasad, 1995; Solomons, 1998), protein synthesis (Prasad, 1995), wound healing (Heyneman, 1996), DNA synthesis (Prasad, 1995; IMFNB, 2001) and cell division (Prasad, 1995).

Most of the concern about excessive zinc concentration in soil relates to its possible uptake by crops and consequent adverse effects on the crops themselves and on livestock and human diets. Excess Zn causes toxicity in plant whose symptoms included chlorosis in young leaves, browning of coralloid roots, and serious inhibition on plant growth (Long et al., 2003). Food grown in highly contaminated soils tends to take up more Zn than normal. Consumption of such food stuffs leads to high Zn levels in blood and Zn accumulation in body organs. Zinc toxicity can occur in both acute and
chronic forms. Acute adverse effects of high Zn intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea and headache (IMFN, 2001). High levels of Zn may disrupt the homeostasis of other essential elements. For example, in adults, subtle effects of Zn on copper utilization may occur at doses of Zn near the recommended level of intake of 15 mg/day and up to about 50 mg/day (IPCS, 2001).

2.3.2 Copper

Copper is found naturally as sulphides, oxides and carbonates. Due to its chemical properties it is one of the few metals to be found naturally in inactive form and it is for this reason that it has been known and used by man for many years (Townshead, 1995). The major sources of Cu in land are mining operations, agriculture, solid waste and sludge from treatment works. Agricultural use of Cu products accounts for 2% of Cu released to the soil (IPCS, 1998). Most Cu deposited on soil is strongly adsorbed and remains in the upper few centimetres of soil. The range of Cu in non-polluted soils is 6-60 µg/g (Kabata-Pendias and Pendias, 1992). The Cu level along the roadside topsoil in a study around Nairobi was reported to have a mean value of 37.8 µg/g and a range of 3.4-238.8 µg/g (Oyaro, 2002) while garden soils from Limuru and Kiambu had levels of Cu ranging from 18.0-36.5 µg/g and 9.9-148.0 µg/g, respectively (Kaara, 1992). Levels of Cu in khat farms from Ethiopia range from 9.13 to 29.8 µg/g (Atlabachew et al., 2011). In soils where high levels of Cu contamination have been concentrated in the surface horizons, deep rooted perennial plant species are rarely affected unlike
shallow rooted plant species (Goyer and Myron, 1997). Xiong and Wang (2005), found that Cu concentration in the shoots was significantly influenced by Cu concentration in soil and increased markedly with an increase in the soil Cu concentration. The mean Cu concentration in pasture grass across the Thika highway varied from ND to 39.2 µg/g during the dry season (Magothe, 2009). However, mean Cu values in pumpkin and kale leaves obtained from garden soils from Kiserian were reported as 4.8 µg/g and 2.25 µg/g respectively, (Kaara, 1992). Copper levels >100 µg/g were found in samples of *Brassicarapa* root and *Murraya koenigii L.* (Kirmani et al., 2011).

Copper is found as component of food eaten by humans and animals. It is widely distributed in foods with organ meat and seafood having the highest concentrations (10-100 mg/kg) and dairy products having relatively low levels (IPCS, 1998). High levels of Cu have been also identified in wheat-bran, beans and seeds (Jorhem and Sundstrom, 1993). Although humans are exposed to Cu from many sources including drinking water, soil and dust, 75-99% of Cu intake is from food (CCME, 1997). Copper is an essential element and adverse health effects are related to its deficiency as well as excess. It is an essential enzymatic element. It is necessary for normal biological activities of amino oxides and tyrosinase enzyme. Tyrosinase is the enzyme that is required for catalytic conversion of tyrosine to melanin, which is a vital pigment located beneath the skin, and thus protects the skin from dangerous radiations (Hashmi et al., 2007). The places where copper
accumulates are the liver first, then the brain and the reproductive organs (Wilson, 2010). Long term exposure to Cu can cause irritation of the nose, mouth and eyes and causes headaches, stomachaches, dizziness, vomiting and diarrhoea (Lenntech, 2009).

2.3.3 Lead

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead exposure accounts for utmost 1% of the global burden of disease with the highest burden in developing regions (Fewtrell et al., 2004). Lead emitting sources include mining and smelting, coal-burning electric power plants and incinerators (Hill, 2007) while the addition of artificial fertilizer and pesticides cause an increase of Pb levels in agricultural soil (Onder et al., 2007). The approximate mean values of Pb in soils have been reported as ranging between 15-25 µg/g while the average levels of Pb in the soil are in the range of 10-70 µg/g (Laugher, 1992), but higher Pb levels of 45.4-263.8 µg/g have been reported in garden soils from areas within and around Nairobi (Kaara, 1992). While in Nigeria, the Pb content in soil along major and minor load ranged from 15.28-76.92 µg/g (Okunola et al., 2007). The mobility of lead is greater in sandy soils, which tend to lack organic matter, than in organic soils (Kirmani et al., 2011) however levels of Pb in uncontaminated soil range from 10 to 70 µg/g (Kabata-Pendias and Pendias, 1992).
Lead is effectively absorbed by both roots and leaves (Tyagi and Mehra, 1990). Lead in plants growing in uncontaminated and unmineralised areas is quite constant with a range of 0.1-10 µg/g (Kaara, 1992), however Mitei (1996), reported levels of Pb in tobacco leaves in Kenya to be between 7.40-25.0 µg/g. In Iran, the mean level of Pb in various vegetables was found to be 13.60 µg/g (Maleki and Zaraswand, 2008), while the mean Pb level in grass, Festuca proensis L was reported as 21.39 µg/g (Buszewski et al., 2000). The levels of Pb in khat of range 4.8-9.1 µg/g were reported in Addis Ababa (Tilahun, 2009). Ghani (2010), noted that the uptake, transport and accumulation of Pb by plants are strongly depended on concentration, soil type, soil properties and plant species. Although Pb is not an essential nutrient for plants, majority of Pb is easily taken up by plants from the soil and accumulated in roots while only a small fraction was translocated upward to the shoots (Patra et al., 2004). Voutsa et al. (1996) reported high accumulation of Pb, Cr, and Cd in leafy vegetables due to atmospheric deposition in the form of metal containing aerosols. These aerosols can enter the soil and be absorbed by plants, or alternatively be deposited on leaves and then adsorbed. Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield (Bigdeli and Seilsepour, 2008). Excess Pb causes a variety of toxicity symptoms in plants, such as reduced growth, chlorosis and darkening of the root system. Inhibition of root growth appears to result from Pb-induced inhibition of cell division of
the root meristem (Eun et al., 2000). Lead inhibits photosynthesis, alters the mineral nutrition and water balance, modifies hormone levels and affects the structure and permeability of the plasma membrane (Sharma and Dubey, 2005).

Food is the highest source of exposure for people living in Pb free or Pb safe homes. About 90% of a person’s lead intake is eventually stored in the skeleton (Hill, 2007). Lead exposure is associated with increased specific childhood internalizing behaviours such as anxiety and social problems (Roy et al., 2009). Other effects include deficiency in cognitive function due to destruction of the central nervous system (Giddings, 1998; Ndinya, 1998), enzyme inhibition, kidney impairment, reproductive problems and teratogenic effects (Sodhi, 2009).

There is also evidence that higher Pb values are associated with hypertension (Navas-Acien et al., 2007), peripheral vascular disease (Navas-Acien et al., 2004) increased adult mortality (Lustberg and Silbergeld, 2002; Schober et al., 2006) and cognitive decline in older people (Weisskopf et al., 2004). Exposure to low levels for a long period results in loss of weight, body weakness and anaemia in children, while severe cases may result in constipation, loss of appetite, nausea and vomiting, insomnia, headache, diarrhoea, irritability and dizziness, coma and even death (Ferguson, 1998). Blood Pb concentrations <10 µg/dL induces adverse effects in humans including elevated blood pressure, impaired nervous system development,
delayed sexual maturation, neurobehavioral effects, depressed renal glomerular filtration rate and reduced heme synthesis (ATSDR, 2007). Lead can potentially impair normal foetal bone growth by competing with Ca for deposition in bone because lead and Ca have similar chemical properties (Potula, 2005).

2.3.4 Cadmium

Cadmium is widely distributed in the earth's crust. In areas not known to be polluted, the median Cd concentration in soil has been reported to be in the range of 0.2 to 0.4 mg/kg. However, much higher values, up to 160 mg/kg soil, are occasionally found (IPCS, 1992). Cadmium is released into air during mining and smelting of Zn, Pb and Cu. Fossil fuel burning especially coal is an ongoing source. Soil receives Cd from two sources. Firstly, phosphate fertilizers invariably contain Cd as a natural contaminant. When land is sprayed with phosphates, Cd becomes incorporated in the soil. Secondly, polluted irrigation water increases the Cd load of soil (Sodhi, 2009). Li et al. (2010), found that pig, dairy cow, and chicken manures contained high Cd due to its presence in their feeds. Cadmium has a limited number of applications but within this range the metal is used in a large variety of consumer and industrial materials. The principal applications of Cd fall into five categories: protective plating on steel; stabilizers for polyvinylchloride (PVC); pigments in plastics and glasses; electrode material in nickel Cd batteries; and as a component in various alloys (IPCS, 1992).
Fergusson (1998), reported that fertilizers contain 0.1-190 µg/g of Cd while nitrogen fertilizer contain 0.1-9 µg/g of Cd. Contamination of agricultural land by Cd from phosphatic fertilizer is a global problem. Hanč et al. (2008), reported that addition of manure increased Cd uptake by plants. High soil levels are a special concern because plants take up Cd more readily than other metals (Hill, 2007). Levels of Cd ranging from 0.73 to 1.23 µg/g were obtained in Ethiopian farms where khat is grown (Atlabachew et al., 2011). The levels of Pb in uncontaminated soil range from 10 to 70 µg/g (Kabata-Pendias and Pendias, 1992). Mitei (1996), reported Cd levels in Kenyan tobacco leaves in the range 0.5-2.33 µg/g while the mean level of Cd in pumpkin and kale leaves from garden soil in which manure and fertilizers had been used to improve soil was found to be 0.81 µg/g and 0.82 µg/g respectively (Kaara, 1992). Mapanda et al. (2007) reported elevated concentrations of Cd, 2.5-6.3 µg/g, in leaf vegetables (Brassica species) irrigated with waste water. The levels of Cd with a range of 1.3-2.9 µg/g were reported in khat samples in Addis Ababa (Tilahun, 2009).

Due to its high rate of soil to plant transfer, Cd is a contaminant found in most human food stuff, which render diet a primary source of exposure among non-smoking, non-occupationally exposed population (Clemens, 2006; Mclaughlin et al., 2006; Franz et al., 2008). Akesson et al. (2008), observed increased endometrial cancer risk in a Swedish cohort among participants who consumed >15 µg/day of Cd mainly from cereals and vegetables. These finding suggested a very large health burden associated
with exposure to Cd at levels experienced by many populations worldwide. Its affinity for sulfhydryl groups induces its solubility in lipids which, in turn, causes it to bio-accumulate in liver and kidneys (Sodhi, 2009). In some middle aged or older individuals not occupationally exposed, Cd was found to have levels almost as high as those known to affect kidney functions (Hill, 2007). Long term exposure to low dose Cd has been linked to tubular impairment with a loss of re-absorptive capacity for nutrients, vitamins and minerals (IPCS, 1992).

Cadmium displaces zinc in many vital enzymatic reactions, resulting in disruption or cessation of activity. This normally leads to acute gastro-enteritis (Sodhi, 2009). Recent epidemiologic studies involving exposure with some effect assessment have linked low level Cd exposure of current populations with some adverse effects that are not restricted to kidney and bone but include almost every organ and where Cd accumulates including eye tissue (Satarug et al., 2010).

### 2.3.5 Chromium

Chromium is a naturally occurring element in rocks, animals, plants, soil and volcanic dust (ATSDR, 1998). In most soils Cr occurs in low concentrations, 2 - 60 mg/kg (IPCS, 1988). The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species although there are various other valence states which are unstable and short lived in biological systems. Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with
oxygen as chromate (CrO$_4^{2-}$) or dichromate (Cr$_2$O$_7^{2-}$) oxyanions. Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer et al., 2003).

The wide use of Cr compounds by modern industries has resulted in the discharge of large quantities into the environment via emission, waste water or solid waste disposal (Townshead, 1995). It is used on a large scale in many different industries, including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, and pulp and paper production (Zayed and Terry, 2003). These industries have become especially large contributors of Cr pollution, which can ultimately have significant adverse biological and ecological effects. Very high levels of Cr (VI) contamination (14,600 mg/kg in ground water and 25,900 mg/kg in soil) were reported at the United Chrome Products site in Corvallis, Oregon (Krishnamurthy and Wilkens, 1994). Another source of concern is the use of municipal sewage and sludge in agriculture. These anthropogenic activities have led to the widespread contamination that Cr shows in the environment and have increased its bioavailability and bio-mobility. The levels of Cr in non contaminated soil range between 5 to 121µg/g (Kabata-Pendias and Pendias, 1992). Chromium levels in garden soil with mean values of 43.4 µg/g and 26.8 µg/g have been reported in Kiserian and Limuru respectively (Kaara, 1992).
Chromium behavior in soil is controlled by soil pH and redox potential (Mondol et al., 2011) while Hossner et al. (1998) suggested that the availability of the soil Cr to the plant depends on the oxidation state of Cr, pH, and the presence of colloidal binding sites and Cr-organic complexes that would influence its total solubility. Chromium concentrations found in plants grown on normal soil seldom exceed 5 mg/kg on a dry weight basis and stays below the soil concentrations. Tilahun (2009), reported Cr in khat ranging 3.1-6.76 µg/kg from Ethiopia while Mapanda et al. (2007) reported Cr levels in the range of 1.5-6.6 µg/g from vegetable leaves irrigated with waste water in the city of Harare. The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulfate (Cervantes et al., 2001). Independent uptake mechanisms for Cr (VI) and Cr (III) have been reported in barley (Shanker et al., 2005). Plants can absorb both Cr (VI) and Cr (III) (Hossner et al., 1998). A number of soil processes and factors may affect the form and biomobilization potential of Cr. Chromium (VI) is readily transformed to Cr (III) as a result of reduction by Fe (II) in solution and at mineral surfaces, by sulfur compounds, or by soil organic matter undermost soil conditions (Fendorf, 1995). Chromium (III) has been found to be readily absorbed by macromolecular clay compounds; also, humic acids contain donor groups forming stable Cr (III) complexes, especially when they produce chelate rings, and adsorption of Cr (III) to humic acids renders it insoluble, immobile and unreactive (James, 1996). Although Cr (III) can be oxidized to Cr (VI), especially in the presence of manganese oxides, oxidation usually occurs.
only in moist conditions, and not appreciably in dry soils. Cr (III) can thus be expected to be the predominant form. Cr (III) is largely present in soil as relatively unavailable, insoluble oxides of Cr (Kotaś and Stasicka, 2000).

The general population is exposed to Cr by eating food, drinking water and inhaling air that contains the chemical (ATSDR, 1998). Trivalent chromium is essential for proper insulin function and is required for normal protein, fat and carbohydrate metabolism, and is acknowledged as dietary supplement (Bagchi et al., 2002). Chromium (VI) is generally considered to pose the greatest human health risk because it is more toxic, more soluble, and more mobile than Cr (III) (James, 1996). Almost all the hexavalent Cr in the environment arises from human activities. It is derived from the industrial oxidation of mined Cr deposits and possibly from the combustion of fossil fuels, wood and paper. In this oxidation state, Cr is relatively stable in air and pure water, but it is reduced to the trivalent state, when it comes into contact with organic matter in biota, soil, and water (IPCS, 1988).

The body has several systems of reducing Cr (VI) to Cr (III). Once absorbed into the bloodstream, Cr (VI) is rapidly taken up by erythrocytes after absorption and reduced to Cr (III) inside the red blood cells. In contrast, Cr (III) does not readily cross red blood cell membranes, but binds directly to transferrin, an iron-transporting protein in the plasma (ATSDR, 1998; EPA, 1998; Dayan and Paine, 2001). This Cr (VI) detoxification leads to
increased levels of Cr (III). The estimated safe and adequate daily dietary intake of Cr is 50 to 200 µg for adult and adolescents (RDA, 1989).

The problems that are associated with Cr involve skin rashes, stomach ulcer, kidney, liver damages, lung cancer and ultimate death (Kirmani et al., 2011). Long term exposure can cause kidney and liver damage and damage to circulatory nerve tissue (Lenntech, 2009). At an elevated concentration it is toxic for both plant and animals. Hexavalent Cr causes marked irritation of the respiratory tract. Studies on experimental animals have reported hexavalent Cr to cause various forms of genetic damage in short-term mutagenicity tests, including damage to DNA, and misincorporation of nucleotides in DNA transcription (IPCS, 1988).

2.4 Heavy metal transfer factor (TF) from soil to khat

The soil-to-plant transfer factor is one of the key components that determine human exposure to metals through the food chain. Generally, transfer factor expresses the bioavailability of a metal at a particular position on a species of plant (Tsafe et al., 2012). This is however, dependant on different factors such as the type of soil, its pH and the nature of the plant itself. TF values are used to describe the accumulation of chemicals in organisms, especially those living in contaminated environments (Wang et al., 2004). The higher the value of TF, the more mobile/available the metal is. Hence, the high TF value may exert potential risk to human health.
2.5 Risk assessment

To protect humans from harmful effects of heavy metals, WHO has set similar values for toxicity, termed provisional maximum tolerable weekly intakes (PMTWI) and Provisional maximum tolerable daily intakes (PMTDI). PMTDI is the end point for contaminants with no cumulative properties while PMTWI is the end point for food contaminants such as heavy metals with cumulative properties. WHO has calculated a PMTDI of 35 mg/day of Cu for a 70 kg adult (0.5 mg/kg/day), while that of Zn is 0.3-1.0 mg/kg/day. Lead and cadmium are well known for their toxicity and adverse effects on human health. The WHO has recommended a PMTWI of Cd and Pb as 0.025 mg/kg body mass (equal to 17.50 mg/week for a 70 kg adult) and 0.007 mg/kg body mass (equal to 0.49 mg/week for a 70 kg adult) respectively (FAO/WHO, 1999).

2.6 Soil pH

Soil pH is a determination of the soil solution’s acidity and alkalinity. By definition, pH is the negative logarithm (base 10) of the hydrogen ion concentration in a solution, that is,

\[ \text{pH} = -\log [\text{H}^+] \] ..................................................................................Eq. 1

Soil pH applies to the H⁺ ions concentrations in the solution present in soil pores which is in dynamic equilibrium with the predominantly negatively charged surface of the soil particles (Alloway, 1990). Hydrogen is added in
the form of ammonia-based fertilizers, urea-based fertilizers and as protein (amino-acids) in organic fertilizers. Transformation of these sources of nitrogen to nitrate releases hydrogen ions (H\(^+\)) to create soil acidity. Therefore, application of large quantities of both chemical and organic fertilizers may eventually make the soil more acidic. The increased use of nitrogenous fertilizers generally increases soil acidity (Ishibashi et al., 2004). Nartey et al., (2012) reported that fertilizer amended soils have generally lower pH than natural soils. Other factors that do affect soil acidity include soil organic matter and rainfall. Soil organisms are continuously decomposing organic matter. The net effect of their activity is that hydrogen ions are released and the soil becomes more acidic. Soils under high rainfall conditions are more acidic than soils under dry conditions.

Soil pH greatly influences the availability of both nutrients and toxins for uptake by plant roots (Brady and Weil, 2002). The solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately pH 5.0 to 7.0) (Kabata-Pendias, 2004; Wang et al., 2006). Evans (1989), explained that pH has a significant effect on metal dynamics because it controls adsorption and precipitation, which are the main mechanisms of metal retention in soils. Metal solubility tends to increase at lower pH. With the exception of Mo, Se and As, the solubility of trace elements is reduced with increasing soil pH because of the precipitation of insoluble hydroxides, carbonates and organic complexes (Silveira et al., 2003). It affects the mobility of pollutants in the soil by influencing the rate
of biochemical breakdown, solubility and adsorption to colloids. Thus soil pH is a critical factor in predicting the likely hood that given pollutant will contaminant ground water, surface water and food chain (Brady and Weil, 2002).

Heavy metals are non-biodegradable and bio-accumulate up the food chain and their levels in khat could affect its users. Therefore monitoring soil pH which is an important factor governing metal solubility from mineral surfaces, transport, and their bioavailability is a task of high priority. There is very little information if any on the levels of heavy metals in khat grown in Kenya and hence need for this study.

2.7 Methods of analysis of heavy metal

Several techniques for determination of metallic elements are currently in use. These include the atomic absorption spectroscopy (AAS) (Taylor et al., 2006), inductively coupled plasma mass spectroscopy (ICP-MS) (Conor, 2004), the inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Pavel et al., 2005) and energy dispersive X-ray fluorescence (EDXRF) spectroscopy (Beckhoff et al., 2006). For this study, the AAS was used because of its availability, reproducibility and time efficiency. The AAS has high sensitivity and selectivity. It is a single elemental method in which one element is determined in a series of samples and instrumental parameters optimized for the next element and can easily be automated. In AAS a substance is vaporized and decomposed into gaseous atoms in flame or
electrothermal atomizer. The concentration of atoms is measured by absorption of specific wavelength of radiation. The atoms exhibit line absorption spectra.

2.8 Principles of AAS

AAS is a method of elemental analysis that works on the principle of absorption of radiation energy by free atoms. The concentration of an element is measured by the absorption of radiation with characteristic frequency by free atoms of an element. Light of certain wavelength produced by monochromatic or hollow cathode lamp emits spectral lines corresponding to energy required for excitation of an element of interest. The analytical signal is obtained from the difference between the intensity of the source in the absence of the element of interest and the decreased intensity obtained when the element of interest is present in the optical path. Absorption of light is associated with transition process from one steady state to another, for instance the case of a steady state O and J where \( E_0 < E_J \), the O-J transition results in the absorption of light with the frequency given in Equation (2).

\[
\nu_{OJ} = \frac{E_J - E_O}{h}
\]

…………………………………………………………………………Eq.2

Where

\( h \) - Planck’s constant
V - Frequency

$E_0$ - Energy at ground state

$E_j$ - Energy at the excited state

O - J – the transition stimulated by absorption of external radiation.

The number of atoms in the excited state relative to the number in the ground state is given by Maxwell–Boltzmann law (Skoog et al., 1998), given by Equation 3.

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} \exp \left( \frac{E_0 - E_1}{KT} \right)$$

Eq.3

Where

$N_1$ - Number of atoms in the excited state

$N_0$ - Number of atoms in the ground state

$g_1$ and $g_0$ – Statistical weight of excited and ground state, respectively

$K$ - Boltzmann’s constant

$T$ - Absolute temperature

$E_0$ - Energy at the ground state

$E_1$ - Energy at excited state

The relative fraction of atoms in excited state is dependent on temperature whereas intensity is independent of temperature. Sample solution is aspirated through nebulizer into the air/acetylene or nitrous oxide/acetylene flame (Taylor et al., 2006). An electrically heated graphite furnace is used when
very high sensitivity is required. The sample solution gets dispersed into mist of droplets and then evaporated into dry salt. The dry salt goes into vapour and dissociates into atoms that absorb resonance radiation from external source. The unabsorbed radiation is allowed to pass through the monochromator which isolates spectral lines. The isolated analyte line falls on the detector and the output is amplified and recorded. The parameter measured is absorbance \( A \) and related to levels by the Equation 4.

\[
A = \log \frac{I_0}{I} = \varepsilon cl \\
\text{..........................Eq.4}
\]

Where

\( A \) - Absorbance

\( I_0 \) - Incidence radiation

\( I \) - Attenuated radiation

\( \varepsilon \) - Molar absorptivity \( \text{(Lmol}^{-1}\text{cm}^{-1}) \)

\( c \) - Concentration \( \text{(moldm}^{-3}) \)

\( l \) - Path length (cm)

Since the relationship between absorbance \( A \) and concentration \( c \) is linear over a wide range of levels (Beer’s law), standards are used to obtain calibration curve from which levels of analyte is established through interpolation method. The most important components of atomic absorption spectroscopy (AAS) are:

(i) A light source (usually a hollow cathode lamp)

(ii) An atom cell (atomizer)
2.8.1 Light source

The light source is usually a hollow cathode lamp of the element that is being measured. It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas (neon or argon). Each element has its own unique lamp which must be used for that analysis. Applying a potential difference between the anode and the cathode leads to the ionization of some gas atoms. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state. The shape of the cathode which is hollow cylindrical concentrates the emitted radiation into a beam which passes through a quartz window all the way to vaporized sample. Since atoms of different elements absorb characteristic wavelength of light, analyzing a sample to see if it contains a particular element means using light from that element or a source that give a broad spectra.

2.8.2 Atomizer

Elements to be analyzed need to be in atomic state. Atomization is separation of particle into molecules and breaking molecules into atoms. This is done
by exposing the analyte to high temperatures in a flame or graphite furnace. The role of the atom cell is to primarily dissolve a liquid sample and then the solid particles are vaporized into their free gaseous ground state form. In this form atoms will be available to absorb radiation emitted from the light source and thus generate a measurable signal proportional to concentration. There are two types of atomization: Flame and graphite furnace atomization. In flame ionization which was used in this study, sample solutions are usually introduced into a nebulizer by being sucked up a capillary tube. In the nebulizer, the sample is dispersed into tiny droplets which can be readily broken down in the flame.

2.8.3 Monochromator

A monochromator is used to select the specific wavelength of light which is absorbed by the sample and to exclude other wavelength. The selection of the specific light allows the determination of the selected element in the presence of others.

2.8.4 Read out devices

The light selected by the monochromator is directed on to a detector that is typically a photomultiplier tube whose function is to convert the light signal into an electrical signal proportional to the light intensity. The signal could be displayed for read out or further fed into a data station for print out by the requested format.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The khat leaf and soil samples were collected from three main khat growing regions in the country namely Mbeere, Embu and Nyambene. Mbeere and Embu were selected because most of the khat for domestic market is distributed from these places while Nyambene area was selected because it
has a long history of khat as part of their traditions. Nyambene is also considered to be the most important producer of quality khat in Kenya for both domestic consumption and for export.

### 3.2 Research design

The experimental design which was used involved the determination of levels of selected heavy metals in both soil and khat samples from three khat growing regions namely Mbeere, Embu and Nyambene. Samples were obtained from different farms randomly selected in each region, then dried, weighed and digested before the digests were analyzed for the chosen elements using AAS.

### 3.3 Reagents

The nitric acid and perchloric acid used in this study were of analytical grade and were supplied by Hopkin and Williams Ltd, England. Commercial 1000 ppm standard solutions of Zn, Cu, Cr, Cd and Pb were purchased from Sigma chemical company. Distilled water was also used for sample preparation, dilution and rinsing apparatus prior to analysis.

### 3.4 Cleaning of glassware and sample containers

All glassware were cleaned with detergent and hot water, rinsed several times with tap water and then soaked for 12 hours in 10% analytical grade nitric acid solution. Finally they were rinsed with distilled de-ionised water.
and dried in the oven at 105 °C. The plastic containers were cleaned with detergent and tap water, soaked in 1:1 nitric acid and water overnight and rinsed thoroughly with distilled de-ionised water. They were then dried in an open rack and stored safely in a locked dust free storage area.

3.5 Sample collection and digestion

3.5.1 Soil samples

The samples were obtained from 45 farms randomly selected in each region. The soil samples were taken from a depth of 0-30 cm which represents the plough layer and average root zone for nutrients uptake and heavy metals burden by khat plants (Nyangababo and Hamya, 1986). In each farm, ten subsamples were randomly collected and then mixed thoroughly in to one composite sample to get a representative sample of that farm weighing about 1 kg. The sample was then placed in a polythene bag, sealed and labelled appropriately. A total of 135 soil samples from the three khat growing regions were collected. In the laboratory, soil samples were dried in an oven at 80 °C to a constant weight, ground using mortar and pestle, then passed through a 2 mm sieve and stored in labelled containers awaiting further analysis.

3.5.2 Khat samples

Khat leaves or tips ready for harvesting were sampled from khat trees grown in the farms where soil was sampled. Ten subsamples from each farm were
then combined to form a representative sample of the farm weighing about half a kilogram. A total of 135 khat samples from three regions were collected and transferred in to the laboratory where they were washed with tap water and then rinsed several times with de-ionised water. They were then dried in an oven at 70 °C for 24 hour. After cooling the samples were ground to fine powder and packed in clean, labelled and decontaminated plastic containers awaiting further analysis.

3.5.3 Sample digestion

The samples were digested following the procedure recommended by the (AOAC, 1990). One gram of dried sample was placed in 250 ml digestion tube and 10 ml of concentrated nitric acid added. The mixture was boiled gently for 30-45 minute. After cooling, 5 ml of 70% perchloric acid was added and the mixture boiled gently until dense white fumes appeared. Then 20 ml of distilled water was added and the mixture was boiled further to release any fumes. The solution was cooled further and filtered through whatman No. 42 filter paper into a 50 ml volumetric flask. The filtrate was made to the mark using distilled water.

3.6 Method detection limit and recovery test

The calibration curves were established by a plot of absorbance readings against the corresponding concentration of ideal standards. The absorbance readings and concentration of ideal standards were used to calculate the
correlation coefficients (r). The method detection limits were calculated using the equation below.

\[
\text{Method detection limit} = 3 \times \text{standard deviation of blank readings} \quad \text{Eq. 5}
\]

The accuracy of the analytical procedure was investigated by spiking a 10 ml aliquot of 5µg of each analyte metal into conical flask containing 1.0g of the khat sample. Then same digestion procedure was followed for non-spiked and spiked samples side by side. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer and the percentage recovery calculated using the following equation.

\[
\% \text{ Recovery} = \frac{\text{Conc. in spiked sample} - \text{conc. in non-spiked sample}}{\text{Amount added}} \quad \text{Eq. 6}
\]

3.7 Determination of the selected heavy metals using AAS

Determination of Zn, Cu, Pb, Cd and Cr was done in replicates using computerized Varian Atomic Absorption Spectrometer model AA-10 (Varian manufacturing co. ltd, Australia). The calibration of the instrument using standards and blank was frequently done between samples to ensure stability of the base line.
3.8 Soil pH determination and Transfer factor

3.8.1 Soil pH determination

The soil pH was determined in accordance to the procedure described by Geotechnical Engineering Bureau (Geotechnical Engineering Bureau, 2007). A soil sample of 30 g was placed into a glass beaker and 30 ml of distilled water added. The mixture was allowed to stand for one hour, stirring after every 15 minutes. Buffer solutions of pH of 4 and 5 were used to calibrate the digital pH meter before taking measurements.

3.8.2 Transfer factor (TF)

The transfer factor is the metal concentration in plant tissue above the ground divided by the total metal concentration in the soil. It signifies the amount of heavy metals in the soil that ended up in the plant. Transfer factor was calculated for each metal according to the following equation (Cui et al., 2004).

\[
TF = \frac{P_s (\mu g \ g^{-1} \ dry \ wt)}{S_t (\mu g \ g^{-1} \ dry \ wt)} \]

Where,

- \(P_s\) is the concentration of metal in plant
- \(S_t\) is the concentration of metal in soil

3.9 Data analysis

Mean values obtained for Zn, Cu, Pb, Cd and Cr from the three regions were compared by One-Way ANOVA at 95% level using SPSS 18 for
windows assuming that there were significant differences among them when the statistical comparison gives $p<0.05$. Pearson’s correlation analysis was used to investigate the existence of linear relationship between metal concentration in khat and in the soil.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction

The levels of Zn, Cu, Pb, Cd and Cr in soil and khat samples obtained from farms in the three main khat growing regions in Kenya were determined in triplicates using computerized Varian Atomic Absorption spectrophotometer. The validity of the atomic absorption spectroscopy (AAS) results was
assessed by spiking of samples with standards of known levels and calculating percentage recoveries.

4.2 Recovery test

The recovery test for all samples was performed in triplicates and the results are as indicated in table 4.1.

Table 4.1: Recovery test results for the metals (percentage).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Conc. In sample (μg/g)</th>
<th>Amount added (μg/g)</th>
<th>Conc. in spiked sample (μg/g)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>10.3±0.01</td>
<td>5.0</td>
<td>15.26±0.04</td>
<td>99.2±0.02</td>
</tr>
<tr>
<td>Zn</td>
<td>37.0±0.02</td>
<td>5.0</td>
<td>41.89±1.2</td>
<td>97.8±0.03</td>
</tr>
<tr>
<td>Cr</td>
<td>15.2±0.01</td>
<td>5.0</td>
<td>20.12±0.12</td>
<td>98.4±0.02</td>
</tr>
<tr>
<td>Cd</td>
<td>0.47±0.02</td>
<td>5.0</td>
<td>5.38±0.13</td>
<td>98.2±0.01</td>
</tr>
<tr>
<td>Pb</td>
<td>7.4±0.02</td>
<td>5.0</td>
<td>12.36±0.2</td>
<td>99.2±0.01</td>
</tr>
</tbody>
</table>

As shown in table 4.1 the percentage recovery for khat samples lie in the range 97.8-99.2 %, which are within the acceptable range for metals (Hight, 1998). This confirms that the method is of good precision and accuracy.

4.3 Method detection limits and equations of the calibration curves

The detection limits, the correlation coefficients, and the equations of the calibration curves for the determination of metals in both soil and khat samples by AAS are given in table 4.2.
Table 4.2: Detection limits, correlation coefficients and equations of the calibration curves.

<table>
<thead>
<tr>
<th>Element</th>
<th>Method detection limit (µg/ml)</th>
<th>Correlation coefficient of calibration curve</th>
<th>Equations for calibration Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.02</td>
<td>0.999</td>
<td>$Y = 0.018x + 0.007$</td>
</tr>
<tr>
<td>Cu</td>
<td>0.027</td>
<td>0.998</td>
<td>$Y = 0.01x + 0.004$</td>
</tr>
<tr>
<td>Cr</td>
<td>0.03</td>
<td>0.999</td>
<td>$Y = 0.018x + 0.001$</td>
</tr>
<tr>
<td>Pb</td>
<td>0.016</td>
<td>0.998</td>
<td>$Y = 0.001x + 0.005$</td>
</tr>
<tr>
<td>Cd</td>
<td>0.008</td>
<td>0.997</td>
<td>$Y = 0.001x + 0.002$</td>
</tr>
</tbody>
</table>

Table 4.2 indicates that the correlation coefficients of all calibration curves were $\geq 0.997$, which shows that there was a very high positive correlation between concentration and absorbance. The method detection limits for all the selected metals were < 0.1 µg/g indicating that the method is applicable for the determination of heavy metals at trace levels. The performance of AAS spectrophotometer used in this study was therefore good and reliable to warrant its use in the analysis of the selected heavy elements in the samples.

4.4 Mean concentration levels of selected heavy metals in soil and khat samples

The mean levels of Zn, Cu, Cd, Pb and Cr in soil and khat samples from khat growing areas of Mbeere, Embu and Nyambene are discussed in the following subsections.
4.4.1 Concentration levels of zinc in soil and khat samples

The mean levels of Zn in soil and khat samples are presented in table 4.4.

Table 4.4: Mean concentration levels (µg/g) of zinc in soil and khat samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mbeere mean±SE</th>
<th>Embu mean±SE</th>
<th>Nyambene mean±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>58.52±8.16</td>
<td>156.65±8.33</td>
<td>103.23±6.96</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>range 25.50-238.90</td>
<td>82.45-440.30</td>
<td>53.00-245.90</td>
<td></td>
</tr>
<tr>
<td>Khat</td>
<td>40.77±1.58</td>
<td>42.93±1.28</td>
<td>36.49±1.40</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>range 28.55-73.95</td>
<td>31.05-60.10</td>
<td>25.15-57.85</td>
<td></td>
</tr>
</tbody>
</table>

Note: mean±SE values followed by the same small letters within the same row are not significantly different at $\alpha = 0.05$

4.4.1.1 Concentration levels of zinc in soil

From table 4.4, Zn levels in the soil ranged from 25.50 µg/g to 440.30 µg/g. The mean concentration of Zn in the soil samples was highest in Embu with a mean of 156.65±8.33 µg/g, a value that was significantly ($p < 0.05$) higher than in Mbeere and Nyambene, respectively. This is attributed to farming methods used by farmers in Embu such as use of fertilizers and application of compost manure. Use of agrochemicals such as fertilizers and pesticides has been reported as one source of Zn in soil (Alloway, 1995) while application of compost manure not only results in Zn accumulation in soil but also causes an increase in Zn mobility and enhances Zn leaching (Asada et al., 2010). The mean value for Nyambene is similar to the mean value of
106.9 µg/g reported in garden soil at kiseriani (Mitei, 1996) implying that similar farming methods were being used. However, the concentration levels of Zn in this study are higher than the values (49.9-131.3 µg/g) obtained in Ethiopian farms where khat is grown (Atlabachew et al., 2011). The reason for this is due to application of different methods of farming, geographical and climatical variation of the two countries. The levels of Zn in this study show that there was no Zn contamination in the soil since the background total Zn concentrations in soil is 10–300 mg/kg (IPCS, 2001) except in some parts of Embu where values were above the range.

4.4.1.2 Concentration levels of zinc in khat

The Zn levels in khat as indicated in table 4.4 range from 25.15 µg/g to 73.95 µg/g. The highest mean concentrations of Zn in khat samples were found in Embu with a mean of 42.93±1.28 µg/g and is attributed to its high concentration in the soil. This value was not significantly different (p<0.05) from the mean value obtained in Mbeere. Since these are adjacent regions it could be due to the farmers in the two regions having similar agricultural practices such as application of Zn based insecticides. The mean value of Zn reported in Nyambene was significantly (p<0.05) different from mean values obtained in Mbeere and Embu. The values of Zn in this study are within the
normal levels of Zn in most crops which range 10-100 µg/g DW (IPCS, 2001).

Lower levels of Zn, 24.1-46.9 µg/g have been reported in Ethiopian khat (Tilahun, 2009). The reason for this variation may be due to cultivation of different khat species in the two countries, different methods of farming and lastly geographical variation of the two countries. Zinc plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phosphohydrolases (Yap et al., 2010) while excess Zn causes toxicity whose symptoms included chlorosis in young leaves, browning of coralloid roots, and serious inhibition on plant growth (Long et al., 2003).

Zinc levels in this study are below permissible values of 60µg/g in vegetables (FAO/WHO, 2001) implying that there was no Zn contamination in khat.

4.4.2 Concentration levels of copper in soil and khat samples

The levels of copper in soil and khat samples were calculated and compared as shown in table 4.5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mbeere</th>
<th>Embu</th>
<th>Nyambene</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Mean±SE 7.86±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.04±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.01±1.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Khat</td>
<td>Mean±SE</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.46±0.57a</td>
<td>0.10-13.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>±SE</td>
<td>13.73±1.66c</td>
<td>5.55-41.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.85±0.44b</td>
<td>3.45-14.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: mean±SE values followed by the same small letters within the same row are not significantly different at $\alpha = 0.05$.

### 4.4.2.1 Concentration levels of copper in soil

The concentration levels of Cu in soil ranged from 0.65 µg/g to 54.40 µg/g. The highest mean concentration values were found in Nyambene with a mean of 40.01±1.19 µg/g. This mean value was significantly ($p < 0.05$) different from mean values obtained in Mbeere and Embu. The difference in Cu levels in the three regions could be attributed to the difference in their parent materials. Similar concentration levels of Cu in garden soil with mean values of 43.4 µg/g and 26.8 µg/g have been reported in Kiserian and Limuru respectively (Kaara, 1992) implying similar soil management methods were being used. However, the concentration values obtained in Nyambene, 28.65-54.40 µg/g are above Cu levels reported in khat farms in Ethiopia which range from 9.13 to 29.8 µg/g (Atlabachew et al., 2011). This is due to difference in the levels of Cu in the parent rocks as well as geographical and climatical variation of the two countries.

Copper concentration levels reported in this study were within the range of Cu in non-polluted soils of 6-60 µg/g (Kabata-Pendias and Pendias, 1992)
and below the permissible limits for agricultural land use of 100 µg/g (FAO/WHO, 2001) implying that there was no Cu contamination in the soil.

4.4.2.2 Concentration levels of copper in khat

The concentration levels of Cu in khat ranged from 0.10 µg/g to 41.80 µg/g as indicated in table 4.5. The mean levels from the three regions differ significantly (P<0.05) with the highest recorded in Embu. High Cu levels in khat samples from Embu could be due to application of Cu based insecticides that are used for spraying coffee. Furthermore, farmers in this region irrigate khat from streams which pass through coffee farms. These streams are likely to be contaminated with agrochemical residues from coffee farms. Agricultural use of Cu products accounts for 2% of Cu released to the soil (IPCS, 1998). Khat from Mbeere had the lowest levels of Cu which is attributed to its low concentrations in the soil. Xiong and Wang (2005), found that Cu concentration in the shoots was significantly influenced by Cu concentration in soil and increased markedly with an increase in the soil Cu concentration. The levels of Cu reported in this study are below the maximum allowable limits of Cu in vegetables of 40 µg/g recommended by (WHO/FAO, 2001). Therefore khat was not contaminated with Cu.

4.4.3 Concentration levels of lead in soil and khat samples

Table 4.6 shows the mean concentration levels of Pb obtained in soil and khat samples from the three khat growing regions.
Table 4.6: Mean concentration levels (µg/g) of lead in soil and khat samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mbeere</th>
<th>Embu</th>
<th>Nyambene</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Mean±SE</td>
<td>10.22±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.76±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.34±3.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>5.00-15.50</td>
<td>10.00-18.50</td>
<td>11.00-119.00</td>
<td></td>
</tr>
<tr>
<td>Khat</td>
<td>Mean±SE</td>
<td>3.28±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.36±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.89±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>0.50-6.00</td>
<td>5.00-12.50</td>
<td>1.50-13.00</td>
<td></td>
</tr>
</tbody>
</table>

Note: mean±SE values followed by the same small letters within the same row are not significantly different at p<0.05.

4.4.3.1 Concentration levels lead in soil

The levels of lead in soil ranged from 5.00 to 119.00 µg/g as shown in table 4.6. The highest mean value of 24.34±3.81 µg/g was recorded in Nyambene. This value was significantly (p<0.05) different from values obtained in Embu and Mbeere, and is attributed to the addition of artificial fertilizer and pesticides to the soil leading to an increase of Pb levels in agricultural soil (Onder et al., 2007). The low levels of Pb in samples from Mbeere may be due to the soil type and soil physical properties since it has been suggested that the mobility of lead is greater in sandy soils, which tend to lack organic matter, than in organic soils (Kirmani et al., 2011). These levels of Pb indicate that there was no Pb contamination in the top soil since they were within Pb levels in uncontaminated soil range from 10 to 70 µg/g.
(Kabata-Pendias and Pendias, 1992) and far below the permissible limits, 50 μg/g for agricultural land (FAO/WHO, 2001).

4.4.3.2 Concentration levels of lead in khat

In khat samples concentration levels of Pb ranged from 5.00 µg/g to 119.00 µg/g as shown in table 4.6. Khat from Embu had the highest concentration mean value which was not significantly (p<0.05) different from the mean value obtained in Nyambene. Low soil pH values in Embu and Nyambene accounts for relatively high levels of Pb in the two regions. The solubility of heavy metals is generally greater as pH decreases (Kabata-Pendias, 2004; Wang et al., 2006). Low levels of Pb reported in Mbeere are due to its low levels in the soil and the soil type. Ghani (2010), noted that the uptake, transport and accumulation of Pb by plants are strongly depended on concentration, soil type, soil properties and plant species.

Similar results of Pb in khat of range 4.8-9.1 µg/g were reported in Addis Ababa (Tilahun, 2009). This could be attributed to the mobility of Pb after its absorption. Patra et al. (2004) reported that majority of Pb is easily taken up by plants from the soil and accumulate in roots while only a small fraction is translocated upward to the shoots. The presence of Pb in khat is due to application of fertilizers and manure containing Pb (Onder et al., 2007). Kenya has also been using leaded gasoline. Therefore Pb emitted from vehicles exhaust could have also contributed to the presence of Pb in khat. Voutsa et al. (1996) reported high accumulation of Pb, Cr, and Cd in leafy vegetables due to atmospheric deposition in the form of metal containing
aerosols. These aerosols can enter the soil and be absorbed by plants, or alternatively be deposited on leaves and then adsorbed. Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield (Bigdeli and Seilsepour, 2008). Excess Pb causes a variety of toxicity symptoms in plants (Eun et al., 2000; Sharma and Dubey, 2005). The mean levels of Pb in khat exceeded the permissible values for vegetables (0.3 µg/g) recommended by the FAO/WHO (FAO/WHO, 2001) implying that khat considered in this study was contaminated by lead.

4.4.4 Concentration levels of cadmium in soil and khat samples

Concentration levels of Cd in soil and khat samples were compared at $\alpha =0.05$ level as shown in table 4.7

Table 4.7: Mean concentration levels (µg/g) of cadmium in soil and khat samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mbeere Mean±SE</th>
<th>Embu Mean±SE</th>
<th>Nyambene Mean±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.63±0.05a</td>
<td>0.95±0.03b</td>
<td>0.58±0.04a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Range</td>
<td>0.10-1.40</td>
<td>0.60-1.30</td>
<td>0.05-1.10</td>
<td></td>
</tr>
<tr>
<td>Khat</td>
<td>*ND</td>
<td>0.48±0.02</td>
<td>*ND</td>
<td>0.743</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0.15-0.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Note: mean±SE values followed by the same small letters within the same row are not significantly different at \( \alpha = 0.05 \), *ND = Below method detection limit

### 4.4.4.1 Concentration levels of cadmium in soil

Cadmium levels in the soil ranged from 0.05 µg/g to 1.40 µg/g as indicated in table 4.7. The highest mean value of 0.95±0.03 µg/g was recorded in Embu. This value was significantly different (\( p < 0.05 \)) from the values obtained from both Mbeere and Nyembene. This is due to difference in the levels of Cd in the parent rocks as well as agricultural practices employed by farmers in each region. High levels in Embu could be attributed to application of large volumes of phosphate fertilizers which are mainly used in coffee farms by farmers. Phosphate fertilizers invariably contain Cd as a natural contaminant (Sodhi, 2009). Similar levels of Cd ranging from 0.73 to 1.23 µg/g were obtained in Ethiopian farms where khat is grown (Atlabachew et al., 2011) implying that farmers could be practising similar soil management methods. Cadmium levels reported in the soil are within levels of Cd in uncontaminated soil of between 0.01 to 11 µg/g (Kabata-Pendias and Pendias, 1992) and far below 3 µg/g, the permissible limits for agricultural land (FAO/WHO, 2001). Therefore, there was no Cd contamination in the soil.

### 4.4.4.2 Concentration levels of cadmium in khat

Cadmium was only reported in khat samples from Embu which ranged from 0.15 to 0.90 µg/g and a mean value of 0.48±0.02 µg/g as indicated in table 4.7. This mean value of 0.48±0.02 µg/g exceeds 0.2 µg/g, the maximum
allowable limits of Cd in vegetables (FAO/WHO, 2001) implying that there was Cd contamination in khat. This could be due to use of manure and phosphate fertilizers that are reported to contain traces of Cd from phosphate rocks. Fergusson (1998), reported that fertilizers contain 0.1-190 µg/g of Cd while nitrogen fertilizer contain 0.1-9 µg/g of Cd. Li et al. (2010), found that pig, dairy cow, and chicken manures contained high Cd due to its presence in their feeds while Hanč et al. (2008) reported that addition of manure increased Cd uptake by plants. Since some khat farmers apply phosphate fertilizer while others apply daily cow and chicken manure or both on their farms to varying levels, this could have contributed to variation in Cd concentration in the soil affecting its bioavailability for plant absorption. The presence of Cd in khat from Embu is also related to its high concentration in the soil while the absence of Cd in khat samples from Mbeere and Nyambene may be attributed to its weak adsorptive nature in the soil (Mido and Satake, 2003).

The levels of Cd in this study, 0.15-0.90 µg/g are lower than 1.3-2.9 µg/g reported in khat samples in Addis Ababa (Tilahun, 2009) and 0.50-2.33 µg/g reported in fresh tobacco leaf in Kenya (Mitei, 1996). The reason for this may be due to cultivation of different khat species in the two countries and application of different methods of farming.

4.4.5 Concentration levels of chromium in soil and khat samples
The concentration levels of Cr in soil and khat from the three khat growing areas are presented as shown in table 4.8.

Table 4.8: Mean concentration levels (µg/g) of Cr in soil and khat samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mbeere</th>
<th>Embu</th>
<th>Nyambene</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Mean±SE</td>
<td>21.41±1.48</td>
<td>44.49±1.03</td>
<td>107.47±4.61</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>7.50-41.00</td>
<td>31.00-55.50</td>
<td>49.50-170.50</td>
</tr>
<tr>
<td>Khat</td>
<td>Mean±SE</td>
<td>*ND</td>
<td>18.62±1.48</td>
<td>18.01±1.12</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>5.00-39.50</td>
<td>3.50-30.00</td>
<td></td>
</tr>
</tbody>
</table>

Note: mean±SE values followed by the same small letters within the same row are not significantly different at \(\alpha=0.05\), *ND =Below method detection limit

4.4.5.1 Concentration levels of chromium in soil

The concentration levels of Cr in soil samples from the three regions range from 7.50 µg/g to 170.50 µg/g as shown in table 4.8. The highest concentration levels with a mean of 107.47±4.61 µg/g were found in Nyambene. This value was significantly (p<0.05) different from values obtained from both Mbeere and Embu. The difference in Cr levels in the three regions could be attributed to the difference in Cr levels in parent rocks as well as agricultural practices employed by farmers in each region. Soils in Mbeere had the lowest levels of Cr and this could be attributed to relatively high soil pH and its speciation. Kotaś and Stasicka (2000), reported that Cr (III) is largely present in soil as relatively unavailable, insoluble oxides of Cr. However, the mean Cr levels reported in this study are within the range of non contaminated soil which is between 5 to 121µg/g (Kabata-Pendias
and Pendias, 1992). Similar levels of Cr in garden soil with mean values of 43.4 µg/g and 26.8 µg/g have been reported in Kiserian and Limuru respectively (Kaara, 1992) implying similar soil management methods were being used.

4.4.5.2 Concentration levels of chromium in khat

Chromium in khat samples was only detected in Embu and Nyambene with mean levels of 18.62±1.48 µg/g and 18.01±1.12 µg/g respectively as shown in table 4.8. These values are higher than the maximum allowable limits in vegetables 2.3 µg/g (FAO/WHO, 2001). Chromium was not detected in Mbeere and this could be attributed to its low concentration levels in the soil, the type and nature of the soil as well as its chemical form in the soil. Hossner et al. (1998) suggested that the availability of the soil Cr to the plant depends on the oxidation state of Cr, pH, and the presence of colloidal binding sites and Cr-organic complexes that would influence its total solubility. Therefore, only a very small fraction of total Cr content in the soil found to be extractable is available to the plant (Zou et al., 2006). Low soil pH in both Embu and Nyambene may also have attributed to the presence of Cr in khat from these regions since Cr behavior in soil is controlled by soil pH and redox potential (Mondol et al., 2011).

The levels of Cr obtained in this study are higher than those obtained from khat in Addis Ababa which ranged from 3.1 to 6.76 µg/g (Tilahun, 2009) and
1.5-6.6 µg/g, found in vegetable leaves irrigated with waste water in the city of Harare (Mapanda et al., 2007). This is because different crops vary in their ability to accumulate Cr in their tissues (Zayed et al., 1998) as well as cultivation of different khat species in the two countries and application of different methods of farming.

4.5 Soil pH

The mean pH values of soils from the three khat growing regions are presented in table 4.9.

<table>
<thead>
<tr>
<th>pH</th>
<th>Mbeere</th>
<th>Embu</th>
<th>Nyambene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>5.26-7.38</td>
<td>5.47-7.07</td>
<td>5.25-6.72</td>
</tr>
<tr>
<td><strong>Mean±SE</strong></td>
<td>6.38±0.13</td>
<td>6.22±0.07</td>
<td>6.15±0.06</td>
</tr>
</tbody>
</table>

The soil pHs of the farms ranged from 5.25 to 7.38 as shown in table 4.9. Soils in Nyambene had lower soil pH than those from Embu or Mbeere. The acidic condition in soil may be due to application of NPK fertilizers. The increased use of nitrogenous fertilizers generally increases soil acidity (Ishibashi et al., 2004). Nartey et al. (2012), reported that fertilizer amended soils have generally lower pH than natural soils. Soil pH is one of the most influential parameters that control conversion of immobile solid-phase metal
forms to more mobile and/or bio-available solution-phase forms. The solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately pH 5.0 to 7.0) (Kabata-Pendias, 2004; Wang et al., 2006). Therefore low pH values of soils in this study could have accounted for the transfer of metals from soil to plant.

4.6 Correlation coefficients between heavy metals in soils and khat

Correlation tests were carried out between concentration of the metals in the soil and khat to establish whether there was any relationship. Their correlation coefficients are summarized in table 4.10.

Table 4.10: Correlation coefficient (r) between metal concentration levels in soils and khat plants

<table>
<thead>
<tr>
<th>Correlation between soil and khat</th>
<th>Mbeere</th>
<th>Nyambene</th>
<th>Embu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.237</td>
<td>0.327*</td>
<td>-0.025</td>
</tr>
<tr>
<td>Cu</td>
<td>0.699**</td>
<td>0.409**</td>
<td>-0.504**</td>
</tr>
<tr>
<td>Pb</td>
<td>0.095</td>
<td>0.164</td>
<td>-0.071</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>0.380*</td>
<td>0.079</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td>0.033</td>
</tr>
</tbody>
</table>

**Correlation is highly significant  *Correlation is significant

As shown in table 4.10, there are positive correlations between concentrations in soil and khat for all metal in the studied regions except for Zn, Cu and Pb in Embu. In Mbeere, these relations were not statistically
significant for Zn and Pb but were significant for Cu (0.699, p<0.01). In Nyambene, correlation coefficients for Zn (0.327, p<0.05), Cu (0.409, p<0.01), and Cr (0.380, p<0.05) were statistically significant while in Embu, only Cu in soil significantly correlated negatively with copper (0.504, p<0.01) in khat. Therefore the concentration of the metals in soil appears to influence the uptake of metals in khat. The results of positive correlation between soil and plants have been supported by earlier findings (Fatoki, 2003; Onder et al., 2007) implying that there was a strong relationship among these heavy metals in soil and plants. However, the negative correlation results indicated by some metals, give a strong suspicion to the fact that some elements might be assimilated through other organs of the plants other than their roots or some plants may have high affinity of assimilation of some elements directly from atmospheric deposition and agrochemicals such as pesticides.

4.7 Heavy metal transfer factor from soil to khat

The amount of heavy metals in the soil that ended up in khat was determined using transfer factors. Table 4.11 shows the transfer factors (TF) of different heavy metals from soil to khat in each region.

Table 4.11: Transfer factors from soil to khat

<table>
<thead>
<tr>
<th>Element</th>
<th>Mbeere</th>
<th>Embu</th>
<th>Nyambene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.99±0.06</td>
<td>0.30±0.02</td>
<td>0.39±0.02</td>
</tr>
<tr>
<td>Cu</td>
<td>0.70±0.13</td>
<td>0.99±0.17</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>Cr</td>
<td>Cd</td>
</tr>
<tr>
<td>----</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>0.34±0.03</td>
<td>0.54±0.02</td>
<td>0.41±0.03</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>-</td>
<td>0.42±0.03</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>Cd</td>
<td>-</td>
<td>0.52±0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

Zinc in Mbeere and Cu in Embu recorded the highest TF values. All TF values were below 1. This implies that it is very difficult for these metals to transfer from the soils to the plant, that is metal bioavailability in the soil is very low. Transfer quotient of 0.1 indicates that the plant is excluding the element from its tissues. The greater the transfer coefficients value than 0.50, the greater the chances for metal contamination by anthropogenic activities (Sajjad et al., 2009). Reference to this statement, only Cd and Cr in Mbeere and Cd in Nyambene were excluded in the khat tissue, while in Mbeere, Zn (0.99) and Cu (0.70) and in Embu, Cu (0.99), Pb (0.54) and Cd (0.52) could be contaminated by anthropogenic activities. High TF of Cu in Mbeere and Embu imply that Cu is more bioavailable to plant than other metals, that is Cu can be transferred from soils to plant more easily. According to the TF calculated in this study, it may be concluded that khat from Mbeere and Embu are high Cu-accumulator. The low TF value for Cu in Nyambene, despite its high concentration level in the soil may be due to its strong adsorption onto the organic matter, which renders it less bioavailable to plants (Oluyemi et al., 2008). The difference in TF of the same heavy metal between regions may be related to soil nutrient management and soil properties (Cui et al., 2004).
4.8 Correlation matrix between Transfer factors of metals and Soil pH

Pearson’s correlation coefficients for relationship between the various metal transfer factors and soil pH are given in table 4.12.

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
<th>Cr</th>
<th>Pb</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.165</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.116</td>
<td>0.500**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.402**</td>
<td>-0.029</td>
<td>0.260*</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.196</td>
<td>-0.198*</td>
<td>-0.238**</td>
<td>0.390**</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.482**</td>
<td>0.081</td>
<td>-0.172**</td>
<td>0.021</td>
<td>-0.134</td>
<td>1.000</td>
</tr>
</tbody>
</table>

** Correlation is highly significant  *correlation is significant.

Significant positive relationship of transfer factors were observed between Cd-Cr, Zn-Cu and Cr-Pb while the transfer factor for Cu had significant but reciprocal interaction with that of Pb at $r = -0.238$. The positive correlation indicates that the absorption of the heavy metals in khat influences the co-absorption of other heavy metals in the same plant. Soil pH had significant but negative correlation with transfer factors for Cd and Cu at $r = -0.482$ and -0.172, respectively. Negative correlation implies that decrease in soil pH go together with increase in the solubility of Cd and Cu in the soil and increase in their bioavailability to the khat.
4.9 Estimation of heavy metal intake through consumption of khat.

The estimated heavy metal intakes through consumption of khat in relation to the PMTDI/PMTWI are presented in table 4.13. Khat from Embu was used since it had all the selected heavy metals studied.

Table 4.13: Estimation of heavy metal intakes (for 70 kg adult) through consumption of khat.

<table>
<thead>
<tr>
<th>Element</th>
<th>Intake from khat in mg/100g khat (FW)</th>
<th>Maximum limits</th>
<th>PMTDI/PMTWI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>1.073 mg/day</td>
<td>70 mg/day</td>
<td>PMTDI</td>
</tr>
<tr>
<td>Cu</td>
<td>0.343 mg/day</td>
<td>35 mg/day</td>
<td>PMTDI</td>
</tr>
<tr>
<td>Cr</td>
<td>0.466 mg/day</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.012 mg/day</td>
<td>0.49 mg/week</td>
<td>PMTWI</td>
</tr>
<tr>
<td>Pb</td>
<td>0.209 mg/day</td>
<td>1.750 mg/week</td>
<td>PMTWI</td>
</tr>
</tbody>
</table>

NR=FAO/WHO has not recommended PMTDI or PMTWI for Cr. Source: (FAO/WHO, 1999)

Zinc is an essential mineral which is involved in numerous aspects of cellular metabolism and is required for catalytic activity of approximately 100 enzymes (Sandstead, 1994; IMFNB, 2001). The mean daily intake of zinc by khat consumers is estimated to be 1.073 mg for 100 g of khat (since fresh khat is chewed for its stimulating property, the result in terms of dry weight basis is converted to fresh (wet) weight basis using a conversion factor of 0.25). These values may not pose a health risk according to PMTDI of 70 mg/day (FAO/WHO, 1999). No adverse, health effects are thus expected.
from Zn intake from khat unless other additional and richer sources of this heavy metal in the diet contribute significantly to its intake.

Copper is an essential trace element, vital to the healthy life of many animals and plants. The average daily intake of Cu from khat is 0.343 mg for 100 g of fresh weight. This value is far much below the current PMTDI of Cu of 35 mg/day (FAO/WHO, 1999). Therefore, no adverse health effects are thus expected from Cu intake from khat.

Chromium is another essential mineral found in khat. The availability of this metal in human is important for many biological activities (Manahan, 2003). It regulates blood sugar, therefore reducing medication and insulin needs in diabetic patients (Bahkru, 2006) and also plays a role in the management of heart diseases by regulating fat and cholesterol synthesis in the liver (WHO, 1996). The average daily intake of Cr by khat consumers of 100 g of khat per day is 0.466 mg fresh weight. The WHO has not recommended PMTDI or PMTWI for Cr. Therefore, no adverse health effects are thus expected from Cr intake from khat.

Lead is the most significant toxin of heavy metals and the inorganic forms are absorbed through ingestion by food (Ferner, 2001) while cadmium has no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Nolan, 2003; Young, 2005). The consumption of khat contributes 0.012 mg for 100 g fresh weight of Cd daily and 0.209 mg for 100 mg fresh weight of Pb daily. The
FAO/WHO has recommended a PMTWI of Cd and Pb of 0.49 mg/week and 1.750 mg/week respectively (FAO/WHO, 1999). According to these values, the daily intake of Cd and Pb by Kenyan consumer for 100 g of khat alone is below the PMTWI levels. However, if above 100 g of khat is consumed and if other sources of Cd and Pb are included then the intake may exceed the recommended levels and continuous exposure to Cd and Pb may result in their gradual accumulation in human vital organs which may cause profound biochemical and neurological changes in the body.

The results of the present study indicate that intakes of the selected heavy metals due to consumption of khat, pose no health risk as the values are lower than the respective permissible intakes. The daily metal intake from khat can however be minimized by reducing the amount of khat consumed.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study the levels of five heavy metals in khat and soil from three main khat growing regions were analysed. The result showed that:

i. Soil samples from the three regions were found to contain Pb, Cd, Zn, Cu, and Cr. The levels of Zn in soil were the highest in Embu and Mbeere while Cr levels were the highest in Nyambene.

ii. There were significant variations in levels of Zn, Cu, and Cr in soils from the three regions.

iii. Levels of selected heavy metals in soil were below the permissible limits recommended by FAO/WHO for agricultural land use.

iv. Khat samples from the three regions were found to contain Zn, Cu and Pb. The levels of Zn were the highest in each region.

v. Khat from Mbeere had the lowest levels of heavy metals.

vi. In Nyambene, the levels of Zn, Cu and Cr in soil positively correlated with the levels in khat.
5.2 Recommendations

5.2.1 Recommendations from the study

i Although the intakes of the selected heavy metals due to consumption of khat pose no health risk, the daily metal intake can however be minimized by reducing the amount of khat consumed.

ii There is need for monitoring the levels of heavy metals in khat to ensure that they do not exceed acceptable levels.

iii Khat under these study regions could be contributing significantly to Pb intake especially to children who are more susceptible to its adverse effects than adults because their major organs, including the brain, are still being developed. The government therefore should come up with policies that will control the distribution, sale and use of khat especially to children.

5.2.2 Recommendations for further work

i This study covered only five elements. More trace elements other than the one considered in this study as well as major elements should be analysed in order to provide a comprehensive data on the metal profile of the khat plant.
ii Similar studies should also be done without washing khat. This will be able to provide information on the total concentration of heavy metals consumed by khat chewers as khat is never washed during chewing. Contamination with heavy metals is likely to occur during handling especially transportation, loading and unloading.

iii Chemical speciation should be carried out in both soil and khat to determine the chemical forms of heavy metals present in the soil and in khat. This is because there are some forms of heavy metals that are very toxic to both plants and animals while others are less toxic.
REFERENCES


Ferner, D. J. (2001). Toxicity. Heavy metals. eMedicine Journal, 2:1


Geotechnical Engineering Bureau (2007). Test method for the determination of the pH value of water or soil by pH meter. State of New York, department of transportation. 1-5.


Figure 1. Levels of zinc in soil and khat samples
Figure 2. Levels of copper in soil and khat samples.

APPENDIX III

Figure 3. Levels of lead in soil and khat samples.

APPENDIX IV

Figure 4. Levels of chromium in soil and khat samples
APPENDIX V

Figure 5. Levels of cadmium in soil and khat samples