Determination of Heavy Metals in Scalp Hair of Selected Kenyan Urban and Rural Population.

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Determination of Heavy Metals in Scalp Hair of Selected Kenyan Urban and Rural Population.

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DECLARATION

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

Signature ……………………………Date…………………………

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3. **The late Dr. Joyce Wanjiru Njenga**

J KUAT, Kenya

DEDICATION

To my parents: Late Father, Francis Abuor and my Mum, Angelina Abong'o Abuor

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LISF OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
ASTDR	Agency for Toxic Substances & Disease Registry
CBD	Central Business District
CRM	Certified Reference Material
KNBS	Kenya National Bureau of Statistics
CNS	Central Nervous System
DAP	Diammonium Phosphate
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
GOK	Government of Kenya
HDL	High-Density Lipoprotein
HGAAS	Hydride Generation Atomic Absorption Spectroscopy
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MAP	Monoammonium Phosphate
NEMA	National Environmental Management Authority
SAS	Statistical Analysis Software
SGS	Societte Generalle de Surveillance
VGP	Vapor Generation Program
WHO	World Health Organization

ABSTRACT

Pollution by heavy metals contamination and the resulting health effects present challenges currently facing both developed and developing countries. Metal poisoning is not only expensive but also difficult to diagnose, particularly in developing countries where resources are limited. Hair samples (N = 240) were obtained with assistance of barbers and hair dressers located in Nairobi, Mombasa and Kisumu. Concentrations of Lead, Cadmium, Copper, Manganese, Zinc and Mercury were determined in scalp hair of male and female of children and adults of ages (5 - 14, 15 - 20, 21 - 40, > 40 years). Concentrations of the elements in the samples investigated were determined by using Atomic Absorption Spectrophotometer (AAS), applying both flame and flameless techniques in determination of the stated elements. As a control, samples were taken from non-industrialized agricultural rural areas in North Rift, Msambweni and Western Kenya. The generated data was analyzed using Statistical Analysis Software System (SAS) focusing on Analysis of Variance by One-Way (ANOVA), Correlation Analysis, Bar Charts and Line Graphs, Significance tests and Confidence Intervals set at 95 % level of confidence. One-Way ANOVA revealed a significant effect of urban and age on heavy metal concentration (p value of 0.0001 and 0.002 respectively at $p < 0.05$). Linear Correlation between paired metals indicated strong positive correlations. The results revealed that the hair samples of Kenyan urban inhabitants had significantly higher concentrations of all metals Pb, Cd, Cu, Mn, Zn and Hg than those from the rural counterparts at 95% confidence level. The concentration of the studied metals were in the increasing order of Hg < Cd < Pb < Cu < Mn < Zn. It was found out that age and geographical location had great influence on metal hair concentration among the Kenyan inhabitants. Comparing the obtained results in this study with the literature data, Kenyan Scalp hair heavy metal concentration emerged the highest among the other regions of the world, particularly with respect to Cd (1.59 mg/kg) and Mn (27.l9 mg/kg). Also, with exception of Nigeria, Libya and Pakistan, Kenya had higher hair Pb (13.52 mg/kg) concentration than the selected countries of the World.

CHAPTER ONE

1 INTRODUCTION

1.1 General Introduction.

Heavy metals are chemical elements with a specific gravity of at least 5 times the specific gravity of water (Lide, 1992). Heavy metals are everywhere in nature as components of the earth's crust as they occur naturally in water, soil and biota. The metals are a unique class of toxicants since they cannot be broken down to non-toxic forms by the biological system and once the ecosystem is contaminated by them, they remain as a potential hazard to human health for many years. Literature sources point to the fact that these metals are released to the environment by both natural and anthropogenic sources, especially mining operations and industrial activities (Ogola *et al.*, 2002, Pinheiro *et al.*, 2007). Toxic heavy metals in air, soil and water are global problems that are a threat to environment and human health. Their concentrations depend also on local geology and globally distributed pollution (Khan *et al.,* 2008; Hang *et al*., 2009).

Heavy metals are common in industrial applications such as their use in manufacture of pesticides, fungicides, batteries, alloys, electroplated metal parts, textiles, pigments, dyes, and steel industries (Mohammad and Shashi, 2006). Other uses of heavy metals include; industrial use in pharmaceuticals, cosmetic, dental amalgam and paints manufacturing. Every part of the human body contains at least a few atoms of every stable element in the periodic table (Gordus, 1973).

Although a large number of these elements are found in detectable amounts in the human tissues; blood, urine and hair, the hair in particular contains higher concentration of many of these elements. Certain metals such as zinc, iron, manganese, copper, at trace levels are nutritionally essential for a healthy life and are commonly found in foodstuffs and commercially available multivitamin products, and their deficiency can lead to various health effects (Goldhaber, 2003). These metallic elements are found in every living organism, where they play a variety of roles. However, at higher concentration heavy metals are dangerous as they tend to bio-accumulate and can lead to poisoning. For example, zinc and manganese are essential elements but higher levels of the two elements can lead to adverse health effects, as in the case of high exposure to manganese that has been linked to Parkinson’s disease (Lucchini *et al.,* 2007). Elevated levels of zinc may cause disease in serum High- Density Lipoprotein (HDL) cholesterol level with subsequent increase in the risk of coronary artery disease, gastrointestinal irritation, anaemia due to zinc- induced copper deficiency, pancreatic and adrenal gland damage (ASTDR, 2007).

1.2 Metals of Greatest Public Health Risk

The three heavy metals commonly cited as being of the greatest public health concern are cadmium, lead and mercury. In the Agency for Toxic Substances and Diseases Registry (ATSDR) priority list of hazardous substances, lead, mercury, arsenic and cadmium appear among the top 10 (ATSDR, 2007). Mercury for example, is a hazardous metal that has caused in the last decades serious episodes of environmental contamination and human intoxication in several locations around the World, such as outbreaks in Minamata and Iraq (Counter and Buchanan, 2004). As regards to toxicity in human, methyl mercury is high on the list of dangerous mercury compounds, being able to bio-accumulate through the food chain to reach human populations (Gochfeld, 2003). Toxicology studies have demonstrated that the Central Nervous System (CNS) represents a target organ for mercury toxicity (Mottet *et al.*, 1994). Kazi *et al*. (2008) found high levels of cadmium in blood and hair of male lung cancer patients, hence the compelling evidence in support of positive associations between cadmium, cigarette smoking and lung cancer risk.

Heavy metals become toxic when they are not metabolised by the body and accumulate in the soft tissues. Heavy metals and some organic poisons cannot be metabolized by the organs responsible in the human body, so over time they bio-accumulate in the fats, tissues, heart, lungs and other sensitive body organs until the level of their toxicities are reached (Roberts, 1999). The metals may enter the human body through food, air, water and absorption through the skin. Industrial exposure accounts for common route of exposure in adults while ingestion is the common route of exposure in children. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, and copper poisoning: gastrointestinal disorders, diarrhoea, paralysis, vomiting and convulsion, depression and pneumonia when volatile vapours and fumes of the metals are inhaled (McCluggage, 1991). The nature of the toxic could be acute or chronic, neurotoxin, carcinogenic, mutagenic or tetratogenic (McCluggage, 1991). Long term exposures to lead in humans can cause acute or chronic damage to the nervous system; Long term exposure to cadmium has been associated with renal dysfunction. A wide rage to metabolic disorders and neuro-psychological defects has been associated with environmental exposure to low levels of lead (Nriagu, 1988).

High exposure to cadmium can lead to obstructive lung disease and has been linked to lung cancer and damage to human respiratory systems. Copper is an essential element to human, but in high dozens can cause anaemia, liver and kidney damage.

1.3 The Human Hair

Human hair is a metabolic by-product that incorporates metals into its structure during the growth process. Hair growth begins in the hair follicle where it is formed from circulating blood and other body fluids. This is the period where heavy metals and contaminants in human fluids enter the hair structure (Azhari *et al*., 1990). As a result of continuous abstraction and growth in the follicle, hair is finally extruded as a fibre above the skin where it is isolated from metabolic events inside the body; hence it provides a unique indication of the prevalent levels of contaminants in the body fluids at the time of its genesis.

Determination of heavy metal content in hair plays an important role in exposure assessment and has been explored as a tool for assessing the impact of environmental pollution by many researchers (Nowak, 1998, Revai, 2001, and Pereira, *et al.*, 2004). Therefore, head hair metal analysis can help in early identification of long tem nutritional deficiencies that can cause illness besides identifying the toxic metals. Hair and other biological material such human milk, blood, urine and nails has been used to determine trace elements concentration in various epidemiological surveys (Feng *et al*., 1997; Ozkaynak *et al.,* 2005). The World Health Organization (WHO) and major international organizations such as Internal Atomic Energy Agency (IAEA), and the United States Environmental Protection Agency (US EPA) have recommended hair analysis for determination of heavy metal levels in certain cases (Druyan *et al.*, 1998; Morton *et al.*, 2002; Smodis, 2005).

Major advantage of using human hair as a bio-indicator of heavy metal exposure is the fact that when compared to other biological materials it represents longer integrator of exposure to the metals hence can reflect exposure of previous months (Okamoto *et al.,* 1985; Nowak, 1996; Rivai, 2001).

Further, hair samples can be easily obtained without injury to the donor, are stable and can be stored for longer periods of time before being analysed and has higher concentration of metals as compared to the conventional biological materials and rest of the body (Suzuki, 1988). Unlike other biological specimens for example blood and urine, trace metal content of hair correlates well with body stores, especially of bones, and reflects for each individual, specific factors genetical and environmental origin (Limic and Valkovic, 1986).

It has been estimated that in a healthy person, the concentration of heavy metals such as lead in scalp hair may be 10 - 50 times higher than in blood and 100 - 500 times than in urine (Hansen, 1981). In opposition, some limitations have been described for hair analysis and use of hair as a biomarker of general metal exposure, mainly due to the occurrence of exogenous contamination that might contribute to differences in metal content (ASTDR, 2001; Frisch and Schwartz, 2002; Barbosa *et al.*, 2005). Some other constraints that have been pointed out (Seidel *et al.,* 2001; Harkins and Susten, 2003) for the use of hair analysis are:

i) The lack of correlation between concentrations of heavy metals in hair and other target organs like kidney and liver or body fluids like urine and blood.

ii) Lack of scientific knowledge about kinetics of incorporation of trace elements in the hair.

iii) The insufficiency of epidemiological data to support predictions of health effects, related with a specific concentration of each element in the human hair (Seidel *et al.,* 2001).

Despite the constraints human hair has been considered a useful screening tool in assessment of likely occurrence of environmental exposures (Masters, 2003). With the notion of all the advantages, human hair samples have been extensively used as a first step in human exposure assessment to heavy metal pollution.

1.4 Heavy Metals in the Environment

The concentration and distribution pattern of heavy metals within the ecosystem is important. Low concentrations of heavy metals occur in natural ecosystems, but recent expansions in human population growth, industry, and peri-urban agricultural activities in African cities have led to an increase in heavy metal occurrence in excess of natural loads (Biney *et al*., 1994; Dickinson *et al.,* 1987). Nairobi, Mombasa and Kisumu are the most industrialised and populated towns in Kenya. High levels of heavy metals have been reported in soil according to Onyari *et al.*, 1991, Lalah *et al.,* 2008, Kamau (2001), Makokha *et al.* (2008). Significantly high concentration of Pb has been reported among school aged children in schools located along the highways in Nairobi by Hussein *et al.* (2008).

The aim of this study was to assess the concentration of Pb, Cd, Cu, Zn, Mn and Hg in the hair among the Kenyan urban and rural inhabitants.

1.5 Statement of the Problem

Heavy metal pollution and the resulting effects represent a challenge currently facing developing countries, Kenya included. This is reinforced by the fact that metal poisoning is difficult and expensive to assess in developing countries due to the limited resources. Hence there is need to increase the level of understanding and assessment of environmental risk exposure to heavy metal pollution in Kenya.

1.6 Rationale

The use of human hair for the study of occupational and environmental exposure to toxic and heavy metals has continued to generate interest among researchers from different parts of the World. Studies have ranked hair as an excellent indicator of past changes in metabolism and environmental exposure to heavy metal pollution. Many researchers have associated heavy metal concentration in human hair with urbanization, vehicular emissions, occupational exposures and dietary habits. In Kenya little work has been done in this area that continue to elicit increased interest amongst researchers, especially those in the field of medical, biological, forensic and environmental science. Last time that hair was used for investigation of heavy metal contamination in Kenya was over twenty years ago (Wandiga and Jumba, 1982).

Kenya like any other developing countries faces serious problems of urbanization, vehicular pollution and industrial discharges that present serious health risks and exposure to the public, particularly the urban population. Given the fact that metal pollution is difficult and expensive to screen in developing countries like Kenya, hair can serve as an alternative biomarker in assessing heavy metal environmental exposure. Therefore there is need for assessment of the level of exposure to metal pollution and the underlying consequences.

1.7 Hypothesis

There is significant exposure to heavy metal pollution among Kenyan population

There is no significant exposure to heavy metal pollution among the Kenyan rural and urban population

1.8 The Objectives of the Study

1.8.1 Main Objective

The main objective of the study was to evaluate the concentration of lead, cadmium, manganese, zinc, mercury and copper in scalp hair samples of selected groups of people in three distant towns of Nairobi, Mombasa and Kisumu and some rural areas of Sondu, Kipsamoite and Msambweni regions in Kenya.

1.8.2 Specific Objectives

The specific objectives were:

i. To analyse scalp hair samples from urban and rural areas for Pb, Cd, Cu, Zn, Mn and Hg.

ii. To evaluate the relationship between age, sex and geographical location with heavy metal content among selected Kenyan population.

iii. To compare heavy metal analysis findings of major cities of Nairobi, Mombasa, Kisumu and rural areas of Kapsabet, Sondu and Msambweni

1.9 Significance of the study

The present data could act as baseline information for future environmental pollution abatement program. Further, biological media such as hair can be used as index of environmental exposure to toxic metals. The data obtained from this study suggests that human hair can be used to identify heavy metals exposure in epidemiological surveys. The levels of heavy metal in the hair tissue were to be significantly influenced by both environmental and nutritional factors.

1.10 Scope and Limitation

This study focused on determination on the levels of selected heavy metals namely: lead, cadmium, copper, manganese, zinc and mercury in scalp hair of the major Kenyan urban centres of Nairobi, Mombasa and Kisumu. The scalp hair samples were collected from children of ages 5-14 years, and adults of ages 15 years and above.

Certified reference materials (CRM) for validation of results for biological samples like hair are not readily available in Kenya. In view of this, adequate quality assurance control was ensured by inter-laboratory comparison of representative samples carried out at JKUAT and SGS Kenya Limited Laboratories.

Cultural beliefs among the sample population led to delay in sample collection durations. The researcher took time to explain to the volunteers on the purpose and benefits of the study and also increased sampling duration.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Hair as Bio-indicator of Heavy Metals

Recent years have seen a lot of interest in using human hair as bio-indicators of toxic and pollution by heavy metal since hair, as metabolic and end product does incorporate heavy metals into their structure during growth process (Mortada *et al.,* 2002; Pereira *et al.,* 2004; Rashed and Hossam., 2007). Hair has been used in many studies as a bio-indicator of mercury exposure for human populations. At the time of hair formation, metals from the blood capillaries penetrate into the hair follicles. As hair grows approximately 1 cm each month, mercury exposure over time is recapitulated in hair strands (Niculescu *et al*., 1983). Metal levels in hair closest to the scalp reflect the most recent exposure, while those farthest from the scalp are representative of previous blood concentrations. Sequential analyses of hair mercury have been useful for identifying seasonal variations over time in hair mercury content (Strumylaite *et al*., 2004). Metal exposure is usually determined by analysis of individual metal in blood and hair.

The suitability of hair analysis as a means of screening for heavy metal poisoning and exposure is well documented making human hair analysis and area of increased interest particularly in the field of forensic, medical, biological and environmental sciences (Nowak, 1993; Bader *et al.,* 1999; Ashraf *et al.,* 1995; Wilhelm *et al.,* 2007).

Hair is an excellent indicator of past changes in metabolism and environmental exposure to metals (Ajayi *et al*., 2001). Indeed many studies have suggested use of human hair as one of the suitable measures of heavy metals pollution (ASTDR, 2001; Sekhar *et al.,* 2003; Pereira et al., 2004; Adekola *et al.,* 2004). Many advantages have been pointed out in use of human hair over the conventional measure of exposure blood, one advantage being that hair represents longer integrator of exposure to elements and hence can reflect exposure of previous months (Okamoto *et al.,* 1985; Aharoni and Tesler, 1992; Revai 2001). Other advantage pointed out include the fact that hair can be obtained without causing injury to the donor and is stable hence can be stored for a longer period before analysis exercise and that hair has higher concentrations of residues of the target analytes as compared to other biological materials i.e. urine and blood (ASTDR 2001).

Many researchers have recommended the use of human hair for monitoring heavy metals (Wenning, 2000; Seidel *et al.,* 2001; Harkins and Susten, 2003; Pereira et al., 2004). Compared with other tissues, hair is easy to achieve complete digestion due to its lipid content (Cheng *et al.,* 1996). Some studies have shown a correlation between heavy metals for instance lead content in hair and blood (Wilhelm *et al.*, 2002; Black *et al.*, 2002; *Sanna et al.*, 2003). A significant correlation was observed between the blood - lead and hair - lead concentrations among occupationally exposed people of Romania (Niculescu *et al.*, 1983). Similar study using human hair also revealed higher geometric mean of lead in the hair of exposed ceramic workers than in the persons that were not exposed to the metal (Strumylaite *et al.,* 2004).

Hair has been used in many studies as a bio-indicator of mercury exposure for human populations (Björnberg *et al.*, 2003; Elhamri *et al.,* 2007; Morrisette *et al.,* 2004; Srogi, 2007). Hair specimens were used in evaluation mercury levels in pregnancy and its relationship with fish consumption among the population living along St. Lawrence River (Morrisette *et al.,* 2004). Sequential analyses of hair mercury have been useful for identifying seasonal variations over time in hair mercury content, as mercury levels in hair closest to the scalp reflect the most recent exposure, while those farthest from the scalp are representative of previous blood concentrations. Individual methyl mercury exposure is often determined by analysis of mercury in blood and hair (Björnberg *et al*., 2003).

2.2 Heavy Metals in Human Hair

In Kenya, a study conducted on exposure of school age children of some heavy metals using human nails revealed elevated levels in urban children (Hussein *et al.,* 2008). The use of human hair for the study of occupational and environmental exposures has been explored by a number of researchers from different parts of the world. For example, level of lead in human hair has been attributed to vehicular emissions and automobile traffic in two cities of Ibadan and Ilorin in Nigeria (Adekola *et al.,* 2004).

Ahmed and Elmubarak (1990) found a strong correlation between urbanization and levels of trace elements in the hair of Saudi Arabian adult male population. A study among the Polish population using human hair (Nowak, 1998) observed a dependence of heavy metal concentration on age and sex. Rashed and Hossam (2007) recorded higher concentration of heavy metals (Pb, Cu, Cd, Zn) in hair of workers and children in populated Kima area in Aswan, Egypt than West Aswan (unpopulated area). A comparative study using hair carried out among the children of Amazon Riverside villages revealed higher concentration of mercury levels in older children of age 7 - 12 years than in babies of 0 - 1 year (Pinheiro *et al.*, 2007). It has also been found out that individuals have different heavy metals detoxification capacity given the varying genetic regulatory mechanism among humans (Gundacker *et al.*, 2007). Heavy metals in human hair therefore, serve as a useful indicator for assessment of the extent of exposure of an individual to environmental metal pollution.

A study in two Nigerian cities of Ibadan and Ilorin indicate high levels of head hair lead (67.7 _+43.3 mg/kg) on average as compared to literature values from the rest of the World that has been attributed to relatively high level of vehicular traffic in the two Nigerian cities (Adekola *et al.*, 2004). A similar study in Nigeria by Nnorin *et al.*, (2005) reported high levels of heavy metals with geometric mean values of 65.4 ug/g (Pb) and 1.2 ug/g (Cd) of the studied population. A study on Egyptians by Mortada *et al.*, (2002) revealed hair metal content of Pb 1.8 – 7 ppm Cd 0.2 - 2.8 ppm; and recent study by Rashed and Hossam (2007) indicate similar results; 0.44 ppm for Cd and 5.95 ppm for Pb. Adekola *et al. (2004),* reported hair metal content of Pb (32-78 ppm); (Zn 462 - 1050 ppm); (Cu 10.7 - 28.0 ppm).

Literature values of some hair metals contents reported in World wide studies include Vienna, Austria were; (Cd 0.041 ppm); Pb (1.17 ppm), Rome, Italy; (Cd 0.05 ppm) and Pb (1.92 ppm), Wolfsperger *et al*., 1994); India (Cd 0.4 ppm); (Pb 8.03 ppm); (Cu 14.76 ppm) and (Zn 152 ppm) (Samata *et al.*, 2004) . In Indonesia hair metal content are as follows; Cd, 0.43 ppm; Pb, 30.6 ppm; Cu , 3.14 ppm and Zn 33 ppm (Rivai 2001) while Japanese hair contains Pb, Cu and Zn in concentration of; 3 ppm, 13.3 ppm and

210 ppm respectively (Gerhardsson *et al.,* 2002). In Korea, reported hair metal contents for Cd, Cu Pb and Zn are 0.11 ppm, 11 ppm, 2.5 ppm and 141 ppm respectively (Yoo *et al.,* 2002) while in Poland human hair contains levels of (Cd 0.55 ppm), (Pb 4.8 ppm), (Cu 7.2 ppm) and (Zn 124 ppm) (Nowak and Chmielnicka, 2000). The variations in hair metal contents have been mainly attributed to the difference in environmental exposure and geographical conditions (Rashed and Hossam, 2007).

Several factors have been reported to be responsible for heavy metal exposure and high levels of metals in human hair. In the study of Cd, Cu and Zn in the hair of people from Portugal (Pereira *et al.,* 2004) found higher metal concentrations in the hair of the mining groups as opposed to the control group. This was also in agreement with other studies among the environmentally exposed people of Aswan in Egypt (Rashed and Hossam, 2007). In Nigeria Adekola *et al.,* (2004) reported increased levels of trace metals in hair of people exposed to vehicular pollution in Nigeria. A similar report is recorded in Ghana by Golow and Kwaansa (1994) where increased levels of metals in hair of school going children was linked to vehicular exhaust emissions.

2.3 Effect of Age, Sex and Location on Heavy Levels in Hair

Many workers have demonstrated dependence of heavy metals on factors such as geographical location of urban population (Ashraf *et al.,* 1995; Feng *et al.,* 1997). Khalique *et al.,* (2005) observed a distinct metal concentration dependence on sex and age. It has also been found that lead accumulates with age and that copper and cobalt of the youth seem to give way to predominance of zinc on maturity (Zakrgysnka *et al.,* 1998). Ozden *et al.,* (2007) in a study of exposed children in Istanbul, Turkey revealed high levels of lead and cadmium among school going children who were situated along main highways and exposed to tobacco smoke (Ozden *et al.,* 2007).

Copper levels in human hair have been found to have no direct relationship with gender and location (Ashraf *et al.,* 1998). Nowak and Chmielnicka (2000) in a study among inhabitants of Katowice, Poland revealed that hair is an environmental marker of exposure to lead in humans and depends on sex and age. The same study also observed that an increase in concentration of the same element leads to decreased in iron and calcium – essential trace elements (Nowak and Chmielnicka, 2000).

2.4 Analytical Methods for Determination of Heavy Metals

The Instruments that are extensively used for heavy metal analysis include AAS, Flame Atomic Spectrometry, Graphite Furnace –Atomic Spectroscopy; Hydride Generation Atomic Absorption Spectroscopy, Atomic Emission Spectroscopy.

The choice of the analytical instrument in this study was mainly been dictated by the expected limits of detection of the analytes under investigation, that is at mg/kg levels AAS instrument suffices while at µg/kg levels, Graphite Furnace-Atomic Absorption technique, ICP- AES would be most ideal. Further, the instrument is readily available in Kenya.

Atomic absorption spectroscopy is now a well-established technique for the determination of trace elements covering a wide range of analyte types. These include the determination of trace elements in areas as diverse as environmental, chemical and industrial analysis. Of these instruments, AAS have been extensively used due to its availability, robustness and ease of operation. Many studies have involving hair metal analysis and other biological samples have used AAS (Nnorin *et al*., 2005; Rashed and Hossam 2007; Rita and Meenu, 2004; Sukamar and Subramanian, 1992; Shah *et al*., 2006; Adekola *et al*., 2004; Nowak *et al*., 2000, Samanta et al., 2004; Pinheiro et al., 2006; Rivai *et al*., 2001).

Similar instrument has been used extensively in Kenya for investigation of heavy metals in soil, water, food, biota and other biological samples (Hussein *et al*; 2008; Lalah *et al*., 2008; Makhokha et al., 2008; Ogola et al., 2002; Oyaro *et al*., 2007; Ochieng *et al*., 2008 Onyari *et al*., 1991).

2.4.1 Flame Absorption Spectroscopy (FAS)

Flame atomic absorption is a very common technique for detecting metals and metalloids in environmental samples. It is very reliable and simple to use. The technique is based on the principle that ground state metals absorb light at specific wavelengths. In this technique a liquid sample is aspirated and mixed as an aerosol with combustible gasses (acetylene and air or acetylene and nitrous oxide.) The mixture is ignited in a flame of temperature ranging from 2,100 to 2,800 degrees C (depending on the fuel gas used.). During combustion, atoms of the element of interest in the sample are reduced to the atomic state. A light beam from a lamp whose cathode is made of the element being determined is passed through the flame into a monochronometer and detector. Free, unexcited ground state atoms of the element absorb light at characteristic wavelengths; this reduction of the light energy at the analytical wavelength is a measure of the amount of the element in the sample.

2.4.2 Graphite Furnace- Atomic Absorption Spectroscopy (GFAA)

Graphite furnace atomic absorption spectrometry is a highly sensitive spectroscopic technique that provides excellent detection limits for measuring concentrations of metals in aqueous and solid samples. The advantages of GFAA spectrometry over other analytical methods include; its sensitivity and lower detection limits, low spectral interference the use of small sample size.

The graphite furnace is an electrothermal atomizer system that can produce temperatures as high as 3,000°C. The heated graphite furnace provides the thermal energy to break chemical bonds within the sample and produce free ground-state atoms. Ground-state atoms then are capable of absorbing energy, in the form of light, and are elevated to an excited state. The amount of light energy absorbed increases as the concentration of the selected element increases.

2.4.3 Hydride Generation Atomic Absorption Spectroscopy (HGAAS)

Atomic Absorption Spectrophotometry (AAS) is the commonest methodology for analysing for metals and some metalloids. But due to interferences, poor reproducibility, and poor detection limits an alternative method for metalloids like antimony, arsenic and selenium are routinely analysed by HGAAS. The main components of the instrument are identical to AAS; hollow cathode lamp, air /acetylene flame and optical system. The reaction of many metalloids with sodium borohydride and HCL produces a volatile hydride for examples reaction of arsenic with sodium borohydride produces H3As However, the main difference is the hydride generation system. The hydride generation system has the functions of:

i. aspiration of liquid sample and mixing of liquid sample with sodium borohydride and HCL

ii. creation of volatile hydride of the analyte metalloid from the reaction

iii. flow the gaseous hydride into the optical cell.

2.4.4 Atomic Emission Spectroscopy

Atomic emission spectroscopy (AES) is a method of chemical analysis that uses the intensity of light emitted from a flame, plasma, arc, or spark at a particular wavelength to determine the quantity of an element in a sample. The wavelength of the atomic spectral line gives the identity of the element while the intensity of the emitted light is proportional to the number of atoms of the element.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) uses an inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. Advantages of this method over other techniques are excellent limit of detection and linear dynamic range, multi-element capability, low chemical interference and a stable and reproducible signal. Disadvantages are spectral interferences (many emission lines), cost and operating expense and the fact that samples typically must be in solution.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Research Design

The sample consisted of children and adults who live in the major cities of Nairobi, Mombasa and Kisumu. The Study areas were chosen on basis levels of industrial activities and population density. Rural locations of Msambweni, Mosop-Kapsabet and Sondu constituted control.

3.2 Sampling Site

The sampling sites and the area of study are as shown in figure 1. The sites in the study areas were chosen based on anthropogenic activity profiles taking into consideration industrial and demographic influences. Nairobi is the capital city of Kenya and is situated along the geographical coordinate's 1° 16' 60" South and 36° 49' East. It is the most populous and industrialized city in Kenya with an estimated population of about 3.1 million people according to the 2009 census (KNBS, 2010).

Mombasa is the second largest city in Kenya, lying on the Coast of Indian Ocean. It is a major sea port and has an international airport. The city is the centre of the coastal tourism industry and has a population of about 900,000 people according to 2009 population census (KNBS, 2010). Mombasa city is situated along geographical coordinates 04^{o} 02'S and 39^{o} 43' E.

Kisumu is the third largest city in Kenya and is located at 00° 03'S and 34° 45'E and sits on Lake Victoria-the second largest fresh water lake in the World. The city has an estimated population of 600,000 people according to 2009 population census (KNBS, 2010).

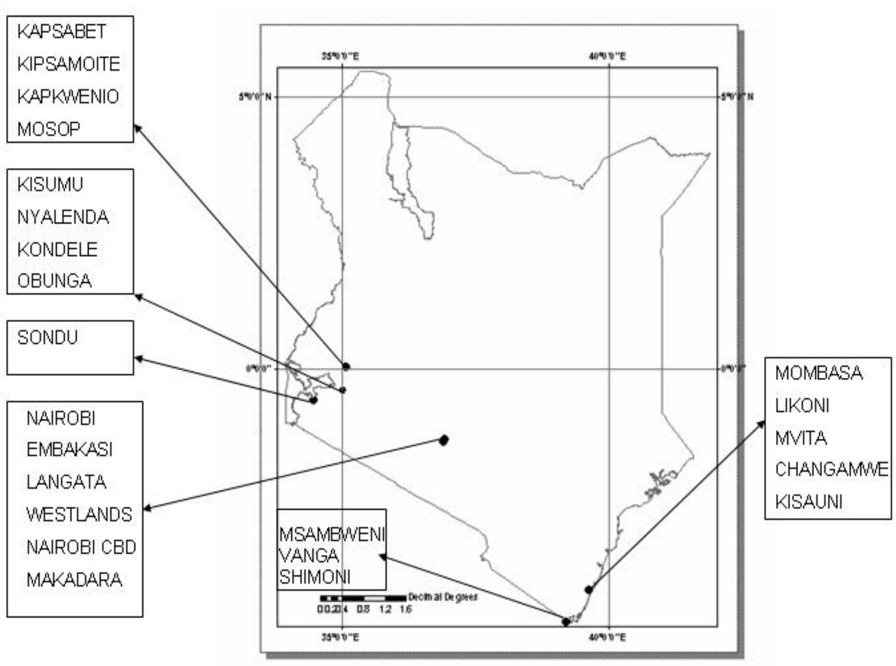


Figure 1. Map of Kenya showing the study areas.

3.3 Ethical Consideration

The Helsinki Protocols of 1996 which stipulate the appropriate ethical considerations for studies involving human samples were adhered to. The donors and volunteers were briefed on the objectives and the benefits of the study and given the assurance regarding confidentiality of their personal information.

3.4 Inclusion and Exclusion Criteria

Nairobi, Mombasa and Kisumu represent the major urban centres in Kenya with the largest vehicle density, industrial activities and population density (KNBS, 2010). This choice of the age was informed by the school going and the working ages of the sampled population.

3.5 Solvents, Reagents and Apparatus

Double distilled water was used throughout for the preparation of samples and dilution of the stock standard solutions. The glassware used in this study was decontaminated by overnight soaking in 5 % HNO_3 and thorough rinsing with deionised water. The water used was distilled and deionised. The reagents used in the analysis were of high quality analytical grade. Liquid soap, nitric acid AR, Acetone, and Perchloric acid were supplied by Sigma Aldrich. All glassware used in this study were soaked overnight in 5% nitric acid and rinsed thoroughly with deionised water before use. The heavy metal working standards were prepared from the stock solution (1000ppm) on a daily basis and checked for stability of absorption before taking the readings.

3.6 Sample Collection and Technique

In order to assess the contamination burden of adults and children in urban and rural Kenya, concentration of toxic metals Pb, Cd, Hg and some essential metals Zn, Cu and Mn were studied in a sample of 240 children and active adults aged 5 to 40 years and above 40 years. The samples were taken from people living in areas with different levels of pollution and industrialization.

Scalp hair was obtained with assistance of barbers and hair dressers located at different parts of Nairobi, Mombasa and Kisumu. Samples were also collected from the inhabitants of three rural areas of Kapsabet in North Rift, Msambweni in South Coast and Sondu in Southern Nyanza. The rural subjects were from areas where residents were engaged in agriculture related occupation. Hair that was bleached, dyed or chemically treated was not sampled. Only hair with natural colour and from individuals residing permanently in the study areas was considered for analysis. The information related to the donor's age, sex, feeding habits and area of residence was obtained orally and indicated on the sampling bags. The hair samples were randomly collected from males and females of different age groups (Table 1 and 2) ranging from:

i. Children aged between 5 - 14 years in both urban areas (Nairobi, Mombasa and Kisumu) and the rural areas.
ii. Adults of ages 15 - 20 years in both urban and rural areas.
iii. Adults between ages 21 - 40 years in both urban and rural areas.
iv. Adults of age > 40 years in both urban and rural areas

Table 1: Hair samples from urban areas of different age groups

Location	No. of Samples	Age in years	Gender
Nairobi	40	5 - 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)
Mombasa	40	5 - 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)
Kisumu	40	5 - 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)

Table 2: Hair samples from rural areas of different age groups

Location	No. of Samples	Age in years	Gender
Mosop-Kapsabet	40	5 – 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)
Msambweni	40	5 – 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)
Sondu	40	5 – 14	M (5), F(5)
		15 - 20	M (5), F(5)
		21 - 40	M (5), F(5)
		> 40	M (5), F(5)

M – Male, F – Female

3.7 Sample Preparation

Composite hair sample was washed with acetone for about 10 min. with continuous stirring, followed by rinsing with distilled water and then again with acetone. Distilled water and acetone were used in this study as washing liquids to remove the exogenous contaminants. Washed samples were then over dried at 60 ^{o}C for a period of 12 hr. As observed by Mikasa *et al.*, (1988) and Samanta *et al.,* (2004), this washing method does not result into loss of heavy metals.

Procedure for collecting and analyzing samples followed those used by Nowak and Kozlowski (Nowak, 1998; Nowak and Kozlowski, 1998)

3.8 Sample Treatment

500 mg pre-treated hair samples were taken in a 50-ml Erlenmeyer flask. 5 ml of concentrated nitric acid (69 %) was added at room temperature. The acid was allowed to react at room temperature to prevent foaming. The contents were heated to boiling point, followed by addition of 1 ml of perchloric acid (70 %). The contents were heated until dense white fumes appeared making the end of digestion process. The contents of the flask were then transferred to 50 ml volumetric flask and made to the mark with 2 % nitric acid. Reagent blank without hair samples was subjected to the same treatment as the samples in order to check for possible reagent contamination.

3.9 Analysis of hair samples using AAS Instrument

The AAS equipment was subjected to calibration and sensitivity tests before use. The samples and the blank were analysed for Cu, Zn, Cd, Pb Mn, and Hg using Buck210 model 210 VGP Atomic Absorption Spectrophotometer (AAS) located at the GOK Chemistry Laboratory (JKUAT) and Cold-Vapour Mercury Analyzer W/200A). The spectrophotometer was calibrated using a range of standards prepared from stock atomic absorption standards (Analytical Grade) and all the main instrumental parameters such as lump current, spectral band width and wavelength optimised for each metal as shown in table 3.

Table 3. Detection limit and absorption lines used for AAS analysis (Buck Model 210 VGP Atomic Absorption Spectrophotometer)

Metal	Spectral bandpass (nm)	Absorption Line (nm)	Detection limit (mg/kg)
Lead	0.7	217.0	1.00
Cadmium	0.7	228.8	0.10
Zinc	0.2	213.9	0.05
Copper	0.7	324.7	0.10
Manganese	0.7	279.8	0.50
Mercury	0.7	253.6	0.05

Triplicate subsamples were analysed separately in order to record average metal concentration with mean metal concentration lying within +_ 2 %. The blank was regularly interjected after every five measurements to ensure normal functioning of the AAS.

3.10 Statistical Analysis

The results obtained were analysed using Statistical Analysis Software System (SAS). The data were subjected to Analysis of Variance (ANOVA). Summary statistical analysis was also done to explore the data. To compare the metal concentrations between different groups of individuals, one-way Analysis of Variance (ANOVA) was conducted and statistical significance set at $P < 0.05$. Pearson's correlation coefficient analysis was also carried out among possible pairs of heavy metals in order to associate metals based on common origins.

The results of head hair heavy metal levels were presented as geometric means ± geometric standard deviations.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Validation of Results

Adequate quality assurance control was ensured by inter-laboratory comparisons of representative samples carried out at Jomo Kenyatta University of Agriculture and Technology and at SGS Kenya limited (Table 4). SGS Kenya limited is ISO 17025 accredited and also NEMA accredited for heavy metal analyses. For comparative check, parallel subsamples processed under similar analytical conditions were subjected to analysis at SGS laboratory.

One-way ANOVA done on comparative test data between two laboratories revealed no significance difference at < 0.05 probability level and the results were found to agree within acceptable margin of error as indicated in Table 4.

Table 4: Inter-laboratory comparison results between JKUAT and SGS Laboratories.

Metal	Minimum Detection Limit (ppm)	JKUAT Lab (mg/kg)	SGS Lab (mg/kg)
Pb	0.10	6.68 ± 1.35	7.10 ± 1.50
Cd	0.01	1.62 ± 0.105	1.81 ± 0.015
Zn	0.05	153.00 ± 10.84	156.00 ± 10.00
Cu	0.01	15.30 ± 0.90	13.25 ± 2.45
Mn	0.05	54.39 ± 10.96	65.50 ± 1.50
Hg	0.005	0.16 ± 0.024	0.20 ± 0.01

4.2 Comparison of Urban and Rural Metal Concentrations

This study was designed to determine heavy metal content in hair of residences from rural and urban areas of Kenya. The average heavy metal concentrations (mg/kg, dry weight) in the scalp hair of the two groups are summarised in Tables 5 and 6 which present urban and rural results respectively.

Table 5: Average metal contents of scalp hair of urban donors

Location	Age (Years)	Heavy Metals (mg/kg), N = 120					
		Pb	Cd	Cu	Mn	Zn	Hg
Nairobi	5-14	9.18 ± 3.45	1.31 ± 0.19	18.97 ± 3.91	12.42 ± 3.88	188.7 ± 9.20	0.24 ± 0.11
	15-20	12.55 ± 4.12	1.32 ± 0.14	13.46 ± 5.15	24.04 ± 5.70	187.0 ± 9.40	0.35 ± 0.18
	21-40	19.11± 3.73	1.46 ± 0.1 7	18.34 ± 3.44	25.36 ± 6.42	179.8 ± 8.90	0.68 ± 0.12
	>40	12.01± 2.99	2.38 ± 0.14	16.88 ± 3.62	29.25 ± 7.34	161.6 ± 7.50	0.39 ± 0.09
Mean		13.21 ± 3.57	1.59 ± 0.18	16.91 ± 4.03	27.77 ± 5.88	178 ± 8.50	0.42 ± 0.27
Mombasa	5-14	10.60 ± 3.13	1.10± 0.07	12.60 ± 3.42	22.32 ± 3.97	173.5 ± 8.1	0.36 ± 0.14
	15-20	10.93 ± 2.98	1.19± 0.08	14.73 ± 4.11	33.54± 8.62	199.0 ± 9.7	0.38± 0.13
	21-40	15.07 ± 3.83	1.83 ± 0.27	13.22 ± 3.55	27.53 ± 6.71	179.0 ± 7.7	0.60± 0.18
	>40	12.72 ± 4.93	2.17 ± 0.22	17.55 ± 4.76	28.87 ± 7.53	157.0 ± 8.4	0.27± 0.06
Mean		12.33 ± 3.72	1.57 ± 0.16	14.53 ± 3.96	26.07 ± 6.71	177.1 ± 8.5	0.40 ± 0.13
Kisumu	5-14	9.23 ± 3.64	1.12 ± 0.11	15.72 ± 4.44	53.42 ± 9.97	162.6 ± 7.3	0.18± 0.05
	15-20	11.77 ± 3.95	1.47 ± 0.14	14.78 ± 4.86	66.43 ± 10.6	148.7 ± 9.5	0.21± 0.13
	21-40	12.55 ± 3.18	1.88 ± 0.21	17.08 ± 3.56	55.63 ± 20.3	182.6 ± 10.8	0.57± 0.19
	>40	10.80± 2.97	2.01± 0.18	15.97 ± 4.52	53.08 ± 10.9	164.8 ± 9.2	0.11± 0.06
Mean		11.09 ± 3.45	1.62 ± 0.16	15.89 ± 4.35	57.14 ± 12.9	164.7 ± 9.20	0.27 ± 0.11

In the two categories of donors, Zn and Mn indicated the highest levels in head hair. Zn showed the maximum average levels of 187.1 mg/kg and 179.3 mg/kg, for rural and urban donors respectively while Mn peaked at an average level of 57.1 mg/kg and 24.9 mg/kg for both categories of donors respectively. The results indicate significantly higher levels of the Zn metal in head hair of urban areas than rural residents (Fig 2 and 3). With exception of Zn, the other trace essential elements (Cu and Mn) were significantly higher in the head of urban residents that their rural counterparts. Of all the urban residents, Kisumu donors had the highest head- hair Mn concentration. This seem to correlate well with the study by Lalah *et al.*, (2008) that found elevated levels of Mn in the rivers Kisat, Nyando and Nyamasaria which drain into Winam Gulf basin of lake Victoria-the main source of drinking water for Kisumu and its environs. The study indicated that the three rivers had their waters surpassing the WHO maximum permissible limits for drinking water with respect to Mn concentration. This can be attributed to the industrial discharges into the lake- the city has not been having a functioning effluent treatment for sometime.

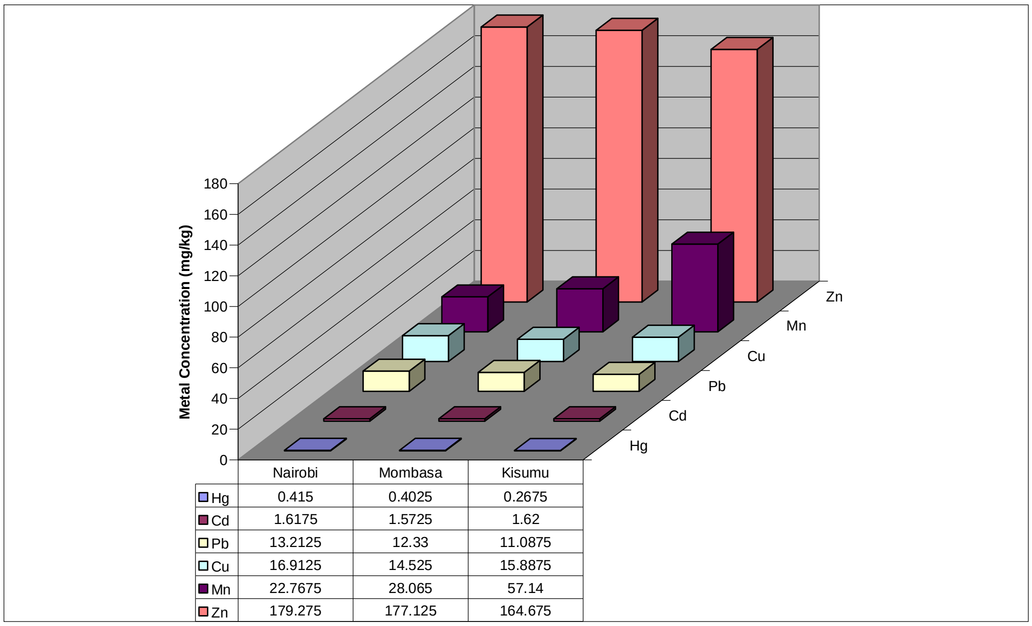
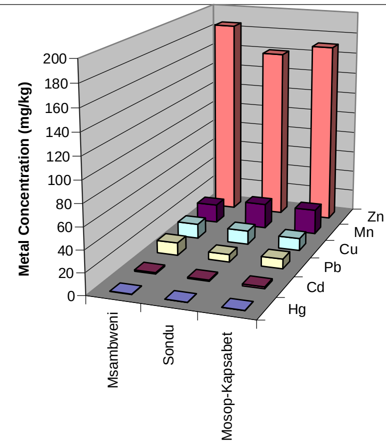


Figure 2. Average metal concentrations in urban location

Table 6: Average metal contents of scalp hair of rural donors

Location	Age (Years)	Heavy Metals (mg/kg), N = 120					
		Pb	Cd	Cu	Mn	Zn	Hg
Msambweni	5-14	8.48±2.55	1.08±0.15	9.21±2.91	11.02±3.93	183.7±9.2	0.19±0.08
	15-20	10.11±3.12	1.15±0.11	11.46±3.72	24.04±5.7	219.0±13.2	0.29±0.08
	21-40	14.12±4.55	1.42±0.25	18.34±3.44	18.22±5.53	201.5±12.4	0.27±0.04
	>40	14.44±4.82	1.65±0.25	15.30±4.33	16.20±4.41	138.18±7.2	0.44±0.10
Mean		11.79±3.76	1.33±0.19	13.58±3.60	17.37±4.89	185.60±10.5	0.30±0.08
Sondu	5-14	4.23±1.45	1.07±0.09	9.88±3.34	27.10±5.9	172.7±11.5	0.12±0.04
	15-20	7.78±1.73	1.09±0.08	11.23±2.51	16.25±3.9	158.8±6.9	0.16±0.06
	21-40	7.73±2.32	1.12±0.14	12.64±3.13	27.53±6.7	162.0±5.6	0.19±0.08
	>40	7.97±2.54	1.53±0.27	17.55±4.76	22.87±7.5	149±3.7	0.18±0.06
Mean		6.93±2.01	1.20±0.15	12.83±3.44	23.47±6.00	160.63±6.93	0.16±0.06
Mosop/	5-14	8.78±2.88	1.17±0.22	10.66±2.88	13.55±3.3	151.7±9.8	0.14±0.05
Kapsabet	15-20	8.57±1.95	1.73±0.23	10.87±2.38	12.23±3.6	196.0±12.8	0.12±0.09
	21-40	8.93±1.99	1.83±0.37	11.77±3.12	13.82±3.1	175.5±9.6	0.16±0.07
	>40	9.88±1.88	2.18±0.39	12.55±3.43	53.08±10.9	159.0±9.3	0.18±0.06
Mean		9.04±2.18	1.73±0.30	11.46±2.95	23.17±5.23	170.55±10.38	0.15±0.07



	Msambweni	Sondu	Mosop-Kapsabet
Hg	0.2975	0.1625	0.15
Cd	1.325	1.2025	1.7275
Pb	11.7875	6.9275	9.04
Cu	13.5775	12.825	11.4625
Mn	17.37	23.4375	23.17
Zn	185.595	160.625	170.55

Figure 3. Average metal concentration in rural locations donors.

4.3 Order of Heavy Metal Concentration

Amongst the heavy metals (Cd, Pb and Hg) analysed, Lead emerged with the highest levels picking at 19.11 mg/kg and 14.44 mg/kg in both urban and rural samples respectively. Of all the heavy metals, Nairobi had the highest average levels (13.2 mg/kg Pb; 1.59 mg/kg Cd; 0.36 mg/kg Hg) followed by Mombasa (11.8 mg/kg Pb; 1.55 mg/kg Cd; 0.36 mg/kg Hg) and Kisumu (10.6 mg/kg Pb; 1.62 mg/kg Cd; 0.27 mg/kg Hg) in that order. This is understandable due to the fact that Nairobi is the largest industrial city of Kenya as compared to Mombasa and Kisumu. Nairobi residents are more exposed to heavy metal pollution with the possible routes of exposure being the vehicular emissions of lead and cadmium. Industrial activity also contributes to the exposure to metals pollutants like zinc, copper and manganese from steel industries, scrap metal smelting factories that are so common in Nairobi Metropolis. The overall order of increase in mean concentration for metals for both urban and rural areas followed the order of Hg < Cd < Pb < Cu < Mn < Zn. However, there was generally higher metal concentration among urban donors as compared to their rural counterparts.

With exception of Cd, the metal concentration was highest in Msambweni followed by Kapsabet and finally Sondu. This may be attributed to the close proximity Msambweni to Mombasa, the second largest industrialised city in Kenya. Among the industries in Mombasa is a petroleum refinery, Cement plants, steel factories, scrap metal recycling factories which are possible sources of metal pollution. Some studies have revealed heavy metal contamination along the Kilindini and Makupa Creeks (Kamau, 2001).

One interesting observation is the significantly high average levels of Cd among Mosop-Kapsabet inhabitants. This could be attributed to the fact that Kapsabet area is an agricultural and livestock active zone with wide range of fertilizer application on especially tea plantations with possibility of accumulation of Cd in the soil- plant system. Extensive use of fertilizers especially phosphatic containing Cd impurities has been reported to result into broad-scale, relatively low level contamination in agricultural soils (Mc Laughlin *et al.*, 1996; Merrington *et al.*, 2001). The persistent nature of Cd in the soil environment and its potential for bio-availability and accumulation in food crops and livestock present potential possible pathway and route of exposure in the study area. Kenya Bureau of Standard specifies heavy metal contamination (Cd) content of DAP and MAP fertilizers of 7 ppm maximum (KS 157: 157 - 2007).

4.4 Heavy Metals Concentration as Per Age Group

The data of heavy metal content according to the age groups in both urban and rural areas (Figs. 4 and 5) shows that the highest of Pb, Cd, Cu, Mn and Hg content in hair were found in groups of adults.

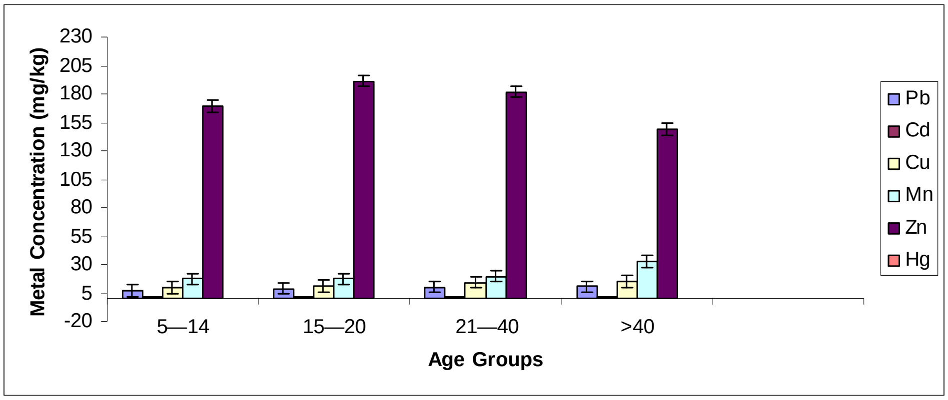


Figure 4. Relationship between age groups and hair metal concentration in rural donors

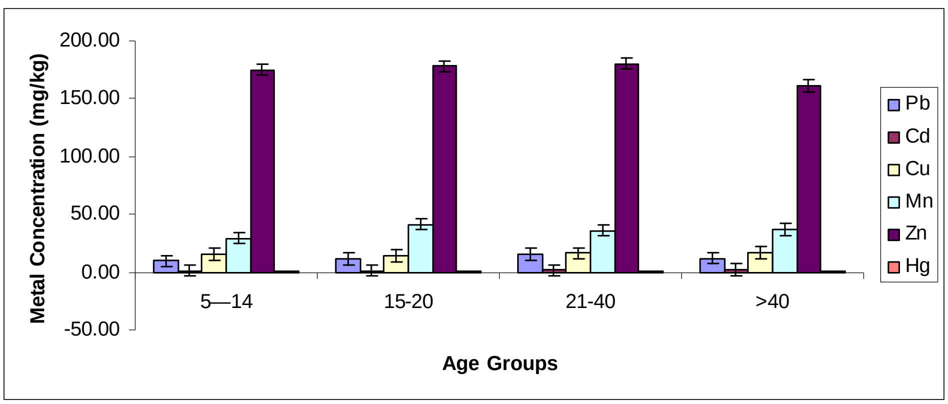


Figure 5. Relationship between age groups and hair metal concentration in urban donors

This was particularly so among the age group of 2 l - 40 years with mean hair metal contents; (Pb, 19.1 ± 3.73 mg/kg, Cd, 1 .40 ± 0. 17 and Hg, 0.68 ± 0.12 mg/kg); (Pb, 15.07 ± 3.83mg/kg, Cd ,1.83 ± 0.27 mg/kg, Hg, 0.6 ± 0.I8 mg/kg); (Pb, 12.55 ± 3.18 mg/kg, Cd, 1.88 ± 0.21 mg/kg, Hg, 0.5 ± 0.19) for Nairobi, Mombasa and Kisumu respectively. Adekola *et al.,* (2004) made similar observation with Pb in cities of Ibadan and form in Nigeria. This can be attributed to the fact this age group is the most active and contributes most to the labour force in the cities of Kenya hence, have higher risk of occupational exposure to heavy metal pollution. Similar results had been earlier reported by Ashraf *et al.,* (1995). The data also shows an influence of age on metal concentration in both rural and urban inhabitants (Fig. 4 and 5). With exception of Zn, heavy metals such as Pb, Cd and Hg contents increase with age which points out the bio-accumulative properties of the heavy metals. The lowest Zn content in hair was found in group of adults of age above 40 years and seemed to decrease with age in both categories of donors. Figures 4 and 5 indicate that Zn content decrease with age. The reduction of Zn levels in adult's hair could be attributed to the use of zinc for the production of the melanin pigment in hair (Adekola *et al.*, 2004). Comparing the values of Zn in similar age groups metal in both urban and rural residents reveals that the metal is significantly higher in the urban population than their rural counterparts. This could be attributed to the fact that urban dwellers are more exposed to occupational zinc pollution than the rural counterparts. This is particularly so with those who work with galvanized metals in the informal sector (in the Jua Kali sector).

4.5 Interaction of Age of Donors and Metal Concentrations.

The levels of trace metals Pb and Cd were observed to increase with age of both urban and rural donors with Zn concentrations showing marginal decreasing trend with age which could be associated with ageing process. Similar trend was observed among Pakistan donors (Khalique *et al.,* 2005) and in the USA (Paschal *et al.,* 1989). The amount of Pb and Cd were found to accumulate in hair of elderly donors among both rural and urban residence. Age seemed to have had an influence on the trace elements Pb and Cd, except for Hg that indicated random occurrence. As shown in Figures 4 and 5 there was an inverse relationship between Pb and Zn among various age groups suggesting possible antagonism between Pb and Zn in human body. The relationship seemed to be in agreement with observation earlier made in study by Meng (1998) with regard to behaviour of the two elements. Influence of gender on head hair metal concentration was insignificant (at $P < 0.405$) in both categories of Kenyan inhabitants (Appendix 1).

4.6 Analysis of Variance (ANOVA)

The analysis of variance of means of hair metal concentrations and their interactions show different levels of significance among the donors, age groups and locations (Appendix 1). The mean separation results are as presented in Appendices 2 and 3.

The mean value for heavy metals Pb, Cd and Hg in the hair was significantly higher (one-way ANOVA, $p < 0.0001$) in the urban group compared to their rural counterparts. Nairobi (Pb 13.21 mg/kg, Cd 1.59 mg/kg, Hg 0.42 mg/kg); Mombasa (Pb 12.33 mg/kg, Cd 1.57 mg/kg, Hg 0.40 mg/kg) and Kisumu (Pb 11.09 mg/kg, Cd 1.62 mg/kg, Hg 0.27 mg/kg) versus rural areas of Msambweni (Pb 11.78, Cd 1.33, Hg 0.30 mg/kg); Mosop-Kapsabet (Pb 9.04 mg/kg, Cd 1.73 mg/kg, Hg 0.15 mg/kg) and Sondu (Pb 6.93 mg/kg, Cd 1.20 mg/kg, Hg 0.16 mg/kg). Essential elements (Mn, Cu and Zn) were found to be randomly distributed in hair of both urban and rural donors. Among the cities sampled, Nairobi had the highest level of hair heavy metals followed by Mombasa and Kisumu in that order. As indicated above, this is can be attributable to the fact that Nairobi is the largest city in Kenya and is the most industrialized with high motor vehicle density and population of about 4 million people as compared to Mombasa and Kisumu. Of the urban locations, Nairobi is considered the most impacted by heavy metal pollution and this is shown by the presence of higher levels of Pb, Cd and Hg. Possible sources may be as a result of contamination due to industrial wastes and air pollution resulting from high traffic. Another possible pathway would be through food chain as indicated by Oyaro *et al.*, (2007) that reported high levels of Pb and Cd in meat sampled in Nairobi.

Comparing these data and those earlier reported in Kenya by Wandiga and Jumba (1982) hair (Pb,52 mg/kg); Cd, (2.22 mg/kg); Cu, (24 mg/kg); Hg, (12.20 mg/kg); Zn, (196 mg/kg) (Table 11), this study finding shows significant reduction in heavy metal levels, particularly with respect to Pb and Hg. This could be attributed, in the case of Pb to the fact that use of leaded gasoline has since been banned in Kenya while reduction in Hg could be due to the sampling approach adopted in the earlier study. The level of other metals such as Cu and Zn are also comparatively lower than the earlier reported values (Wandiga and Jumba, 1982). The latest study of heavy metal contamination in Kenya was undertaken using nails as biomarker (Hussein *et al.* (2008).

4.7 Comparison of metal concentration with Other Worldwide Studies

Comparison of this data with other Worldwide studies on similar metals (Table 7) indicated mixed information. Cd levels in hair from the present study, though lower than those earlier reported for Kenya are higher than those reported in the literature and other regions of the World while Hg is the lowest among the selected countries. Heavy metals such as Pb and Cd levels in hair from this study were higher than in people's hairs from Aswan in Egypt with Pb of 5.95 mg/kg and Cd of 0.44 mg/kg) (Rashed and Hossam, 2007), Bandarlampung City, Indonesia (Pb 3.14 mg/kg and 0.43 mg/kg) (Rivai, 2001). Indian hair shows Cd and Pb levels of 0.40 mg/kg, Cd 8.03 mg/kg (Samanta *et al.*, 2004) while a study by Shah *et al*., (2006) on head hair metal content of people of Islamabad, Pakistan revealed metal levels of Pb, (15.97 mg/kg); Cd, (0.38 mg/kg); Zn, (226.1 mg/kg) and Mn, (1.93 mg/kg) (Shah *et al*., 2006). Libyan hair contains Pb, Cd. Zn and Mn in concentration of 24.95, 0.530, 190.3 and 1.73 mg/kg respectively (Shah *et al*., 2006). In a comparative study of age and location dependence on metal concentration of Pb, Zn Cu conducted in two Nigerian cities of Ibadan and Ilorin (Adekola *et al.*, 2004) reported head hair contents of Pb, (57 - 67 mg/kg); Zn, (32 -914 mg/kg) and Cu, (18 - 19 mg/kg) that are comparatively much higher than the values of this study. The high levels of the three metals were attributed to vehicular pollution in the two Nigerian cities.

The highest level head hair mercury (2.23 – 16.1 mg/kg Hg) was recorded in the children of Amazon Riverside Villages in Brazil that was associated with fish eating (Pinheiro *et al.*, 2006) that is about ten to seventy times the levels of our current study (0.24 mg / kg Hg).

Table 7: Comparison of hair metal levels (mg/kg, dry weight) from the present study with those reported for other regions of the world

Location	Pb	Cd	Zn	Cu	Mn	Hg
Nairobi, Kenya[1]	13.52	1.59	188.7	15.12	27.19	0.24
Kenya[2]	52	2.22	196	24	-	12.2
Islamabad, Pakistan[3]	15.97	0.38	226.1	-	1.93	-
Tripoli, Libya[3]	24.95	0.53	190.3	-	1.73	
India[4]	8.03	0.4	152	14.7	-	-
Ibadan, Nigeria[5]	57-67	-	532- 914	18-19	-	-
Aswan, Egypt[6]	5.95	0.44	172	10.6	-	-
Poland[7]	4.8	0.55	124	7.2	-	-
Indonesia[8]	3.14	0.43	33	30.6		
Portugal[9]		0.17-1.52	141	7.7-27.2	1.2-11.2	-
Amazon Riverside, Brazil[10]	-	-	-	-	-	2.23-16.1
Banglades[11]	-	-	-	-	-	0.02-95

[1]Present study; [2] Other study from Kenya; [3]Shah *et al*., (2006); [4]Samanta *et al*., (2004); [5]Adekola *et al*; (2004); [6]Rashed and Hossam, (2005); [7]Nowak *et al.*, (2000); [8]Rivai , (2001); [9]Pereira *et al*., (2002); [10]Pinheiro *et al*, (2006); [11]Holsbeek *et al*, (1996)

As compared with the earlier study in Kenya, the values obtained in this study with those earlier published by Wandiga and Jumba (1982) (Pb, 52 mg/kg; Cd, 2.22 mg/kg; Cu 24 mg/kg; Hg 12.2mg/kg) the study shows lower concentration in all the metals with exception of Zn values that seem to be in the same ranges. This can be possibly attributed to the fact that a lot of developments particularly with regard to phasing out of leaded motor gasoline (major source of lead in air) have taken place since the earlier study was conducted more than two decades ago. More so, there was slight difference in approach on the methodology as in the earlier study, hair samples were collected regardless of whether it was treated or not as opposed to the present study where only natural hair was sampled.

Comparison of the data in the present study of heavy metals Pb, Cd, Zn, Cu, Mn, Hg with those that have been reported in the literature and other parts of the world indicates lower values except for Cd and Mn where elevated levels are observed. In Libya, human hair contains levels of Cd**,** Mn and Pb, 0.53 mg/kg, 1.73 mg/kg and 24.95 mg/kg respectively with leaded gasoline fuel still in use in Libya (Shah *et al.,* 2006). In Poland, head hair contains Cd and Mn levels of about 0.55 mg/kg and 2.5 mg/kg respectively (Nowak and Chmielnicka, 2000). Other locations that have recorded lower Cd concentration than Kenya include; Vienna, (Austria) (Cd, 0.04 mg/kg) and Rome, (Italy) (Cd, 0.05 mg/kg (Wolfsperger *et al.,* (1994); India with Cd 0.40 mg/kg (Samanta *et al.,* 2004); Indonesia with Cd, 0.43 mg/kg (Rivai, 2000**).**

4.8 Linear Metal - Metal Correlation Analysis

A linear metal-metal correlation analysis was done to find out whether there is any relationship among various metal pairs (Table 8).

Table 8: Correlation coefficient matrix for metal-to- metal in scalp hair of the donors

	Cd	Cu	Pb	Mn	Hg	Zn
Cd	1.00000					
	0.79588					
Cu	<.0001	1.00000				
	0.95897	**0.76294**				
Pb	<.0001	<.0001	1.00000			
	0.69942	**0.54428**	**0.75878**			
Mn	<.0001	<.0001	<.0001	1.00000		
	0.52157	**0.42156**	**0.46090**	0.26401		
Hg	0.0002	0.0035	0.00130	0.07620	1.00000	
Zn	-0.2570	0.2862	0.27584	0.28740	0.66890	1.00000

Bold values are significant at $p < 0.05$

Strong positive correlations were found to exist between Pb - Cd (r = 0.959), Cu - Cd (r = 0.796), Pb - Cu (r = 0.763), Mn - Pb (r = 0.759), Mn - Cd (r = 0.699), Zn - Hg (r = 0.670), Mn – Cu (r = 0.544) and Hg – Cd (r = 0.522). In addition, some significant correlations were also observed between metal pairs, Hg - Cu (r = 0.422) and Hg – Pb (r = 0.461). Some literature indicates the tendency of metals to increase or decrease in hair tissue as a result of metabolic or physiological factors that are responsible for distribution of metals among different individuals (Ashraf *et al.*, 1995; Nowak and Chmielnicka., 2000). Hence the hair metal concentrations were grouped in pairs in order to find any possible correlation.

Table 11 shows cases where these correlations are statistically significant. The results indicate that despite variable environmental exposure, there is a statistically significant high positive correlation (r = 0.544 - 0.959, $p < 0.0001$) between Cd, Pb, Cu and Mn in the hair of the Kenyan inhabitants, which suggest a common source of these particular metals. A similar Pb - Cd relationship in hair was also reported by Zachwieja *et al.*, (1995) and D'souza *et al.*, (2003). A weak negative correlation Zn and other metals, particularly with Pb / Zn (r = -0.257) seems to reflect a well known physiological antagonism of the two elements. As has been observed by researchers, cadmium has been found to compete for gastrointestinal absorption with zinc and interferes with the metabolism of the same element (Peraza *et al.*, 1998; Nowak and Chmielnicka, 2000). Similar interaction has also been recorded with Pb – Zn pair. Mercury seems to interact mainly with selenium (D'souza *et al.*, 2003) with no interaction recorded with the metals under this study. It should also be emphasized that interactions between toxic and essential metals can result in disturbances of processes regulated by these same elements which can also lead to a number of pathological states (Peraza *et al.*, 1998). However, there is no readily available explanation how element content changes in hair tissue with various internal factors. Presumably, environmental contaminants through synergy and antagonism with bio-elements may affect metabolic pathways that results in altered concentration of metals in some body tissues (Nowak and Chmielnicka, 2000).

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the present investigation shows location and age dependence for trace metals in Kenyan population. Higher concentrations of heavy metals were obtained in urban centers and in different age groups indicating individual metabolic activity, industrial activity, occupational exposure and geographical location of the donors as influencing factors in metal concentration. Head hair accumulated metal concentration in the following order of Zn > Mn > Cu > Pb > Cd > Hg. Age and location strongly influenced metal concentration. No significant relationship was found between gender and metal concentration in Kenya.

This study has further demonstrated the use of human hair as a biomarker in determination of different levels of heavy metal exposure of communities with varied environmental backgrounds. The presence of the selected and studied heavy metals in human hair depends on environmental exposure as demonstrated by significantly higher concentrations of the metals in the largest Kenyan cities as compared to the relatively less industrialized agricultural rural areas.

Comparing the obtained result with the literature data reveal that Kenyan head hair metal concentration are among the highest when compared to the available data from various regions of the World, particularly with respect to Cd (1.59 mg/kg) and Mn (27.1 mg/kg). With exception of Nigeria, Libya and Pakistan, Kenya had higher hair concentration of Pb (13.52 mg/kg) than the selected countries of the World. Results obtained from this study will assist in the development of preventive strategies and direct government actions to face the problem of heavy metal contamination in urban areas of Kenya.

5.1 Recommendations.

i. From the results obtained, we recommend that farther investigation be done in Kapsabet- Mosop area with view of finding out the possible sources of high Cd occurrence among residents of this area. As a first step, further additions of Cd through fertilizers need to be regulated for its sustainable management with consideration given to using of fertilizers that are low in Cd and avoiding overuse of phosphorus pentoxide based fertilizers.

ii. The study reveals that lead is still prevalent in the Kenyan. It is therefore recommended that further study be conducted to appraise the impact of the administrative policies and the government efforts hitherto put in place to limit lead pollution, for example use of unleaded motor gasoline.

iii. It is recommended that further studies be done to evaluate the levels of other heavy metals and metalloids like arsenic, selenium, nickel, chromium and vanadium that were covered in the present study.

iv. Similar study should be conducted for other industrial towns like Athi River, Thika and Nakuru.

v. Further, the data obtained from this study suggest that human hair can be used for identification and monitoring of exposure to heavy metals in Kenya.

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APPENDICES

Appendix 1: Means of hair metal concentration at p < 0.05

Source of Variance	DF	Sum of Squares	Mean Square	F Value	Pr >F
Model	67	1144644.39	17084.24	57.42	<0.0001
Location	5	3593.09	718.62	2.42	0.0371
Gender	1	285.21	285.22	0.93	0.3373
Age group	3	3002.35	1000.78	3.36	0.0195
Metal	5	980734.93	196146.99	659.22	< 0.0001
Location metal	23	18268.01	794.26	2.67	< 0.0001
Location age	15	3134.63	208.98	0.68	0.08046
Location gender	5	1143.37	228.67	0.74	0.5930
Gender metal	5	1577.61	315.52	1.02	0.4049
Age metal	15	11462.92	764.19	2.57	0.0015

Appendix 2: Means Separation – age and metal interaction

Age of donors	Metal	LSMEAN	Error	Pr
21 - 40	Zn	181.2121	4.559832	<.0001
15 - 20	Zn	175.9513	4.655051	<.0001
5 - 14	Zn	168.3925	4.812415	<.0001
> 40	Zn	138.2085	5.712725	<.0001
21 - 40	Cu	15.08931	4.386587	0.0007
15 - 20	Pb	10.62662	3.036669	0.036

Appendix 3: Means separation –location and metal interaction

Location	Metal	LSMEAN	Error	Pr
Sondu	Mn	23.86194	4.175609	0.001
Sondu	Zn	152.008	5.786632	<.0001
Kapsabet	Zn	164.1098	5.784197	<.0001
Mombasa	Cu	17.8654	3.393187	0.00057
Mombasa	Zn	177.7339	5.891696	<.0001
Nairobi	Cu	14.58537	3.590919	0.0279
Nairobi	Pb	13.64719	3.583163	0.0393
Nairobi	Mn	28.93691	4.757286	<.0001
Nairobi	Zn	186.9888	5.782385	<.0001
Msambweni	Mn	20.9807	4.7552911	0.0021
Msambweni	Zn	174.353	5.782385	<.0001
Kisumu	Pb	11.9371	2.978844	0.0471
Kisumu	Mn	55.49183	5.795183	<.0001
Kisumu	Zn	140.4529	6.979696	<.0001