DETERMINATION OF CADMIUM, CHROMIUM, LEAD AND MERCURY IN HONEY SAMPLES FROM MBEERE, MERU, AND KIRINYAGA DISTRICTS

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DECLARATION

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I declare that this thesis is my original work and has not yet been presented for a degree in any other university.

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DEDICATION

This work is dedicated to my family, not forgetting K.G.M. who all saw me through the good and tough times of this course.

ABSTRACT

This study focuses on quantitative analysis of toxic heavy metals; cadmium, chromium, lead and mercury in unrefined and refined honey, honeycombs, bees, flowers, soils and water samples from selected districts in Kenya; namely Mbeere, Meru and Kirinyaga. The work is based on the use of atomic absorption spectrophotometric technique. The mean concentrations of the metals in honey have been compared to their acceptable concentration limits of cadmium (0.008 to 0.010 ppm), chromium, (less than 1 ppm), lead (0.02 to 0.6 ppm), and mercury (less than 0.001 ppm) proposed by World Health Organization (WHO), Food Agricultural Organization (FAO), and Kenya Bureau of Standards (KBS). The mean concentration levels of cadmium, chromium, lead and mercury in all 60 unrefined honey samples from the three districts showed that, while the concentration of chromium and cadmium in all honey samples fall within the acceptable levels, those of lead in some areas were slightly higher than the WHO, FAO and KBS recommendations. This is particularly so for honey samples from Mbeere and Meru districts where the highest concentrations of lead in Mbeere unrefined honey collected from Ishiara approximately 0.5 km from a major road was 1.39 ppm while unrefined honey collected from Kangoro in Meru had 0.73 ppm of lead. With cadmium, the concentration was above the WHO, FAO and KBS limit in all districts.

Refined honey samples from USA and Kenya bought from Kenyan Supermarkets were also analyzed and found to contain 0.022 ppm, 0.027 ppm, 0.45 ppm and 0.8 ppb of cadmium, chromium, lead and mercury respectively for the USA honey; while refined honey from Kenya contained 0.018 ppm of chromium, 0.032 ppm of cadmium, 0.10 ppm of lead and 0.5 ppb of mercury.

The two commonly used digestion methods; the wet oxidation and dry ashing oxidation methods were used to digest the samples. Comparison of the effectiveness of the two methods was performed by subjecting them to *t*-tests at 95 % confidence level. The *t*-critical which was found to be 2.06 was compared to the *t*-calculated for chromium, lead and cadmium which were 3.22, 10.53 and 11.5 respectively. These values were higher than *t*-critical values suggesting that the two digestion methods gave significantly different results.

The effect of cation interferences on the determination of lead, cadmium and chromium showed that the presence of Cu^{2+}, Mn^{2+}, Ni^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+} and Na^{+} at concentration levels below 4 ppm had no significant effect on the concentration levels of the heavy metals. However, it was observed that concentration higher than 4 ppm of interfering cations enhanced or depressed concentration levels of the heavy metals.

Good recoveries ranging from 95.5%—100.4 % for lead, 92.5%—101.5 % for chromium, and 98 %—100.5 % for cadmium, were obtained from unrefined honey samples.

Correlation studies of cadmium, chromium and lead concentrations in honey, honeycombs, bees, flowers and soil samples were performed using excel software. Results indicate that chromium showed positive correlation between the soil and the flower samples, the honey and the bee samples and between the honeycombs and the flower samples. For cadmium, the significant correlation was observed between the soil and the flower samples. For lead, positive correlations were observed between all the samples.

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SYMBOLS AND ABBREVIATIONS

AAS:	Atomic Absorption Spectrometry
CTA 2000:	Chem Tech Analytical Atomic Absorption Spectrophotometer
FAO:	Food and Agricultural Organization
WHO:	World Health Organization
KBS:	Kenya Bureau of Standards
Pb:	Lead
Cr:	Chromium
Cd:	Cadmium
Hg:	Mercury
*u*g:	Microgram
ng:	Nanogram
nm:	Nanometers
ppm:	Parts per million
ppb:	Parts per billion
Stdev:	Standard deviation
C.V.:	Coefficient of variation
R_{xy}:	Correlation coefficient
cm^3:	cubic centimeters
dm^3:	cubic decimeters
mA:	milliamps

CHAPTER ONE

INTRODUCTION

1.0 GENERAL INTRODUCTION

Heavy metal pollution of the environment has in the recent past captured the attention of many researchers. Studies have shown that the presence of heavy metals in the environment and in consumable foodstuffs pose harmful effects to humans and animals (Goyer and Mehlmain, 1976). As a result of this, there is need to monitor concentration levels of heavy metals more closely since they are being added to soil, water and air in increasing amounts through accelerated agricultural and industrial activities. Toxic heavy metals pose more serious problems than pollution by pesticides, sulfur dioxide, nitrogen oxides, carbon oxides and other known contaminants since no metal is degradable (Calvin and Monroe., 1972: UNEP, 1980). However, analysis of pesticide pollution should not be ignored since certain pesticides have been known to wipe out whole populations of honey producing bees (Anderson and Glowa, 1984).

The major cause of environmental pollution in Kenya is mainly due to a large variety of industrial effluents. Since Kenya is endeavoring to be industrialized by the year 2020, technological advances are being implemented which have lead to an increase in the use of heavy metals such as lead, copper, cadmium, etc in the industrial sector. Once the heavy metals enter the environment, bio-degradation does not occur. Over time they accumulate and pollute the environment by interfering with the

photosynthetic balance and the stability of the ecosystem (Mertz and Underwood, 1987).

Heavy metals in the ecosystem mostly find adsorption sites in the soil where they are retained very strongly either on the organic or inorganic colloids. Some are less strongly adsorbed and thus have a higher likelihood of entering the food chain. The main processes involved in the movement of the heavy metals from the soil into the food chain via the plants roots are: (a) mass flow with the soil solution as it moves to and is absorbed by the roots, and (b) diffusion through the soil solution down the concentration gradient created by the absorption of the heavy metals by the roots (Greenland and Hayes, 1981).

In general, the elemental composition of the plant will reflect the composition of the soil or any other growing medium. However, the concentrations of the elements in the plant will not necessarily be the same as those in the soil or in the nutrient solution. Thus plants, to some degree, are selective in their absorption of elements (Cannon *et. al.*, 1972). The uptake of the heavy metals from the soil to the plants is determined by several factors such as:

a) The abundance of the metals in the lithosphere
b) The form of the element (oxidation states, inorganic or organic forms)
c) The pH of the soil
d) The physical conditions of the soil (temperature, moisture content)
e) The genetic constitution of the plant species

Since plants provide the main source of minerals to animals and to most human beings, the factors influencing the trace element content of the plant also determine the dietary intakes of these elements.

Most of the heavy metals reach humans through water and food chains (Mastinu & Clemente, 1974). Thus, honey which is a sweetener and high energy yielding food can act as an agent for heavy metal accumulation in human beings and animals (Jones, 1987). Honey, which is manufactured by honey bees (*Apis mellifera*) is mainly composed of fructose, glucose, and water in varying proportions (table 1.0); it also contains several enzymes, such as invertase which converts sucrose to fructose and glucose, and lysozyme, which has both a bacteriostatic and a lytic effect on most Gram positive bacteria in honeys (Gojmerac, 1980; Frazer and Weshoff, 1978).

Table 1.0: Composition of Honey

Principal Components	**Percent**
Water (Natural moisture)	17.20
Levulose (d-fructose and first sugars)	38.19
Dextrose (d-glucose and grape sugars)	31.28
Sucrose	1.31
Maltose and other reducing dissaccharides	7.31
High sugars	1.50
Total Sugars	**79.59**
Acids	0.57
Proteins	0.26
Ash	0.17
Subtotal	**97.79**
Minor constituents	2.21
Total	**100.00**

Source: Adapted from White, Riethof and Subers, 1962

The age of the honey and the source of the nectar greatly influence the color and the flavor of the honey. It is important to note that light-colored honeys are usually of higher quality than darker honeys (Encarta , 1997a).

The contamination of honey can occur through the following modes: (a) fumes from burnt leaded petrol, (b) agricultural materials such as fertilizers, pesticides, insecticides, etc. (c) mining and smelting of non-ferrous metals, (d) metallurgical industries, and, (e) urban and industrial waste products (Alloway, 1990). Once honey is contaminated and humans ingest it, trace amounts of heavy metals and other contaminants can then accumulate in the body organs and tissues.

The heavy metals investigated in this study are cadmium, chromium, lead and mercury. Among the most dangerous are the mercuric compounds which, have a high lipid solubility. These can easily penetrate and accumulate in human and animal tissues where they block the mechanism for oxygen supply (Boldt and Brugenwert, 1978). Chronic mercuric poisoning, which occurs when small amounts of the metal or its fat-soluble salts, particularly methyl mercury, are repeatedly ingested over long periods of time causes irreversible brain, liver and kidney damage. The mercuric vapor is also extremely dangerous, but the ingestion of metallic mercury is not as toxic since most of it passes through the human system unchanged (Encarta, 1997 b). Chronic poisoning from other heavy metals such as cadmium, chromium and lead can cause serious health complications such as: high blood pressure caused by cadmium, paralysis and heart weakness caused by lead and lung cancer caused by chromium.

1.1 JUSTIFICATION OF STUDY

According to the National Development Plan, the demand for honey in Kenya is expected to be more than double from 30,000 kg in 1992 to 65,000 kg by this year 2000 (National Development Plan 1997-2001). Since honey is used as medicine for some ailments, as a sweetener for beverages, as a bread-spread, as a moistening agent for tobacco manufacture, as a preservative for meat and in the manufacture of cosmetics, it is essential to monitor the concentration of toxic heavy metals in honey and compare their levels with those recommended by the Food and Agricultural Organization (FAO), the World Health Organization (WHO), and the Kenya Bureau of Standards (KBS). The recommended ranges for the metals of interest in this study are Cadmium 0.001 ppm, (μg/kg of body weight per day), Chromium less than one ppm, i.e. < 1 ppm, Lead 0.02—0.6 ppm, and Mercury less that 0.001 ppm (Fennema, 1985: Hegarty, 1988: KBS, 1996). Such studies on Kenyan honey are few and there is therefore, need to investigate the relationships between the amount of heavy metals in honey and in honey-combs, bees, soil, water and flowers from a given locality. There is an assumption in rural and urban areas of Kenya that honey in its unrefined form is pure and good for consumption. For this reason, most people are unaware of the dangers the suspected heavy metals in honey can cause to health hence the need for this study. Furthermore, with the expected increase in the demand for honey in Kenya the export potential for this commodity is also anticipated to go up. This calls for screening honey from known pollutants such as toxic heavy metals and pesticides to ensure its competitiveness in the world markets.

1.2 OBJECTIVES OF STUDY

The specific objectives of this study are as follows:

i. To determine the concentration levels of cadmium, chromium, lead, and mercury in honey from Mbeere, Meru, and Kirinyaga districts using Atomic Absorption Spectroscopic technique (AAS), and to compare their concentration levels with those in honey from other studied areas in Kenya.

ii. To compare levels of heavy metals from this study with the standards as prescribed by the FAO, WHO and KBS organizations.

iii. To establish if there is a relationship between heavy metal concentration in honey, honeycombs, bees, flowers and soil from the above districts of Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 HEAVY METALS IN THE ENVIRONMENT

2.1.1 RELATIVE ABUNDANCE OF ELEMENTS IN THE EARTH'S CRUST

Heavy metals is a term widely used to refer to a group of metals and metalloids which have densities >5 g/cm^3 and are in most cases associated with pollution and toxicity (Tarr and Miessler, 1991). The most notable in the list being Lead, Mercury, Zinc, Cobalt, Nickel, Copper, Cadmium, Iron, Chromium and Manganese. However, this study focuses on the determination of the concentration levels of Cadmium, Chromium, Lead, and Mercury in honey. These metals occur naturally on the Earth's layers in different concentrations as shown in table 2.0.

Table 2.0: Elementary composition of rocks and soils (ppm)

Element	Igneous Rocks	Sedimentary Rocks	Earth Soil	Lunar Soil
Mg	2.6×10^3	1.34×10^4	6.3×10^3	64×10^3
Cr	100	160	200	$1\text{-}3\times10^3$
Mn	950	670	850	6×10^3
Fe	56.3×10^4	3.33×10^4	38×10^3	128×10^3
Co	25	23	8	24-58
Ni	75	95	40	185-420
Cu	55	57	20	4.5-9.0
Zn	70	80	50	6.7-24.9
Cd	0.2	0.3	0.5	0.3-1
Hg	0.08	0.4	0.01	<0.04
Pb	12.5	20	10	2.2-10.0

Source: Valkovic, 1975

The Earth can be considered as three concentric layers known as the core, mantle and the crust. It is believed that the core consists mainly of iron and a small percent of nickel, and the mantle is composed of mainly impure magnesium silicate. The crust consists of all the other elements but in varying degrees as shown below in table 2.1

Table 2.1: Average Abundance of Elements in The Earth's Crust

Element	Abundance (%)
O	46.60
Si	27.70
Al	8.13
Fe	5.00
Ca	3.36
Na	2.83
K	2.51
Mg	2.09
Others	1.43

Source: Vlasov, 1966

2.1.2 TRACE ELEMENTS IN SURFACE WATERS

Trace elements can be defined as elements that occur naturally in nature and are found in minute concentrations. Hence, heavy metals that are present in small concentrations can be regarded as trace elements.

Most natural waters at some stage make contact and react with the minerals of the Earth's crust. Since water is the primary agent in rock weathering, the chemical composition of the weathered rocks determines the elemental composition of natural

waters. The chemical mechanisms involved in the transportation of the elements in rivers and streams after they have been weathered from the rock are as follows (Gibbs, 1973):

- dissolving of ionic species
- formation of complexes with organic molecules in solution,
- adsorption on solids,
- precipitation and co-precipitation on solids (metallic coatings),
- incorporation in solid biological material,
- incorporation in crystalline structures.

Studies performed by Gibbs (1973), revealed that trace elements transported by the Amazon and Yukon rivers found in Brazil for instance were distributed among the above listed transport phases. The distribution for both rivers was found to be similar although the two rivers are in extremely different climatic regions. It was found that copper and chromium were transported mainly in crystalline solids and manganese in coatings; while iron nickel and cobalt were equally distributed between precipitated metallic coatings and crystalline solids.

Other surveys performed by Durum and Hem (1972) showed that heavy metals such as arsenic, cadmium, chromium, lead, zinc, and mercury, are widely distributed in low concentration in surface-water sources of public water supply. Distribution of some of the metals showed a distinct regional pattern and thus it was difficult to estimate their relation of the concentration levels to anthropogenic sources. Another study of surface-water in Canada performed for the MacKenzie drainage basin, which was virtually unpolluted at the time, showed that the concentrations of strontium, manganese, iron, zinc and copper were of the order of 1μg dm^{-3} (Reeder, *et al.*, 1972).

Most trace element concentrations in the world's principal river waters are around or below 100μg dm^{-3} (0.001ppm). Only aluminum, iron, manganese, barium and strontium range much over 100μg dm^{-3}. Significant differences with respect to area, in trace element concentrations have also been reported for rivers in North America. It was found that Atlantic river waters on the whole are slightly more enriched in concentrations of silver, chromium, molybdenum, nickel, strontium titanium, and slightly deficient in barium and lithium as compared to the median values for North America river waters (Durum and Hafty, 1963).

2.2 SOURCES OF HEAVY METAL POLLUTANTS

The distribution of elements in Nature, their concentration and their movements can be seriously affected by human activities. Environmental pollution occurs when humans introduce different substances into the marine, atmospheric or soil environment. By definition, a chemical pollutant is a substance that produces any undesired effects. If the pollutant reaches its most toxic level it can kill or give rise to serious health complications to both humans and animals. However, if the heavy metals are in trace amounts, both animals and plants can benefit (Purves, 1977). The accumulation of trace elements in the environment is what causes them to be termed as pollutants.

2.2.1 CADMIUM: SOURCES AND USE

Cadmium, which is a naturally occurring trace element, has its major source in the atmosphere from volcanic action although some is derived from ocean sprays, forest fires and the release of metal enrichment particles from terrestrial vegetation (Fulkerson *et al.*, 1973). Cadmium is geochemically associated with zinc and is found as an impurity (up to 3 %) in zinc ores such as zincblende and sphalerite (ZnFe)S or calamine ($ZnCO_3$). Cadmium is also found in the rare mineral greenockite (CdS). In

uncontaminated soils, the cadmium/zinc ratio is usually in the range 1:100 to 1:1000 (Fulkerson, *et. al.*, 1973).

Zinc ores constitute the main industrial source of cadmium and the metal is fractionated during the smelting or electrolytic processes employed for the refining of Zinc. There is therefore, the possibility of a considerable release of cadmium into the environment during Zinc-refining operations, and to a lesser extent during lead and copper smelting. Thus the anthropogenic sources of cadmium are from metal refining and electroplating works (Alloway, 1990).

Cadmium is used industrially as an anti-friction agent, as a rust inhibitor, in plastic manufacture, as an orange coloring agent in enamels and paints, and in alkaline batteries. For instance, several hundred tons of solder containing cadmium are used in the UK every year, and since this is a relatively volatile metal, soldering operation inevitably lead to high concentrations of this metal in the atmosphere (Purves, 1977). Cadmium is also released into the environment through its dissipative uses such as cadmium fungicides, cadmium-based enamel, and cadmium pigment. It is also used as a stabilizer for plastics in nickel-cadmium dry-cell batteries, and finds large-scale use of materials in which cadmium acts as an impurity, for example, in zinc products, phosphate fertilizers and coal.

2.2.2 HEALTH AND TOXICITY EFFECTS OF CADMIUM

As a trace element, cadmium is important in both aquatic and terrestrial environments. However, small concentrations of 1.1 ppm in the blood are extremely toxic (Baselt, 1978). Cadmium absorbed in the human body is only slowly excreted and as a consequence cadmium toxicity is markedly cumulative in nature. Hence, there is the possibility of chronic cadmium poisoning among industrial workers regularly exposed

to this metal or its compounds, such as workers in factories that manufacture alkaline accumulators (Nordberg, 1972). Kidney disease has been reported in Glasgow among a group of coppersmiths working with cadmium-containing solder. In an epidemiological survey of workers exposed to cadmium dust, Lauwerys and co-workers found excessive proteinuria due to kidney damage in 68 % of male workers with over 20 years exposure, compared with an incidence of 15 % in men with less than 20 years exposure (Lauwerys *et al*, 1974). The level of cadmium in airborne dust at this study area was less that the American threshold limit of 200$\mu g/m^3$.

The most notorious case of cadmium toxicity was the disorder known as Itai-Itai disease, which occurred in Japan after World War II (Baselt, 1978). This was essentially an osteomalacia associated with serious kidney damage. The disease was largely confined to post-menopausal women who had borne several children. In some cases of the disease the osteomalacia led in the terminal stages of the illness to rapid progressive reduction in stature, multiple fractures and finally to skeletal collapse. The associated levels of cadmium in the area's rice diet were up to 3.4 ppm in the dry matter of brown rice and a mean level of 0.5 ppm cadmium in the white rice (Purves, 1977). The fatal cadmium concentrations in blood, brain, liver, kidney cortex and kidney nerve are 1.1 ppm, 0.5 ppm, 80 ppm, 80 ppm and 8.9 ppm respectively (Baselt, 1978).

The toxic nature of cadmium, is no doubt, to some extent due to its similarities in atomic structure to zinc. This similarity allows cadmium to replace zinc in enzyme systems in living organisms. For example, it has been reported that zinc can be replaced by cadmium in carboxypeptidase enzyme systems which catalyze peptide degradation (Mahler and Cordes, 1966). Other effects caused by cadmium poisoning

include severe abdominal pain, vomiting, diarrhea, nausea, headache, rapid pulse, liver damage convulsion and pulmonary edema (Thompson, 1977).

2.3.1 CHROMIUM: SOURCES AND USE

In nature, chromium occurs in a combined state with rocks of high magnesia and low silica content. It is also associated with iron, alumina, calcium, copper and magnesium (table 2.2) (Furman, 1989). Chromite ($FeO.Cr_2O_3$) is the only commercially important ore of chromium, and its three principal uses are in metallurgy, refractory and chemical manufacture. In metallurgy it is used to form alloys with iron, nickel, cobalt, alumina and copper. As a refractory compound it is used in x-rays, and in chemical manufacture it is used in the field of leather tanning and as a pigment. The pigments of chromium compounds are used as coloring agents in ceramic glaziers and in glass making. Other uses of chromium include fungicides, in wood preservation and in rust inhibition in cooling-tower recirculating water systems (Nurnberg and West, 1988). Data of some industrially important inorganic chromium compounds is listed in table 2.3

The nutritional need for chromium is well established, but as yet not quantified. It is known however, that chromium is bound to the globulin fraction of the hemoglobin. The few studies done on chromium in the human body show that it is widespread in low concentrations without special concentration in any organ (Anderson and Mertz, 1987).

Table 2.2 Chromium Distribution in The Environment

Source	Range	Average
Crust	80 - 200μg/g	125μg/g
Clays	30 - 590μg/g	120μg/g
Soils	10 - 150μg/g	40μg/g
Coals	10 - 1000μg/g	20 - 60μg/g
Petroleum	--	0.3μg/g
Sea water	1μg/dm^3	--
River water	1 -10μg/dm^3	--
Municipal water	0 - 35μg/dm^3	--
Air in Urban Station	0.001 - 0.003μg/dm^3	--
Air in Rural Station	0.001μg/dm^3	--

Source: Wanninen & West, 1988.

Table 2.3 Some Industrially Important Chromium Compounds

Formula	Industrial Use
$Cr(CH_3COO)_3$	tanning leather, catalysis
$CrBr_3$	in catalysis for polymerization of olefins
Cr_2O_3	pigments, latex paints, catalyst, dyeing polymers
CrO_2	high energy magnetic tapes
CrO_3	corrosion inhibitor, oxidant, catalyst, plating metals
$BaCrO_4$	anticorrosive pigment
$CaCrO_4$	corrosion inhibitor, depolarizer in batteries
$CuCrO_4$	in fungicides, seed protectants, wood preservative
K_2CrO_7	tanning leather, dyeing, painting, oxidizer
$PbCrO_4$	pigment
$ZnCrO_4$	in anti-corrosive pigments

Source: Wanninen and West, 1988

2.3.2 HEALTH AND TOXICITY EFFECTS OF CHROMIUM

Chromium is widely distributed in human tissues in extremely low and variable concentrations (total body content <6 mg). As a trace element it is not harmful, but high concentrations are very toxic. Healthwise, studies have shown that chromium is involved in glucose tolerance in humans. A deficiency of chromium in the body is characterized by disturbances in glucose, lipid and protein metabolism (Anderson and Mertz, 1987).

As a toxin chromium is most toxic in its hexavalent state, Cr (VI). When the hexavalent state enter the body it is irreversibly reduced to the trivalent state, Cr (III), which is less toxic. Chromium toxicity appears to be a very limited problem, while marginal dietary intakes, usually from beer, meats and yeast, are widespread (Anderson and Mertz, 1987; Wanninen and West, 1988). It should be noted however, that chronic exposure to high chromium levels has been correlated to lung cancer in humans and kidney damage in animals. Dermatitis among homemakers has also been correlated to chromium in detergents and bleaches, and the high incidences of allergies among construction workers may be related to the presence of chromium in cement.

2.4.1 LEAD: SOURCES AND USE

Lead is an element which is extensively used and is one of the most widespread metal in the environment largely due to human activities (Tarr and Miessler, 1991). The main lead ores that are mined are galena (PbS), cerrusite ($PbCO_3$), and anglesite ($PbSO_4$). On a global basis, about a quarter of total production of lead is from lead scrap. The world main producers of lead are the USA, Australia, and Canada but, other industrialized countries import large quantities of lead ore for smelting and

refining. Other sources of lead in the environment include smelting and refining of lead, the burning of lead based petroleum fuels containing lead additives and to a lesser extent the smelting of other metals and the burning of coal and oil.

Since lead is easily smelted, it has been used by humans for hundreds of years and can therefore be regarded as a longstanding environmental contaminant. In the nineteenth century lead was widely used for conducting water in cisterns, but its use for such purposes has now become unpopular because of the toxicity hazard associated with the enhancement of lead levels in drinking water (Conway and Pretty, 1991). Lead and inorganic lead compounds are now used in a variety of commercial products and industrial materials including plastics, storage batteries, bearing alloys, insecticides, ceramics, cable sheathings, sheeting, radiation shields, and even in some paints. However, there is some health hazard associated with the manufacture and eventual disposal of these products, thus alternatives are being sought. For example, white lead or basic lead carbonate ($2PbCO_3Pb(OH)_2$), used to be employed extensively as a pigment for paints, have been replaced to some extent with titanium-based pigments. Red lead or triplumbic tetraoxide (Pb_3O_4), calcium plumbate ($CaPbO_4$) and lead chromate ($PbCrO_4$) are still being used and the erosion of these lead pigmented paints must still be regarded as a major source of environmental pollution.

As mentioned earlier, numerous compounds of lead are used in the manufacture of plastics, ceramic glaziers and "leaded" glass. For example, lead bisilicate is used in ceramic glazing and tribasic lead sulfate is used as a stabilizer in the production of PVC plastics. In such products lead is usually chemically combined in a highly insoluble form so that their final disposal as waste does not lead to any significant release of lead in the environment. Thus, ceramic tableware and cooking and storage

vessels do not normally present any risk of acute lead poisoning, although small quantities of lead may be introduced into the diet from this source over a long period of time.

2.4.2 HEALTH AND TOXICITY EFFECTS OF LEAD

Since lead is absorbed and retained by living organisms, the lead content of the human body reflects the extent to which lead is present as a contaminant in the environment. Presently, human beings are exposed to enhanced levels of lead in food, drink and in the air they breathe, and for most of those who are not industrially exposed, food and drink is probably the major source of intake (Smith *et al.*, 1989). Thus lead enters the human body system through three major pathways: (I) inhalation, (ii) ingestion, and (iii) skin contact.

Inhaling lead based gasoline or petrol can lead to lead poisoning if the gasoline has tetraethyllead and tetramethyllead. For instance, the inhalation or ingestion of 1g of tetraethyllead may be sufficient to cause death within 3 to 30 days due to the slow degradation of the compound to triethyllead (Baselt, 1978). The fatal concentrations of tetraethyllead in the brain, lung, liver and spleen are 10 mg/kg, 22 mg/kg, 41 mg/kg, and 12 mg/kg respectively.

Lead also adversely affects many enzyme systems, but most noteworthy in regard to clinical indices of lead poisoning are the effects on heme synthesis. In the excess presence of lead, the heme, which is the oxygen carrier in hemoglobin, cannot be synthesized, thus oxygen cannot be bound in the human blood leading to death. Death may occur in 1 to 2 days after the ingestion of 10 to 30g of a lead salt by an adult. For young children a dose of 7.5mg of lead can easily lead to death (Hill, 1984). The fatal

inorganic lead concentrations in the brain, liver, kidney, flat bone, and long bone are 5.8 mg/kg, 40 mg/kg, 8.8 mg/kg, 268 mg/kg, and 132 mg/kg respectively.

Other health hazards attributed to lead poisoning include a lower rate of development in prenatal and postnatal stages in humans, nervous systems disorders, inhibition of enzymes necessary for the formation of hemoglobin, mitochondria damage, brain damage (insomnia, lack of ambition) exhaustion, frequent colds, lower intelligence, muscle and heart weakness, impotence, infertility, still births, premature senility, convulsion, coma, blindness, paralysis and mental retardation (Hodgson, 1987: Calvin and Monroe, 1972).

2.5.1 MERCURY: SOURCES AND USE

Mercury is a non-essential trace element the presence of which in human tissues represents uptake from both natural and anthropogenic sources. At ordinary temperatures element mercury is shiny mobile liquid, silvery-white in color with an appreciable vapor pressure. The world production of mercury, which is largely centered in Spain, the USA, Italy and China was estimated as being greater than 9,000 tons per annum in 1985. (Higgins and Burns, 1992). The main ore source of mercury is cinnabar (HgS), although more recently Livingstonite ($HgSb_4S_7$) has also become an important ore source (Higgins and Burns, 1992). To obtain the metal from cinnabar or Livingstonite, the ore is roasted with air and the gases produced passed through a condensing system. However, a substantial amount, may be 50 % is lost to the environment during this process. Mercury can also occur in its pure form or combined with silver in small amounts (Higgins and Burns, 1992).

The current use of mercury is mainly industrial. Inorganic mercury is employed in the chloro-alkali industry (where chlorine is produced by electrolysis of sodium chloride using a mercury cathode), in the production of electric batteries, mercury vapor lamps, electrical relays and liquid switches. It is also used in manufacturing of paint as a catalyst in the form of mercuric chloride ($HgCl_2$), in the manufacture of vinyl chloride, urethane plastics and acetaldehyde. Elemental mercury is also used in chemical and physical laboratories and in barometers and thermometers. Some laboratories still use mercury in the form of mercuric oxide (HgO) as a catalyst in Kjeldahl procedures for determining total nitrogen in soils and plant tissues. This is no longer recommended since mercury may find its way into laboratory drains from this source.

2.5.2 HEALTH AND TOXICITY EFFECTS OF MERCURY

Inorganic mercury released by industries into waterways can be converted to organo-mercury compounds, which are much more toxic than inorganic mercury. Among the organo-mercury compounds, alkyl mercury compounds such as methyl or ethyl mercury are the most dangerous (Encarta, 1997 b). When absorbed by humans methyl mercury has a half-life of about 70 days, and can thus cause serious health complications before it is excreted. (Baselt, 1978).

The average lethal dose of mercury is about 100 mg for organic mercury and 1g for inorganic mercuric salts. Several mass poisonings involving over 8000 people have been caused by accidental ingestion of organic mercury in contaminated sea food (Minimata disease), or by ingesting mercury treated seed grain (Baselt, 1978). The lethal doses for methyl mercury poisoning and inorganic mercury poisoning are shown in tables 2.4 and 2.5.

Table 2.4 Concentration of Fatal Methylmercury Poisoning (mg/l or mg/kg)

	Blood	Brain	Liver	Kidney	Urine
Average	2.6	27	30	22	0.8
(Range)	0.6 - 6.0	18 - 35	4.2 - 78	2.4 - 41	0.8

Source: Baselt, 1978

Table 2.5 Concentration of Fatal Inorganic Mercury Poisioning (mg/l or mg/kg)

Period of Surviving	Blood	Brain	Liver	Kidney
2 hours	22	3	56	136
8 days	0.8	1.1	33	47

Source: Baselt, 1978

Mercury in elemental form can also enter the blood circulation directly from inhaled mercuric vapor. Exposure to the vapor produces toxic effects on the nervous system which include anxiety, depression, lack of concentration and characteristic tremors which affect the hands. Long exposure to the mercuric vapor causes irreversible brain, liver and kidney damage (Encarta , 1997 b).

Thus, toxicological implications of mercury depend heavily on the chemical form involved (Owen, 1978). Exposure to organo-mercury compounds is more dangerous than exposure to inorganic mercuric salts. However, both forms of mercury are extremely toxic, as depicted in tables 2.5 and 2.6. Extreme caution should always be observed when dealing with mercury.

2.6 THE HONEY-BEE AND THE PRODUCTION OF HONEY

In the world of bees there are 11 families(see table 2.6). The honeybee belongs to the family Apidae, and within this family there are four species namely: *Apis florea, Apis*

dorsata, Apis mellifera, and *Apis indica. Apis florea* is referred to as the little honeybee, while *Apis dorsata* is called the giant honeybee. Both of these species are considered to be more primitive than *Apis mellifera.* Bee colonies of *Apis florea* are small by comparison *to Apis mellifera* and *Apis dorsata. Apis florea* usually have an exposed comb, thus they do not gather and store a large surplus of honey. *Apis dorsata,* which are bigger than *Apis florea,* also build exposed combs, thus they also store very little honey. *Apis mellifera* is the remarkable honey bee. It builds nests in cavities and it is capable of gathering large quantities of nectar and pollen. These bees are the ones responsible for most honey production worldwide.

Table 2.6 Family of bees and their common examples

Family of Bees	Common Examples
Colletidae	Plasterer and yellow faced bees
Halicitidae	Halichid and alkali bees
Andemidae	Andemid bees
Meditillidae	Meditillid bees
Megachilidae	Leaf cutting bees
Fraeliidae	
Anthoporidae	digger and cuckoo bees
Xylocopidae	Carpenter bees
Oxaeidae	Oxaeid bees
Bumbidae	Bumble bees
Apidae	Honey and stingless bees

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Source: Gojmerac, 1980

The species *Apis mellifera* is found mainly in Euro-Asia, and Africa. The genotype of the bees found in the three continents is different, each especially adapted to its environment. These different species of bees are sometimes races or sub-species. In the African continent the most important race is the *Adanosanii,* commonly known as the African honeybee (Gojmerac, 1983).

The African honey bee, of which there are two major strains in Kenya, the yellow bees and the dark bees, are good workers and easily adapt themselves to various local conditions making them extremely drought resistant (Mann, 1973). Out of the two strains, the yellow bees, which have two yellow bands on the abdomen, are very prolific and are usually good-tempered. The darker bees are much less prolific, but are much better producers of comb honey. They regulate their breeding program to suit the season, thus performing better in times of drought or prolonged dry spells. These bees are bad-tempered compared to the light bees and they can attack both humans and animals.

Table 2.7 Development Time for Honey bees

	Days		
Stage	**Worker**	**Queen**	**Drone**
Egg	2 ½ to 3	2 ½ to 3	2 ½ to 3
Immature larvae	4	4	4
Mature larvae	1	2	3
Pre-pupal	1	1	4
Pupa	9	5	8
Sealed brood	12	7 ¼	13 ½
Egg to Adult	20 - 21	14 - 15	22 - 29
Life expectancy	weeks	years	months

Source: Gojmerac, 1983

A bee worker that is three weeks can start foraging for pollen, nectar, or water depending on the weather and the needs of the colony. The foraging activities are usually confined to a specific geographical location depending on the availability of plants, prevailing winds and barriers. The foraging area is usually between 1 and 3 km

from the hive, but if necessary foraging can be done up to 12 km away from the hive. Once nectar is collected it is taken to the hive and deposited into the house bees. These house bees digest the pollen and nectar, then deposit the unripened honey from their honey sacs (found in the abdomen) into the cells. After deposition of nectar, moisture is evaporated and invertase converts sucrose to glucose and fructose in the ripening honey. The ripened honey contains traces of proteins, probably derived from honey bees, and varying amounts of acetic, butyric, formic, gluconic, lactic, mallic, pyroglutanic, phosphoric and succinic acids (see table 1.0 for the composition of honey).

2.7 PAST WORK ON HONEY

2.7.1 HEAVY METALS IN HONEY AND OTHER BEE PRODUCTS

Research on heavy metals in honey and its by-products has been done mainly in Europe and America. Honey has been used as a bio-indicator for heavy metal contamination, smelter pollution and for mineral prospecting (Jones, 1987). Most of the data acquired revealed high concentrations of metal pollutants in honey and other bee products harvested near industrial and town centers, as compared to rural or non-urban centers. Honeys harvested near major roads and waste incineration plants also showed high levels of heavy metal pollution.

Potential use of honey as an indicator in mineral prospecting and environmental contamination studies has been exploited in the measurement of copper and lead levels in honey from twenty six sampling sites in England (Jones, 1987). Soil samples were also taken from the same sites and information on the nearness of the sampling sites to major roads and possible contamination sources tabulated. Samples of honey collected and sealed in 1950-51 were also analyzed for silver, cadmium, copper and lead

contents. The following concentrations were found: (i) silver from 0.1 to 6.5 *n*g/g, (ii) cadmium from 0.3 to 300 *n*g/g, (iii) copper from 35 to 6510 *n*g/g, and (iv) lead from 2 to 200 *n*g/g.

Zelewiski *et al* (1989) and coworkers have analyzed heavy metals in bee products collected in Poland. The concentration of iron, zinc, manganese, copper, nickel, lead, cadmium and cobalt were determined in six samples of honey, five samples of pollen loads and eleven samples of stored pollen (bee bread) collected in 1989 from five districts within Poland. Their results are tabulated in table 2.8.

Table 2.8 Concentration levels (ppm) of some heavy metals in bee products from Poland

	Iron	Zinc	Manganese	Copper	Nickel	Lead	Cadmium	Cobalt
Pollen loads	80.2	48.5	50.0	7.43	0.4	2.38	0.05	0.007
Bee Bread	106.3	48.0	50.7	5.73	0.62	0.25	0.11	0.08
Honey	2.8	4.0	4.6	0.01	0.48	0.05	0.01	0.03

Source: Zelewiski *et. al*, 1989

Their data showed that the heavy metal pollution was relatively low and this seemed to suggest that in general the hive products from these particular areas were only slightly contaminated by industrial pollution.

Miguel *et al* (1989) have also studied heavy metals in insects found in industrialized regions of southern Poland. Samples of honeybees were collected between 1986-87 and the heavy metal content of zinc, copper, cadmium, and lead determined. These researchers found that the metals accumulated in the honeybee's digestive tract far more than on the rest of the body. However, lower concentrations levels of the metals

were found in the honey samples, while higher concentrations were present in the pollen. This suggested that it might be better to use the pollen as an indicator of heavy metal contamination in the environment as opposed to the honey.

Studies performed in Czechoslovakia by Veleminsky *et al* (1990) and coworkers used honeybees and their products to monitor environmental pollution caused by heavy metals; lead, cadmium, copper and zinc. Data from worker bees obtained at various periods of the winter season, and also from the drones, larvae, honey and wax combs were analyzed and compared with data drawn from pollen. The information obtained from the worker bees was found to be most significant. Over winter, dead workers taken from the hives showed relatively low values of heavy metals. Forager workers returning to the hive manifested low values because the heavy metals were reduced during the course of development.

Stein *et al* (1986) analyzed lead, cadmium and manganese in honey and sugar. The honey samples collected from locations in Munster, Federal Republic of Germany, showed that heavy metal contents were highest in samples gathered from a town center and near a busy road. Concentration levels of lead and cadmium in particular showed seasonal variation. Thus the mean lead and cadmium contents in honey harvested in spring was 130μg/kg and 15 μg/kg respectively. The mean concentration of lead and cadmium levels in honeys harvested in the summer was 270μg/kg and 20μg/kg respectively.

In another part of Europe, Bogdanove *et al* (1986) analyzed Swiss and imported honeys by atomic absorption spectrometry (AAS). The mean concentration values with the standard deviation for lead, cadmium and zinc in nectar honey were found to

be 0.09μg/g ± 0.10, 0.005μg/g ± 0.005 and 2.78μg/g ± 1.07 respectively. Corresponding values in twenty-one samples of honeydew honey were 0.21μg/g ± 0.17, 0.019μg/g ± 0.014, and 4.53μg/g ± 4.01 respectively. These values were below the minimum WHO and FAO acceptable levels for these metals.

In a study performed by Roggi *et al* (1990) values for organochlorine, organophosphorus and polychlorinated biphenyl pesticide residues and heavy metal in twenty six samples from agricultural and industrial areas in Italy were tabulated. The researchers found that in many cases the values were close to or, as in the case of lead, exceeded the maximum acceptable daily intake for infants.

Cimino *et al* (1984) studied the effects of volcanic activity on heavy metal concentrations in Italian honey. Twelve samples of honey were taken from apiaries on the slopes of Mount Etna, Sicily and were compared with one sample from Volcano Island, seven samples from a non-volcanic area, two samples from the Italian Alps and samples from Greece. It was found that higher concentrations of heavy metals were found in honey harvested near volcanic mountains than in other places.

Gabriel *et al* (1994) studied the distribution of copper smelter emissions in southern Arizona using honey mesquite as a bioindicator. The honey mesquite was considered as a possible bioindicator for industrial smelter pollution after analysis was performed on soils, leaves and bark sampled at various distances and altitudes from a large operational copper smelter in Arizona. The honey mesquite was found to reflect the degree of copper emissions from the smelter and thus used as a bioindicator of copper concentration in the area.

In another study performed in the United States, Bromenshenk *et al* (1985) monitored pollution in the Puget Sound district of Washington using sixty-four beekeepers. This area has over 130 pollutant sources such as smelters and chemical plants. The distribution of these pollutants' sources was then used to draw maps showing where arsenic, fluoride and cadmium were in abundance. In fact, in one area concentrations of arsenic and fluoride in bees were as high as 12.5 ppm and 182 ppm respectively, whereas at another area they were less than 0.5 ppm and 4 ppm respectively. The permissible limits for these two elements are 1ppm for arsenic and 20 mg for fluorine. Distribution patterns of the pollutants were not identified, but it was observed that high lead concentration was found near roads

Table 2.9 summarizes concentration levels of notable heavy metals in honey and other bee products from various countries of the world.

Table 2.9 **A Summary of Concentration levels (ppm) of heavy metals in honey and other bee products from various countries**

Country	Source	Zinc	Lead	Copper	Cadmium
Poland	Honey	4.0	0.05	0.01	0.01
	Pollen Load	4.85	2.38	7.43	0.05
	Bee Bread	48.0	0.25	57.3	0.11
England	Honey	-----	0.002—2	0.35—6.50	0.003—3
Germany	Honey (SPR)	-----	0.13	-----	0.05
	Honey (SUM)	-----	0.27	-----	0.02
Switzerland	Honey	2.78	0.04	-----	0.005
	Honey dew	4.53	0.21	-----	0.17
Kenya	Honey	0.508	0.065	0.312	0.001

2.8 THEORETICAL BACKGROUND OF ANALYTICAL TECHNIQUE USED IN THIS STUDY

2.8.1 ATOMIC ABSORPTION SPECTROPHOTOMETRIC TECHNIQUES

2.8.1.1 INTRODUCTION

Many analytical methods, both qualitative and quantitative, involve the interaction of matter and radiant energy. Radiant energy may be defined as energy, the propagation and transfer of which are accomplished as a wave motion without transfer of matter (Fischer and Peters, 1985). The term radiant energy is generally used with reference to the energy of electromagnetic radiation. The interaction of free atoms with various forms of energy results in three very closely related spectroscopic phenomena; absorption, emission and fluorescence (the latter two are not of importance in this study).

2.8.1.2 PRINCIPLE OF ATOMIC ABSORPTION SPECTROMETRY (AAS)

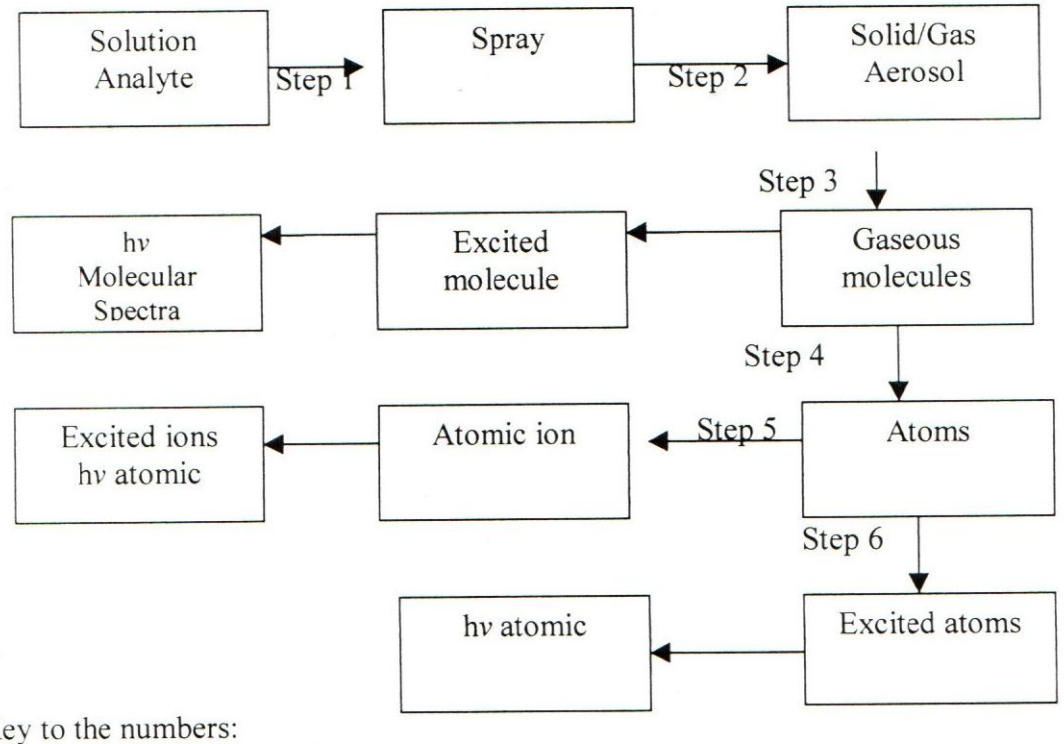
Atomic absorption spectrometry (AAS) is an analytical method for the determination of elements based upon the absorption of radiation. In its simplest sense AAS techniques make use of the fact that free atoms of an element absorb light at wavelengths characteristic of that element (Alloway, 1990). Upon absorption of the light energy, the atoms get excited and the absorbance readout is then converted to concentration readings.

2.8.1.3 PRODUCTION OF ATOMIC VAPORS

Usually samples are introduced to the AAS as water aerosols, but for those elements that form volatile hydroxides, the hydroxide itself can be introduced to the flame. The function of the flame in atomic absorption spectrometry is to produce excited atoms

from the sample components. The processes occurring during atomization are shown in figure 2.1.

The fuel/oxidant mixtures used in AAS are both safe to handle and produce a range of temperatures from about 2000 to 3000K. Although the flame temperature plays a significant role in the production of atoms other parameters such as, the chemical effects of radicals and other substances in the flame also play a major part.



Key to the numbers:
Step 1: Nebulization
Step 2: Dissolution
Step 3: Volatilization
Step 4: Dissociation
Step 5: Ionization
Step 6: Atomization
Source: Price, 1974

Figure 2.1 Processes Occurring During Atomization

Fuel gases used in the production of the flame are propane, hydrogen and acetylene while the oxidants include air and nitrous oxide. Pure oxygen is rarely used as an

oxidant since it provides a mixture whose burning velocity is high and difficult to control. When it is used, oxygen is usually mixed with an inert gas such as nitrogen or argon. Fuel/oxidant mixtures may be combined in ratios which may be stoichiometric, lean or rich.

The most widely used of the fuel/oxidant mixtures is air/acetylene. This flame enables about 30 of the common metals to be determined. Acetylene mixed with nitrous oxide is also used and this produces higher temperatures than the acetylene air mixture. The success for this mixture as an atomizing gas in atomic absorption can be ascribed to the high temperature produced by nitrous oxide as an oxidant and the high critical carbon/oxygen ratio contributed by acetylene. This high carbon/oxygen ratio produces more metal atoms in the flame.

2.8.1.4 QUANTITATIVE LAWS OF ATOMIC ABSORPTION

I: **Lambert's Law**: This law relates the thickness of the test sample to the ratio of the intensities of radiation transmitted through the sample and radiation incident to the sample. It should be anticipated that other factors being equal, the intensity of transmitted radiation should decrease as the thickness of the sample is increased.

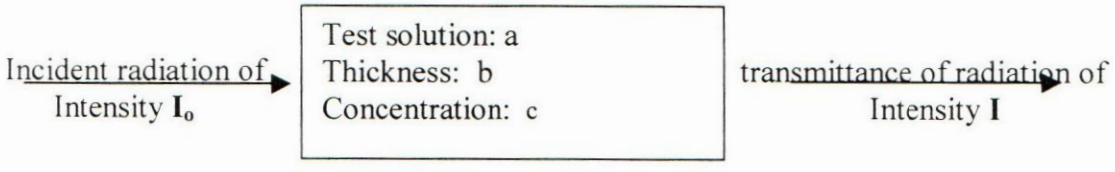


Figure 2.2 Schematic representation of radiation absorption

Lambert's law is written as follows: (Fisher and Peters, 1985)

$$A = \log I_0/I = kb$$

Where I_0: is the intensity of incident light

I: is the intensity of transmitted light

A: is the absorbance

k: is a constant characteristic of the nature of the absorbing medium

b: is the thickness or path length of the medium.

II: **Beer's Law**: This law applies to substances which absorb radiant energy by relating the concentration of the absorbing species to the ratio of the intensities of radiation transmitted through the absorbing medium, and the radiation incident upon the medium. That is to say, any increase in the concentration of the absorbing species should increase the number of photons absorbed and, correspondingly, decrease the intensity of the incident radiation.

Beer's Law can be written as: Log I_0/I = k"c where k" is a constant and c is the concentration. The two laws can be combined to have Beer-Lambert's Law which describes the linear relationship between absorbance (A), concentration (c), and pathlength (l). This is written as follows: (Fisher and Peters, 1985)

$$A = \log I_0/I$$

$$A = \varepsilon cl$$

Where A = Absorbance
l = pathlength
c = concentration
I_0 = intensity of incident light
I = intensity of transmitted light
ε = absorption coefficient.

From the Beer-Lambert's law one can see that absorbance is directly proportional to the concentration or the number of absorbing atoms. With this in mind one can establish analytical working curves by plotting absorbance values against

concentration values and this forms the basis of quantitative analytical technique in atomic absorption spectrophotometry.

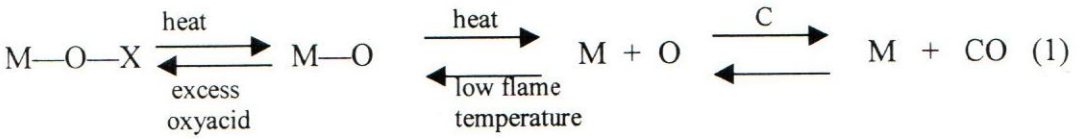
2.8.2 INTERFERENCES IN ATOMIC ABSORPTION SPECTROMETRIC TECHNIQUE

Atomic absorption is a very powerful technique for analysis which has very few interferences. The main interferences are as follows: 1) Chemical, 2) Ionization, 3) Incomplete volatilization, 4) Matrix effect, and 5) Scatter and Background absorption.

I Chemical Interferences:

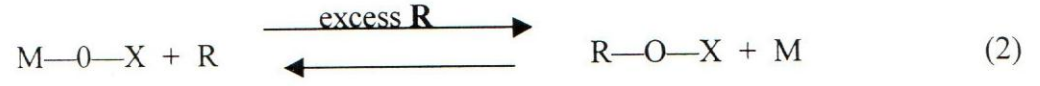
It has been the practice among atomic absorption analysts to differentiate between 'enhancing' and 'depressive' chemical interferences. With the former type of interference, the chemicals enhance the sensitivity, while with the latter the chemicals lower the sensitivity of the instrument. In all cases however, the presence of substances which produce an improved sensitivity for a given element actually do so by reducing an existing depressive interference. Thus it would seem more logical to look upon any effect which causes an element to give less than its maximum possible absorption as a chemical depressive interference.

Stable compound formation is the best known of the interferences and it is responsible for many of the depressive effects both in absorption and emission flame photometry (Price, 1974). It arises because compound or radicals containing the elements being analyzed are not broken down into individual atoms at the temperature of the flame being used. The stable compounds formed are usually refractory oxides which give low sensitivity at low flame temperatures. An increase in the flame temperature usually results in the formation of free atoms of the wanted metal as shown in the following equation 1.



Lower temperatures and/or excess oxyacids thus favor the persistence of the stable oxysalt M—O—X, or stable refractory oxides M—O. The use of a fuel rich flame, especially with acetylene, produces a high carbon (C) count which increases the carbon/oxygen ratio. The presence of carbon in the flame produces more of the metal atoms and carbon monoxide.

Stable compounds (metal oxides) formed in the flame can also be destroyed by the use of releasing agents. The action of a releasing agent is to influence the chemical equilibrium in equation 2 in the desired direction.



R is the releasing agent:

Good releasing agents are those metals which themselves form stable oxysalts, and for this reason strontium and lanthanum salts have been most used. Another good releasing agent is ethylenediamine tetraacetic acid (EDTA) which behaves as a pre-flame releasing agent. Sodium sulfate is also commonly used as a releasing agent.

II Ionization Interferences:

This occurs when an element is easily ionized instead of atomizing in the flame. When this is the case an ionization buffer is required. This is a second easily ionizable metal added in excess to the solution. For example, in the determination of potassium (K), the element ionizes easily. To overcome this effect, lithium (Li) which ionizes more easily than potassium is added in excess. Lithium produces enough electrons to prevent the ionization of potassium.

$$Li \overset{heat}{\rightleftharpoons} Li^{+} + e \quad (3)$$

$$K^{+} + e \rightleftharpoons K \quad (4)$$

III Incomplete Volatilization:

This implies that, at the temperature of the flame, the droplets produced by the nebulizer have given rise to solid particles. These particles, because of their high vaporization temperature, and their speed through the flame, are not completely converted to vapors. The degree of atomization is thus lower than would be expected, given the other chemical conditions of the flame. This type of interference effect is usually experienced under reducing conditions, and then is caused by the formation of metal/metal solutions of high boiling points.

IV Matrix effects:

These influence the number of atoms actually entering the radiation beam rather than their effectiveness there. They usually arise from the differences in the physical properties of the samples and the so-called matching standards (Price, 1974). A difference in acid concentration for example, causes a difference in viscosity or surface tension. The viscosity affects the rate of up-take of the sample by a given nebulizer and, the surface tension affects the size distribution of the droplets formed, and hence the nebulizer. To reduce this type of interference the method of standard additions is recommended.

V Scatter and Background Absorption:

These types of interferences actually 'enhance' the reading obtained by adding a spurious absorbance to the true value. The magnitude of this effect varies considerably with the wavelength at which measurements are being taken. For

particles having a diameter of less than one tenth of the wavelength of the incident radiation, the amount of scatter according to Rayleigh theory is proportional to λ^{-4}, so that towards the lower wavelengths, a very sharp increase in scatter is experienced (L'vov, 1980). Light scattering therefore affects particularly those elements that absorb at the lower wavelengths, especially arsenic, selenium, zinc, cadmium and lead.

The presence of the scatter and background absorption is easily detected. All calibration curves should pass through the origin. If a particular one doesn't, either there is a reagent blank present in the standards or there is a scatter. To avoid scatter and background absorption, one must optimize the instrument in use by trying to reproduce the manufacturer's conditions.

2.8.3 BASIC COMPONENTS OF AN ATOMIC ABSORPTION SPECTROPHOTOMETER

The atomic absorption spectrophotometer consists of a light source, which is a hollow-cathode lamp made of the element to be determined, an atom reservoir (flame), a wavelength selector (monochromator), a radiation detector, and a tuned amplifier and read-out system as illustrated in figure 2.3. The cathode lamps are designed to emit atomic spectra for a specific element, although some are designed to be multi-element. The light emitted from the lamp passes through the atomizer in the form of a flame, into which a fine mist of a solution of the analyte is sprayed by a nebulizer. This light then proceeds into a monochromator which is set to transmit radiation. Light emerging from the monochromator exit falls on the photocathode of a photomultiplier detector, and an output current proportional to the incident light intensity, is amplified, processed electronically and finally presented to a readout

device such as a moving coil-meter, a pen recorder (in the present case a printer), or a digital display (Smith, 1983).

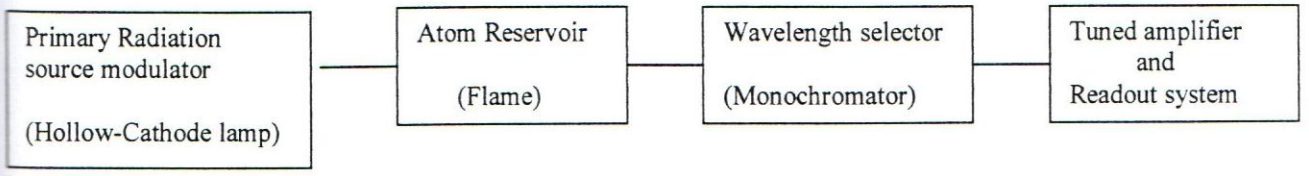


Figure 2.3 Schematic block diagram of an Atomic Absorption Spectrophotometer

2.8.3.1 INSTRUMENTAL PERFORMANCE

The performance of the atomic absorption spectrophotometer is determined by two parameters, namely: 1) the detection limit, and 2) the sensitivity. Both of these parameters are different for each element since they depend on the absorbance observed for each element.

I Detection Limit:

In atomic absorption spectrometry the detection limit is the concentration corresponding to three times the standard deviation of a series of not less than ten readings taken close to the blank level (Miller and Miller, 1994). The determination of the detection limit is then the statistical evaluation of the smallest quantity of analyte detectable with a 95 % certainty. Since the detection limit does not depend directly on the instrument noise it can be used in respect of all types of instruments including those with an integrating read-out. The detection limit is also directly related to sensitivity (for if the slope of the calibration curve is doubled, the concentration corresponding to a given absorbance is halved), but it is lower (better)

than the sensitivity by a factor which depends on the stability and reproducibility of the read-out system (Smith, 1978).

II Sensitivity:

Sensitivity is the concentration (quoted usually in ppm in aqueous solutions) which will absorb 1 % of the incident resonance radiation of a given element (Caulcutt and Boddy, 1994). In atomic absorption spectroscopy, sensitivity is the concentration of the analyte that produces an absorbance of 0.004436 as long as measurements are made in the linear region of the calibration curve. Alternatively, sensitivity is in fact the reciprocal of the slope of the calibration curve in the vicinity of the origin (Price, 1974). One can also determine the sensitivity of an instrument using the formula

$$\text{Sensitivity} = \frac{\text{Concentration of Std (ppm)} \times 0.0044}{\text{Measured absorbance}}$$

The sensitivity helps an analyst to know if all instrumental condition are optimized and if the instrument is working up to the manufacturer's specifications.

2.8.4 COLD VAPOR METHOD IN AAS TECHNIQUES (FLAMELESS MERCURY DETERMINATION)

An extremely sensitive method for determination of mercury is accomplished by a non-flame atomization procedure at room temperature. In this technique mercury (e.g. in air, water or occupational samples) is collected in a potassium permanganate ($KMnO_4$) solution. The permanganate oxidizes the organomercury compounds as well as elemental mercury to produce mercuric ions. For analysis the excess permanganate is destroyed with hydroxylamine (NH_2OH) for example, and a reducing agent such as stannous chloride ($SnCl_2$) or sodium borohydride ($NaBH_4$) is added to reduce the mercury ions to elemental mercury (Godden and Thomerson, 1980). This elemental mercury has an appreciable vapor pressure at room temperature. By

bubbling a gas such as argon or nitrogen through the solution in an Erlenmeyer flask, the mercury vapors can be swept into a glass tube aligned in the path of the atomic absorption spectrophotometer. The mercuric vapor absorbs the lines of mercury just as atomic mercury does in a flame. The atomization efficiency of this process is greatly improved over flame atomization, with detection limits of a few nanograms or less. This method is preferred over electrothermal ones because of volatility losses of mercury during drying and charring cycles before atomization. Care must be taken to avoid the presence of UV absorbing organic solvent vapors. Many of these solvents strongly absorb at the same wavelength (252.7 nm) as mercury (Christian, 1980). The following equations portray how elemental mercury is produced.

$$5Hg + 2MnO_4^- + 16H^+ \longrightarrow 5Hg^{2+} + 2\,Mn^{2+} + 8H_2O \quad (5)$$

$$6MnO_4^- + 5NH_2OH + 13H^+ \longrightarrow 6Mn^{2+} + 5NO_3^- + 14H_2O \quad (6)$$

$$Hg^{2+} + SnCl_2 + 2Cl^- \longrightarrow Hg^0 + SnCl_4 \quad (7)$$

2.8.5 SAMPLE DIGESTION PROCEDURES USED IN AAS ANALYSIS

Trace elements in foodstuff and the environment are rarely found in free form. They are predominantly bound to proteins or low molecular weight ligands such as amino acids (Wolf *et al.*, 1981). In order to characterize these compounds using the atomic absorption spectrophotometer, the compounds must be separated from their biological or environmental matrices. This means that the organic matter must be destroyed through sample digestion. The two techniques usually employed for sample digestion are: 1) dry oxidation (ashing), and wet digestion (oxidation).

2.8.5.1 DRY ASHING OXIDATION METHOD

This method involves the complete combustion of all organic matter followed by the dissolution of mineral contents using an acid. Combustion is usually carried out in a muffle furnace which allows better control of digestion temperature than combustion using gas burners. The destruction of the organic matter is accomplished by the high temperatures of the muffle furnace.

This method is attractive because large samples can readily be handled thus making it advantageous for trace element work. Ashing at low temperatures is usually employed when one wants to retain the more volatile elements during the destruction of organic matter. Thus, mineral elements that are subject to volatilization losses can be oxidized at minimum temperatures. However, dry ashing is seldom suitable for sensitive trace analysis, such as the case of mercury and selenium (Benton & James, 1984; Allen, 1989). Ashing temperatures exceeding 550°C should not be used for more than two hours since the local overheating can lead to volatilization losses. Thus many workers prefer to ash samples at 450° C although it is better for each researcher to determine the optimum ashing temperature for the elements being determined.

2.8.5.2 WET DIGESTION (ACID OXIDATION)

There are numerous wet oxidation procedures which have been proposed, but they all fall into four categories which differ only in the digestion mixture used: 1) sulfuric acid and hydrogen peroxide, 2) nitric acid and sulfuric acid, 3) perchloric acid and nitric acid or sulfuric acid, and 4) nitric acid alone (see table 2.10). All these different methods are used for digestion in the initial stages of sample decomposition, but their end result is the same. When digesting, the use of perchloric acid or hydrogen

peroxide speeds up the process of the destruction of organic matter. However, the use of these chemicals is very dangerous since they are quite explosive (Allen, 1989). The main advantage of this procedure is that it is fast and it is less prone to either volatilization and retention loses. This makes wet oxidation the preferred mode of digestion over dry ashing when analyzing for sensitive trace elements in foodstuff. The advantages and disadvantages of the wet oxidation and dry ashing procedures are shown in table 2.11.

Table 2.10 Characteristics of Wet Oxidation Mixtures

Digestion Reagent	Applicability to Organic Matrix	Remarks
H_2SO_4/HNO_3	Vegetable origin	The most used, danger of volatilization as in the determination of As, Se and Hg
$HCLO_4$ alone	Biological origin	Catalysts$(NH_4)_2MoO_4$
$H_2SO_4/HClO_4$	Biological origin	Suitable only for small samples, danger of explosion
$HNO_3/HClO_4$	Protein, Carbonate (No fat)	less explosive, loss of Lead
$H_2SO_4/HClO_3$/ $HClO_4$	Universal, (Also fat and carbon black	No danger with exact temperature control. As, Sb, Au and Fe are volatile under some reflux conditions
HNO_3 alone	General: also fat	Applied directly to most materials.

Source: Benton & Jones, 1984

Table 2.11 Comparison of Wet Oxidation and Dry Ashing Methods of Digestion

WET OXIDATION	DRY ASHING
More rapid	Rather slow
Temperature lower, less volatilization	Temperature higher, more volatilization
Less sensitive to nature of sample	More sensitive to nature of sample
Needs relatively more supervision during digestion process	Needs relatively less supervision during digestion process
Inconvenient for large samples	Large samples easily handled

Source: Benton & Jones, 1984

CHAPTER THREE
EXPERIMENTAL

3.0 MATERIALS AND METHODS

3.1 REAGENTS USED IN THIS STUDY

All the chemical reagents used in this study were of analytical grades (AR) and were acquired from Kobian Kenya Ltd. Gases used for the atomic absorption spectrometer (AAS) were obtained from the British Oxygen Company (BOC) Kenya Ltd. The chemical reagents used were Hydrochloric acid (HCl), Nitric acid (HNO_3), Sulfuric acid (H_2SO_4), Cadmium chloride ($CdCl_2 \cdot 2½H_2O$), Chromium (III) chloride ($Cr(Cl_3) \cdot 3H_2O$), Lead nitrate ($Pb(NO_3)_2$), Mercuric chloride ($HgCl_2$), Calcium chloride ($CaCl_2 \cdot 2H_2O$), Manganese chloride ($MnCl_2 \cdot 4H_2O$). Sodium chloride (NaCl), Copper nitrate, ($Cu(NO_3)_2 \cdot 3H_2O$), Magnesium chloride ($MgCl_2 \cdot 6H_2O$), Nickel chloride ($NiCl_2 \cdot 6H_2O$), Iron chloride ($FeCl_3 \cdot 6H_2O$), Stannous chloride ($SnCl_2$) and Sodium sulfate (Na_2SO_4). The gases used for the atomic absorption spectrometer (AAS) were acetylene (C_2H_2), argon (AR) and compressed air. Deionized water was also used for the preparation of all the standard solutions.

3.2 EQUIPMENT AND GLASSWARE

Atomic absorption spectrophotometer model CTA 2000 double beam instrument was used. It was driven by a computer and the acquired data printed by an Epson printer. A deuterium (D_2) lamp was used for the automatic background correction (ABC) of the signal. The equipment's burner height could be adjusted up to a maximum 10 cm and a transverse dial was available to align the burner in the path of the beam from the hollow-cathode (HCL) lamp. Other apparatus such as analytical balance, muffle

furnace, Bunsen burner, digestion block, rotary evaporator, and a mercury detection unit for the CTA 2000 were used.

The glassware used consisted of beakers, volumetric flasks, conical flasks, measuring cylinders, pipettes, micro-pipettes, graduated cylinders, and digestion flasks. Other items used in this work included spatulas, sample storage bottles, deionized water bottles and filter papers.

3.3 SAMPLING METHODS

3.3.1 SAMPLING, STORAGE AND TREATMENT OF HONEY, HONEYCOMBS, BEES, FLOWERS, SOILS AND WATER SAMPLES

Basically, sampling was done in three Kenyan districts, namely; Kirinyaga, Meru, and Mbeere districts. Samples were also collected from the Mt. Kenya Forest. Samples were to be collected during the dry and wet seasons, but because of the prolonged dry weather in the country samples were collected only during the dry season.

I: Sampling of Honey, Honeycombs and Bees

In each district sampling stations were selected randomly. In Kirinyaga district the stations where sampling was carried out were Sagana, Kagio, Tetu Gacheru and Kiritiini; in Meru district the sampling stations were Gaitu, Chuka, Kangoro, and Kambandi; while in Mbeere district the sampling stations were Ishiara, Kiamsita Hill , Ciakago, and Kambati Village. In each stations three samples each of honey, honeycombs and bees weighing approximately 300—400 grams were collected within a 5 km radius. The distance between sampling stations varied from 10 km to 15 km. This was based on the fact that bees have been known to travel up to 12 km from the hive during their foraging activities. The samples collected were stored in air tight plastic containers and transported to the analytical laboratory where they were refrigerated before analysis. Sampling from Mt. Kenya forest was similar to that from

the districts except that samples were collected within a radius of about 50 km. Permission had to be granted by the local forest warden before venturing into the forest for sampling.

II: Sampling of Flowers

The flower samples were collected randomly from the same stations where honey was collected. At each station flowers were picked randomly starting from the vicinity of the hive up to a few kilometers from the hive. The flower samples were oven dried at about 60^0C—70^0C and crushed before analysis.

III: Sampling of Soils

Soil samples were collected in a similar manner except that from each sampling station, two soil samples were collected at 6 inches and 12 inches from the surface. The weight of the collected soil samples were approximately 500—600 grams per sample. Before analysis the soils were air dried and all foreign matter, such as leaves, stones, etc., removed by sieving.

IV: Sampling of Water

Water samples were collected from streams, ponds, and even rivers within the vicinity of beehives. The samples were stored in plastic bottles which had been previously rinsed with deionized water. Acidification of the water samples using nitric acid was done to prevent the metabolic processes of microorganisms which might cause changes in the sample.

3.3.2 SAMPLING OF REFINED HONEY

Refined Kenyan and USA honey samples were used in this study. These samples were bought from Uchumi supermarket in Nairobi. The Kenyan honey samples were

packed by Baston Ltd. Nairobi, while honey from the US was packed by the Bessonet Bee Co. Inc. in Donaldsonville, Los Angeles, and it was the Gulf Breeze brand labeled as pure US grade A honey with only natural additives.

3.4 SAMPLE PREPARATION AND DIGESTION

In this study, collected honey samples were classified as liquid honey and comb honey. The liquid honey samples, which were free from granulation, were mixed thoroughly by stirring and shaking. For the comb honey, the honeycombs had to be dripped in order for the comb honey to be collected. This honey was then mixed by stirring and shaking until a homogeneous mixture was obtained.

The soil samples were air dried to remove all moisture and were further sifted to remove large particles before digestion. Flower samples were oven dried between 60°C and 70°C and the water samples acidified before digestion.

3.4.1 DIGESTION OF SAMPLES

Two techniques were used for sample digestion, namely; dry ashing oxidation and wet oxidation methods..

3.4.1.1 OPTIMIZATION FOR DRY ASHING PROCEDURE

Honey samples obtained from Meru district were used to optimize the ashing temperatures to be used in dry ashing oxidation method. Five grams each of unrefined honey were weighed separately into pre-weighed silica dish ashing vessels. A Bunsen burner was used to char the samples to dryness until foaming and smoking stopped. The dishes were placed in a muffle furnace and ashed at separate temperatures of 200°C, 250°C, 300°C, 350°C, 400°C, 450°C, 500°C, 550°C, 600°C, 650°C, 700°C and 750°C. The ash obtained from each ashing temperature was then

dissolved in HCl (10 ml), digested to obtain a clear solution, allowed to cool and made up to 100 ml with deionized water. However, the samples that were to be analyzed for lead were dissolved in nitric acid (10 ml) as opposed to hydrochloric acid (Omoga & Kagwanja, 1999). These samples were then analyzed for cadmium, chromium, and lead and their concentrations were plotted against the ashing temperature to get the optimization graphs. The highest concentration reading for cadmium, lead, and chromium were between 450°C and 460°C. The optimization graphs are depicted in fig 3.0 to fig 3.2

3.4.1.2 DRY ASHING PROCEDURE

Five grams each of unrefined honey, refined honey, honey combs, whole bee, flower and soils samples were weighed separately into pre-weighed silica dish ashing vessels. They were then treated using the procedure described in section 3.4.1.1. The temperatures used were between 450°C—460°C for 3 hours until carbon free ash was obtained. All the samples were ashed in triplicate. The solutions were then analyzed for cadmium, chromium and lead using an atomic absorption spectrophotometer.

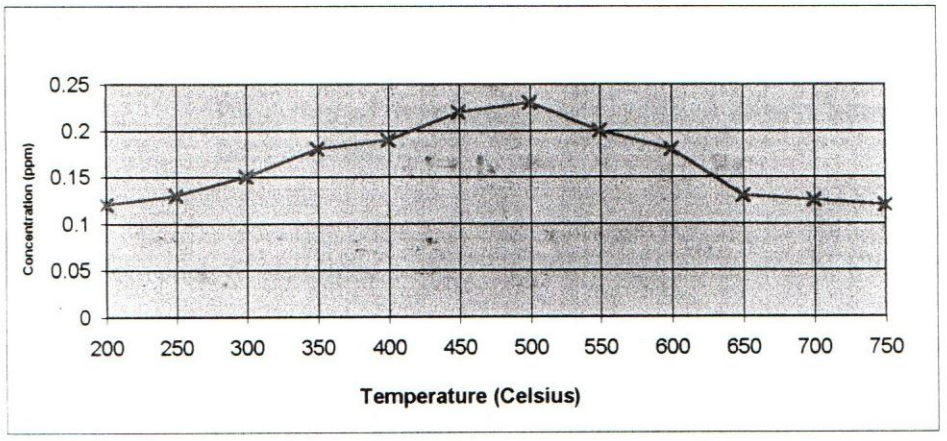


Figure 3.0 Concentration levels (ppm) of chromium versus ashing temperature

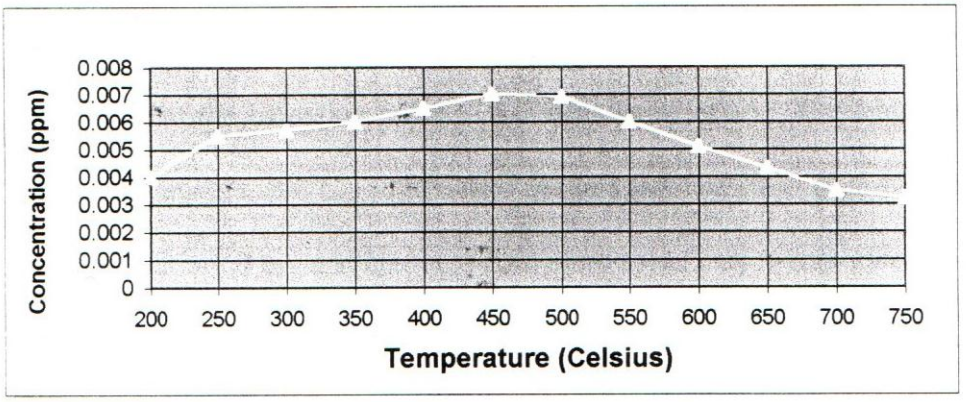


Figure 3.1 concentration levels (ppm) of cadmium versus ashing temperature

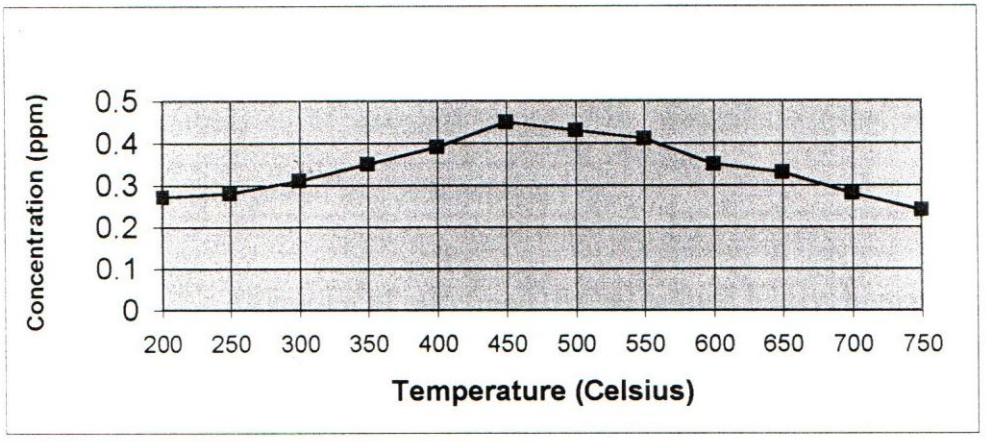


Figure 3.2 Concentration levels (ppm) of lead verusu the ashing temperature

3.4.1.3 WET OXIDATION PROCEDURE

3.4.1.3.1 Digestion of unrefined honey, refined honey, honeycombs, whole bees and flowers

One gram each of unrefined honey, refined honey, honeycomb, whole bee, and flower samples were weighed separately on filter papers and put into separate digestion flasks. 10 cm^3 of concentrated nitric acid was added into each sample, stirred thoroughly and placed on a digestion block. The temperature of the digestion block was raised gradually to about 130^0C and maintained at this point for two hours. This ensured that the oxidation process was near completion. The temperature was then raised to between 180^0C —200^0C and the samples heated to near dryness to complete the destruction of organic matter. More nitric acid (2 cm^3) was added and the reaction mixture heated to a final volume of 5 cm^3. The mixture was cooled, filtered, and made to 100 cm^3 with de-ionized water. Each sample was digested in triplicate and a blank solution was also prepared. This was achieved by subjecting 1 g of filter paper through a similar process (Omoga & Kagwanja, 1999). The samples and the blank were now ready to be analyzed using AAS. The samples that were analyzed for mercury were digested in a different manner.

3.4.1.3.2 Digestion of unrefined honey, refined honey, honeycombs, whole bees, soil and flowers for analysis of mercury

Two grams each of unrefined honey, refined honey, honeycomb, whole bee, flower and soil samples were weighed separately on filter papers and put into separate digestion flasks placed in a digestion block. Concentrated sulfuric acid (10 cm^3), and concentrated nitric acid (10 cm^3) were then added to each separate flask on the digestion block. The temperature of the block was raised to about 100^0C and samples

left to digest till all the organic matter was destroyed. The temperature of the digestion block was monitored carefully because the mixture of sulfuric acid and nitric acid is very explosive. When the final digest was brought to 5 cm^3, the solution was cooled, filtered, into 100 cm^3 volumetric flask and brought to the mark with de-ionized water. The blank solution was prepared by subjecting 1 g of filter paper through the same process. Each sample and the blank were digested in triplicate and were then analyzed for mercury using the cold vapor method (Stux and Parker, 1989).

3.4.1.3.3 Digestion of soil samples

One gram each of soil samples were weighed and transferred into separate digestion flasks and placed on a digestion block. Fifteen mls of acid mixture consisting of (4 vol) nitric acid and (2 vol) hydrochloric acid was added to each digestion flask. The temperature of the digestion block was raised gradually to 100^0C and maintained at this temperature for two hours to ensure that the oxidation process was complete. After two hours the temperature of the block was raised to between 180^0C —200^0C then finally to 240^0C. The sample residue was heated to near dryness, cooled, and 10 cm^3 of nitric acid was added.. The mixture was then boiled for ten minutes and allowed to cool, filtered in to 100 cm^3 volumetric flask and brought to the mark. The blank solution was prepared by subjecting the digestion acid mixture through the same process (Omoga & Kagwanja, 1999).

3.5 PREPARATION OF STOCK SOLUTIONS

1000 ppm standard stock solutions of chromium, cadmium, lead and mercury were prepared from analar grade salts of the elements. These solutions were subsequently diluted to make standard solutions of appropriate concentrations (see Appendix VII).

The dilute solutions were used in the drawing of the calibration curves shown in figures 3.3 to 3.6.

3.5.1 PREPARATION OF STANDARD SOLUTIONS FROM STOCK SOLUTIONS

Calibrating standard solutions of appropriate concentrations of cadmium, chromium, lead and mercury were prepared by diluting the 1000 ppm stock solution of each element (see Appendix VIII).

3.6. INSTRUMENTAL CALIBRATION

Atomic absorption spectrophotometer model CTA 2000 working conditions had to be optimized for cadmium, chromium, lead and mercury determinations before analysis of samples commenced. For each element to be analyzed parameters such as the burner height, the wavelength, the fuel and oxidant flow rates, had to be set according to the manufacturers guideline.. Some parameters were set automatically by switching on the spectrophotometer, while others had to be set manually by the operator. The instrumental conditions recommended by the manufacturer are summarized in table 3.0.

Table 3.0 Instrumental conditions for determination of cadmium, chromium, lead and mercury

Spectrophotometric parameters				
Metal	Cd	Cr	Pb	Hg
Wavelength (nm)	228.8	357.9	217	253.7
Slit Width (nm)	3	2	3	2
Lamp Current (mA)	3	4	4	4
Sample flow rate (ml/min)	8	8	8	1
Oxidant flow rate (l/min)	7.2	7.2	7.2	n/a
Fuel flow rate (l/min)	2.2	4.2	2	n/a
Burner Height (cm)	7	5	7	n/a
Detection Limit (ppm)	0.002	0.002	0.001	0.00001
Flame	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Argon Gas

Once these conditions were set in the instrument, various parameters such as the flow rate, and the lamp current were adjusted daily to ensure the manufacturers specifications were obtained.

3.6.1 CALIBRATIONS CURVES

In the analysis of samples, calibration curves play an important role in determining the concentrations of the element of interest. A series of working metal solutions were prepared by diluting the stock solutions with deionized water as described in section 3.5.1. The calibration curves were acquired by plotting the absorbance values versus the concentration values of the working standard solutions for cadmium, chromium, lead and mercury. In our case, the analytical working curves for cadmium, chromium, lead and mercury were obtained automatically by the machine Their graphs are shown in figures 3.3, 3.4, 3.5, and 3.6.

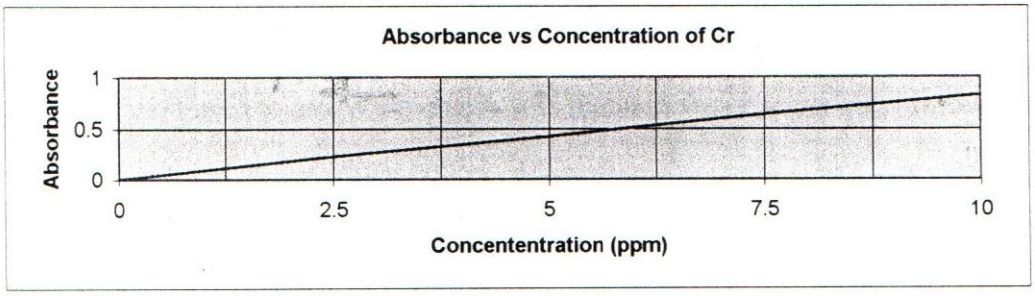


Figure 3.3 Calibration working curve of chromium showing absorbance versus concentration (ppm) of standard chromium solutions

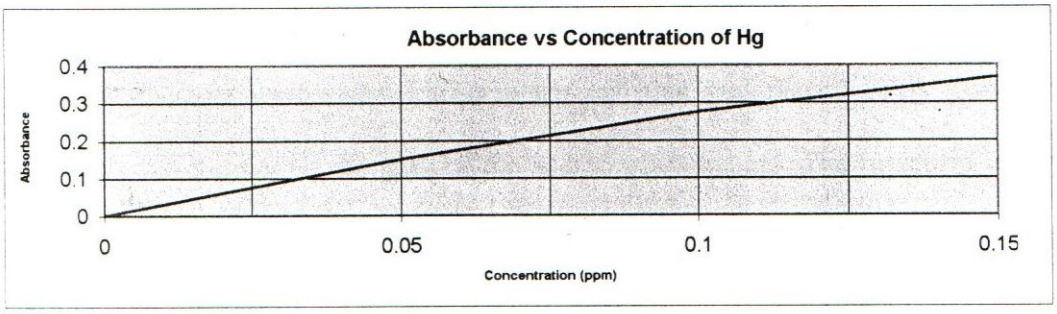


Figure 3.4 Calibration working curve of mercury showing absorbance versus concentration (ppm) of standard mercury solutions

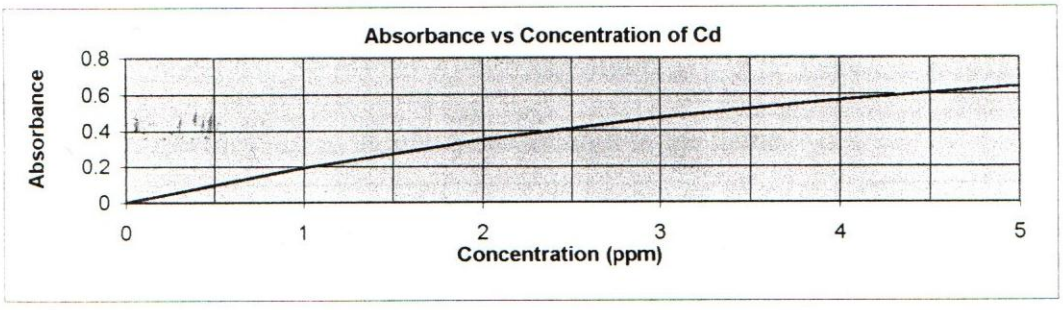


Figure 3.5 Calibration working curve of cadmium showing absorbance versus concentration (ppm) of standard cadmium solutions

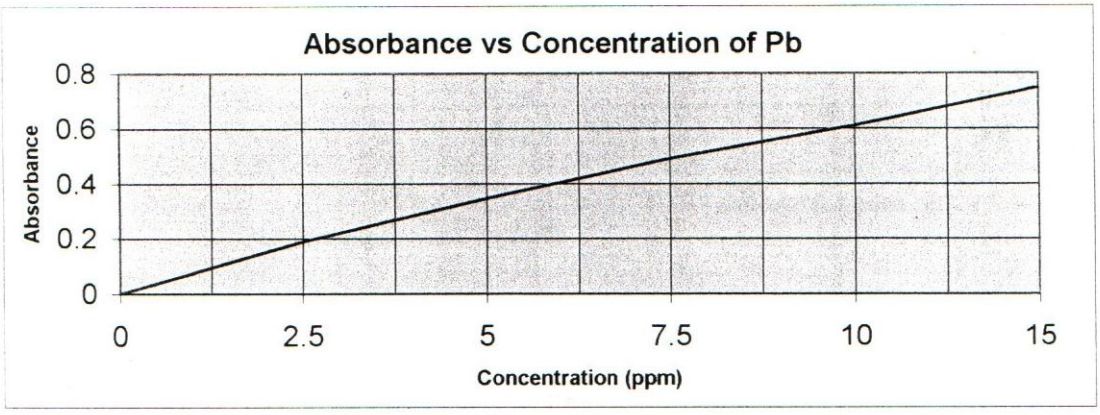


Figure 3.6 Calibration working curve of lead showing absorbance versus concentration (ppm) of standard lead solutions

3.6.2 DETECTION OF MERCURY USING THE COLD VAPOR METHOD

The method used to analyze mercury was the cold vapor technique. In this procedure, the CTA 2000 AAS was optimized using the conditions described in table 3.0. Working standards for mercury as described in section 3.5.1 were also prepared to calibrate the spectrophotometer to the manufacturers requirements for linearity.. However, in this procedure the flame was not used as the atomizer, instead elemental mercury vapors were swept into a quartz cell that was placed in the position of the burner, and aligned in the path of the spectrophotometer. The mercuric vapors were obtained after 1 ml of stannous chloride ($SnCl_2$) was mixed with 1ml of the standards or the sample solutions in the mercury detection unit provided for the model CTA 2000 AAS. The mercuric vapors produced were then swept into a glass tube provided for the model 680 Shimadzu AAS using argon gas. The mercuric vapors absorb the lines of mercury just as atomic mercury does in the flame.

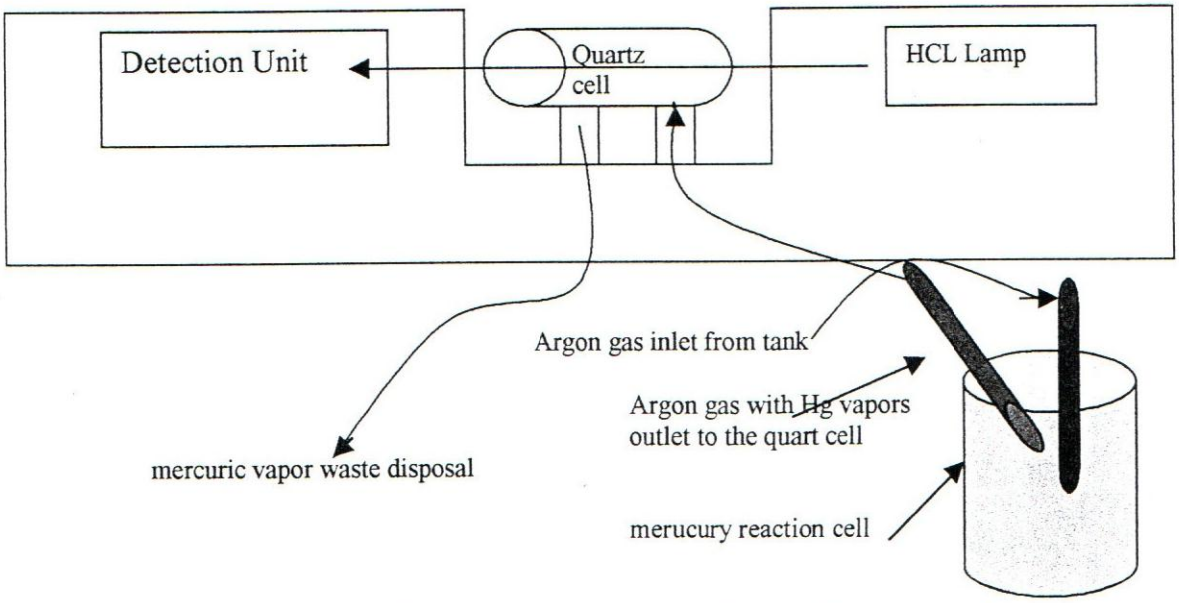


Figure 3.7 Schematic Block diagram for the set up of the cold vapor method

3.6.3 RECOVERY STUDIES

It is well known that complete extraction or separation of an analyte from a given matrix is never possible. At most, extraction or separation minimizes the concentration of the analytes. In order to assess the completeness of the recovery of the analyte from a matrix, recovery studies have to be performed.

In this study, recovery studies were performed on honey samples by adding known quantities of each metal to the honey samples prior to digestion step. In this way, potential losses of the sample during treatment is avoided.

Samples of honey weighing 1 g each were obtained and to this was added 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1.0 ppm of cadmium, chromium, and lead. These samples were then digested following the same procedure as described in section 3.4.1.3. They were then analyzed for the respective elements. The recoveries were then calculated using the following formula (Omoga & Kagwanja, 1999):

$$\% \text{ Recovery} = \frac{\text{Amount Recovered—Amount initially present}}{\text{Amount added}} \times 100\%$$

3.6.4 INTERFERENCE STUDIES

The effect of Ca^{2+}, Mn^{2+}, Cr^{2+}, Cd^{2+}, Na^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Fe^{2+}, and Pb^{2+}, on the determination of cadmium, chromium and lead were performed by spiking honey samples with known concentrations of the ions. Standard solutions of 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm of calcium, manganese, chromium, cadmium, sodium, copper, lead, magnesium, nickel and iron salts were prepared . Six digested honey samples were then spiked separately with 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm of each of the above ions and then analyzed for cadmium, chromium and lead. After concentrations of the metals were acquired, a releasing agent, Na_2SO_4,

(2%) was added to the already spiked samples and their concentrations were determined. This was to check if any of the above elements either enhanced or depressed the cadmium, chromium, and lead signals at different concentrations.

3.6.5 STANDARD ADDITION METHOD

This method was performed on honey samples to find out whether there were any matrix effects on the determination of cadmium, chromium, lead and mercury in honey. Working standard solutions consisting of 0.01ppm, 0.02 ppm, 0.04 ppm, 0.06 ppm, 0.08 ppm, and 1.0 of cadmium, chromium, and lead diluted from the stock solution, and standard solutions of 0.01 ppm, 0.02 ppm, 0.03 ppm, 0.04 ppm, and 0.05 ppm, of mercury diluted from the stock mercury solution were added to digested honey samples and the absorbances at each concentration were recorded. One of the honey samples was not spiked with standard solution prior to digestion step, and its absorbance was considered as being at zero concentration. After the absorbances were acquired, they were plotted against the concentrations and a straight line was extrapolated to cross the X axis at a position considered to be the true concentration of the digested honey sample.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.0 OPTIMIZATION OF MEASUREMENT CONDITIONS

The instrument's working parameters were optimized based on the manufacturer's detection limits. As shown in table 4.0 the working detection limits were lower for cadmium and chromium than the manufacturers levels. These low levels were achieved by changing parameters such as the transverse dial, photomultiplier voltage, lamp current, fuel flow rate and the alignment of the hollow cathode tubes and are based on taking ten readings of the blank and multiplying the standard deviation by 3 (i.e. $3\sigma_{blank}$).

Table 4.0 Optimized Parameters used in atomic absorption spectrophotometer (model CTA 2000) for low detection limits of cadmium, chromium, lead and mercury

Element	Cd	Cr	Pb	Hg
Lamp Current (mA)	3	6	5	3
Photomultiplier voltage	280	260	440	500
Fuel flow rate (l/min)	4.2	4.8--5.0	2	n/a
Oxidant flow rate (l/min)	7.2	7.2	7.2	n/a
Author's detection limit	0.0005	0.001	0.001	0.00001
Manufacturer detection limit	0.002	0.002	0.001	0.00001

4.1 CALIBRATIONS CURVES

According to the procedure described in section 3.6.2, the standard calibration curves for the heavy metals determined in this study were obtained and are shown in figures 3.3, 3.4, 3.5, and 3.6. The linear range for chromium standard solution is between 0 ppm and 10 ppm and any point beyond 10 ppm starts to curve off. For cadmium standard solution, the linear range was observed to lie between 0 ppm and 2 ppm while standard lead solution exhibits linearity in the range between 0 ppm and 15 ppm. Standard mercury solution had a linear curve from 0 ppm to 0.1 ppm. The sensitivities for cadmium, chromium, lead and mercury were found to be 0.178, 0.082, 0.0502 and 0.407 respectively.

4.2 CONCENTRATIONS OF CADMIUM, CHROMIUM ,LEAD AND MERCURY IN SAMPLES

The concentration of chromium, cadmium, and lead obtained from unrefined honey, honeycombs, bees, flowers, soil, and water samples from Meru district, Kirinyaga district, Mbeere district and Mt. Kenya forest, and refined honeys from Kenya and USA are tabulated in tables 4.1 to 4.27.

Table 4.1 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Gaitu obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MGH	0.30	0 04	13.33	0.003	0.0006	20.00	0.51	0.09	17.65	ND
MGHC	0.45	0.05	11.11	0.004	0.0005	12.50	0.52	0.18	34.62	ND
MGB	0.27	0.09	33.33	0.004	0.001	25.00	0.19	0.05	26.32	ND
MGF	0.39	0.02	5.13	0.010	0.002	20.00	0.20	0.05	25.00	ND
MGS	0.40	0.04	10.00	0.015	0.001	6.67	0.24	0.08	33.33	ND
MGW	0.03	0.02	66.67	0.001	0.00056	56.00	0.09	0.02	22.22	ND

MGH=Meru Gaitu Honey, MGHC=Meru Gaitu Honeycombs, MGB=Meru Gaitu Bees,
MGF=Meru Gaitu Flowers, MGS=Meru Gaitu Soil, MGW=Meru Gaitu Water, ND=Not Detected.

The mean concentration levels of chromium, cadmium, lead and mercury in samples from Gaitu station are depicted in table 4.1. It was observed that comparing results from other stations in Meru district (Table 4.2—4.8), honey collected from this station had the second highest amount of chromium with the mean concentration of 0.3 ppm. Similarly, concentration of chromium in honeycombs, bees, flowers, soils and water samples obtained from this station were higher than similar samples from other Meru stations. The high chromium concentrations levels in samples collected from this station can be related to its abundance in the environment due to possible use of chromium based fungicides or as seed protectant in this agricultural area. This source of chromium is most probable because there are no chromium based industries which could be another possible source of this metal in this area.

Close examination of the data depicted in table 4.1 indicates that, within this station, flower and soil samples exhibited close concentration levels of chromium, while bees

and flower samples had close concentration of lead. Bees and honeycombs had the same concentration of cadmium, while the concentration levels of cadmium was highest in the soil samples from Chuka station with concentration 0.029 ppm (Table 4.7). This is the highest concentration level of cadmium recorded in all samples analyzed from Meru district and reflects the extensive use of phosphate fertilizers in this region. It was noted that of all the samples investigated, water samples from this station had the lowest concentration of chromium, cadmium and lead, while honeycombs had the highest accumulation of the three heavy metals. Of the four elements monitored, lead exhibited the highest concentration of 0.51 and 0.52 ppm which was recorded in honey and honeycombs from Gaitu respectively. Mercury could not be detected in any of the samples analyzed from this station.

Table 4.2 Concentration levels (ppm) of Cd, Cr, Pb, and Hg in honey, honeycombs, bees, flowers and soils samples from Gaitu obtained using dry ashing method

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MGH	0.25	0.04	16.00	0.002	0.001	50.00	0.47	0.01	2.13 ND
MGHC	0.37	0.02	5.41	0.003	0.001	33.33	0.48	0.02	4.17 ND
MGB	0.20	0.05	25.00	0.002	0.0006	30.00	0.12	0.02	16.67 ND
MGF	0.25	0.01	4.00	0.008	0.001	12.5	0.18	0.01	5.56 ND
MGS	0.38	0.04	10.53	0.009	0.002	22.22	0.22	0.07	31.82 ND

MGH=Meru Gaitu Honey, MGHC=Meru Gaitu Honeycombs, MGB=Meru Gaitu Bees, MGF=Meru Gaitu Flowers, MGS=Meru Gaitu Soil, ND=Not Detected.

Table 4.2 shows data obtained when samples from Gaitu station were subjected to dry ashing oxidation procedure. Samples exhibited similar concentration trends as indicated in table 4.1 except that the concentration of cadmium, chromium and lead

were lower than when the samples were digested using wet oxidation method. This lowering of metal concentrations when dry oxidation procedure was employed can be attributed to volatility losses during the digestion step of the samples. Comparison of the two procedures will be discussed in section 4.6.

Table 4.3 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kambandii obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MKH	0.21	0.06	28.57	0.011	0.002	18.18	0.73	0.01	1.37	ND
MKHC	0.37	0.10	27.03	0.010	0.004	40.00	0.81	0.19	23.46	ND
MKB	0.17	0.04	23.53	0.006	0.001	16.67	0.21	0.02	9.52	ND
MKF	0.20	0.03	15.00	0.012	0.001	8.33	0.69	0.09	13.04	ND
MKS	0.22	0.02	9.09	0.003	0.001	33.33	0.77	0.08	10.39	ND
MKW	0.01	0.006	60.00	0.002	0.001	50.00	0.25	0.05	20.00	ND

MKH=Meru Kambandii Honey, MKHC=Meru Kambandii Honeycomb, MKB=Meru Kambandii Bees, MKF=Meru Kambandii Flowers, MKS=Meru Kambandii Soil, MKW=Meru Kambandii Water, ND=Not Detected.

Results of heavy metal concentration in samples collected from Kambandii station are summarized in table 4.3. It was observed that honey, honeycombs, bees, flower, soil and water samples from this station had the highest concentration of lead compared to the rest of the stations in Meru district. The highest concentration of lead recorded in honeycombs, soils, honey and flower samples were 0.81, 0.77, 0.73 and 0.69 ppm respectively. The high concentration of lead can be attributed to the location of a major road passing through the sampling station. This major road has been in operation longer than the other major roads found near the other sampling stations in Meru district. Motor vehicles which use leaded petrol can be regarded as the major

sources of lead contamination in the environment which is reflected in the higher levels of this metal in honeycombs (0.81 ppm) and the soil (0.77 ppm). The high concentration of lead in the honeycombs may be due to the fact that the lifetime of honeycombs is longer in a hive than honey and thus accumulate lead over time. Concentration levels of chromium and cadmium in samples from Kambandii were relatively low except in honeycomb samples which had a high concentration of chromium (0.37 ppm).

Table 4.4 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kambandii obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MKH	0.16	0.02	12.50	0.007	0.002	28.57	0.71	0.02	2.82 ND
MKHC	0.30	0.04	13.33	0.005	0.002	40.00	0.75	0.01	1.33 ND
MKB	0.15	0.04	26.67	0.004	0.001	25.00	0.18	0.05	27.78 ND
MKF	0.09	0.04	44.44	0.010	0.002	20.00	0.55	0.05	9.09 ND
MKS	0.18	0.05	27.78	0.018	0.005	27.78	0.71	0.05	7.04 ND

MKH=Meru kambandii Honey, MKHC=Meru Kambandii Honeycomb, MKB=Meru Kambandii Bees, MKF=Meru Kambandii Flowers, MKS=Meru Kambandii Soil, ND=Not Detected

As noted before, the concentration levels of Cr, Cd and Pb were lower after the samples were subjected to dry ashing procedure due to volatility losses. The coefficients of variation (CV) calculated for samples subjected to dry ashing procedures were observed to be quite high in comparison to those obtained after the samples were subjected to the wet oxidation procedure. The high CV values portray

the fact that the ashing procedure for the samples from Kambandii yielded a lot of volatility losses.

Table 4.5 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kangoro obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MKGH	0.19	0.05	26.32	0.009	0.001	11.11	0.26	0.08	30.77 ND
MKGHC	0.24	0.07	29.17	0.008	0.004	50.00	0.31	0.06	19.35 ND
MKGB	0.09	0.05	55.56	0.002	0.001	50.00	0.11	0.07	63.64 ND
MKGF	0.09	0.02	22.22	0.018	0.005	27.78	0.18	0.02	11.11 ND
MKGS	0.14	0.03	21.43	0.024	0.006	25.00	0.29	0.03	10.34 ND
MKGW	0.01	0.006	60.00	0.002	0.001	50.00	0.08	0.05	62.50 ND

MKGH=Meru Kangoro Honey, MKGHC=Meru Kangoro Honeycombs MKGB=Meru Kangoro Bees MKGF=Meru Kangoro Flowers, MKGS=Meru Kangoro Soil, MKGW=Meru Kangoro Water, ND=Not Detected.

Bee and flower samples from Kangoro station exhibited equal concentration of chromium as shown in table 4.5. However, it was observed that the percentage of coefficient of variation calculated for the two samples differed significantly, bee samples exhibiting calculated CV of 55.56 %, while flower samples showed 22.26 %. The higher CV value for bee samples shows that the concentration of chromium in the samples varied significantly which implies the possibility of bees travelling long distances for foraging purposes. Soil samples from Kangoro had a relatively high concentration of cadmium compared to the other samples. In fact it was 12 times higher than that found in bees. This high concentration can be attributed to the frequent use of cadmium based fertilizers in the soils.

Table 4.6 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soil samples from Kangoro obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MKGH	0.16	0.02	12.50	0.007	0.002	28.57	0.71	0.02	2.82	ND
MKGHC	0.30	0.04	13.33	0.005	0.002	40.00	0.75	0.01	1.33	ND
MKGB	0.15	0.04	26.67	0.004	0.001	12.50	0.18	0.05	27.78	ND
MKGF	0.09	0.04	44.44	0.010	0.002	20.00	0.55	0.05	9.09	ND
MKGS	0.18	0.05	27.78	0.018	0.005	27.78	0.71	0.05	7.04	ND

MKGH=Meru Kangoro Honey, MKGHC=Meru Kangoro Honeycombs MKGB=Meru Kangoro Bees
MKGF=Meru Kangoro Flowers, MKGS=Meru Kangoro Soil, ND=Not Detected

It was generally found that honey from Kangoro station had less than the threshold maximum limits of chromium, cadmium and lead required by WHO, FAO and KBS standards. Concentration levels of these elements were also found to be lower in samples digested by dry ashing oxidation method when compared to samples digested by wet oxidation method. Data for the two methods are depicted in tables 4.6 and 4.5 respectively. The difference is again attributed to volatility losses of those elements at the ashing temperature of 450^0 C.

Table 4.7 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Chuka obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MCH	0.49	0.04	8.16	0.005	0.001	10.00	0.21	0.04	19.05	ND
MCHC	0.22	0.09	40.91	0.005	0.003	60.00	0.22	0.04	18.18	ND
MCB	0.10	0.01	10.00	0.003	0.001	33.33	0.08	0.02	25.00	ND
MCF	0.10	0.05	50.00	0.019	0.007	36.84	0.21	0.07	33.33	ND
MCS	0.20	0.06	30.00	0.029	0.007	24.14	0.24	0.06	25.00	ND
MCW	0.02	0.01	50.00	0.001	0.0006	60.00	0.09	0.02	22.22	ND

MCH=Meru Chuka Honey, MCHC=Meru Chuka Honeycombs, MCB=Meru Chuka Bees, MCF=Meru Chuka Flowers, MCS=Meru Chuka Soil, MCW=Meru Chuka Water, ND=Not Detected.

Samples from Chuka station in Meru district exhibited low concentrations of lead in honey, honeycombs, bees, soil and water compared to similar samples from other stations in Meru district. These results are summarized in tables 4.7 and 4.8. It was observed that the lead concentrations were relatively low, especially for the bee samples. High concentrations of cadmium were observed in soil and flower samples with the concentrations being 0.029 and 0.019 ppm respectively. In fact, the cadmium level in soil from this station was the highest compared to the other sampling stations in Meru district. These high concentrations can be attributed to cadmium based fertilizers which are regularly used during planting seasons.

Results obtained when dry ashing procedure was used as a digestion method (table 4.8) follow the same trend as those obtained when wet oxidation method was utilized, except that concentration values are lower due to volatility losses.

Table 4.8 **Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, and soil samples from Chuka obtained using dry ashing procedure**

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MCH	0.48	0.05	10.42	0.003	0.001	33.33	0.11	0.02	18.18	ND
MCHC	0.10	0.02	20.00	0.001	0.0007	70.00	0.16	0.02	12.50	ND
MCB	0.08	0.01	12.50	0.002	0.001	50.00	0.06	0.01	16.67	ND
MCF	0.12	0.01	8.33	0.017	0.002	11.76	0.17	0.03	17.65	ND
MCS	0.20	0.04	20.00	0.022	0.001	4.55	0.21	0.02	9.52	ND

MCH=Meru Chuka Honey, MCHC=Meru Chuka Honeycombs, MCB=Meru Chuka Bees, MCF=Meru Chuka Flowers, MCS=Meru Chuka Soil, ND=Not Detected

Table 4.9 **Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Sagana obtained using wet oxidation procedure**

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
KSAH	0.59	0.15	25.42	0.007	0.001	14.29	0.23	0.09	39.13	ND
KSAHC	0.54	0.16	29.63	0.006	0.001	16.67	0.24	0.10	41.67	ND
KSAB	0.27	0.04	14.81	0.008	0.002	25.00	0.12	0.05	41.67	ND
KSAF	0.45	0.08	17.78	0.008	0.001	12.50	0.27	0.02	7.41	ND
KSAS	0.52	0.08	15.38	0.014	0.001	7.14	0.30	0.04	13.33	ND
KSAW	0.03	0.01	33.33	0.001	0.0005	50.00	0.06	0.01	16.67	ND

KSAHC=Kirinyaga Sagana Honey, KSAHC=Kirinyaga Sagana Honeycombs, KSAB=Kirinyaga Sagana Bees, KSAF=Kirinyaga Sagana Flowers, KSAS=Kirinyaga Sagana Soil, KSAW=Kirinyaga Sagana Water, ND=Not Detected.

Samples from Kirinyaga district were collected from four stations namely Sagana, Kagio, Tetu Gacheru and Kiritiini. Results from the four stations are given in table 4.9 to table 4.16. The highest concentration detected was that of chromium in honey samples from Tetu Gacheru station (table 4.13). In all honey samples from the four Kirinyaga stations the concentration of chromium ranged between 0.59—0.89 ppm, except honey samples from Kiritiini station which had chromium concentration of 0.32 ppm. It was however observed that Kiritiini soil samples had chromium concentration of 0.53 ppm, while the mean concentration of chromium in flower and bee samples from this station stood at 0.39 ppm and 0.25 ppm respectively (table 4.15). This observation implies that most of chromium in this area is retained in the soil where it is extensively used in fungicides and as seed dressers. Concentrations of cadmium in all honey, honeycomb, bees and water samples from the four stations of Kirinyaga district were very low and < 0.009 ppm. The highest cadmium concentration was recorded in soil samples from Kiritiini station with 0.026 ppm of Cd. Concentration of lead in samples collected from Kirinyaga districts were relatively low when compared to those in samples from Meru district. Lead concentration in honey samples from Kirinyaga district fall in the range 0.18—0.23 ppm, the highest lead concentration being recorded in soil samples from Sagana and Kagio stations with 0.20 ppm of lead. It was also observed that concentration of chromium cadmium and lead in honey and honeycomb samples from the four stations in Kirinyaga are closely related (table 4.9—table 4.16).

Results of heavy metal concentration in samples collected from Sagana station in Kirinyaga district and digested using the wet oxidation procedure are summarized in table 4.9. It was observed that the concentration values for cadmium in all the

samples, except in the soil (0.014 ppm) are close to uniform and are relatively low. This can be attributed to the lack of mobility of cadmium from one environment to the next. However, the higher concentration of cadmium in the soil samples can be due to the use of phosphate fertilizers which contain cadmium.

Table 4.10 shows the results obtained when samples from Sagana station were subjected to dry ashing oxidation procedure. It was observed that the cadmium levels in all the samples were uniform at 0.005 ppm except for honeycomb and soil samples which were 0.002 and 0.009 ppm respectively. These concentrations of cadmium, as well as those of chromium and lead are lower than those found in samples digested using the wet oxidation method, and this can be attributed to volatility losses during dry ashing procedure.

Table 4.10 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soil samples from Sagana obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KSAH	0.52	0.10	19.23	0.005	0.001	20.00	0.18	0.09	50.00 ND
KSAHC	0.49	0.05	10.20	0.002	0.001	50.00	0.18	0.04	22.22 ND
KSAB	0.21	0.02	9.52	0.005	0.001	20.00	0.09	0.04	44.44 ND
KSAF	0.39	0.05	12.82	0.005	0.001	20.00	0.20	0.01	5.00 ND
KSAS	0.49	0.07	14.29	0.009	0.001	11.11	0.25	0.02	8.00 ND

KSAHC=Kirinyaga Sagana Honey, KSAHC=Kirinyaga Sagana Honeycombs, KSAB=Kirinyaga Sagana Bees, KSAF=Kirinyaga Sagana Flowers, KSAS=Kirinyaga Sagana Soil, ND=Not Detected

Table 4.11 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kagio obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
KKH	0.75	0.02	2.67	0.007	0.002	25.43	0.20	0.05	25.00	ND
KKHC	0.60	0.14	23.33	0.007	0.002	28.57	0.22	0.05	22.73	ND
KKB	0.41	0.09	21.95	0.006	0.001	16.67	0.08	0.05	62.50	ND
KKF	0.75	0.04	5.33	0.015	0.002	13.33	0.19	0.02	10.53	ND
KKS	0.83	0.08	9.64	0.014	0.011	7.14	0.30	0.04	13.33	ND
KKW	0.02	0.01	50.00	0.002	0.0005	25.00	0.09	0.02	22.22	ND

KKH=Kirinyaga Kagio Honey, KKHC=Kirinyaga Kagio Honeycombs, KKB=Kirinyaga Kagios Bees, KKF=Kirinyaga Kagio Flowers, KKS=Kirinyaga Kagio Soil, KKW=Kirinyaga Kagio Water, ND=Not Detected.

Bee, flowers and soil samples from Kagio station in Kirinyaga district had the highest concentration of chromium of 0.41, 0.75 and 0.83 ppm respectively compared to other sampling stations in Kirinyaga district as depicted in table 4.11. The highest chromium concentration was observed in honey samples from Tetu Gacheru (0.89 ppm) followed by honey samples from Kagio, which had a mean concentration value of 0.75 ppm of chromium. It was also observed that honey samples from Tetu Gacheru, Sagana and Kagio stations had similar mean concentration values of cadmium at 0.008, 0.007 and 0.007 ppm respectively. This same trend was also observed with the samples from Kagio station which had been subjected to the dry ashing procedure. The concentration values for all the metals analyzed were lower due to volatility losses as is depicted in table 4.12.

Table 4.12 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flower, and soil samples from Kagio obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
KKH	0.68	0.02	2.94	0.004	0.001	25.00	0.16	0.04	25.00	ND
KKHC	0.57	0.10	17.54	0.004	0.001	25.00	0.17	0.04	23.53	ND
KKB	0.33	0.05	15.15	0.002	0.001	25.00	0.05	0.04	80.00	ND
KKF	0.66	0.04	6.06	0.009	0.001	11.11	0.11	0.05	45.45	ND
KKS	0.77	0.07	9.09	0.013	0.002	15.38	0.19	0.02	10.53	ND

KKH=Kirinyaga Kagio Honey, KKHC=Kirinyaga Kagio Honeycombs, KKB=Kirinyaga Kagios Bees, KKF=Kirinyaga Kagio Flowers, KKS=Kirinyaga Kagio Soil, ND=Not Detected.

Table 4.13 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Tetu Gacheru obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
KTH	0.89	0.04	4.49	0.008	0.002	23.63	0.18	0.01	5.56	ND
KTHC	0.72	0.27	37.50	0.006	0.004	66.67	0.22	0.03	13.64	ND
KTB	0.37	0.18	48.65	0.005	0.001	20.00	0.05	0.02	40.00	ND
KTF	0.18	0.02	11.11	0.016	0.002	12.50	0.18	0.05	27.78	ND
KTS	0.25	0.04	16.00	0.022	0.014	63.64	0.23	0.02	8.70	ND
KTW	0.02	0.009	45.00	0.002	0.0008	40.00	0.10	0.01	10.00	ND

KTH=Kirinyaga Tetu Gacheru Honey, KTHC=Kirinyaga Tetu Gacheru Honeycombs, KTB=Kirinyaga Tetu Gacheru Bees, KTF=Kirinyaga Tetu Gacheru Flowers, KTS=Kirinyaga Tetu Gacheru Soil, KTW=Kirinyaga Tetu Gacheru Water, ND=Not Detected

Honey samples from Tetu Gacheru station in Kirinyaga district had the highest mean concentration of chromium at 0.89 ppm compared to other samples from the rest of

Kirinyaga district. This high amount can be attributed to industrial effluents from a nearby town. However, the soil samples were not as highly contaminated as the honey samples suggesting that the contamination of honey samples occurred through air pollution.

The soil and flower samples from Tetu Gacheru station were noted as having similar concentrations of chromium and lead. The mean chromium concentrations in flower and soil samples were 0.18 and 0.25 ppm respectively, while the mean lead concentrations were 0.18 ppm in flower samples and 0.23 ppm in soil samples. The presence of these two metals in the soil and the flower samples in approximately equal amounts could be attributed to the presence of a major town near the sampling station.

Table 4.14 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soil samples from Tetu Gacheru obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KTH	0.81	0.02	2.47	0.004	0.001	25.00	0.12	0.02	16.67 ND
KTHC	0.69	0.18	26.09	0.005	0.003	60.00	0.20	0.02	10.00 ND
KTB	0.31	0.10	32.26	0.002	0.001	50.00	0.06	0.01	16.67 ND
KTF	0.10	0.05	50.00	0.009	0.002	22.22	0.10	0.02	20.00 ND
KTS	0.21	0.02	9.52	0.015	0.002	13.33	0.20	0.01	5.00 ND

KTH=Kirinyaga Tetu Gacheru Honey, KTHC=Kirinyaga Tetu Gacheru Honeycombs, KTB=Kirinyaga Tetu Gacheru Bees, KTF=Kirinyaga Tetu Gacheru Flowers, KTS=Kirinyaga Tetu Gacheru Soil, ND=Not Detected

Table 4.14 shows the results of samples from Tetu Gacheru station which were subjected to dry ashing oxidation procedure. The concentration values of chromium, cadmium and lead in all the samples digested using the dry ashing procedure were

lower than those in samples subjected to the wet oxidation procedure. This lowering of the concentration values can be attributed to volatility losses occurring during dry ashing procedure.

Table 4.15 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kiritiini obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KKIH	0.32	0.09	28.13	0.004	0.001	22.50	0.20	0.02	10.00 ND
KKIHC	0.33	0.08	24.24	0.006	0.001	16.67	0.20	0.04	20.00 ND
KKIB	0.25	0.11	44.00	0.005	0.001	20.00	0.11	0.02	18.18 ND
KKIF	0.39	0.02	5.13	0.015	0.006	40.00	0.13	0.07	53.85 ND
KKIS	0.53	0.06	11.32	0.026	0.003	11.54	0.17	0.02	11.76 ND
KKIW	0.02	0.008	40.00	0.001	0.0005	50.00	0.11	0.02	18.18 ND

KKIH=Kirinyaga Kiritiini Honey, KKIHC=Kirinyaga Kiritiini Honeycomb, KKIB=Kirinyaga Kiritiini Bees, KKIF=Kirinyaga Kiritiini Flowers, KKIS=Kirinyaga Kiritiini Soil, KKIW=Kirinyaga Kiritiini Water, ND=Not Detected

Results of heavy metal concentration in samples collected from Kiritiini station are summarized in table 4.15. It was observed that the mean concentration values of chromium in honey and honeycomb samples were similar with values at 0.32 and 0.33 ppm respectively. These concentrations were the lowest compared to similar samples collected from other sampling stations in Kirinyaga district. It was further noted that the mean concentration of cadmium in soil samples from Kiritiini station was 0.026 ppm which was the highest recorded value of cadmium in Kirinyaga district. This high cadmium value can be explained by the fact that Kiritiini is an agricultural area where cadmium based fertilizers are frequently used.

Table 4.16 Concentration levels (ppm) of Cd, Cr, Pb, and Hg in honey, honeycombs, bees, flowers and soils samples from Kiritiini obtained using dry ashing p procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KKIH	0.35	0.05	14.29	0.002	0.001	50.00	0.15	0.01	6.67 ND
KKIHC	0.29	0.07	24.14	0.002	0.0007	35.00	0.19	0.02	10.53 ND
KKIB	0.17	0.09	52.94	0.001	0.0005	50.00	0.09	0.01	11.11 ND
KKIF	0.25	0.12	48.00	0.010	0.002	20.00	0.09	0.02	22.22 ND
KKIS	0.49	0.02	4.08	0.018	0.003	16.63	0.14	0.02	14.29 ND

KKIH=Kirinyaga Kiritiini Honey, KKIHC=Kirinyaga Kiritiini Honeycomb, KKIB=Kirinyaga Kiritiini Bees, KKIF=Kirinyaga Kiritiini Flowers, KKIS=Kirinyaga Kiritiini Soil, ND=Not Detected

Table 4.16 depicts the results obtained after samples from Kiritiini station were subjected to dry ashing oxidation. It was noted that the mean concentration of cadmium in honey and honeycomb samples from this station were equal at 0.002 ppm, but their CV percentages were different with honey being at 50.00 % and honeycombs 35.00%respectively. The difference in the coefficients of variation suggests that the samples experienced different volatility losses during ashing at 450^{O}C.

Table 4.17 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Ishiara obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBIH	0.42	0.09	21.43	0.003	0.001	33.33	1.39	0.80	57.55 ND
MBIHC	0.41	0.05	12.20	0.007	0.002	28.39	1.39	0.83	59.71 ND
MBIB	0.18	0.09	50.00	0.003	0.002	66.67	0.34	0.05	14.71 ND
MBIF	0.51	0.04	7.84	0.010	0.001	10.00	1.17	0.35	29.91 ND
MBIS	0.64	0.05	7.81	0.021	0.001	4.46	1.22	0.65	53.58 ND
MBIW	0.03	0.007	23.33	0.003	0.001	33.33	0.07	0.02	28.57 ND

MBIH=Mbeere Ishiara Honey, MBIHC=Mbeere Ishiara Honeycombs, MBIB=Mbeere Ishiara Bees, MBIF=Mbeere Ishiara Flowers, MBIS=Mbeere Ishiara Soil, MBIW=Mbeere Ishiara Water, ND=Not Detected

Samples from Mbeere district were collected from four station; Ishiara, Kiamsita Hill, Kambati Village and Ciakago. The mean concentrations of the three heavy metals are tabulated in tables 4.17—4.24. As in other districts, mercury was not detected in any of the samples. The highest concentration of chromium, which ranged between 0.03—0.64 ppm, was recorded in soil samples (0.64 ppm) while the lowest concentration levels of chromium was found in water samples (0.03 ppm). Concentration of cadmium was relatively low, the highest concentration being found in Kambati soil samples which recorded 0.039 ppm (table 4.21). It should be noted that the concentration of cadmium in honey samples collected from the four Mbeere stations fall in the range 0.001—0.012 ppm. These values of cadmium are all below WHO and FAO limits. High concentration values of cadmium in soil samples from Kambati area can be attributed to the use of phosphate fertilizers by farmers in this area.

It was observed that of all the samples investigated from Mbeere stations samples from Ishiara station had the highest lead concentration of 1.39 ppm (table 4.17). Sampling from Ishiara station was carried out in a site near a major road. Thus the main source of lead pollution in samples collected from this station is mainly from the burning of leaded gasoline used by motor vehicles plying along this road. Concentrations of lead were higher in honey and honeycomb samples in comparison to soil and flower samples from the same station (table 4.17). It was also observed that although sampling from Kiamsita Hill was carried out in a site far away from the main road, concentration of lead in samples from this region are rather high especially in soil samples where lead concentration was recorded as high as 0.83 ppm (table 4.19). This high lead content in the soil may be due to Jua-Kali activities around this area.

Table 4.18 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soils samples from Ishiara obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBIH	0.39	0.06	15.38	0.001	0.0006	60.00	1.29	0.05	3.88 ND
MBIHC	0.33	0.04	12.12	0.003	0.001	33.33	1.37	0.21	15.33 ND
MBIB	0.13	0.04	30.77	0.001	0.0005	50.00	0.27	0.04	14.81 ND
MBIF	0.47	0.03	6.38	0.006	0.002	33.33	1.17	0.05	4.27 ND
MBIS	0.61	0.04	6.56	0.017	0.001	5.88	1.17	0.51	43.59 ND

MBIH=Mbeere Ishiara Honey, MBIHC=Mbeere Ishiara Honeycombs, MBIB=Mbeere Ishiara Bees, MBIF=Mbeere Ishiara Flowers, MBIS=Mbeere Ishiara Soil, ND=Not Detected

Table 4.18 shows data obtained when samples from Ishiara station were subjected to dry ashing procedure. The trend of the concentration of metals in the samples was similar to those of samples subjected to the wet oxidation procedure except that they were lower. This lowering of metal concentration when dry oxidation procedure was employed can be attributed to volatility losses during the digestion step.

Table 4.19 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kiamsita Hill obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBKHH	0.47	0.08	17.02	0.012	0.001	8.23	0.47	0.07	14.89 ND
MBKHHC	0.28	0.06	21.43	0.010	0.002	20.00	0.50	0.07	14.00 ND
MBKHB	0.14	0.04	28.57	0.007	0.001	14.29	0.13	0.03	23.08 ND
MBKHF	0.46	0.05	10.87	0.018	0.001	5.56	0.80	0.13	16.25 ND
MBKHS	0.55	0.03	5.45	0.018	0.003	16.67	0.83	0.59	71.08 ND
MBKHW	0.02	0.006	30.00	0.002	0.001	50.00	0.01	0.005	50.00 ND

MBKHH=Mbeere Kiamsita Hill Honey, MBKHHC=Mbeere Kiamsita Hill Honeycombs, MbKHB=Mbeere Kiamsita Hill Bees, MBKHF=Mbeere Kiamsita Hill Flowers, MBKHS=Mbeere Kiamsita Hill Soil, MBKHW=Mbeere Kiamsita Hill Water, ND=Not Detected

As was noted earlier, soil samples from Kiamsita Hill station had a relatively high concentration of lead which was recorded at 0.83 ppm as is depicted in table 4.19. This lead content in soil samples was high compared to other samples from the same station, and this can be attributed to the small Jua-Kali sectors found around this area. However, the lead concentration in flower samples was 0.80 ppm and this was similar to the lead concentration found in soil samples (0.83 ppm). This similarity suggests that either the flowers were able to uptake the lead from the soils, or the flowers were

polluted from aerosols coming from the Jua-Kali sectors. Honey samples from Kiamsita Hill station recorded the highest concentration of cadmium at 0.012 ppm compared to samples from Ishiara, Kambati Village and Ciakago which all recorded cadmium at 0.003, 0.004 and 0.007 ppm respectively. Data in table 4.20 portrays the same trend as the data in table 4.19. However, the samples that were subjected to the dry ashing oxidation procedure had lower heavy metal concentrations due to volatility losses (table 4.20).

Table 4.20 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soils samples from Kiamsita Hill obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBKHH	0.42	0.06	14.29	0.009	0.002	22.22	0.42	0.03	7.14 ND
MBKHHC	0.22	0.04	18.18	0.003	0.001	33.33	0.49	0.09	18.37 ND
MBKHB	0.10	0.04	40.00	0.002	0.001	50.00	0.09	0.02	22.22 ND
MBKHF	0.39	0.03	7.69	0.009	0.002	22.22	0.77	0.04	5.19 ND
MBKHS	0.49	0.03	6.12	0.012	0.002	16.67	0.77	0.21	27.27 ND

MBKHH=Mbeere Kiamsita Hill Honey, MBKHHC=Mbeere Kiamsita Hill Honeycombs, MBKHB=Mbeere Kiamsita Hill Bees, MBKHF=Mbeere Kiamsita Hill Flowers, MBKHS=Mbeere Kiamsita Hill Soil, ND=Not Detected

Table 4.21 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Kambati Village obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	
MBKVH	0.27	0.08	29.63	0.004	0.001	16.13	0.50	0.11	22.00	ND
MBKVHC	0.61	0.13	21.31	0.009	0.002	22.22	0.54	0.04	7.41	ND
MBKVB	0.22	0.03	13.64	0.007	0.002	28.57	0.22	0.02	9.09	ND
MBKVF	0.33	0.02	6.06	0.020	0.005	25.00	0.49	0.03	6.12	ND
MBKVS	0.45	0.08	17.78	0.039	0.004	10.26	0.57	0.13	22.81	ND
MBKVW	0.01	0.008	80.00	0.001	0.0002	20.00	0.05	0.01	20.00	ND

MBKVH=Mbeere Kambati Village Honey, MBKVHC=Mbeere Kambati Village Honeycombs, MBKVB=Mbeere Kambati Village Bees, MBKVF=Mbeere Kambati Village Flowers, MBKVS=Mbeere Kambati Village Soil, MBKVW=Mbeere Kambati Village Water, ND=Not Detected

Tables 4.21 and 4.22 show results obtained from samples in Kambati Village station which were subjected to wet oxidation and dry ashing oxidation procedures respectively. It was observed that soil samples from Kambati village station had the highest mean concentration value of cadmium (0.039 ppm) and the lowest mean concentration of chromium (0.27 ppm) compared to other soils samples from Mbeere district (table 4.21). The high cadmium values can be attributed to the use of phosphate fertilizers by farmers in that area. It was also noted that honey, honeycomb and soil samples had similar lead concentrations of 0.50, 0.54 and 0.57 ppm respectively. These close concentrations could be attributed to pollution caused by leaded gasoline from motor vehicles. Table 4.22 exhibits a similar trend in heavy metal concentrations except that the values are lower. This difference in the concentration of the metals is due to volatility during ashing at $450^{O}C$.

Table 4.22 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soil samples from Kambati Village obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBKVH	0.25	0.07	28.00	0.001	0.0007	70.00	0.45	0.05	11.11 ND
MBKVHC	0.58	0.09	15.52	0.007	0.002	28.57	0.52	0.07	13.46 ND
MBKVB	0.17	0.02	11.76	0.003	0.001	16.67	0.19	0.01	5.26 ND
MBKVF	0.21	0.01	4.76	0.010	0.003	30.00	0.42	0.04	9.52 ND
MBKVS	0.32	0.03	9.38	0.031	0.002	6.45	0.49	0.10	20.41 ND

MBKVH=Mbeere Kambati Village Honey, MBKVHC=Mbeere Kambati Village Honeycombs, MBKVB=Mbeere Kambati Village Bees, MBKVF=Mbeere Kambati Village Flowers, MBKVS=Mbeere Kambati Village Soil, ND=Not Detected

Table 4.23 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers, soils and water samples from Ciakago obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBCH	0.41	0.07	17.07	0.007	0.001	12.86	0.35	0.04	11.43 ND
MBCHC	0.49	0.09	18.37	0.007	0.002	28.57	0.36	0.08	22.22 ND
MBCB	0.19	0.04	21.05	0.003	0.002	66.67	0.15	0.03	20.00 ND
MBCF	0.42	0.02	4.76	0.022	0.004	18.18	0.25	0.04	16.00 ND
MBCS	0.55	0.08	14.55	0.034	0.009	26.47	0.31	0.04	13.90 ND
MBCW	0.01	0.006	60.00	0.003	0.001	33.33	0.02	0.006	30.00 ND

MBCH=Mbeere Ciakago Honey, MBCHC=Mbeere Ciakago Honeycombs, MBCB=Mbeere Ciakago Bees, MBCF=Mbeere Ciakago Flowers, MBCS=Mbeere Ciakago Soils, MBCW=Mbeere Ciakago Water, ND=Not Detected

Data for the heavy metal concentration in samples from Ciakago station in Mbeere district is shown in table 4.23. It is observed that honey, honeycomb, flowers and soil

samples from Ciakago have mean concentrations of 0.35, 0.36, 0.25, and 0.31 ppm of lead respectively. These were the lowest recorded levels of lead in similar samples from other sampling stations in Mbeere district. This seems to suggest that Ciakago station was the least polluted area with lead from leaded gasoline or Jua-Kali stations. It was also noted that honey, honeycomb and soil samples from Ciakago station had similar lead concentrations of 0.35, 0.36 and 0.31 ppm respectively probably due to aerosol pollution. Comparing chromium levels in honey, honeycomb and bee samples from Ishiara station to those from Ciakago station, a similarity is observed. Honey, honeycomb and bee samples from Ishiara exhibited chromium levels of 0.42, 0.41 and 0.18 ppm (table 4.17) respectively, while samples from Ciakago station had 0.41 ppm of chromium in honey, 0.49 ppm of chromium in honeycombs and 0.19 ppm of chromium in bee samples.

Table 4.24 Concentration levels (ppm) of Cd, Cr, Pb, and Hg in honey, honeycombs, bees flowers and soil samples from Ciakago obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb		Hg
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBCH	0.38	0.07	18.42	0.005	0.001	20.00	0.32	0.02	6.25 ND
MBCHC	0.40	0.05	12.50	0.002	0.001	50.00	0.31	0.08	25.81 ND
MBCB	0.15	0.03	20.00	0.001	0.0005	50.00	0.11	0.04	36.36 ND
MBCF	0.36	0.02	5.56	0.013	0.003	23.08	0.18	0.03	16.67 ND
MBCS	0.49	0.05	10.20	0.027	0.005	18.52	0.23	0.09	39.13 ND

MBCH=Mbeere Ciakago Honey, MBCHC=Mbeere Ciakago Honeycombs, MBCB=Mbeere Ciakago Bees, MBCF=Mbeere Ciakago Flowers, MBCS=Mbeere Ciakago Soils, ND=Not Detected

The sample concentrations in table 4.24 follow a similar pattern as those in table 4.23. The only difference is that the metal concentrations are lower in table 4.24. This is due to volatility losses occurring during dry ashing.

Table 4.25 Concentration levels (ppm) of Cd, Cr, Pb, and Hg in honey, honeycombs, bees, flowers and soil samples from Mt. Kenya Forest obtained using wet oxidation procedure

Sample Code	Cr			Cd			Pb			Hg**		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
Mt.KH	0.61	0.09	14.75	0.006	0.001	16.67	0.86	0.13	15.12	0.10	0.005	5.00
Mt.KHC	0.65	0.07	10.77	0.011	0.003	27.27	0.92	0.14	15.22	0.11	0.006	5.45
Mt.KB	0.65	0.07	10.77	0.010	0.003	30.00	0.45	0.11	24.44	ND	ND	ND
Mt.KF	0.61	0.05	8.20	0.069	0.005	7.25	0.80	0.15	18.75	0.10	0.007	7.00
Mt.KS	0.77	0.07	9.09	0.075	0.012	16.00	0.87	0.21	24.14	0.10	0.008	8.00
Mt.KW	0.05	0.01	20.00	0.002	0.0005	5.00	0.09	0.02	22.22	ND	ND	ND

Mt.KH=Mt.Kenya Forest Honey, Mt.KHC=Mt.Kenya Forest Honeycombs, Mt.KB=Mt.Kenya Forest Bees Mt.KF =Mt.Kenya Forest Flowers, Mt.KS=Mt.Kenya Forest Soil, Mt.KW=Mt.Kenya Forest Water, ND=Not Detected

** Concentration of Mercury (Hg) is in ppb

Table 4.26 Concentration levels (ppm) of Cd, Cr, Pb and Hg in honey, honeycombs, bees, flowers and soil samples from Mt. Kenya obtained using dry ashing procedure

Sample Code	Cr			Cd			Pb			Hg		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
Mt.KH	0.55	0.07	12.73	0.003	0.001	33.33	0.79	0.10	12.66	ND	ND	ND
Mt.KHC	0.57	0.05	8.77	0.008	0.002	25.00	0.88	0.09	10.23	ND	ND	ND
Mt.KB	0.55	0.05	9.09	0.005	0.002	40.00	0.41	0.12	29.27	ND	ND	ND
Mt.KF	0.52	0.04	7.69	0.052	0.004	7.69	0.73	0.10	13.70	ND	ND	ND
Mt.KS	0.69	0.03	4.35	0.067	0.009	13.43	0.75	0.15	20.00	ND	ND	ND

Mt.KH=Mt.Kenya Forest Honey, Mt.KHC=Mt.Kenya Forest Honeycombs, Mt.KB=Mt.Kenya Forest Bees Mt.KF =Mt.Kenya Forest Flowers, Mt.KS=Mt.Kenya Forest Soil, ND=Not Detected

To act as a control, samples were also collected and analyzed from Mt. Kenya forest where human activities involving use of chromium, cadmium, lead and mercury are minimal. Results from Mt. Kenya forest are depicted in tables 4.25 and table 4.26. Concentrations of cadmium in soil and flower samples from Mt. Kenya forest are high being 0.075 and 0.069 ppm respectively. This may be due to its release from volcanic action, forest fires and from metal enrichment particles from terrestrial vegetation. However, concentrations of cadmium in honey samples from this station is by no means low; being 0.006 ppm. The forest samples also had high levels of lead in the soil and the honey. The source of this lead could be from natural deposits in the forest. Samples of honey honeycombs, flowers and soils also contained mercury in very trace amounts. This trace amount could be the amount of mercury found naturally in nature, hence its small amount in the samples.

From table 4.26 it is evident that the dry ashing procedure caused volatilization losses of mercury in the Mt. Kenya Forest samples. In fact, dry ashing is not recommended for the determination of trace elements such as mercury. The rest of the samples showed a decrease in cadmium, chromium and lead concentrations due to volatilization losses.

As can be seen in tables 4.1 to 4.26, the levels of lead, chromium and cadmium vary from station to station and all their mean concentrations fall within the recommended levels set by the WHO, FAO, and the KBS. However, in Mbeere district, the levels of lead exceed the recommended limit which is set between 0.02—0.6 ppm. The high levels of lead in Mbeere District were mainly encountered in samples collected from Ishiara station. As mentioned before, this sampling site has a major road passing

through it, and combustion of leaded gasoline petrol accounts for the elevated lead levels in this area. Other high lead concentrations levels were also detected in samples from Meru District where sampling sites were near major roads.

Concentrations of chromium, cadmium, lead and mercury were determined in refined honeys bought randomly from Uchumi supermarkets. The results are shown in table 4.27 and table 4.28.

Table 4.27 Concentration levels (ppm) of Cd, Cr and Pb in Refined Honeys obtained after wet digestion

Source of Refined Honey	*Cadmium(ppm)*	*Chromium (ppm)*	*Lead (ppm)*
Kenya	0.032 ± 0.004	0.018 ± 0.001.	0.10 ± 0.004
USA	0.022 ± 0.005	0.027 ± 0.009	0.45 ± 0.004

It is observed that refined honey from Kenya has lower chromium and lead levels compared to American honey. Kenyan honey has higher cadmium concentration. This can be attributed to contamination of the honey during packing.

Determination of the presence of mercury in most of the samples from various stations proved difficult. In fact, of all the areas investigated only the samples from the Mt. Kenya forest proved to have some trace amounts of mercury as follows: Honey 0.1 ppb, Honeycombs 0.11 ppb, Bees, no trace found, Flower 0.1ppb, and the soil had 0.1 ppb. The absence of mercury in samples from other areas can be attributed to the lack of industries which utilize mercury or from natural source. However, some mercury is still expected to occur naturally in the environment, and this was probably the amount observed in samples from the Mt. Kenya Forest.

Table 4.28 Concentration levels (ppb) of mercury in refined honey from Kenya and the USA obtained after wet oxidation procedure

Source of Refined Honey	Concentration of Mercury (ppb)
Kenya	$0.5 \pm 4.97 \times 10^{-2}$
USA	$0.8 \pm 8.6 \times 10^{-2}$

From table 4.28, one can see that honey form USA is more contaminated in mercury than the one from Kenya. The reason for this cannot be inferred directly from the table. These honey samples may be contaminated, but the level of mercury is below that set by the FAO, WHO, and the KBS, which requires foodstuffs to contain less than 0.001 ppm of mercury.

Recovery studies were performed in each of six honey samples from Gaitu station in Meru district. Recoveries of each metal was assessed by adding known quantities of analar grade salts of respective metals to honey samples prior to subjecting them to wet oxidation process. In this way potential losses which may occur during the sample treatment and digestion procedure were avoided. Triplicate determinations were carried out for each sample. The results of recoveries of various heavy metals of interest in this study are given in tables 4.29 to table 4.31. The mean recoveries varied between 95.5 to 100.4 % for lead, 92.5 to 101.5 % for chromium and 98.0 to 100.5 % for cadmium. These values indicate that satisfactory recoveries were achieved for the samples tested.

Table 4.29 Recovery Data For Lead Obtained In Honey by Wet Oxidation

Amount of Salt Added (ppm)	Amount of Lead in Sample (ppm)	Amount Recovered (ppm)	Recovery %
0.0	0.74	0.740	----
0.2	0.74	0.931	95.50
0.4	0.74	1.139	99.75
0.6	0.74	1.342	100.33
0.8	0.74	1.543	100.38
1.0	0.74	1.732	99.20

Table 4.30 Recovery Data For Chromium Obtained In Honey by Wet Oxidation

Amount of Salt Added (ppm)	Amount of Chromium in Sample (ppm)	Amount Recovered (ppm)	Recovery %
0.0	0.30	0.300	
0.2	0.30	0.485	92.50
0.4	0.30	0.675	93.75
0.6	0.30	0.909	101.50
0.8	0.30	1.110	101.25
1.0	0.30	1.297	99.70

Table 4.31 Recovery Data For Cadmium Obtained In Honey by Wet Oxidation

Amount of Salt Added (ppm)	Amount of Cadmium in Sample (ppm)	Amount Recovered (ppm)	Recovery %
0.0	0.004	0.004	
0.2	0.004	0.200	98.00
0.4	0.004	0.405	100.25
0.6	0.004	0.607	100.50
0.8	0.004	0.803	99.88
1.0	0.004	1.002	99.80

4.4 INTERFERENCE STUDIES

The effect of various cation interferences suspected to occur naturally in the honey matrix were investigated by spiking honey samples with 1, 2, 4, 6,8 and 10 ppm of Ca^{2+}, Mn^{2+}, Cr^{2+}, Cd^{2+}, Na^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Fe^{2+} and Pb^{2+} and performing triplicate measurement in solutions with and without the interfering cations. The effect of releasing agent was also assessed by adding 2 % Na_2SO_4 to the sample and observing the concentration of recovered chromium, cadmium and lead. As seen in tables 4.32 to tables 4.34 chromium at concentrations greater than 6 ppm enhanced the concentration of lead significantly while concentrations of chromium less than 4 ppm had no effect on the determination of lead. The addition of 2 % Na_2SO_4 had no effect on the concentration levels of lead in honey samples while also the presence of magnesium, nickel, sodium at all concentrations had no effect on the concentration of lead. Cadmium and iron at concentrations greater than 6 ppm depressed the amount of lead in the sample. The addition of cadmium, sodium, copper and manganese to honey samples had no affect on the determination of chromium. It was observed that the addition of calcium, magnesium nickel and iron depressed the amount of chromium in the sample. At concentrations greater than 6 ppm, this depression in the concentration values was readily observed. It interesting to note that low concentrations of lead (less than 6 ppm) depressed the amount of chromium in the sample, while concentrations greater than 6 ppm slightly enhanced the amount of chromium in the honey (table 4.33). The addition of chromium, sodium, and copper to honey samples depressed the amount of cadmium in the honey samples. The addition of the releasing agent sodium sulfate returned the concentration readings of cadmium in the honey samples back to normal. On the other hand, the addition of lead, calcium, magnesium, nickel and iron

at concentrations greater than 6 ppm enhanced the cadmium concentration in the honey samples significantly (table 4.34).

Table 4.32 Effect of various concentrations levels (ppm) of cations on the determination of lead (ppm) in honey samples

Amount of Lead in Sample	Concentration of Cd added	Recovery with no releasing agent	Recovery with 2% Na_2SO_4	Amount of Lead in Sample	Concentration of Cr added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.43	1	0.45	0.42	0.43	1	0.43	0.43
0.43	2	0.45	0.43	0.43	2	0.43	0.43
0.43	4	0.44	0.43	0.43	4	0.45	0.43
0.43	6	0.44	0.43	0.43	6	0.47	0.43
0.43	8	0.44	0.42	0.43	8	0.47	0.42
0.43	10	0.44	0.43	0.43	10	0.47	0.43

Amount of Lead in Sample	Concentration of Na added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Lead in Sample	Concentration of Ca added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.43	1	0.43	0.43	0.44	1	0.43	0.44
0.43	2	0.42	0.43	0.44	2	0.42	0.44
0.43	4	0.42	0.43	0.44	4	0.42	0.44
0.43	6	0.43	0.43	0.44	6	0.41	0.44
0.43	8	0.43	0.42	0.44	8	0.41	0.43
0.43	10	0.43	0.43	0.44	10	0.41	0.43

Amount of Lead in Sample	Concentration of Cu added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Lead in Sample	Concentration of Mg added	Recovery with no releasing agent	Recovery with 2 % Na2SO4
0.47	1	0.46	0.46	0.47	1	0.47	0.47
0.47	2	0.47	0.46	0.47	2	0.47	0.47
0.47	4	0.49	0.47	0.47	4	0.46	0.47
0.47	6	0.49	0.47	0.47	6	0.46	0.46
0.47	8	0.49	0.47	0.47	8	0.47	0.47
0.47	10	0.48	0.46	0.47	10	0.47	0.47

Amount of Lead in Sample	Concentration of Ni added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Lead in Sample	Concentration of Fe added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.44	1	0.44	0.44	0.47	1	0.46	0.47
0.44	2	0.44	0.44	0.47	2	0.45	0.47
0.44	4	0.43	0.44	0.47	4	0.45	0.47
0.44	6	0.44	0.43	0.47	6	0.42	0.47
0.44	8	0.43	0.44	0.47	8	0.42	0.46
0.44	10	0.44	0.44	0.47	10	0.42	0.46

Amount of Lead in Sample	Concentration of Mn added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.44	1	0.44	0.44
0.44	2	0.44	0.44
0.44	4	0.45	0.44
0.44	6	0.45	0.44
0.44	8	0.44	0.44
0.44	10	0.44	0.44

Table 4.33 Effect of various concentrations levels (ppm) of cations on the determination of chromium (ppm) in honey samples

Amount of Chromium in Sample	Concentration of Cd added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Chromium in Sample	Concentration of Pb added	Recovery with no releasing agent	Recovery with 2% Na_2SO_4
0.52	1	0.52	0.51	0.52	1	0.51	0.51
0.52	2	0.51	0.52	0.52	2	0.51	0.51
0.52	4	0.51	0.52	0.52	4	0.53	0.52
0.52	6	0.52	0.52	0.52	6	0.54	0.52
0.52	8	0.52	0.52	0.52	8	0.54	0.51
0.52	10	0.52	0.52	0.52	10	0.54	0.51

Amount of Chromium in Sample	Concentration of Na added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Chromium in Sample	Concentration of Ca added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.43	1	0.43	0.43	0.51	1	0.50	0.50
0.43	2	0.42	0.43	0.51	2	0.50	0.50
0.43	4	0.42	0.43	0.51	4	0.50	0.51
0.43	6	0.43	0.43	0.51	6	0.49	0.51
0.43	8	0.43	0.42	0.51	8	0.49	0.51
0.43	10	0.43	0.43	0.51	10	0.49	0.50

Amount of Chromium in Sample	Concentration of Cu added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Chromium in Sample	Concentration of Mg added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.51	1	0.51	0.510	0.51	1	0.50	0.50
0.51	2	0.52	0.510	0.51	2	0.50	0.50
0.51	4	0.52	0.510	0.51	4	0.50	0.51
0.51	6	0.52	0.500	0.51	6	0.49	0.51
0.51	8	0.53	0.500	0.51	8	0.49	0.51
0.51	10	0.52	0.510	0.51	10	0.49	0.50

Amount of Chromium in Sample	Concentration of Mn added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Chromium in Sample	Concentration of Ni added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.44	1	0.44	0.44	0.44	1	0.430	0.44
0.44	2	0.44	0.44	0.44	2	0.430	0.44
0.44	4	0.45	0.43	0.44	4	0.410	0.44
0.44	6	0.45	0.43	0.44	6	0.410	0.43
0.44	8	0.45	0.44	0.44	8	0.400	0.43
0.44	10	0.46	0.44	0.44	10	0.400	0.44

Amount of Chromium in Sample	Concentration of Fe added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.47	1	0.45	0.46
0.47	2	0.45	0.46
0.47	4	0.44	0.47
0.47	6	0.44	0.47
0.47	8	0.44	0.46
0.47	10	0.44	0.46

Table 4.34 Effect of various concentrations levels (ppm) of cations on the determination of cadmium (ppm) in honey samples

Amount of Cadmium in Sample	Concentration of Cr added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Cadmium in Sample	Concentration of Pb added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.012	1	0.011	0.011	0.012	1	0.013	0.011
0.012	2	0.011	0.011	0.012	2	0.015	0.011
0.012	4	0.011	0.012	0.012	4	0.015	0.012
0.012	6	0.011	0.012	0.012	6	0.016	0.012
0.012	8	0.011	0.012	0.012	8	0.015	0.012
0.012	10	0.011	0.012	0.012	10	0.015	0.011

Amount of Cadmium in Sample	Concentration of Na added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Cadmium in Sample	Concentration of Ca added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.012	1	0.011	0.012	0.009	1	0.009	0.009
0.012	2	0.011	0.012	0.009	2	0.009	0.009
0.012	4	0.010	0.012	0.009	4	0.010	0.009
0.012	6	0.010	0.012	0.009	6	0.010	0.009
0.012	8	0.010	0.010	0.009	8	0.010	0.090
0.012	10	0.010	0.011	0.009	10	0.010	0.010

Amount of Cadmium in Sample	Concentration of Cu added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Cadmium in Sample	Concentration of Mg added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.009	1	0.008	0.008	0.009	1	0.010	0.090
0.009	2	0.007	0.008	0.009	2	0.010	0.090
0.009	4	0.007	0.009	0.009	4	0.010	0.010
0.009	6	0.007	0.009	0.009	6	0.013	0.010
0.009	8	0.006	0.008	0.009	8	0.013	0.010
0.009	10	0.006	0.008	0.009	10	0.013	0.010

Amount of Cadmium in Sample	Concentration of Mn added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4	Amount of Cadmium in Sample	Concentration of Ni added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.009	1	0.010	0.09	0.009	1	0.009	0.009
0.009	2	0.010	0.09	0.009	2	0.009	0.009
0.009	4	0.010	0.08	0.009	4	0.010	0.009
0.009	6	0.090	0.09	0.009	6	0.011	0.008
0.009	8	0.090	0.09	0.009	8	0.011	0.008
0.009	10	0.090	0.09	0.009	10	0.012	0.009

Amount of Cadmium in Sample	Concentration of Fe added	Recovery with no releasing agent	Recovery with 2 % Na_2SO_4
0.009	1	0.010	0.009
0.009	2	0.011	0.009
0.009	4	0.011	0.010
0.009	6	0.011	0.010
0.009	8	0.012	0.009
0.009	10	0.012	0.009

4.5 CORRELATION STUDIES

According to Microsoft Excel 1997 computer software the correlation coefficient (R_{xy}), which has values between –1 and +1, determines whether two ranges of data move together either positively or negatively. When large values of one set are associated with large values of the other set, or vice versa, there is a positive correlation. On the other hand, when small values of one set are associated with large values from another set, then there is negative correlation. When sets are unrelated, then the correlation is close to zero, and when the samples are completely related the correlation is one.

The correlation coefficient (r_{xy}) is determined by taking the covariance of two data sets, and dividing it by the product of their standard deviations as is shown below.

$$r_{xy} = \frac{COV\ (X,Y)}{S_X S_Y}$$

Where r_{xy} is the correlation coefficient
COV(X,Y) is the covariance of two data ranges X and Y
$S_X S_Y$ are the standard deviations of data X and data Y

Table 4.35 Correlation Coefficient (r_{xy}) for the chromium concentration in honey, honeycombs, bees, soil and flower samples

	Honey	*Honeycombs*	*Bees*	*Soil*	*Flowers*
Honey	1				
Honeycombs	0.566985	1			
Bees	0.803136	0.720373	1		
Soil	-0.12611	-0.32101	0.048376	1	
Flowers	0.017546	-0.23479	0.151119	0.983893	1

Table 4.35 shows calculated correlation coefficients for chromium in various samples. The best correlation between the samples is that of soil and the flowers (r= 0.984). A positive correlation is also observed between the honey and the bees, and between the

honeycombs and the bees. Negative correlations are observed between the honey and the soil samples, between the honeycombs and soil samples, and between honeycombs and flower samples. Poor correlations are also observed between the flowers and honey, and flowers and soils.

Table 4.36 Correlation Coefficent (r_{xy}) for the cadmium concentration in honey, honeycombs, bees, soil and flower samples

	Honey	*Honeycombs*	*Bees*	*Soil*	*Flowers*
Honey	1				
Honeycombs	0.197158	1			
Bees	0.316887	0.311086	1		
Soil	-0.37587	0.244867	-0.28244	1	
Flowers	0.152807	0.593891	-0.12121	0.750096	1

For cadmium the only significant correlation is between soil and flower samples. The rest of the samples have relatively small r_{xy} values and some small negative correlations. Since the r_{xy} values are close to zero one can conclude that there is no correlation between the samples for the metal cadmium.

Table 4.37 Correlation Coefficient (r_{xy}) for the lead concentration in honey, honeycmbs, bees, soils and flower samples.

	Honey	*Honeycombs*	*Bees*	*Soil*	*Flowers*
Honey	1				
Honeycombs	0.997217	1			
Bees	0.949955	0.94082	1		
Soil	0.927035	0.950702	0.850911	1	
Flowers	0.987477	0.99336	0.934934	0.96315	1

For lead, there was significant positive correlation between all the samples. The best correlation however, was between the honey and the honeycombs with a correlation coefficient of 0.997217. The smallest r_{xy} value was 0.850911 and this was between the bees and the soil. Thus, one can conclude that there is definite correlation of lead in all the samples. This can be attributed to the abundance of lead in the environment due to anthropogenic sources.

4.6 COMPARISON OF WET OXIDATION PROCEDURE AND DRY ASHING PROCEDURE

The concentrations of chromium, cadmium, lead and mercury obtained from unrefined honey, honeycombs, bees, flowers, soil and water samples from Meru, Kirinyaga and Mbeere districts are tabulated in tables 4.1 to 4.26. In all the tables a general trend of decreasing concentration of metals is observed when samples are subjected to dry ashing. The reason for this is that the high temperatures used in dry ashing procedure gave rise to volatility metal losses. Thus a need to compare the two methods of oxidation was essential; and this was achieved through the *t*-test.

The *t*-test was performed with the null hypothesis being held to be true, i.e. assuming that the two methods of digestion do not give significantly different results. The t-critical at P = 0.05 was 2.06, and the t-calculated for chromium, lead and cadmium were 3.22, 10.53, and 11.5 respectively. Thus, from the t-test the null hypothesis was rejected and the conclusion was that the two methods of digestion do give significantly different results at the 95 % confidence level.

Comparing the concentration values of chromium, cadmium, and lead in honey samples from this study to other honey samples from different sampling stations in Kenya one notices that the values of the heavy metals are different. For instance, in a study performed by Omoga and Kagwanja (1999), unrefined honey samples from I.C.P.E., Marigat, Machakos, and Kitui were analyzed for cadmium, lead, chromium, zinc and copper. Their concentrations were found to be 0.001, 0.065, 0.005, 0.508 and 0.312 ppm respectively. These concentration values of the heavy metals were different from the data obtained in this study (see tables 4.1—4.26). The difference in the concentration values can be due to the different environmental zones these honey samples were collected.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.0 CONCLUSION

In this study, the concentration levels of cadmium, chromium, lead and mercury were determined in unrefined honey samples from Kirinyaga District, Mbeere District, Meru District and Mt. Kenya Forest. The concentration levels were compared to the recommended limits set by the WHO, FAO, and KBS which are 1) cadmium: 0.008 ppm to 0.010 ppm, 2) chromium: less than one ppm (< 1 ppm), 3) lead: between 0.02—0.6ppm and 4) mercury: less than 0.001 ppm. Generally, it was found that the concentration of these heavy metals in Kenyan honey fell within the recommended ranges (tables 4.2 to 4.24). In Meru district the average concentrations of chromium, cadmium and lead in honey samples were 0.30 ± 0.12 ppm, 0.008 ± 0.003 ppm, and 0.43 ± 0.23 ppm respectively. From Kirinyaga district the values were 0.30 ± 0.12 ppm, 0.007 ± 0.003 ppm , and 0.20 ± 0.06 ppm for chromium, cadmium and lead respectively. Finally in Mbeere district the concentrations were 0.40 ± 0.02 ppm for chromium, 0.007 ± 0.003 ppm for cadmium, and 0.68 ± 0.54 ppm for lead. The concentration levels of the same elements were also determined in refined honeys from Kenya and USA randomly bought from the supermarkets. It was found that refined Kenyan honeys had less chromium and lead concentrations when compared to unrefined honeys. However, concentration of mercury in refined Kenyan honey is more than in unrefined honey. The refined honey from Kenya had 0.032 ± 0.004 ppm of cadmium, 0.018 ± 0.001 ppm of chromium and 0.10 ± 0.004 ppm of lead. Honey from the USA had 0.022 ± 0.005 ppm of cadmium, 0.027 ± 0.009 ppm of chromium and 0.45 ± 0.004 ppm of lead. It was found that unrefined

honeys from Mt. Kenya Forest were contaminated with mercury, while the rest of the unrefined honeys were not. Concentration levels of mercury in unrefined honey from Mt. Kenya Forest, and refined honey from Kenya and the USA were 0.10 ppb, 0.5 ppb and 0.8 ppb respectively.

Other studies performed in this investigation included the comparison of the wet oxidation method versus the dry ashing procedure for sample digestion, interference studies, recovery studies and correlation studies. By performing the *t*-test at P = 0.05 it was established that the two methods of digestion yielded significantly different results at the 95 % confidence level. Thus, the wet oxidation procedure was deemed to be a better method of digestion, and this was reinforced through the recovery studies which gave results of high percentage recoveries. Interference studies showed that most cations at low concentrations did not affect the concentration of chromium, cadmium, and lead in honey but slight changes of concentration of metals in the samples were observed at higher concentrations of the foreign cations.

Finally, with the correlation studies, it was established that the concentration of chromium correlated well only in soil and flower samples. This also applied to cadmium concentrations while for lead, there was positive correlation between all its concentrations values in honey, honeycombs, bees, flower and soil samples.

5.1 RECOMMENDATIONS

Since Kenya is aiming to be fully industrialized by the year 2020 there is going to be an increase in amounts of heavy metals released into the environment. This being the case, it would be strongly advisable that the government takes firmer steps in assuring that the contamination of the environment is reduced to a minimum. As of now, pollution of the environment is increasing, as can be affirmed by the higher heavy metal concentrations of lead and chromium in unrefined honey as compared to refined honeys. This contamination does not only affect honeys, but other food stuffs as well. The pollution of the unrefined honeys, especially with lead, is due to wastes from industries, the Jua-Kali sectors, gasoline emissions from motor vehicles and other anthropogenic sources. Thus, the government, NGOs and the private sector should strive to make the industrial sector more environmental friendly by introducing such measures as recycling of waste products or disposing of metal wastes at the point of production so as to avoid their entrance into the environment.

Finally, the consumers of unrefined honey should be made aware of the possible presence of heavy metal which may pose dangers to health. Armed with this kind of knowledge, the local consumer can make sound decisions on which honey products to purchase and consume which are less contaminated. This will force the producers of honey and other foodstuffs to be more conscientious in their production, and hence the quality of Kenyan honey will improve. Thus, more studies should be performed in the rest of Kenya covering other heavy metals which are also toxic to both humans and animals.

Furthermore, it is also recommended that during the packing of honey products the manufacturers should include in the label of contents of the honey the amounts of minerals, vitamins, and heavy metals in the products. This would ensure that consumers make sound decisions on which products to buy.

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APPENDICES

Appendix I: Concentration levels (ppm) of chromium, cadmium and lead in samples from Meru district obtained using wet oxidation procedure

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MGH	0.30	0.04	13.33	0.003	0.0006	20.00	0.51	0.09	17.65
MKH	0.21	0.06	28.57	0.011	0.002	18.18	0.73	0.01	1.37
MKGH	0.19	0.05	26.32	0.009	0.001	11.11	0.26	0.08	30.77
MCH	0.49	0.04	8.16	0.005	0.001	10.00	0.21	0.04	19.05
MGHC	0.45	0.05	11.11	0.004	0.0005	12.50	0.52	0.18	34.62
MKHC	0.37	0.10	27.03	0.010	0.004	40.00	0.81	0.19	23.46
MKGHC	0.24	0.07	29.17	0.008	0.004	50.00	0.31	0.06	19.35
MCHC	0.22	0.09	40.91	0.005	0.003	60.00	0.22	0.04	18.18
MGB	0.27	0.09	33.33	0.004	0.001	25.00	0.19	0.05	26.32
MKB	0.17	0.04	23.53	0.006	0.001	16.67	0.21	0.02	9.52
MKGB	0.09	0.05	55.56	0.002	0.001	50.00	0.11	0.07	63.64
MCB	0.10	0.01	10.00	0.003	0.001	33.33	0.08	0.02	25.00
MGF	0.39	0.02	5.13	0.010	0.002	20.00	0.20	0.05	25.00
MKF	0.20	0.03	15.00	0.012	0.001	8.33	0.69	0.09	13.04
MKGF	0.09	0.02	22.22	0.018	0.005	27.78	0.18	0.02	11.11
MCF	0.10	0.05	50.00	0.019	0.007	36.84	0.21	0.07	33.33
MGS	0.40	0.04	10.00	0.015	0.001	6.67	0.24	0.08	33.33
MKS	0.22	0.02	9.09	0.025	0.009	36.00	0.77	0.08	10.39
MKGS	0.14	0.03	21.43	0.024	0.006	25.00	0.29	0.03	10.34
MCS	0.20	0.06	30.00	0.029	0.007	24.14	0.24	0.06	25.00
MGW	0.03	0.02	66.67	0.001	0.00056	56.00	0.09	0.02	22.22
MKW	0.01	0.006	60.00	0.002	0.001	33.50	0.25	0.05	20.00
MKGW	0.01	0.006	60.00	0.002	0.001	44.50	0.08	0.05	62.50
MCW	0.02	0.01	50.00	0.001	0.0006	60.00	0.09	0.02	22.22

M-Meru, G-Gaitu, K-Kambandii, KG-Kangoro, C-Chuka, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil, W-Water

Appendix II: Concentration levels (ppm) of chromium, cadmium and lead in samples from Meru district obtained using dry ashing method

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MGH	0.25	0.04	16.00	0.002	0.001	50.00	0.47	0.01	2.13
MKH	0.16	0.02	12.50	0.007	0.002	28.57	0.71	0.02	2.82
MKGH	0.18	0.03	16.67	0.007	0.001	14.29	0.18	0.02	11.11
MCH	0.48	0.05	10.42	0.003	0.001	33.33	0.11	0.02	18.18
MGHC	0.37	0.02	5.41	0.003	0.001	33.33	0.48	0.02	4.17
MKHC	0.30	0.04	13.33	0.005	0.002	40.00	0.75	0.01	1.33
MKGHC	0.18	0.06	33.33	0.003	0.001	33.33	0.27	0.05	18.52
MCHC	0.10	0.02	20.00	0.001	0.001	70.00	0.16	0.02	12.50
MGB	0.20	0.05	25.00	0.002	0.0006	30.00	0.12	0.02	16.67
MKB	0.15	0.04	26.67	0.004	0.001	25.00	0.18	0.05	27.78
MKGB	0.06	0.03	50.00	0.002	0.001	50.00	0.07	0.03	42.86
MCB	0.08	0.01	12.50	0.002	0.001	50.00	0.06	0.01	16.67
MGF	0.25	0.01	4.00	0.008	0.001	12.50	0.18	0.01	5.56
MKF	0.09	0.04	44.44	0.010	0.002	20.00	0.55	0.05	9.09
MKGF	0.05	0.01	20.00	0.015	0.002	13.33	0.11	0.03	27.27
MCF	0.12	0.01	8.33	0.017	0.002	11.76	0.17	0.03	17.65
MGS	0.38	0.04	10.53	0.009	0.002	22.22	0.22	0.07	31.82
MKS	0.18	0.05	27.78	0.018	0.005	27.78	0.71	0.05	7.04
MKGS	0.11	0.04	36.36	0.021	0.002	9.52	0.25	0.02	8.00
MCS	0.20	0.04	20.00	0.022	0.001	4.55	0.21	0.02	9.52

M-Meru, G-Gaitu, K-Kambandii, KG-Kangoro, C-Chuka, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil

Appendix III: Concentration levels (ppm) of chromium, cadmium and lead in samples from Kirinyaga district obtained using wet oxidation procedure

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KSAH	0.59	0.15	25.42	0.007	0.001	14.29	0.23	0.09	39.13
KKH	0.75	0.02	2.67	0.007	0.002	25.43	0.20	0.05	25.00
KTH	0.89	0.04	4.49	0.008	0.002	23.63	0.18	0.01	5.56
KKIH	0.32	0.09	28.13	0.004	0.001	22.50	0.20	0.02	10.00
KSAHC	0.54	0.16	29.63	0.006	0.001	16.67	0.24	0.10	41.67
KKHC	0.60	0.14	23.33	0.007	0.002	28.57	0.22	0.05	22.73
KTHC	0.72	0.27	37.50	0.006	0.004	66.67	0.22	0.03	13.64
KKIHC	0.33	0.08	24.24	0.006	0.001	16.67	0.20	0.04	20.00
KSAB	0.27	0.04	14.81	0.008	0.002	25.00	0.12	0.05	41.67
KKB	0.41	0.09	21.95	0.006	0.001	16.67	0.08	0.05	62.50
KTB	0.37	0.18	48.65	0.005	0.001	20.00	0.05	0.02	40.00
KKIB	0.25	0.11	44.00	0.005	0.001	20.00	0.11	0.02	18.18
KSAF	0.45	0.08	17.78	0.008	0.001	12.50	0.27	0.02	7.41
KKF	0.75	0.04	5.33	0.015	0.002	13.33	0.19	0.02	10.53
KTF	0.18	0.02	11.11	0.016	0.002	12.50	0.18	0.05	27.78
KKIF	0.39	0.02	5.13	0.015	0.006	40.00	0.13	0.07	53.85
KSAS	0.52	0.08	15.38	0.014	0.001	7.14	0.30	0.04	13.33
KKS	0.83	0.08	9.64	0.021	0.003	14.29	0.21	0.04	19.05
KTS	0.25	0.04	16.00	0.022	0.014	63.64	0.23	0.02	8.70
KKIS	0.53	0.06	11.32	0.026	0.003	11.54	0.17	0.02	11.76
KSAW	0.03	0.01	33.33	0.001	0.0005	50.00	0.06	0.01	16.67
KKW	0.02	0.01	25.00	0.002	0.0005	39.00	0.09	0.02	22.22
KTW	0.02	0.01	49.00	0.002	0.0008	40.00	0.10	0.01	10.00
KKIW	0.02	0.008	40.00	0.001	0.0005	50.00	0.11	0.02	18.18

K-Kirinyaga, SA-Sagana, K-Kagio, T-Tetu Gacheru, KI-Kiritiini, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil, W-Water

Appendix IV: Concentration levels (ppm) of chromium, cadmium and lead in samples from Kirinyaga district obtained using dry ashing method

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
KSAH	0.52	0.10	19.23	0.005	0.001	20.00	0.18	0.09	50.00
KKH	0.68	0.02	2.94	0.004	0.001	25.00	0.16	0.04	25.00
KTH	0.81	0.02	2.47	0.004	0.001	25.00	0.12	0.02	16.67
KKIH	0.35	0.05	14.29	0.002	0.001	50.00	0.15	0.01	6.67
KSAHC	0.49	0.05	10.20	0.002	0.001	40.00	0.18	0.04	22.22
KKHC	0.57	0.10	17.54	0.004	0.001	25.00	0.17	0.04	23.53
KTHC	0.69	0.18	26.09	0.005	0.003	60.00	0.20	0.02	10.00
KKIHC	0.29	0.07	24.14	0.002	0.001	34.00	0.19	0.02	10.53
KSAB	0.21	0.02	9.52	0.005	0.001	12.00	0.09	0.04	44.44
KKB	0.33	0.05	15.15	0.002	0.001	25.00	0.05	0.04	80.00
KTB	0.31	0.10	32.26	0.002	0.001	50.00	0.06	0.01	16.67
KKIB	0.17	0.09	52.94	0.001	0.001	50.00	0.09	0.01	11.11
KSAF	0.39	0.05	12.82	0.005	0.001	20.00	0.20	0.01	5.00
KKF	0.66	0.04	6.06	0.009	0.001	11.11	0.11	0.05	45.45
KTF	0.10	0.05	50.00	0.009	0.002	22.22	0.10	0.02	20.00
KKIF	0.25	0.12	48.00	0.010	0.002	20.00	0.09	0.02	22.22
KSAS	0.49	0.07	14.29	0.009	0.001	11.11	0.25	0.02	8.00
KKS	0.77	0.07	9.09	0.013	0.002	15.38	0.19	0.02	10.53
KTS	0.21	0.02	9.52	0.015	0.002	13.33	0.20	0.01	5.00
KKIS	0.49	0.02	4.08	0.018	0.003	16.67	0.14	0.02	14.29

K-Kirinyaga, SA-Sagana, K-Kagio, T-Tetu Gacheru, KI-Kiritiini, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil

Appendix V: Concentration levels (ppm) of chromium, cadmium and lead in samples from Mbeere district obtained using wet oxidation procedure

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBIH	0.42	0.09	21.43	0.003	0.001	16.67	1.39	0.80	57.55
MBKHH	0.47	0.08	17.02	0.012	0.001	8.23	0.47	0.07	14.89
MBKVH	0.27	0.08	29.63	0.004	0.001	16.13	0.50	0.11	22.00
MBCH	0.41	0.07	17.07	0.007	0.001	12.86	0.35	0.04	11.43
MBIHC	0.41	0.05	12.20	0.007	0.002	28.39	1.39	0.83	59.71
MBKHHC	0.28	0.06	21.43	0.010	0.002	20.00	0.50	0.07	14.00
MBKVHC	0.61	0.13	21.31	0.009	0.002	22.22	0.54	0.04	7.41
MBCHC	0.49	0.09	18.37	0.007	0.002	28.57	0.36	0.08	22.22
MBIB	0.18	0.09	50.00	0.003	0.002	66.67	0.34	0.05	14.71
MBKHB	0.14	0.04	28.57	0.007	0.001	14.29	0.13	0.03	23.08
MBKVB	0.22	0.03	13.64	0.007	0.002	28.57	0.22	0.02	9.09
MBCB	0.19	0.04	21.05	0.003	0.002	66.67	0.15	0.03	20.00
MBIF	0.51	0.04	7.84	0.010	0.001	10.00	1.17	0.35	29.91
MBKHF	0.46	0.05	10.87	0.018	0.001	5.56	0.80	0.13	16.25
MBKVF	0.33	0.02	6.06	0.020	0.005	25.00	0.49	0.03	6.12
MBCF	0.42	0.02	4.76	0.022	0.004	18.18	0.25	0.04	16.00
MBIS	0.64	0.05	7.81	0.021	0.001	4.76	1.22	0.65	53.28
MBKHS	0.55	0.03	5.45	0.018	0.003	16.67	0.83	0.59	71.08
MBKVS	0.45	0.08	17.78	0.039	0.004	10.26	0.57	0.13	22.81
MBCS	0.55	0.08	14.55	0.034	0.009	26.47	0.31	0.04	12.90
MBIW	0.03	0.01	21.67	0.003	0.001	33.33	0.07	0.02	28.57
MBKHW	0.02	0.01	29.45	0.002	0.001	50.00	0.01	0.01	50.00
MBKVW	0.01	0.01	78.00	0.001	0.000	20.00	0.05	0.01	18.00
MBCW	0.01	0.01	56.79	0.003	0.001	33.33	0.02	0.01	30.00

MB-Mbeere, I-Ishiara, KH-Kiamsita Hill, KV-Kambati Village, C-Ciakago, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil, W-Water

Appendix VI: Concentration levels (ppm) of chromium, cadmium and lead in samples from Mbeere district obtained using dry ashing method

Sample Code	*Cr*			*Cd*			*Pb*		
	Mean	Stdev	CV %	Mean	Stdev	CV %	Mean	Stdev	CV %
MBIH	0.39	0.06	15.38	0.001	0.001	60.00	1.29	0.05	3.88
MBKHH	0.42	0.06	14.29	0.009	0.002	22.22	0.42	0.03	7.14
MBKVH	0.25	0.07	28.00	0.001	0.001	70.00	0.45	0.05	11.11
MBCH	0.38	0.07	18.42	0.005	0.001	20.00	0.32	0.02	6.25
MBIHC	0.33	0.04	12.12	0.003	0.001	33.33	1.37	0.21	15.33
MBKHHC	0.22	0.04	18.18	0.003	0.001	33.33	0.49	0.09	18.37
MBKVHC	0.58	0.09	15.52	0.007	0.002	28.57	0.52	0.07	13.46
MBCHC	0.40	0.05	12.50	0.002	0.001	50.00	0.31	0.08	25.81
MBIB	0.13	0.04	30.77	0.001	0.001	50.00	0.27	0.04	14.81
MBKHB	0.10	0.04	40.00	0.002	0.001	50.00	0.09	0.02	22.22
MBKVB	0.17	0.02	11.76	0.003	0.001	16.67	0.19	0.01	5.26
MBCB	0.15	0.03	20.00	0.001	0.001	50.00	0.11	0.04	36.36
MBIF	0.47	0.03	6.38	0.006	0.002	33.33	1.17	0.05	4.27
MBKHF	0.39	0.03	7.69	0.009	0.002	22.22	0.77	0.04	5.19
MBKVF	0.21	0.01	4.76	0.010	0.003	30.00	0.42	0.04	9.52
MBCF	0.36	0.02	5.56	0.013	0.003	23.08	0.18	0.03	16.67
MBIS	0.61	0.04	6.56	0.017	0.001	5.88	1.17	0.51	43.59
MBKHS	0.49	0.03	6.12	0.012	0.002	16.67	0.77	0.21	27.27
MBKVS	0.32	0.03	9.38	0.031	0.002	6.45	0.49	0.10	20.41
MBCS	0.49	0.05	10.20	0.027	0.005	18.52	0.23	0.09	39.13

MB-Mbeere, I-Ishiara, KH-Kiamsita Hill, KV-Kambati Village, C-Ciakago, H-Honey, HC-Honeycombs, B-Bees, F-Flowers, S-Soil

Appendix VII: Procedures for the preparation of stock solutions used in this study

a) Chromium Stock Solution

7.695g of analytical grade $Cr(NO_3)_2.3H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

b) Cadmium Stock Solution

2.052 g of analytical grade $CdCl_2 2½H_2O$ of were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

c) Lead Stock Solution

1.599 g of analytical grade $Pb(NO_3)_2$. were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

d) Mercury Stock Solution

1.352 g of analytical grade $HgCl_2$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

e) Calcium Stock Solution

3.688 g of analytical grade $CaCl_2 2H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water

and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

f) Manganese Stock Solution

3.602 g of analytical grade $MnCl_2 \cdot 4H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

g) Copper Stock Solution

3.802 g of analytical grade $Cu(NO_3)_2 \cdot 3H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HNO_3 and the contents brought to the mark. This constituted a 1000 ppm stock solution.

h) Sodium Stock Solution

2.542 g of analytical grade NaCl were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HCl and the contents brought to the mark. This constituted a 1000 ppm stock solution.

i) Magnesium Stock Solution

8.363 g of analytical grade $MgCl_2 \cdot 6H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HCl and the contents brought to the mark. This constituted a 1000 ppm stock solution.

j) Nickel Stock Solution

4.049 g of analytical grade $NiCl_2 \cdot 6H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water

and 2 % HCl and the contents brought to the mark. This constituted a 1000 ppm stock solution.

k) Iron Stock Solution

4.840 g of analytical grade $FeCl_3 \cdot 6H_2O$ were weighed on a filter paper and transferred in a 1000 cm^3 volumetric flask. The salt was dissolved in 10 cm^3 of distilled water and 2 % HCl and the contents brought to the mark. This constituted a 1000 ppm stock solution.

Appendix VIII: Preparation of standard solutions from stock solutions used in this study

A: Cadmium standard solutions:

From the 1000 ppm stock solution, dilutions were carried out to make standards of 1 ppm, 2 ppm, 4 ppm, and 5 ppm. This was achieved by pipetting 0.1 cm^3, 0.2 cm^3, 0.4 cm^3, and 0.5 cm^3 from the stock solution into 100 cm^3 volumetric flasks and filling them to the mark with deionized water to make the corresponding concentrations.

B: Chromium standard solutions

From the 1000 ppm stock solution, dilutions were carried out to make standards of 2 ppm, 5 ppm, 7 ppm, and 10 ppm. This was achieved by pipetting 0.2 cm^3, 0.5 cm^3, 0.7 cm^3, and 1.0 cm^3 from the stock solution into 100 cm^3 volumetric flasks and filling them to the mark with deionized water to make the corresponding concentrations.

C: Lead standard solutions

From the 1000 ppm stock solution, dilutions were carried out to make standards of 2 ppm, 5 ppm, 7 ppm, 10 ppm and 15 ppm. This was achieved by pipetting 0.2 cm^3, 0.5 cm^3, 0.7 cm^3, 1.0 cm^3 and 1.5 cm^3 from the stock solution into 100 cm^3 volumetric flasks and filling them to the mark with deionized water to make the corresponding concentrations,

D: Mercury standard solutions

From the standard 1000 ppm stock solution a standard solution of 10 ppm was prepared by pipetting 1 cm^3 of the standard solution into a 100 cm^3 volumetric flask and diluted to the mark. The 10 ppm solution was used to prepare 0.05 ppm, 0.1 ppm, and 0.15 ppm standard solutions. This was achieved by pipetting 0.5 cm^3, 1 cm^3 and 1.5 cm^3 of the 10 ppm stock solution into 100 cm^3 volumetric flasks and filling them to the mark with deionized water.

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