DETERMINATION OF HEAVY METALS IN KENYAN CIGARETTES, TOBACCO LEAVES AND INTERCROPPED PLANTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY.

BY

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DECLARATION

I declare that this thesis is my original work and that it has not been presented to any other University for the award of a degree.

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This thesis has been submitted with my approval as University Supervisor.

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DEDICATION

This work is dedicated to my dear parents, brothers and sisters without whose understanding, patience and encouragement this work would not have been completed.

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ABBREVIATIONS

AW - Ash weight

FW - Fresh weight

DW - Dry weight

CN - Cyanide

LL - Large leaved

ABSTRACT

Recent studies have indicated an increasing concern for the role that cadmium and lead play or are suspected to play in biological and physiological disorders such as emphysema of the lungs, renal tubular damage, cancer and cardiovascular diseases. Studies have revealed that cigarettes contain some heavy metals and that smoking involves the inhalation of small amounts of metals such as cadmium and lead. Since absorption from the lungs is usually complete, heavy smokers tend to absorb more of these metals. Given that both metals are cummulative in nature with long biological half-lives, smokers accumulate twice the amount accumulated by non-smokers.

The study set out to find out whether Kenyan smokers (passive and active) are exposed to high levels of these heavy metals, to compare the levels in the cigarette tobacco with those in fresh tobacco leaves and to compare the levels in the fresh tobacco leaves with those in other crop plants grown in the same locality.

In this study levels of cadmium, lead and zinc were determined in fresh, cured/processed tobacco leaves, soil and

other crop plants growing in the same locality. Levels in eight Kenyan cigarette brands currently in the market were also determined. For this the Spectr AA-10 Varian Atomic Absorption Spectrophotometer (AAS) (Varian Techtron Pty. Ltd. Australia) was utilized. The results reveal that the cadmium levels in Kenyan cigarettes compare well with those reported in the literature; However, the levels of lead and zinc were found to be higher than those reported in the literature. Also evident is the fact that the levels of the metals in cigarettes are higher than in the fresh tobacco leaves. This may indicate contamination during processing.

The results also show that there is no significant differences in cadmium and lead levels in fresh tobacco leaves and in the cured/processed leaves, thus the curing process does not seem to affect the metal levels in the tobacco leaves. This is however not the case with zinc. On the contrary the levels of zinc in fresh leaves are significantly higher than in the cured/processed leaves. Therefore the curing process seems to lower its levels. The levels of these heavy metals in other crop plants are either higher or lower than those in tobacco leaves.

CHAPTER 1

1.0: INTRODUCTION

The development of metal-based industries has contributed a great deal to contamination of the environment with heavy metals. As a result of this contamination, heavy metals have found their way into food-chains through water, land and atmospheric systems. There are a number of routes through which heavy metals reach the environment; in agriculture they are used as fertilizers and pesticides, and at home as water softeners, disinfectants, beauty aids, cleaners and insecticides. Some metals such as lead are utilized in industrial processes and in the transport sector as fuel additives.

The problem with heavy metals is that they are toxic to biological organisms and tend to accumulate in the environment. In fact they have been found to be responsible for a number of well publicised health disasters[1]. Therefore, accumulation of metals and their compounds in the environment is of great concern to researchers.

There are numerous studies[2] which have been carried out on the effects of metals on various biological processes. It has been found that most metals tend to exert toxic effects

when present in excess[3], but when present in low concentrations, they have important roles in metabolic processes. Metals of this type are said to be essential. Others are toxins even at trace levels and yet have no known biological function. They are generally referred to as non essential.

The levels at which metals become toxic depend on two factors. First is the role the particular metals play in biological processes. Secondly, their effects on other essential elements. Concerning the role a metal plays, it is known that the toxicity of a metal and its compounds is influenced by a number of factors. These include: the metal's intrinsic toxicity, its form of entry, its combining capacity and the ability of the biological system to absorb and transport it to the target organ most susceptible to metal intoxication[3]. Other factors include the capacity of the metal to undergo biotransformation to a less or more toxic form at the target organ or during transfer, its ability to bind to the essential macromolecules and the homeostatic mechanisms of the organism to excrete or sequester the metal.

Some of the more sensitive and susceptible activities and systems affected by metal intoxication are: the structure and function of nucleic acids and proteins, permeability of the membranes of the cells and subcellular organelles,

release of potent substances such as histamine and biosynthetic formation of hormones.

As far as the effects on other essential elements is concerned, toxic metals basically tend to compete with essential elements for naturally occuring chelating molecules. These chelating molecules when in association with essential elements play specific important functions. The most important of these is the regulation of metal ion concentration[4]. Potentially harmful metals with physicochemical properties similar to the essential ones compete with the latter for chelates, thus preventing their normal functioning by forming irreversible and stable bonds. For instance iron, an essential element, becomes biologically very active by incorporation into the porphyrin nucleus. By competition for active sites, a potentially harmful or toxic element such as cadmium might become toxic in an analogous way[4].

Unlike animals, plants can accumulate elements especially heavy metals in or on their tissues[5]. This is due to their great ability to adapt to variable chemical properties of the environment. This makes plants to be important intermediate reservoirs through which trace elements from soils and partly from waters and air move to animals and especially to man. For example, vegetables when

consumed by man can act as a source of these metals. Therefore, accumulation of high quantities of metals in plant parts that can be used as food or beverage is an important environmental problem. This form of metal pollution has potential biological and health effects some of which are described in section 1.0.2 of this thesis.

1.0.1 ESSENTIAL ELEMENTS

As has been observed in section 1.0, essential elements even in small amounts play important roles as far as healthy plant and animal life is concerned. They are in fact the inorganic counterparts of essential biological organic nutrients namely, vitamins and proteins. Some like zinc (Zn) and copper (Cu) are known to be components of enzymes which play key roles in the growth and health of both plants and animals. For instance, Zn is a component of the enzymes alkaline phosphatase, carboxy peptidase and alcohol dehydrogenase among others. Copper on the other hand is a component of the enzymes tyrosinase, ascorbic acid oxidase, uricase and cytochrome oxidase. Because of the importance of essential metals we will now briefly discuss some of them.

Potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) are quite abundant in biologic systems. These metals have been shown to play vital roles in maintaining the

electrical balance in the body, as well as in transportation of essential metabolites across cell membranes[6,7]. Indeed, it is known that maintenance of cell integrity depends largely on the presence of Ca and Mg.

Copper also plays an important role in oxidation processes that occur in both plants and animals[7]. It is involved in mobilization of iron from the liver[8] and is necessary for blood formation. It also plays a role in elastin and collagen formation in animals[9]. Its deficiency in animals is known to lead to ruptured aorta. Diseases such as cirrhosis, Wilson's, thalassemia, tuberclosis and carcinomas are characterized by increased liver Cu and decreased serum Cu[3,10]. On the other hand patients suffering from Hodgkin's disease, leukemia and hyperthyroidism have shown increased levels of serum Cu[3,11].

Another important metal is iron. Iron is essential to all living organisms. In the body, it is found bound to proteins as either porphyrins or as heme compounds in hemoglobin and myoglobin[3]. These are known oxygen carriers[3,12]. It is also found as non-heme protein complexes in ferritin, transferrin and hemosiderin which act as iron transport and storage proteins[3]. Iron containing enzymes such as cytochromes in mitochondria[13] are involved in electron transport[14] while catalases in red blood cells[3] are involved in peroxidase

breakdowns. Even though iron is an essential element, it also has a wide margin of toxicity. Ingestion of iron salts in excess of one gram can produce severe toxic reactions. An excess of two grams has been found to show fatal effects within six hours in children[3,8].

Manganese (Mn) is another important metal that is required in trace amounts. It is necessary for normal reproductive function in mammals and poultry[3]. Basically Mn is involved in enzymatic reactions related to metabolism of organic acids, carbohydrates, nitrogen and phosphorous[3]. Plants require it for photosynthesis[3,15]. About 3-7 mg of Mn is ingested daily in a well-balanced diet. However, in high doses, it is toxic. Symptoms of poisoning in man include psychomotor instability, hallucinations and limb rigidity.

Molybdenum (Mo) is present in all marine as well as land plants and animals[3,15]. Normally it is required in trace amounts to all organisms except for algae. It is a component of the enzymes xanthine oxidase[16], aldehyde dehydrogenase[17], nitrate reductase[18] and nitrogenases[19]. It has been postulated that Mo containing enzymes play a role in inorganic sulfate metabolism[20]. High doses however, result in disruption of phosphorous metabolism leading to bone fracture, joint abnormalities[21] and thyroid malfunction in animals[22].

Selenium (Se) is found in both marine and terrestrial plant and animals[3]. Its deficiency in humans has not been clearly established. However, there is some evidence that its deficiency may be a complicating factor in certain types of kwashiorkor in children[3]. It is known to be toxic to animals even in relatively low concentrations. Chronic intoxication is manifested by dullness, lack of vitality and emaciation of the animal. Hair loss, soreness of joints and sloughing of hooves are also some of the symptoms. In acute selenosis, animals suffer from blindness, abdominal pain and some degree of paralysis, and death due to respiratory failure, starvation and thirst[3].

Chromium (Cr) is considered an essential element whose deficiency leads to glucose intolerance[23]. It is involved in cholesterol synthesis[3]. In its high oxidation states (+4 and +6), Cr is very toxic[24]. It causes bronchitis, dermatitis, and cancer in both man and other animals[3,25]. Absorption of alkali dichromates leads to renal damage[3].

Unlike vitamins and proteins, essential metals cannot be synthesized by living organisms. Their only source is the environment[26]. For animals and particularly humans, these are obtained through consumption of natural food and other food products that contain these metals. It is therefore necessary that foods and especially vegetables that are consumed in

large quantities contain some amount of these essential metals.

1.0.2 NON ESSENTIAL ELEMENTS

As already explained in section 1.0, non essential elements are those with no known biological function. These include heavy metals such as arsenic (As), mercury (Hg), silver (Ag), lead (Pb), antimony (Sb) and cadmium (Cd). Food and non-food plants grown on contaminated soil tend to take up high levels of heavy metals. Such foods when consumed result in metal accumulation in certain body organs where they cause biological as well as physiological disorders owing to their toxicity.

Their toxicity is attributed basically to the role they play in biological processes as well as the way they interact with essential elements both at intestinal and organ level[4].

Arsenic is a cumulative protoplasmic poison[3]. Trivalent arsenals react with sulfhydryl groups in cells and so inhibit sulfhydryl containing enzyme systems which are essential to cellular metabolism. Workers exposed to As-containing dusts have been found to develop dermatitis and conjunctivitis as well as myocardial damage[3,27].

Mercury is quite toxic. Inhalation of its vapor even in minimal concentrations is hazardous. Inhaled Hg rapidly leaves the lungs for the circulatory system. Studies have demonstrated the rapid biotransformation *in vitro* of Hg from the elemental form to the mercuric ion[3]. Following exposure, it is partly distributed in the blood as mercury vapor and so penetrates the blood-brain barrier more readily[3]. Chronic exposure results in permanent injury to the brain[3,28] and hence paralysis and loss of mental function[3,29]. It is an intense kidney poison[3] which also causes pneumonitis[3].

Antimony is another toxic metal which has been known since approximately 4000 BC. Symptoms of acute poisoning are similar to those induced by As, although compounds of the former are more caustic than of the latter. Industrial exposures to Sb can result in irritation of the skin, mucous membranes of the mouth, nose and throat. Eczema and other forms of dermatitis occur. Pneumonitis has also been reported[3].

Silver has no known function in plant and animal metabolism. It is toxic, though to a lesser extent when compared to As, Sb, or Pb. The metal inactivates sulfhydryl enzymes and also combines with amino, imidazole, carboxyl and phosphate groups. Since Ag is absorbed slowly its systemic action is not extensive[3]. It causes argyria, a grey to bluish

pigmentation on the skin.

Lead may be absorbed into the body by ingestion, inhalation and through the skin[4,30]. It is a general protoplasmic poison that is cumulative, slow acting and subtle. It produces a variety of symptoms such as encephalopathy, lead colic, arthralgia and paralysis. Like other heavy metals it has an affinity for sulfur.

Cadmium is known to be toxic to almost all living systems[31,32] particularly at high doses. It inhibits sulfhydryl enzymes and has an affinity for other ligands in cells such as hydroxyl, carboxyl, phosphatyl, cysteinyl and hystidyl side chains of proteins, purin and porphyrin[3]. It can disrupt oxidative phosphorylation pathways and has been shown to interfere with the metabolism in bones of rats which were fed with calcium-deficient diet[33].

This study will only be concerned with cadmium, lead and zinc. The levels of these three heavy metals in tobacco leaves, intercropped plants and Kenyan cigarette brands currently available on the market will be determined. Instead we will briefly describe tobacco. The economic importance of tobacco and how cigarette smoking contributes to ingestion of Cd, Pb and Zn will be discussed. A description of the chemistry, occurrence, uses and biochemical roles of these

heavy metals is presented in chapter two of this thesis.

1.1. TOBACCO AND ITS PLACE IN THE WORLD.

It is known that the toxicity of a metal depends among other factors on the mode of entry. A number of studies have shown that for most toxic metals, entry into the body through inhalation is of more importance than ingestion. It is also known that plants are the main intermediate reservoirs through which trace elements from soils and partly from waters and air move to man and animals. Man consumes plants for food, medicinal and beverage purposes basically by ingestion.

There are however, some plants that do not fall in this category. An example is tobacco which is not used for food purposes. It is a plant whose leaves are mainly consumed in three ways; smoking, chewing and inhalation of its dust (snuff). Its leaves contain among other substances, nicotine which is addictive. Consumption of tobacco is also believed to lead to diseases such as cancer, and respiratory problems. Despite this, tobacco leaves have had and continue to have a tremendous impact on the world.

The botanical genus *Nicotiana* contains over 64 species, yet the only one which has never been found growing wild is

Nicotiana tobacum. Its various guises are collectively called tobacco.

Tobacco holds an unparalleled position among crop plants in several individual particulars[34]. It is one of the few crops that enters the world market entirely on a leaf basis. It is also the most widely grown commercial non-food plant in the world. In many countries, Kenya included, it is an instrument of very high importance in financial and economic policy. Overall, the economic status achieved by this single non food crop is remarkable.

Tobacco leaf has always had a high unit value, which is important on its own, but also enables it to bear transport costs that would cripple other commodities. This position has been maintained very well and even relatively improved from time to time *vis-a-vis* other crops.

Tobacco occurs in about seven varieties namely: Flue-cured, dark air-cured, light air-cured, fire-cured, burley and oriental tobacco. In Kenya three varieties of tobacco are grown for commercial purposes. These are the burley, flue and fire-cured tobacco grown mainly in the regions of Eastern, Western and Nyanza provinces. Other regions include Central province and Rift Valley which grow the indigenous or traditional varieties that are used for chewing and making

snuff.

Another point of economic importance is the fact that the most widely grown sorts, flue-cured and oriental cigarette tobacco, are highly suited to coarse-textured soils of low inherent fertility which often yields relatively poor returns from any other crop. It is therefore likely for tobacco to dominate any locality in which it becomes established. When this is the case, it becomes responsible for the growth of an agricultural community whose fortunes vary directly with those of the tobacco crop. In certain parts of Kenya this scenario is quite evident. This is especially so in Siakago and Malakisi. Exceptions are the fire-cured and dark-air-cured zones where the soils are suited to other forms of profitable agriculture. In Kenya fire-cured and air-cured (burley) tobacco are confined to a few farmers in Western and Nyanza provinces.

Flue-cured or bright cigarette tobacco is used mainly for cigarette making and is consumed less in pipes or chewing. The dark-fire-cured tobacco on the one hand is used principally in snuff, chewing tobaccos and pipe mixtures. On the other hand burley is used mainly for blending cigarette tobacco and less for chewing.

1.1.1: Smoking of the cigarette.

During a puff, the periphery of the cigarette burns faster than the apex. The maximum temperature attainable at the periphery is about 900 ^{0}C when a cigarette is puffed. The recorded temperatures in the centre of the coal (some 8-10 mm from the line of the paper burn) exceed 800 ^{0}C[34]. This coal temperature has a significant effect on smoke composition[35]. The higher the coal temperature the more gaseous the smoke and the less particulate it is.

The actual combustion of a single cigarette is dependent upon several influences. These include width of tobacco cut, density of cigarette, moisture content of the tobacco, diameter of the cigarette, shape of the cigarette (round or oval), permeability of the paper used (increase in permeability results in decrease in coal temperature), filter dilution or ventilation (increase in dilution decreases coal temperature) and puff volume (with higher volumes increasing coal temperature). Cigarette smoke penetrates deep into the lungs and reaches the small airways and alveoli. The fraction of smoke deposited is high because most smokers employ some breath-holding following inhalation of a puff. Their attempt to enhance deposition of smoke results in increased lung burdens of toxic smoke products. Non-smokers are also exposed

to tobacco combustion products when other people smoke. These are termed as passive or involuntary smokers. Studies estimate that non-smokers exposed to passive smoke absorb an equivalent of 0.1-1.0 cigarrete per day[36].

Tobacco smoke in the environment is derived from two sources; Mainstream (MS) and Sidestream (SS). Mainstream smoke emerges into the environment after having first been drawn through the cigarette which filters some of the active constituents. The smoke is then filtered by the smokers own lungs and exhaled. Sidestream smoke arises from the burning end of the cigarette and enters directly into the environment. Differences in the temperature of combustion, the degree of filtration and the amount of tobacco consumed all lead to marked differences in concentration of the constituents of MS and SS smoke[37]. Many potentially toxic gas phase constituents are present in higher concentrations in SS than in MS smoke.

The particulate phase of cigarette smoke, commonly known as tar is inhaled as an aerosol into the smoker's respiratory tract. This aerosol contains ambient air as well as the gases, liquids and solids produced during tobacco combustion. The particulates include a variety of organic and metallic compounds many of which are toxic to lung tissues. Some of the metallic compounds include cadmium chloride and lead

compounds.

In literature Cd concentrations of ~0.5-5 μg/g are reported[38]. Scherer *et al*[38] found that German cigarette tobacco contained ~0.5-1.5 μg Cd/cigarette. In Romania Kovats and Silvia[39] found mean Cd concentrations of 18 different cigarette trademarks to be 1.75 μg Cd/cigarette, while that of 21 commonly smoked Swiss[40] cigarette brands with filters was ~1.2 μg Cd/cigarette or 1.7 μg Cd/g. The mean metal content of 13 brands of cigarettes in the Italian[41] market ranged from traces to 0.585 μg Cd/g, traces to 7.39 μg Pb/g and 0.03 to 25.74 μg Zn/g. On the basis of a small number of available measurements the W.H.O.[42] has estimated the Pb content of tobacco to be in the range 2.5 to 12.2 μg/cigarette. In Kenya the levels of these metals in cigarettes has not been reported. It is therefore the intention of this work to fill this gap. This arises out of the realization that for developing countries such as Kenya, cigarette smoking especially among the youth has been increasing. This has been reflected in an upsurge in tobacco related diseases inspite of its economic importance. It is therefore important that levels of metals in Kenyan cigarettes is determined in a bid to find out whether these levels conform to the W.H.O. standards, and whether tobacco users and involuntary smokers are exposed to unnecessarily high levels of these metals.

1.2: OBJECTIVES OF THE STUDY

To achieve these aims, this work was set out with the following objectives:

1. Determine levels of cadmium, lead and zinc in tobacco leaves and other crop plants grown in the same locality and in the soil.

2. Determine levels of cadmium, lead and zinc in eight cigarette brands currently on the Kenyan market.

3. Compare levels in the tobacco with those in other crop plants grown in the same locality in a bid to find out if tobacco accumulates more of these metals than the latter.

4. Compare levels in cigarettes with those in tobacco leaves.

5. Compare levels in Kenyan cigarettes with those reported in the literature.

6. Compare the levels in the cured and fresh tobacco leaves

to find out whether or not the method of curing affects the levels.

To achieve these objectives, samples were obtained from some parts of Western, Eastern, Central and Rift Valley provinces. These were treated and analysed as is described in chapter three. The results and discussion are presented in chapters four and five respectively.

CHAPTER 2

2.0: LITERATURE REVIEW.

This chapter discusses the chemistry, uses, occurrence and biochemical roles of the heavy metals of interest. We shall begin with zinc.

2.1: ZINC

2.1.1: Chemistry of zinc.

Zinc is a group 2B element with electronic configuration $[Ar]3d^{10}4s^2$. It boils at 907°C[43]. It is not regarded as a transition element because it forms compounds in which the d-shell is full. However, it resembles the latter in its ability to form complexes particularly with ammonia, amines, halides, cyanides and many other ligands. Even with CN-, a strong ligand according to the spectrochemical series, the possibility of $d\pi$-bonding between the metal and the ligand is very much lowered compared to the d-transition elements, owing to its electronic structure. Also no carbonyl, nitrosyl or olefin complexes of the type given by the latter is known. This is true also for cadmium[44]. Since Zn^{2+} and Cd^{2+} have completed d-shells their stereochemistry is determined solely by considerations of size, electrostatic forces, and covalent

binding forces. Due to the effect of size Zn^{2+} is less likely to assume a coordination number of 6 compared to Cd^{2+}, eg. ZnO crystallizes in lattices where the Zn^{2+} ion is in tetrahedral holes surrounded by four oxide ions, whereas CdO has a rock structure.

Zinc does not exhibit variable valency and many of its compounds are white. The most stable oxidation state is +2.

2.1.2: Sources and uses of zinc.

Zinc is obtained from Zn ores such as zincite, franklinite and calamine. It has various industrial uses. The most ancient being the manufacture of bronze and brass. It is employed as a coating on iron and steel. Its sheets are utilized for building purposes and in the manufacture of dry batteries. It is also used in lithography and in viscose rayon manufacture.

Zinc salts are astringent(bitter), mildly antiseptic and corrosive in action. Because of this they have limited application in medicine. Zinc chloride, a soluble salt, is more irritating than the oxide. The oxide, stearate, oleate and sulfate are incorporated into lotions for the relief of puritic conditions. Zinc acetate is employed as a styptic (a

substance used to stop bleeding), while zinc sulfate has been utilized as an emetic[3](a substance used to induce vomiting).

2.1.3: Occurrence of zinc in the environment.

2.1.3.1: Zinc in soil, air and water.

The average concentration of zinc in soils ranges from 50-100 $\mu g/g$[45], though higher values have been reported[5] elsewhere in cases of contamination. The solubilization of Zn minerals during weathering produces mobile Zn^{2+}, especially in acid oxidizing environment. This accumulates in the surface horizon in most soil types. Mean total Zn contents in surface soils of different countries and that of the U.S. range from 17 to 125 $\mu g/g$[5]. The Zn balance in surface soils of different ecosystems shows that the atmospheric input of this metal exceeds its output due to both leaching and the production of biomass.

Zinc dust may find its way into the air from industries manufacturing zinc. It may enter waters from leaching of contaminated soils. The W.H.O. maximum permissible level is 15 $\mu g/g$[46].

2.1.3.2: Zinc in plants.

Soluble forms of Zn are readily available to plants and its uptake has been reported to be linear with concentration[5]. It is an essential element for plant growth. Normal Zn levels in plants are 25-150 μg/g with concentration above 400 μg/g being toxic[47]. In vegetables the normal levels have been reported as; cabbage leaves 2.2 μg/g, potato tubers 3.4 μg/g and in carrots undetected (FW)[48]. Values of 64 μg/g in spinach, 24 μg/g in tomatoes have also been reported[49].

The rate of Zn absorption differs greatly among both plant species and growth media. This is evident from Table 2.0[50] which shows the mean Zn content of vegetable samples from Kiserian and Limuru in μgg^{-1}.

Table 2.0[50]: Mean zinc content for vegetable samples in μg/g.

Plant	Kiserian	Limuru
Pumpkin leaf	17.2	27.06
Spinach	8.84	30.38
Amaranthus (L.L)	8.13	42.3
Kale leaf	6.58	14.84
Soil	106.90	243.4

2.1.3.3: Zinc in man.

A normal human body contains between 1.4 and 2.3 g of zinc[51]. The element is present within all body cells. The highest concentrations in normal tissue are found in the eye. Adult human diets supply about 5-22 mg Zn/day, but most mixed diets supply up to 15 mg/day[52]. Engel *et al*[53]. observed daily zinc intakes ranging from 4.6 to 9.3 mg/day and suggested that 6 mg Zn/day is adequate for normal needs of pre-adolescent girls. In the U.S. the daily recommended allowance is 5 mg for infants, 8 mg for children of four years and above, and 15 mg for pregnant or lactating women[54].

For zinc, it has been established that levels between 150 and 600 mg/day are toxic to man[55]. An excess of it in the body results in reduced growth, anaemia, poor production and reduced activity of the liver catalase and cytochrome oxidase. Acute poisoning leads to vomiting, dehydration, nausea, electrolyte imbalance, dizziness and muscular incoordination[3]. Unlike other heavy metals Zn is not cumulative.

2.1.3.4: Absorption and excretion of zinc.

From the total dietary intake of zinc, 5-10% is usually absorbed[56]. This is however, dependent upon dietary composition. The main part where absorption occurs is the duodenum. The intestines also serve as the major excretory organ of ingested Zn[56]. A small amount is excreted in the urine [15]. Significant quantities are also lost in sweat[57].

2.1.3.5: Interaction with other elements.

Zinc is relatively active in biochemical processes. It is known to be involved in several biological and chemical interactions with a number of elements. Zinc-cadmium interactions appear somewhat controversial. Some reports indicate there is both antagonism and synergism between these elements in the uptake-transport processes. Kitagashi and Yamane[57] explained the observed synergism in terms of Cd competition for Zn active sites. Other findings show[5] antagonism between these cations in the uptake-transport process.

Zinc-copper antagonistic interactions have also been observed. This is thought to indicate same carrier sites in absorption mechanism of both metals[6].

Zinc-iron antagonism is apparently similar to the depressing effects of other heavy metals on Fe uptake. Iron has been found to decrease Zn absorption and the toxicity of Zn that is absorbed[5].

Zinc-calcium and zinc-magnesium interactions vary for a given plant and media. Other factors such as pH control the antagonistic and synergistic character interactions between these elements.

2.2: CADMIUM

2.2.1: Chemistry of cadmium.

Cadmium is a group 2B element below zinc and above mercury in the periodic table. It is a volatile metal with a boiling point of 765°C[58]. Its electronic configuration is [Kr] $4d^{10}$ $5s^2$. The energy for promotion of its electron from s to p orbital is less than that of Zn and Hg. Therefore it can easily be excited to valence state followed by formation of two collinear covalent bonds[58]. Valencies higher than two are not formed presumably because removal of more electrons would destroy the symmetry of a completed d-shell.

Cadmium has an ionic radius similar to that of calcium but it is more polarizable than the latter because of its

greater number of electrons i.e the $4d^{10}$ electrons of Cd do not shield the nucleus as well as the p-electrons of Ca[59]. Its stable oxidation state is +2 but +1 Cd complexes are known[59,60]. Cadmium forms stable compounds with many anions including halides and pseudo-halides such as cyanides[16]. Some of its compounds resemble those of zinc and to a lesser extent those of mercury[59,60]. Complex cations with ammonia and amine ligands are well-defined. Some neutral complexes are formed with chelating agents. For instance it forms complexes with agents such as EDTA, dithizone, and 8-hydroquinoline[59,61]. Complexes of Cd from two to eight coordination number are known[59], although most stable complexes are those in which the coordination number is either four or six. The relative strength of most Cd complexes is similar to that of Zn complexes except with soft ligands such as I^- and SeO_3^{2-} [59] which are stronger than those of Zn. Cadmium forms stronger complexes with sulfur containing compounds particularly those with sulfhydryl groups[59,61].

2.2.2: Sources and uses of cadmium.

Cadmium is extracted during the roasting of Zn ores with sodium chloride. Its chloride, formed during the roasting of the ores, is volatile, and therefore is easily recovered from smelting process.

Thin films of Cd are sometimes applied by electroplating to ferrous metal surfaces to provide a coating that is resistant to corrosion by alkalis, salt water or the atmosphere. It is an important constituent of various types of alloys especially bearing metals and solders. It is used in the manufacture of standard Weston cells, alkaline batteries and is used in the control of atomic fission in atomic reactors. Cadmium sulfide is used as a pigment while the halides are used in photography, lithography and process engraving. Some Cd salts have fungicidal properties and are used as antiseptics or astringents, a substance used to reduce swelling, eg. $CdSO_4$. The oxide is utilized in glass manufacture, in ceramic glazes as well as in the manufacture of silver alloy.

2.2.3: Occurrence of cadmium in the environment.

2.2.3.1: Cadmium in air, water and soil.

Due to its wide industrial use, Cd contamination is found in air, water and soil. The mean background levels of Cd in air is estimated to be between 0.01 and 0.05$\mu g/M^3$ [59,62]. Its mean background level in soil is in the range of 0.06 to 1.0 $\mu g/g$[63]. The amount varies according to the soil and rock type. Soils containing sludge from industrial wastes contain much higher concentrations of Cd[63]. Cadmium also occurs in

surface waters. The cadmium in surface waters arises mainly from natural sources as well as agricultural practices i.e. application of phosphatic fertilizers which have been found to contain traces of Cd. Srikant and Vasant[64] observed that application of excess doses of superphosphate in vineyards 5-35 years old led to elevated levels of Cd in ground water in the vineyards. The background levels in water is less than 1 ug/liter.

2.2.3.2: Cadmium in plants.

Although Cd is considered to be a non-essential element for plants, it is effectively absorbed by both the root and leaf systems. Several soil and plant factors have been found to affect the plant uptake of Cd. The soil pH is listed as the major factor controlling both total and relative uptake of Cd[5]. A comparison of the Cd contents of plant foodstuffs produced under uncontaminated conditions of various countries shows the highest Cd concentration in spinach leaves (0.11 μg/g FW) and lettuce leaves (0.66 μg/g DW, 3.00 μg/g AW)[65].

Because Cd is readily available to plants from both air and soil sources, its concentration is usually more in plants grown in polluted areas. For example lettuce grown on polluted or sewage sludge amended soil was found to contain 34.5 μg/g Cd dry weight [64].

Phosphorous levels as well as other trace elements affect soil absorption, but this effect on Cd uptake is influenced by soil pH[66]. Decreasing pH increases Cd uptake by plants. Leafy vegetables like spinach and root vegetables such as turnip are considered the main routes of Cd supply to man[5].

2.2.3.3: Cadmium in man.

Cadmium may find its way into the human population through food and beverage, drinking water, air and tobacco[67]. In the general population the main exposure is via food and except in the vicinity of cadmium emitting industries, cadmium in ambient air does not contribute significantly. The daily intake via drinking water is generally low, but may be higher in contaminated areas or when Cd-containing material contaminates tap-water. Smoking involves the inhalation of relatively small amounts of Cd, but since it is highly absorbed from the lungs, in heavy smokers, the absorbed amounts may be of same magnitude as that absorbed from food. In man the element accumulates in certain body organs. The kidney is the critical organ of intoxication after exposure to Cd by inhalation or ingestion.

Since all foods contain Cd[3], leafy vegetables having the highest and grains the least, the daily ingestion from all sources for the world population ranges from 2 to 75 μg/day[5]. When the concentration in the human body reaches levels considered to be harmful (>200 μg/g wet weight) in the kidney cortex, renal dysfunction, mainly proteinuria, skeletal disorders and other complications arise[67]. The disturbance in mineral metabolism may eventually lead to osteomalacia[3]. The itai-itai disease may also occur. This is a form of chronic cadmium poisoning manifested by renal dysfunction in combination with osteomalacia or severe osteoporosis[3]. This disease which was quite prevalent in some parts of Japan in the mid 1950's[1], was caused by consuming rice grown on nearby paddies which had been irrigated with water from a river contaminated by zinc mining operations. Hagino and Yashioka[68] reported high concentration of Cd, Zn and Pb in autopsy tissues from people with itai-itai disease and in the everyday foods of the endemic area.

In accumulator workers, emphysema of the lungs, mild liver damage, anaemia, proteinuria, renal tubular damage, some dental changes and anosmia(loss of sense of smell) have been observed[69]. Prostatic cancer has also been reported[70].

The role Cd may play in causing hypertension is in dispute. But a study by Vivoli *et al*[71] revealed that Cd levels

in blood of hypertensives was higher (0.58 μg/l) than of normotensives (0.44 μg/l) with greater difference in non-smokers i.e 0.41 vs 0.25 μg/l.

Animal studies have linked it to cardiovascular diseases. Murungi[72], however, found that the Cd content of kidneys from subjects with cardiovascular diseases did not differ much from those dying from other causes. This study, however, did not consider the subjects history with respect to their smoking habits, place of occupation and exercise.

Cadmium is a cumulative element with a possible biological half-life of about 30 years[3]. The newborn is practically free from it, but there is a steady increase in Cd levels upto the 60th decade[73]. Smokers have been found to have almost twice the amount of renal Cd as non-smokers[61,74,75]. This is due to the fact that cigarettes contain some amount of Cd. One cigarette is reported to contain 1.0-1.5 μg/g[38,76]. The calculated daily consumption of 20 cigarettes results in the absorption of an additional dietary intake of 25-40 μg Cd[77]. Individuals who smoke two or more packets will of course absorb correspondingly more Cd depending on contents in the cigarette tobacco. A smoker inhales about 2 μg/pack of cigarettes.

Inhalation of fumes or dusts of Cd primarily affects the respiratory tract. The kidneys may also be affected. Even a brief exposure to high doses may result in pulmonary edema, and death. The lethal inhalation dose of CdO in man is estimated to be 2500 mg/M^3 [73] for a minute exposure. In fatal cases fatty degeneration of the liver and acute inflammatory changes in the kidneys have been noted. Symptoms of poisoning as a result of ingestion are; sudden nausea, salivation, vomiting and diarrhea, and abdominal pain and discomfort[3]. These are experienced almost immediately. Inhalation of dust or fumes may cause dryness of the throat, cough, headache, a sense of constriction in the chest, shortness of breath (dyspnea) and vomiting. More severe exposure results in marked lungs changes, with persistent cough, pain in the chest, severe dyspnea and prostration which may terminate fatally[3].

2.2.3.4: Absorption and excretion of cadmium.

Animal studies have shown that 98% of ingested Cd is excreted through feces and only 1-2% is absorbed through the intestinal mucosa[61,62]. The amount absorbed depends on the nutritional status of the individual. 25-50% of inhaled Cd is absorbed through the lungs. Once it is in the bloodstream Cd is transported to the liver where it combines with metallothionein and is transported to the kidney where it is

firmly retained[61,62]. The amount absorbed through the skin is negligible.

The major routes of excretion of Cd is through the feces, the amount of which is unknown, and therefore it is not clear whether or not this is a major mode of excretion for absorbed Cd. For unexposed populations, Cd excreted through urine is reported to be in the range of 1 to 20 ppb[78]. Smaller amounts are through sweat, hair (0.2 μg/day) and nails[78]. These modes of excretion are related to the body burden of Cd. Smokers generally excrete more than non-smokers. The slow excretion corresponds to the long biological half-life of Cd, which is estimated to be between 10 and 30 years[79,80].

2.2.3.5: Interaction with other elements.

Studies done *in vivo* have shown interaction between essential metals and Cd[81-83]. Also *in vitro* studies in isolated perfused liver[84], intestinal sacs[85], and red blood cells[86] suggest that Cd and the essential elements compete for common transport pathways.

Kinetic studies have also indicated that Cd competes

with essential metals for membrane binding sites, carriers and ion channels. Other studies suggest that Cd competes with Ca, Cu and Zn for common uptake pathways[87-89]. It is suspected that the interaction between Cd and Fe is only at intestinal level and does not affect its distribution in tissues. It interferes with Zn both at intestinal and organ level[90]. Zinc plays an important role in enzymes involved in the metabolism of carbohydrates[7]. It has been shown that Cd alters carbohydrate metabolism in the liver, and has been implicated in cardiovascular diseases, and a better correlation between the latter and Cd/Zn ratio can be found[91,92].

Selenium plays a role in the immunological system[93]. But Cd has been shown to interfere with Se metabolism, hence affecting the immuno system[94].

2.3: LEAD

2.3.1: Chemistry of lead.

Lead is a group 4B element, with a boiling point of 1744°C[95]. Its electronic configuration is [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$. Thus it is a p-block element. Like other elements in the same group, it has a very high ionization potential. It occurs

mainly as Pb^{2+}, but its +4 oxidation state is also known, though this state is unstable. The lower valency is more ionic. This is in agreement with Fajan's rules since the radius of Pb^{2+} is greater than that of Pb^{4+}.

Lead forms many compounds, most of which are insoluble in water, eg.$PbSO_4$, $PbCrO_4$, or sparingly soluble such as PbF_2 and $PbCl_2$. The oxidation state +2 forms numerous complexes that are mostly octahedral although a phosphorodithioate, $Pb(S_2PPr_2)_2$, is polymeric with six Pb-S bonds and a stereochemically active lone pair.

2.3.2: Sources and uses of lead.

Lead is obtained from its sulfide ore or galena (PbS). Its ores are frequently found in combination with other recoverable metals such as Cu, Zn, Cd and Ag. It is used as a component of lead-acid storage batteries, in cable sheathings, as sheeting and radiation shields. The tetraalkyllead is used as an additive to gasolines to prevent or reduce engine knock. Its compounds are used in ceramic glazes in making pigments and 'leaded' glass. It is also used in ammunition and in making solders.

2.3.3: Occurrence of lead in the environment.

2.3.3.1: Lead in soil, air and water.

The natural lead content of soil is inherited from parent rocks. However, due to widespread Pb pollution, most soils are likely to be enriched in this metal, especially in the top horizon. The approximate mean values in soils have been reported as 15-25 $\mu g/g$[42,96]. High Pb levels have been reported only in Denmark, Japan, Great Britain and Ireland. This most probably reflects the impact of pollution. The stated upper limit for Pb content of a normal soil is 70 $\mu g/g$. According to Kaara[50], the Pb levels in garden soils from areas within and around Nairobi are in the range 45.4-263.8 $\mu g/g$.

A major source of Pb in air is pollution from auto exhaust. Tetralkyllead is added to most high-test gasolines to improve acceleration and as an antiknock. The concentration of Pb in the atmosphere of large cities, where there is heavy traffic, and near highways is so high as to cause toxic reactions in some particularly toxicity-prone individuals[42]. Another source of Pb in ambient air is cigarette smoke[97]. This arises from lead arsenate applied to the tobacco as an insecticide. It may also enter through burning of coal and through the fumes and ash produced by

burning lead battery castings.

Elevated levels of Pb in water arise principally from industrial discharges, highway runoff and weathering processes in areas of natural lead mineralization. Soft water being acidic will erode Pb from lead piping and become contaminated with this heavy metal. Concentrations in ground waters are generally low, most of them having concentrations of less than 0.01 mg/litre. Kimei[98] has reported levels in the range of <0.01-0.38 ppm and 0.05-0.80 ppm during the wet and dry seasons respectively, in underground waters in and around Nairobi.

2.3.3.2: Lead in plants.

Although Pb occurs naturally in all plants, it has not been shown to play any essential roles in their metabolism. Studies indicate that plants are able to take up Pb from soils to a limited extent[5,42]. This uptake varies over the concentration ranges currently present in soils and with various forms of Pb that occur in soils.

Natural Pb in plants growing on uncontaminated and unmineralized areas appears to be quite constant, with a range of 0.1 to 10 μg/g (DW) and an average of 2 μg/g (DW)[50]. The contents in edible portions of plants grown on

uncontaminated areas range from 0.001 to 0.08 μg/g (FW), and 0.05 to 3.0 μg/g (DW)[5,99].

In an analysis of vegetables grown on uncontaminated soils[97] spinach was found to have a mean Pb content of 12.2 μg/g in leaves, with values ranging from 9.7 to 15.9 μg/g. Kale had lower levels in leaves than the former. The mean value was 9.1 μg/g with a range of 6.4 to 10.8 μg/g. Table 2.1[100] gives results obtained from a preliminary survey of Pb and Cd concentration in Kenyan soil and plant samples done by Kinoti[100]. The data in this table reflects a picture similar to that described above[97].

It has been reported[5] that there is a relatively minor effect on the Pb concentrations in plants as a result of soil contamination by agricultural activities. Utilization of Pb-enriched sludges[50] has however, led to elevated Pb levels in soil and consequently increased levels in vegetable plants.

2.3.3.3: Lead in man.

Man is simultaneously exposed in varying degrees to Pb in food, drink and air, as well as a number of other sources which include cigarette smoke. Food becomes contaminated with Pb either at source or during preparation for consumption. Liquids are consumed directly as drinking water, milk and

Table 2.1[100] :Pb and Cd concentrations in Kenyan soil and plant samples in $\mu g/g$.

PLACE AND SAMPLE	LEAD	CADMIUM
Limuru		
Arrowroot leaves	73.07	12.04
Arrowroot roots	108.02	8.69
Kale leaves	73.07	5.34
soil	20.63	5.34
Nairobi		
Spinach leaves	55.59	12.04
Spinach roots	125.50	5.34
Kale leaves	20.63	7.02
Arrowroot leaves	38.11	7.02
Arrowroot roots	20.63	5.34
Soil	125.54	5.34
Kiserian		
Spinach leaves	90.54	5.34
Spinach roots	108.02	10.37
Kale leaves	38.11	5.34
Soil	3.15	10.37

prepared beverages and indirectly in prepared food. There is a high degree of variability in dietary Pb intake from individual to individual as well as from country to country. Typical intakes are thought to lie in the range 100-200 μg/day for adults[97].

Intake of Pb into the body via inhalation depends upon the daily respired volume and the concentration in the inhaled air. This is not to imply that all the Pb in the atmosphere occurs as particulate inorganic lead, a form which is readily inhaled into the alveolar region of the lungs. Only part of this is deposited, the other being exhaled.

Lead is a cumulative poison. Increasing amounts build up in the body and eventually a point is reached where symptoms of disability occur. It normally accumulates in the liver, kidney and a higher percentage in the bones. It produces a continuum of effects primarily on the haematopoietic systems, the nervous systems and the kidneys. Rats fed on diets low in Ca exhibited a markedly increased Pb retention in various tissues such as blood, soft tissues and bone[33]. Normal blood levels are considered to be 20 μg % (200 ng/ml) or less. Levels above 400 ng/ml indicate increased exposures. American cigarette smokers normally show levels around 300 ng/ml[5]. Studies by Elinder *et al.*[101] showed that the blood Pb levels were significantly influenced by sex, smoking habits and

alcohol consumption.

Table 2.2[101] shows the levels in male and female smokers and non-smokers.

Table 2.2[101]: Lead levels in blood of smokers and non-smokers μg/l.

	Smokers	Non-smokers
Male	92.00	77.00
Female	69.00	57.00

Another study by Ander *et al*[102] found that the concentration of Pb in blood of children whose parents were non-smokers was 30 μg/l. The mean level in children with one parent smoker 39 μg/l (smoking father 37 μg/l, smoking mother 41 μg/l), while in children with both parents smokers the level was 47 μg/l.

Lead in soil presents a health risk to young children[103], but exposure can also originate from paint, air, water and food. Table 2.3[104] gives the daily Pb exposure to the general adult population.

A few cases of Pb poisoning, two ending fatally, were traced to snuff[3] which contained 16-20% lead. A body of large scale surveys suggests that Pb is causally related to deficiency in cognitive functioning[3]. Symptoms following prolonged exposure include abdominal discomfort and pain. Severe cases may present colic, others constipation, loss of appetite, nausea and vomiting, insomnia, headache, irritability and dizziness. Lead encephalopathy is the most severe but the rarest manifestation of Pb poisoning[29].

2.3.3.4: Absorption and excretion of lead.

The major routes for Pb absorption are the respiratory and gastrointestinal tracts. Most of the Pb exposure that occurs among non-industrial populations results from the food and drink consumed. About 5-10%[3] of that ingested is absorbed into the body the remainder is excreted via the feces, and is of little concern. Studies indicate that 300 μg are ingested daily by an adult under normal circumstances. The W.H.O.recommends a maximum daily intake of 5 μg/Kg (body weight).

Table 2.3[104]: Daily lead exposure to general adult population.

Source	Amount
Diet	200-300μg/day
Water	20μg/day
Air	15μg/day in urban
Tobacco	10μg/pack of cigarette smoked
Dust, soil and paint	upper limit of 10μg/day, otherwise negligible

Following absorption through the respiratory tract,lead in the blood stream disappears into the tissues. In the stable state more than 90% of the total body lead is stored in the skeleton. There is evidence to show that the half-life for its excretion from the body is not constant, but is a function of the blood lead concentration.

2.3.3.5: Interaction with other elements.

A number of animal studies have suggested that low dietary Ca and phosphorous increase the absorption of lead from the gut[105]. Calcium intake has been found to be inversely related to both absorption and retention of lead in infants[106].

The deficiency of iron has been observed to act synergistically with lead in the impairment of haem synthesis[107]. Lead interferes with copper metabolism which secondarily leads to dysfunction of iron metabolism and haem synthesis[108].

Zinc has been well recognized as a potent antagonist to Pb intoxication[109] and its administration has been shown to decrease the accumulation of Pb in the body organs. It reduces the severity of Pb poisoning by restoring Pb-induced biological alterations in urinary and blood parameters in animals.

The next section deals with the analytical techniques available for trace metal analysis.

2.4: ANALYTICAL TECHNIQUES

Several techniques for determining trace amounts of elements are currently in use or under investigation[110]. They are as varied as the type of samples being analysed. However many of these techniques, either because of complexity of operation, high cost, limited field of application or other reason, are not readily utilized by the average chemist.

Atomic spectroscopic methods, particularly atomic

absorption spectroscopy (AAS), do not suffer from many of these shortcomings and therefore their use has increased rapidly. The two techniques commonly used in trace element analysis are flame AAS and plasma atomic emission spectroscopy (PAES). In this study flame AAS was preferred due to its favourable detection limits, freedom from spectral interference, ease of operation and availability of the instrument.

Atomic absorption spectrometry has been utilized for the determination of several metals. With this technique cadmium appears not to be subject to interference from other metals and constituents of biological materials enhance the absorption signal manyfold. For accurate analysis Cd must be isolated from its matrices. The sodium diethyldithiocarbamate (NDDC)[3] chelate of the element is quantitatively extracted into methyl isobutylketone (MIBK) at pH 5.5-6.5. Lead which is coextracted with Cd does not interfere. For both Zn and Pb analytical sensitivity can be augmented by employing chelation-extraction procedures and/or by use of flameless atomization techniques. Some of the chelating agents are thenoyltrifluoroacetonate[111] in chloroform, dithizone[3], benzoylacetonate[112], ammonium pyrrolidine dithiocarbamate[113], etc.

Another commonly reported technique is anodic stripping

voltametry (ASV). This is a technique in which the analyte is first collected by electrodeposition at a mercury or solid electrode and then redissolved from the electrode to produce a more concentrated solution than originally existed. It is sensitive to nanograms per gram or less and is very useful in the determination of cadmium in aqueous matrices[3]. Potentiometric stripping analysis (PSA), a technique involving the time-potential behavior of a working electrode as it undergoes oxidation, has in recent years found application as an alternative to ASV for the determination of trace metals in different substances[114]. It has also been used for the determination of Pb and Cd in samples such as urine, wine, beer, sea water and biological tissues. This is because it is free from interference from electroactive species.

Other methods reported include neutron activation analysis (NAA)[115-117], atomic fluorescence spectroscopy (AFS), X-ray fluorescence[118], X-ray emission[110] and colorimetry[119] . For most of these methods extraction into organic solvents, ion exchange separation or other methods of sample pre-treatment are often necessary. Previous methods used to study trace elements included dry ashing of the sample. However, this technique has been subject to error due to contamination as well as sample loss as some like Cd are volatile. Wet ashing has been preferred and it is now known that complete decomposition can be achieved by perchloric acid procedure[120].

In fact many such decomposition showed no residual carbon. Since perchloric acid reacts significantly with organic substances at temperatures above 160°C[121] only, the completeness of the procedure is a function of the decomposition time at or above this temperature[122]. Other acids have been used for decomposition. These include nitric acid alone, nitric-sulfuric acid mixture and nitric-perchloric acid mixture.

Several techniques have been used for the simultaneous determination of Pb and Cd[122] or Cd and Zn[123]. Franke and de Zeeuw[120] have described the use of differential pulse anodic stripping voltametry (DPASV) as a screening procedure to identify Zn, Pb, Cd and other heavy metals.

For the three elements the method that was used to determine them was AAS. A short description of this technique will now be presented in section 2.4.1.

2.4.1: ATOMIC ABSORPTION SPECTROMETRY (AAS).

This is one of the most widely used techniques for the determination of metals at trace levels. Its popularity compared to that of flame emission is due to its relative freedom from interference by inter-element effects and its relative insensitivity to variations in flame temperature.

Except for routine determination of alkali and alkaline earth metals, for which flame photometry is often preferred, over sixty elements can be determined in almost any matrix by atomic absorption[3]. Examples include heavy metals in body fluids, polluted waters, foodstuffs, soft drinks and beer; the analysis of metallurgical and geochemical samples and the determination of many metals in soils, crude oils, petroleum products and plastics.

This technique is based upon the absorption of radiation by free atoms. Since the atoms of each element absorb radiation of their own characteristic wavelength, spectral interferences rarely occur.

2.4.1.1: Theory of AAS

The emission and absorption of light are associated with the processes of transition of atoms from one energy state to another. Considering a case of steady states m and n with energies E_m and E_n, where $E_n > E_m$, the m → n transition results in the absorption of light, and the n → m transition results in the emission of (radiation) light with frequency ν_{mn}

$$\nu_{mn} = \frac{E_n - E_m}{h} \qquad (2.0)$$

where h is the Planck's constant.

The m → n transitions are always stimulated by external radiation and this forms the integral part of atomic absorption spectroscopy.

When an atom is in its lowest electronic energy level, it is said to be in its ground state. The ground state atoms of a particular element absorb the radiation of their own specific resonance wavelength and get excited to a higher energy state. The proportion of excited to ground state atoms in a population at a given temperature can be considered with the aid of the Maxwell-Boltzmann relation.

$$\frac{N_n}{N_m} = \frac{G_n}{G_m} \; EXP \; \left\{\frac{E_m - E_n}{KT}\right\} = \frac{G_n}{G_m} \; EXP \; \left\{\frac{-\Delta E}{KT}\right\} \qquad (2.1)$$

Where N is the number of atoms in a state m or n, G is the statistical weight for a particular state and K is the Boltzman constant (1.38×10^{-23} J K^{-1}) and T is the thermodynamic temperature. The ratio N_n/N_m for a number of common atoms over a range of temperature may therefore be calculated. In this case n refers to the first excited state and m is the ground state.

Absorption by atoms takes place within very narrow spectral regions of the order of hundredth of angstrom. In the sun's spectrum many such absorptions show up as Fraunhafer lines, but in the laboratory experiments and

certainly on the analytical scale, only absorptions involving the ground state are normally observed. Absorptions involving the ground state are for this reason known as resonance lines. The absorption spectra of most elements produced under laboratory conditions are extremely simple and there is very little possibility of coincidence and therefore very rare spectral interference.

The extent to which radiation of a particular frequency is absorbed by an atomic vapor is related to the length of the path traversed and to the concentration of absorbing atoms in the vapor. This is analogous to the Beer-Lambert Law for samples in solution. Thus for a collimated, monochromatic beam of radiation of incident intensity I_0 passing through an atomic vapor of thickness l, the intensity of light transmitted may be given as

$$I_\nu = I_o \; e^{-K\nu l} = I_o \; e^{-\epsilon c l} \qquad (2.2)$$

Where I_υ is the intensity of transmitted radiation at frequency υ and k_υ is the corresponding absorption coefficient. ϵ is the molar absorptivity, c is the concentration of analyte and l is the path length.

The absorption coefficient or molar absorptivity characterizes absorption lines just as intensity

characterizes emission lines. For analytical purposes the parameter which is normally measured is Absorbance (A). This is related to concentration by equation (2.3).

$$A = \log \frac{I_o}{I} = \epsilon\, c\, l \qquad (2.3)$$

In AAS, the analytical signal is obtained from the difference between the intensity of the source in the absence of metallic elements present in the liquid and the decreased intensity obtained when metallic elements are present in the optical path. The relationship between absorbance and concentration is nearly linear i.e Beer's Law is obeyed over a wide range of concentration. Therefore in actual practice one uses standards to obtain a calibration curve from which the unknowns are determined.

The next section gives a brief discussion on the instrumental principles of the AAS.

2.4.1.2: Instrumental principles of AAS.

The basic principles involved in instrumentation are similar to those of other spectroscopic absorption methods. Light of a certain wavelength, produced by a special kind of monochromatic lamp, which is able to emit the spectral lines

corresponding to the energy required for an electronic transition from the ground state to an excited state, is allowed to pass through the flame. Meanwhile the sample solution is aspirated into the flame. Before it enters the flame, the solution gets dispersed into a mist of very small droplets which evaporate into the flame to give first the dry salt, and then the vapor of the salt. A part of this vapor dissociates into atoms of the element to be measured, which then absorb resonance radiation from an external source. The unabsorbed radiation from the flame is allowed to pass through a monochromator which isolates the exciting spectral lines of the light source. From the monochromator the unabsorbed radiation is led into the detector (photodetector), the output of which is amplified and measured on a recorder. Absorption is measured by the difference in the transmitted signal in the presence and absence of test element. Some of the most important components of an atomic absorption spectrometer are shown in Figure 2.0

Schematic diagram of an AAS

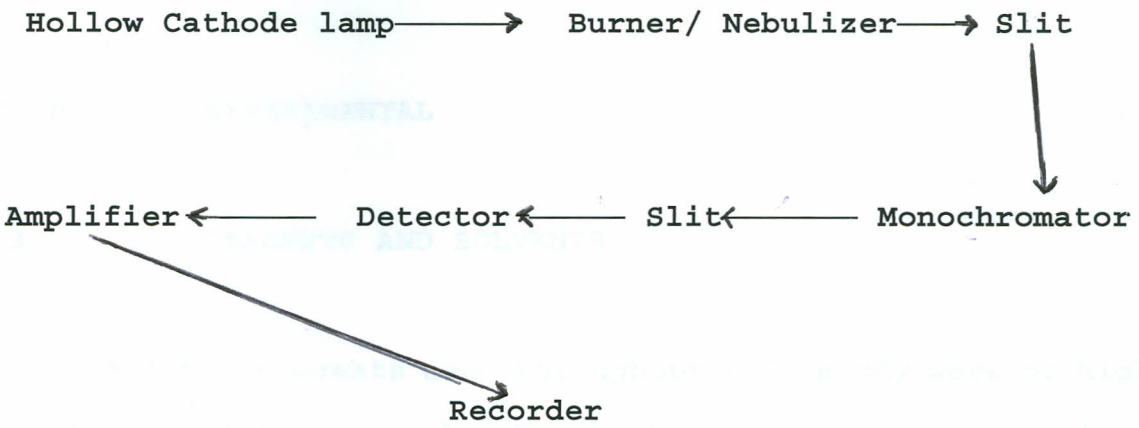

Figure 2.0: Schematic diagram of the most important components of an atomic absorption spectrometer.

In summary this chapter has been concerned with the brief discussion of metals and the analytical techniques available for their analysis. The next chapter will be concerned with the experimental methods that were used in this work.

CHAPTER 3

3.0: EXPERIMENTAL

3.1: REAGENTS AND SOLVENTS.

All the reagents used throughout this study were of high quality analytical grade. The particular reagents are given in section 3.4.

The water used was distilled in an all pyrex distiller and then de-ionised in an Elegestat Micromeg de-ioniser Cartidge type-MC-DC into a plastic container to give distilled-deionised water. This water was stored in a polyethylene container.

3.2: Cleaning of glassware.

The glassware used included pipettes, measuring cylinders, volumetric flasks, filter funnels, weighing bottles and conical flasks. These were thoroughly washed with detergent and tap water. They were then rinsed with distilled water and then with 1:1 nitric acid solution. Later they were rinsed with deionized water and left overnight to dry in the oven at 80°C. Volumetric flasks were left to dry in a rack overnight.

3.3. Calibration of glassware,balance and pH meter.

3.3.1: Glassware.

5 ml, 10 ml and 25 ml pipettes (20°C Goldline MBI, Gt. Britain) were calibrated by weighing samples of de-ionized water delivered by pipettes at a temperature of 22.8-20°C (the pipettes were originally calibrated at 20°C by the manufacturer). The weighings were done on an electronic weighing balance model AC 100. Five weighings were done for each pipette and the average volume was determined using the density of water at 20°C corrected for bouyancy (1.00284 g/ml).

The volumetric flasks used were 1000 ml and 50 ml (20°C MBI, Gt. Britain and Din West Germany). The results for calibration were as follows:

For pipettes

5 ± 0.02 ml

10 ± 0.02 ml

25 ± 0.03 ml

For volumetric flasks

1000 ± 0.10 ml

50 ± 0.04 ml

3.3.2: The Balance.

All weighings were done using an analytical balance (Gebr. Bosch W. Germany) model S-2000.

3.3.3: The pH meter.

The pH meter used in this study was a Chemtrix type 40 (Chemtrix Inc. Hillsboro, ORE. USA). It was calibrated using buffer solutions of pH 4.0,7.0 and 9.0. The buffers were made by dissolving the respective pH tablets in distilled-deionised water and making to the one liter mark in a volumetric flask as specified by the manufacturer.

3.4: Preparation of standard solutions.

To make zinc stock solution, 1 g of Analar grade granulated zinc metal (purity 99.9%) (BDH Chemicals Ltd. Poole England) was dissolved in 40 ml of 1:1 dilute hydrochloric acid. This was then diluted to the 1 litre mark using distilled deionised water. The desired concentrations were prepared by serial dilution using the formula

$$C_1V_1 = C_2V_2 \qquad (3-0)$$

Where V_1 is the volume of the initial solution to be diluted and C_1 is its concentration. V_2 is the expected volume after

dilution and C_2 is the expected concentration. For example, to prepare 50 ml of 100 ppm from 1000 ppm we require 5 ml of the latter. That is:

$$1000\ ppm\ x\ V_1 = 100\ ppm\ x\ 50\ ml \qquad (3.1)$$

$$V_1 = \frac{100\ ppm\ x\ 50\ ml}{1000\ ppm} = 5\ ml \qquad (3.2)$$

Lead stock solution was obtained from spectroscopic grade lead nitrate ($PbNO_3$) (BDH Chemicals Ltd. Poole England). The desired concentrations were prepared by serial dilution as described for zinc.

To preparee the stock solution of cadmium, 1 g of metallic Cd of analytical grade (BDH Chemicals Ltd. Poole England) was dissolved in 10 ml of 68% nitric acid and then diluted to 1 liter with distilled- deionized water. From the stock solution, standards were prepared by serial dilution. The standards ranged from 0.05 to 5.00 ppm. In each of these cases the stock solutions were stored in polyethylene bottles to minimize loss of metal through adsorption. Standards were freshly prepared each time an analysis was to be carried out.

3.5: Blank Preparation.

In order to account for the background effects from the acids and to correct for changes resulting from the digestion procedure, 6% nitric acid solution used in the preparation of standards and sample solutions was prepared and treated in a similar way as the samples. This was used to calibrate the instrument before samples were introduced into the flame. Section 3.6 will now describe how the sampling and sample preparation was carried out.

3.6: SAMPLING.

3.6.1: Tobacco, other crop plants and soils.

Samples were obtained from Muranga (three farms), Othaya (two farms), Kitui (two farms), Uasin-Gishu (two farms), Nandi (three farms), Bungoma (two farms), Embu (three farms), and Meru (three farms). Snuff was bought from shops in Meru town and in Siakago town in Embu. Processed chewing tobacco was bought in Nandi but was not available in the other regions visited. The samples were picked and placed in separate clean polyethylene bags sealed and taken for pre-treatment.

Cigarettes were obtained from shops in Embu, Kenyatta

University and Ruiru. Samples were treated as is described in section 3.7.

3.7: SAMPLE PREPARATION.

3.7.1: Tobacco.

The sample preparation was done as follows. Fresh tobacco leaves and flue-cured samples were washed using tap water, then distilled water and lastly with distilled-deionized water. They were then dried at 70°C for 36 hours. The fire-cured samples were not washed because they were already too dry and were quite brittle. Instead they were dried in the oven at 70°C for 24 hours after which their midribs were removed, crushed and weighed out before acid digestion. 2.0 g of each sample was weighed and placed in separate digestion flasks. 10 ml of concentrated nitric acid was added. They were then let to digest for 10 minutes. 5 ml of 60 % perchloric acid was then added. Further digestion was allowed to proceed until white fumes of perchloric acid vapor were liberated. The samples were then cooled, diluted with ~6 % nitric acid and filtered into 50 ml volumetric flasks. These were made up to the mark with distilled-deionised water and analysed as is described in section 3.8.

3.7.2: Snuff and cigarettes.

Snuff (2.0 g) was weighed and put into 100 ml conical flasks. From each of the eight cigarette brands currently available on the Kenyan market five cigarettes were randomly picked and placed in separate 100 ml flasks. These were then digested as described in section 3.7.1 and analysed as described in section 3.8.

3.7.3: Other crop plants.

Crop plants that were on leaf basis were washed in the same way as the tobacco leaves and dried similarly. Those which were in the form of seeds, cereals or fruit were threshed out and dried in the oven. All these crop plants were then weighed and put into vials before being transferred into appropriate digestion flasks. They were digested the same way as the tobacco and similarly analysed.

3.7.4: Soil.

Soils obtained from various places were dried in the open air for 2-3 days. They were then crushed into fine powder and dried in the oven overnight at 80°C before being stored in dry polythene bags. 1 g samples of each of these were later weighed and put in the appropriate digestion

flasks. 15 ml of 68% nitric acid and 10 ml of 60% perchloric acid were added and digested for 5-6 hours at about 110°C. The samples were then diluted with 6% nitric acid solution and filtered into 50 ml volumetric flasks and made to the mark. Analysis was carried out as for the plant samples.

3.7.4.1: Preparation of soil for pH analysis

Soil pH was determined following the procedure by Black (1965)[124]. 10 g samples of oven dry finely powdered soil were weighed and put into plastic specimen bottles and 25 ml of distilled-deionised water added. The bottles were then tightly stoppered and shaken for 30 minutes with a mechanical reciprocating shaker. After shaking, the suspension was allowed to stand for 10 minutes and the pH of the partly settled suspension determined with a pH meter(Chemtrix type 40). This was then reported as 'soil pH'.

3.8: PROCEDURE FOR AAS ANALYSIS.

Analysis of samples in this work was carried out using a single beam Varian atomic absorption spectrophotometer (Spectr AA-10 Varian Techtron Pty Ltd. Australia) Interfaced with a computer. The samples and standards were aspirated into an air acetylene flame and the concentrations recorded on a computer. The detailed theory of AAS has been described

in section 2.4.1.1. The experimental parameters that were used are presented in table 3.0.

3.8.1: Percentage recovery.

In order to determine recovery, three sets of tobacco samples were weighed out accurately. Into two of these were added known aliquots of a standard solution, so that the concentration of one was close to that of the lowest concentration of the sample and the other had concentration close to that of the highest sample. Each of these were digested and filtered as described in section 3.7.1 then diluted to the mark. AAS readings were taken for the three samples and the percentage recovery was calculated. Recovery efficiency averaged 95%, 94% and 100% for lead, zinc and cadmium respectively.

Table 3.0: Instrumental conditions for the AAS in the determination of Cd, Pb and Zn.

Operating parameters	Cadmium	Lead	Zinc
Wavelength (nm)	228.8	217.0	213.9
Slit width (nm)	0.2	0.3	0.2
Lamp current (mA)	3.0	4.0	3.0
Sensitivity (ppm)	0.02	0.015	0.009
Detection limit (ppm)	0.015	0.02	0.003

This chapter has been concerned with a basic description of how the samples were obtained, treated and analysed. Chapter 4 will be concerned with the results obtained in this work and the discussion. A summary of the conclusions arising from this study will be presented in chapter 5.

CHAPTER 4

4.0: RESULTS AND DISCUSSION.

The levels of the heavy metals zinc, lead and cadmium were determined in Kenyan cigarettes,fresh, cured and processed tobacco, other crop plants that grow in the same locality as tobacco and in soils from the sample areas. The soil pH was also determined and the results are reported in the tables appearing in the following sections.

4.1: LEVELS OF Cd, Pb AND Zn IN FRESH TOBACCO LEAVES.

Table 4.0 below gives the heavy metal content of fresh tobacco leaves from various regions in Kenya. From the table it is evident that the cadmium levels in the tobacco leaves fall within the range reported in literature. The levels in this study were in the range of 0.50-2.33 $\mu g/g$. The reported Cd concentrations in tobacco range from ~0.5 to 5 $\mu g/g$[38].

All the lead levels in the table (7.40-20.83 $\mu g/g$) fall above the range of plants grown on uncontaminated soils (0.1-10.0 $\mu g/g$)[50]. This may suggest that tobacco takes up more lead from the soil than other crop plants reported in literature, and that tobacco was growing in lead contaminated soils resulting in higher metal uptake.

Table 4.0: Levels of Cd, Pb and Zn in fresh tobacco leaves from various regions in Kenya, $\mu g/g$ (DW) [mean ± SD].

REGION	CADMIUM	LEAD	ZINC
Kiahiti (M)*	0.75 ± 0.00	7.50 ± 0.00	-
Kihingu (W)*	1.17 ± 0.29	10.00 ± 0.00	18.75 ± 1.77
Kihingu (N)*	1.19 ± 0.39	7.50 ± 0.00	18.75 ± 1.77
Gatugi (N)*	0.92 ± 0.09	16.67 ± 2.89	66.25 ± 1.77
Gatugi (W)*	1.33 ± 0.09	11.67 ± 3.82	96.25 ± 5.3
T	0.50 ± 0.04	20.83 ± 0.45	64.38 ± 1.77
Mwimbi	1.33 ± 0.29	10.00 ± 0.00	53.75 ± 1.77
Siakago	0.58 ± 0.14	7.40 ± 1.32	26.00 ± 2.12
Chepkoiyo	1.13 ± 0.14	11.00 ± 1.74	76.13 ± 1.05
Cheplengu	1.38 ± 0.14	11.88 ± 2.89	123.75 ± 1.46
Cheplengu(K)*	1.00 ± 0.00	10.25 ± 1.59	90.00 ± 0.41
Samoo	1.13 ± 0.25	8.25 ± 2.02	116.81± 7.49
K1	1.25 ± 0.00	7.50 ± 0.00	-
K2	2.33 ± 0.29	11.00 ± 0.00	169.00 ±1.00
Malakisi (B)*	1.38 ± 0.33	8.13 ± 1.25	29.38 ± 0.15
Malakisi (J)*	0.81 ± 0.375	8.13 ± 1.25	37.50 ± 3.54

* The letters appearing in the brackets stand for the initials of the farmers from whose farms the samples were picked. The letters appearing in subsequent tables will have the same meaning.

Those of zinc fall within the normal concentrations in plants (25-150 mg/Kg), though there is one exception. The tobacco from Kitui was found to be 169.00±1.0 mg/Kg. This may be due to the soil type coupled with soil conditions. The soil could be contaminated with Zn containing compounds.

This study demonstrates that tobacco takes up heavy metals to varying degrees depending on the growth medium. This can be seen as a confirmation of studies as reported in the literature[4] that medium determines metal uptake by plants.

4.2: SOIL pH AND HEAVY METAL CONTENT.

Table 4.1 gives the pH and the heavy metal content of soils from the sample areas. The ranges for normal levels of the heavy metals are given in Table 4.2. From the two tables, one observes that the soils used in this study had higher levels of cadmium than normal. The normal levels being in the range 0.07-1.1 μg/g, while those obtained in this study range from 0.25-2.19. Most of the samples have levels above the normal range. This could probably be due to heavy application of fertilizers used to improve crop yields. These fertilizers contain cadmium as an impurity and possibly this has contaminated the soil. The highest levels are obtained in Kitui (2.50±0.00) and the lowest levels occur in Malakisi

(0.25±0.00). The low pH of the soils studied could also account for high Cd levels in the soils. This is because low pH causes leaching of metals from bedrocks leading to increased amounts in the soils.

It is also evident that most of the soils had higher levels of lead. This is more so in Uasin-Gishu and Nandi districts whose levels are 369.38±1.3 and 331.88-350.00 μg/g respectively. Uasin-Gishu has the highest Pb levels while Siakago (13.33±1.44) has the lowest. The normal levels in the soils being 15-25 μg/g. The high levels in soils from Nandi and Uasin-Gishu could be attributed to the soil type in the sample areas. The soils found in Uasin-Gishu and Nandi are mainly ferralsols and nitisols. According to a study by Norrish[125] ferralsols contain high concentrations of Pb.

In the case of zinc, half of the soil samples have levels over the normal range of 50-100 μg/g and the other half fall within or below this range. This indicates high levels of contamination in areas where the levels are above this range, such as Nyeri (Othaya), Meru, Uasin-Gishu and Nandi districts. This could also result from the parent rocks which are mainly volcanics in these regions. The highest levels occur in Othaya and the lowest in Siakago. The soils in Siakago had the lowest levels of all the three and it also has the highest pH (7.4). At high pH metals are not easily

Table 4.1:pH and levels of Cd, Pb and Zn in soils from various regions in Kenya; μg/g (DW) [mean ± SD].

REGION	CADMIUM	LEAD	ZINC	pH
Muranga				
Kiahiti (M)	0.69±0.24	15.00±0.61	-	4.5
Kihingu (W)	1.50±0.54	35.00±2.40	50.00±0.00	4.7
Kihingu (N)	1.63±0.25	35.00±0.00	83.75±5.30	5.9
Othaya				
Gatugi (N)	2.19±0.66	53.13±0.15	283.75±5.30	7.0
Gatugi (W)	2.19±0.72	29.38±3.75	290.00±3.54	6.0
Meru				
T	1.42±0.52	30.83±0.04	146.25±1.77	6.0
Embu				
Siakago	0.67±0.29	13.33±1.44	32.50±0.00	7.4
Uasin-Gishu				
Chepkoiyo	1.69±0.24	369.38±1.3	139.20±0.26	5.6
Nandi				
Cheplengu(P)	1.44±0.13	341.13±0.06	173.63±3.09	5.6
Cheplengu(K)	1.38±0.14	331.88±1.75	118.06±0.40	5.3
Samoo	1.50±0.00	350.00±0.71	79.38±0.42	4.9
Bungoma				
Malakisi (B)	0.50±0.00	20.00±0.00	37.50±0.00	5.8
Malakisi (J)	0.25±0.00	32.50±3.54	36.25±1.77	6.6
Kitui				
K2	2.50±0.00	14.75±0.71	-	-

Table 4.2[50]: Normal levels of Cd, Pb and Zn in soils.

Metal	Cadmium	Lead	Zinc
Normal levels in soil range in μg/g	0.07-1.1	15-25	50-100

leached from the bedrock therefore thier levels remain low. This may be supported by the fact that Meru which is adjacent to Embu and therefore has similar rock formation has higher levels of the metals with a lower pH of 6.0.

4.3: COMPARISON OF LEVELS OF Cd, Pb AND Zn IN FRESH TOBACCO LEAVES AND SOILS.

4.3.1: Cadmium.

Table 4.3 below gives the levels of Cd in fresh tobacco leaves and in the soils on which they grow. The correlation coefficient between the Cd levels in tobacco leaves and in the soils is +0.168 (t = 0.879, n = 28). There is therefore a positive correlation but this is not statistically significant at confidence intervals above 95%. Earlier studies[4] indicate a significant positive correlation between the levels in plant leaves and in the soil on which they grow, that is a dose-response relationship exists. This indicates that other factors other than concentration affect Cd uptake by the tobacco plant. It is known that low pH release heavy metals from their complexes making them more available to plants. For instance samples from Gatugi the levels in the soil (2.19μg/g) are twice the levels in fresh tobacco leaves(0.92μg/g). This is due to the soil pH (7.0) which is high therefore, Cd is less available to the plant. The tobacco (0.81μg/g) from Malakisi had thrice the levels

Table 4.3: Levels of cadmium in fresh tobacco leaves and in soil, $\mu g/g$ [mean ± SD].

REGION	FRESH TOBACCO	SOIL
Muranga		
Kiahiti (M)	0.75 ± 0.00	0.69 ± 0.24
Kihingu (W)	1.77 ± 0.29	1.50 ± 0.54
Kihingu (N)	1.19 ± 0.39	1.63 ± 0.25
Othaya		
Gatugi (N)	0.92 ± 0.09	2.19 ± 0.66
Gatugi (W)	1.33 ± 0.09	2.19 ± 0.72
Meru		
Mwimbi	1.33 ± 0.29	1.42 ± 0.52
Embu		
Siakago	0.58 ± 0.14	0.67 ± 0.29
Uasin-Gishu		
Chepkoiyo	1.25 ± 0.14	1.69 ± 0.24
Nandi		
Cheplengu (P)	1.38 ± 0.14	1.44 ± 0.13
Cheplengu (K)	1.00 ± 0.00	1.38 ± 0.14
Samoo	1.13 ± 0.25	1.50 ± 0.00
Bungoma		
Malakisi (B)	1.38 ± 0.33	0.50 ± 0.00
Malakisi (J)	0.81 ± 0.38	0.25 ± 0.00
Kitui		
K2	2.32 ± 0.29	2.50 ± 0.00

of Cd in the soil (0.25) due to lower pH. There is a significant negative correlation (r = - 0.334 significant at 90% confidence interval) between the levels of Cd in fresh tobacco leaves and the soil pH. This confirms the results of earlier studies[4] that indicate that soil pH and cadmium level in plants are inversely related.

4.3.2: Lead.

Table 4.4 gives the levels of lead in fresh tobacco leaves and in the soil on which they grow. This table will be discussed in relation to Table 4.1. The correlation coefficient between the levels in the leaves and in the soils is +0.147. The correlation is not statistically significant (t = 0.7578, n = 28) at confidence limits more that 95%. This is in confirmation of results of earlier studies that Pb in soil is relatively unavailable for plant uptake. Hence plants only take it up to a limited extent from the soils[4]. The correlation between the levels in the fresh tobacco leaves and soil pH is positive (r = +0.040) but small. The concentration of Pb is relatively stable in the range 8-11 μg/g for all acidic soils (pH < 7.0) but abnormally high for Gatugi (W), 16 μg/g whose pH (7.0) is neutral.

4.3.3: Zinc.

Table 4.5 gives the zinc levels of fresh tobacco leaves and in the soil on which they grow. In this case there is a positive correlation between the levels in the leaves and in the soil(r = +0.208). This correlation is significant at the 99% confidence interval (t = 10.41, degrees of freedom(v) = 26). However there is also a negative correlation between the levels in the fresh tobacco leaves and the soil pH. These results are in agreement with those reported in literature[4] and therefore are as expected.

The zinc levels in tobacco (66.25μg/g) from Gatugi (N) (soil Zn 283.75, pH 7.0) is lower than 96.25μg/g from Gatugi (W) (soil Zn 290.00, pH 6.0). Similarly the Zn leaves in tobacco from Chepkoiyo 76.13μg/g with soil pH 5.6 and soil Zn 139.19μg/g were higher that those from Gatugi (N) (soil Zn 283.75μg/g, pH 7.0).

This clearly brings out the inverse relationship between Zn uptake by tobacco with pH.

Table 4.4: Levels of lead in fresh tobacco leaves and in the soils on which they grow, μg/g [mean±SD]

REGION	FRESH TOBACCO	SOIL
Muranga		
Kiahiti (M)	7.5 ± 0.00	15.00 ± 6.61
Kihingu (W)	10.00 ± 0.00	35.00 ± 2.40
Kihingu (N)	7.50 ± 0.00	35.00 ± 0.00
Othaya		
Gatugi (N)	16.67 ± 2.89	53.13 ± 5.15
Gatugi (W)	11.67 ± 3.82	29.38 ± 3.75
Meru		
Mwimbi	10.00 ± 0.00	30.83 ± 8.04
Embu		
Siakago	7.40 ± 1.32	13.33 ± 1.44
Uasin-Gishu		
Chepkoiyo	11.00 ± 1.74	369.38 ± 1.31
Nandi		
Cheplengu (P)	11.88 ± 2.39	341.13 ± 6.06
Cheplengu (K)	10.25 ± 1.59	331.88 ± 1.75
Samoo	8.25 ± 2.02	350.00 ± 0.71
Bungoma		
Malakisi (B)	8.13 ± 1.25	20.00 ± 0.00
Malakisi (J)	8.13 ± 1.25	32.50 ± 3.54
Kitui		
K2	11.00 ± 0.00	14.50 ± 0.71

Table 4.5: Levels of zinc in fresh tobacco leaves and soil μg/g

[mean ± SD].

REGION	FRESH TOBACCO	SOIL
Muranga		
Kihingu (W)	18.75 ± 1.77	50.00 ± 0.00
Kihingu (N)	18.75 ± 1.77	83.75 ± 5.30
Othaya		
Gatugi (N)	66.25 ± 1.77	283.75 ± 5.30
Gatugi (W)	96.25 ± 5.30	290.00 ± 3.54
Meru		
Mwimbi	53.75 ± 1.77	146.25 ± 1.77
Embu		
Siakago	26.00 ± 2.12	32.50 ± 0.00
Uasin-Gishu		
Chepkoiyo	76.13 ± 1.05	139.19 ± 18.26
Nandi		
Cheplengu (P)	123.75 ± 1.46	173.63 ± 3.09
Cheplengu (K)	90.00 ± 0.41	118.06 ± 6.40
Samoo	116.81 ± 7.49	79.38 ± 4.42
Bungoma		
Malakisi (B)	29.38 ± 5.15	37.50 ± 0.00
Malakisi (J)	37.50 ± 3.54	36.25 ± 1.77

4.4: DIFFERENCE IN HEAVY METAL LEVELS IN SAMPLES FROM THE SAME REGIONS.

The levels of these heavy metals in samples from the same regions were compared and found to be different. The differences were found to be statistically significant in some cases but not in others.

4.4.1: Tobacco

The differences in Cd levels in samples from Othaya (t = 1.93, v =2), Muranga (t = 0.064, v = 3) and Nandi (t = 2.03, v = 3) are not statistically significant in each case. Those of lead in samples from Muranga (t = 0, v = 3) and Nandi (t = 1.78, v = 3) districts are not statistically significant while the difference in lead levels in samples from Othaya (t = 5.094, v = 2) is significant at 95% confidence interval. The differences in zinc levels (t = 18.42, v = 1) are statistically significant for samples from Othaya at 95% confidence level and Nandi (t = 11.487, v = 3) at 99% confidence level. The samples from Muranga are not

different at all. For cases in which samples from the same area show significant difference, this could be attributed to some contaminationn in soil or variation in application of fertilizers by diferent farmers.

4.4.2: Soil

The differences in Cadmium levels in samples from Othaya (t =1.19, v = 1), Muranga (t = 1.90, v = 2) and Nandi (t = 0.333, v = 3) are not statistically significant. The individual regions may be having similar soil types with little differences in their pH. The difference in lead levels in samples from these same regions are statistically significant at 99% confidence interval (t = 21.243, 25.820 and 21.054 : v = 2, 2 and 3 respectively), that of samples from Malakisi (t = 13.287, v = 1) is only significant at 95% confidence interval. Anthropogenic factors may have contributed to the differences in lead levels in the same regions. These differences in Pb levels could also be due to different levels of contamination from automobiles. The zinc levels in samples from Muranga (t = 20.73, v = 1) and Nandi (t = 63.225, v = 3) are significantly different while those from Othaya (t = 3.839, v = 1)and Malakisi (t = 1.879, v = 3) are not significantly different. The significant difference could result from other factors other than rock type. For

instance the use of fertilizers may influence the levels.

4.5: DIFFERENCES IN HEAVY METAL CONTENT OF SAMPLES FROM ADJACENT REGIONS.

Samples from adjacent regions were compared and found to have different levels of heavy metals. These differences are statistically significant in some cases but not in others.

4.5.1: Tobacco

The difference in cadmium levels between samples from Uasin-Gishu and Nandi ($t = 0.33$, $v = 14$), and the difference in lead levels ($t = 0.52$, $v = 14$) are not statistically significant.The difference in zinc levels ($t = 4.34$, $v = 14$), is statistically significant at the 99% confidence level. The difference in cadmium levels between samples from Embu and Meru ($t = 2.23$, $v = 7$) is statistically significant at 90% confidence limit.That between samples from Muranga and Othaya ($t = 0$, $v = 12$) is not statistically significant. The difference in lead levels between samples from Embu and Meru ($t = 1.58$, $v = 7$) is not statistically significant, while that between samples from Othaya and Muranga ($t = 3.77$, $v = 12$) statistically significant at 99% confidence limit. The difference in zinc levels between samples from Embu and Meru

(t = 6.93, v = 4) and that between samples from Muranga and Othaya (t = 6.03, v = 6) are statistically significant at 99% confidence limit. The fact that the Pb levels in adjacent regions are not significantly different confirms that Pb is relatively unavailable to plants and therefore uptake by plants is only to a small extent.

4.5.2: Soil

In samples from Muranga and Othaya the difference in cadmium levels (t = 3.32, v = 18) is significant at the 99% confidence limit. Those from Embu and Meru (t = 2.19, v = 4) are significantly different at the 90% confidence limit. The difference in cadmium levels between samples from Uasin-Gishu and Nandi (t = 2.76, v = 4) is significant at the 98% confidence limit. The lead levels in samples from Muranga and Othaya are significantly different (t = 2.40, v = 18) at the 95% confidence limit. The difference in zinc levels from these adjacent regions (t = 12.49, v = 8) is significant at the 99% confidence limit.In samples from adjacent regions Embu and Meru, the difference in their lead levels (t = 21.01, v = 4) and that in their zinc levels (t = 91, v = 4) are significant at the 99% confidence limit. The difference in lead levels between samples from Uasin-Gishu and Nandi (t = 7.29, v = 12) is statistically significant at the 99% confidence limit. This indicates that the adjacent regions

may not necessarily have same levels of the heavy metals. This is probably due to their differences in soil types. Other factors such as fallout, soil pH and fertilizers may bring about differences in the levels of heavy metals also.

4.6: COMPARISON OF LEVELS OF Cd, Pb AND Zn IN FRESH TOBACCO LEAVES AND CURED OR PROCESSED TOBACCO LEAVES.

A comparison was made between the heavy metal levels of fresh and cured tobacco leaves. The results are given in the following sections.

4.6.1: Cadmium.

Table 4.6 gives the levels of cadmium in fresh tobacco leaves and in processed or cured tobacco leaves. Significance test indicate that there is no significant difference between the cadmium levels in the fresh and in the cured or processed leaves. This is probably because the temperatures used during curing are not high enough to effect any significant Cd loss. On the other hand the curing process may not be contributing much cadmium contamination to the leaves. In case there was surface contamination of the leaves this was washed out during the sample pre-treatment as in the case of flue cured tobacco. In the case of fire cured tobacco the fact that it

was not washed did not seem to have any significant contribution. Samples from Meru (cured/processed) and Siakago (cured) were obtained from the farmers. The way these samples were previously handled may have contributed to the high levels in Cd. This is more so in samples from Meru which had previously been cured and crush by the farmer.

Table 4.6: Levels of cadmium in fresh and cured/processed tobacco leaves $\mu g/g$ [mean±SD].

REGION	FRESH LEAVES	CURED/PROCESSED LEAVES
Kiahiti (M)	0.75 ± 0.00	1.00 ± 0.00
Kihingu (W)	1.17 ± 0.29	1.08 ± 0.09
Kihingu (N)	1.19 ± 0.39	1.33 ± 0.09
T	0.50 ± 0.04	1.58 ±0.52
Mwimbi	1.33 ± 0.29	1.75 ± 0.25
Malakisi (B)	1.37 ± 0.33	1.06 ± 0.13
Malakisi (J)	0.81 ± 0.38	0.69 ± 0.38
Siakago	0.58 ± 0.14	1.19 ± 0.24
Cheplengu (P)	1.00 ± 0.00	0.88 ± 0.14

4.6.2: Lead.

Table 4.7 gives the levels of lead in fresh and in cured or processed tobacco leaves. For all but one case, there is no significant difference between the levels in the fresh leaves and in the processed/cured tobacco leaves. This is in the samples from Nandi (10.25 μg/g-fresh and 289.38 μg/g-processed). The reason for the significant difference could be because the processed tobacco was purchased from an open air market which is prone to contamination fron exhaust fumes released by traffic transversing the town since most vehicles use leaded petrol. The handling of the tobacco by the trader may also have contributed to contamination.

4.6.3: Zinc.

Table 4.8 gives the levels of zinc in cured/processed tobacco leaves. For all the samples there is a significant difference between the zinc levels in the processed/cured and in the fresh tobacco leaves. In most of the samples the levels in the cured/processed leaves are higher than those in the fresh leaves. This could be due to contamination introduced during the curing process. Seven of the samples

were flue cured leaves. During flue curing pipes are heated and this supplies the necessary heat used to drive out moisture from the leaves and to speed up the conversion of chlorophyll into sugars. It is possible that some of the zinc may have come from the zinc coated iron pipes used to supply the necessary heat.

Table 4.7: Levels of lead in fresh and cured/processed tobacco leaves $\mu g/g$ [mean ± SD].

REGION	FRESH LEAVES	CURED/PROCESSED
Kiahiti (M)	7.50 ± 0.00	7.50 ± 0.00
Kihingu (W)	10.00 ± 0.00	10.00 ± 0.00
Kihingu (N)	7.50 ± 0.00	7.50 ± 0.00
T	20.83 ± 9.46	12.50 ± 4.33
Mwimbi	10.00 ± 0.00	11.67 ± 2.89
Malakisi (B)	8.13 ± 1.25	8.13 ± 1.25
Malakisi (J)	8.13 ± 1.25	9.38 ± 1.25
Siakago	7.40 ± 1.32	8.88 ± 1.00
Cheplengu (P)	10.25 ± 1.59	289.38 ± 0.38

Table 4.8: Levels of zinc in fresh and cured/ processed tobacco leaves μg/g [mean ± SD].

REGION	FRESH TOBACCO	CURED/PROCESSED LEAVES
Kihingu (W)	18.75 ± 1.77	60.00 ± 3.54
Kihingu (N)	18.75 ± 1.77	33.75 ± 1.77
T	64.38 ± 0.88	132.50 ± 2.07
Mwimbi	53.75 ± 1.77	102.50 ± 3.54
Malakisi (B)	29.38 ± 0.15	41.25 ± 0.22
Malakisi (J)	37.50 ± 3.54	41.88 ± 3.75
Siakago	26.00 ± 2.12	45.00 ± 0.00
Cheplengu (P)	90.00 ± 0.41	63.188 ± 4.60

4.7: COMPARISON OF LEVELS OF Cd, Pb AND Zn IN TOBACCO LEAVES AND OTHER CROP PLANTS GROWN IN THE SAME LOCALITY.

Table 4.9 gives the levels of Cd, Pb and Zn in fresh tobacco leaves and in other crop plants that grow in the same locality. From the table we notice that there is no uniformity in the type of crops grown together with tobacco. In cases where there is more than one region with the same type of crops, there are both occasions of significance and lack of significance in the differences in their Cd levels. In all cases except samples from Bungoma and Runyenjes the lead levels in the tobacco leaves are lower than those in other crop plants. The zinc levels of crop plants from Runyenjes, Nandi and Bungoma are higher than those in tobacco while in other regions tobacco had higher levels.

4.7.1: Cadmium.

On comparing the levels of Cd in tobacco and maize there is no significant difference. On the other hand there is a significant difference at the 99% confidence level between the Cd levels of sorghum seeds and tobacco from Bungoma and Siakago. In the case of tobacco and pumpkin leaves, the

difference in Cd levels of samples from Nandi is statistically significant at 99% confidence level but not so for samples from Runyenjes. In all these cases tobacco has higher levels of cadmium than the crop plants.

4.7.2: Lead.

A comparison of lead levels in tobacco and maize leaves from Runyenjes indicates an insignificant difference. The levels in tobacco and sorghum from Bungoma are the same. On the other hand the differences between Pb levels in tobacco and maize, and in tobacco and sorghum from Siakago are significant at the 99% confidence level. In areas growing pumpkin together with tobacco, there is a statistically significant difference in their Pb levels. In these cases pumpkin has higher levels than tobacco.

4.7.3: Zinc.

A comparison of zinc levels in tobacco and maize leaves from Siakago, and tobacco and sorghum seeds from the same place indicates a significant difference at the 99% confidence level. The difference in levels of zinc in tobacco and pumpkin from Runyenjes and Nandi is significant at the 99% confidence level. In these cases tobacco has higher zinc levels than pumpkin.

Table 4.9: Levels of Cd, Pb and Zn in fresh tobacco leaves and other crop plants from the same locality μg/g, [mean ± SD].

REGION/PLANT	CADMIUM	LEAD	ZINC
Meru			
Tobacco	1.33±0.29	10.00±0.00	53.75±1.77
Wandering Jew	0.42±0.38	340.9±7.33	105.94±2.45
Runyenjes			
Tobacco	0.92±0.14	9.17±1.44	101.08±1.20
Irish potato (l)	1.00±0.00	7.50±0.00	-
Pumpkin leaves	0.83±0.14	27.58±2.50	67.63±0.18
Maize leaves	1.00±0.00	7.50±0.00	-
Nandi			
Tobacco	1.38±0.14	11.88±2.39	123.75±1.46
Kale leaves	0.56±0.24	19.81±10.75	61.44±5.03
Pumpkin leaves	0.58±0.41	512.5±17.68	79.25±1.06
Bungoma			
Tobacco	0.81±0.38	8.13±1.25	37.50±3.54
Sorghum seeds	0.13±0.018	8.13±1.25	18.75±1.77
Siakago			
Tobacco	0.58±0.14	7.40±1.32	26.00±2.12
Passion fruit	0.38±0.043	28.19±3.13	30.13±3.36
Passion leaves(F)	0.75±0.00	30.42±1.91	22.50±3.54
Passion leaves(D)	0.83±0.14	25.08±0.63	20.25±3.18
Cowpea seeds	0.17±0.014	298.33±4.81	30.38±0.53
Cowpea leaves	0.88±0.18	248.33±3.29	28.88±1.59
Sorghum seeds	0.25±0.25	263.33±10.58	53.00±2.83
Sorghum leaves	0.17±0.14	28.25±3.58	25.25±0.35
Sweet potato (l)	0.42±0.14	28.83±2.02	19.38±0.88
Maize leaves	0.25±0.25	264.17±1.44	14.75±0.00

KEY: F=FRESH, D=DRY, L=LEAF

A general look at the table reveals that in samples from Siakago, most of the crop plants considered had lower levels of cadmium than tobacco. This indicates that tobacco accumulates more cadmium than these other crop plants. Passion and cowpea leaves have higher levels of cadmium than tobacco. The lead levels in all the crop plants, leaves or seeds (28.83-298.33), are higher than that in tobacco (7.40±1.32). This is an indication that other crop plants accumulate more lead than tobacco does. It is evident also that most of the leaf samples from Siakago have lower Zn levels (14.75-25.25) than tobacco (26.00), while passion fruit (30.125 μg/g), cowpea seeds (30.375 μg/g), cowpea leaves (28.875 μg/g) and sorghum seeds (53.00 μg/g) have higher levels than tobacco. This indicates that other than soil factors plant species play a part in determining the amount of heavy metals taken up by these plants. This is because different plants tend to take up metals to varying degrees even when soil conditions are similar.

The results obtained for cadmium in Table 4.9 are similar to those obtained for vegetables from Limuru[50] (0.43-0.82 μg/g) though some have higher levels than this range. Those of lead are much higher than those obtained in the same study (2.40-4.15 μg/g). The zinc levels in samples analysed in this study are similar to those obtained for the same vegetables[50] (14.84-42.30 μg/g).

In the study by Kinoti[100] the Cd and Pb levels in the vegetables are much higher than those obtained in this study.

4.8: LEVELS OF Cd, Pb AND Zn IN CIGARETTES.

Table 4.10 gives the levels of Cd, Pb and Zn in Kenyan cigarettes. The levels of heavy metals in cigarettes that have been reported in the literature are given in Table 4.11.

Table 4.10: Levels of Cd,Pb and Zn in Kenyan cigarettes (μg/cigarette), [mean ± SD].

BRAND	CADMIUM	LEAD	ZINC
Champion	1.47 ± 0.40	10.20 ± 2.74	16.00 ± 1.2
Sweet menthol	1.65 ± 0.41	16.35 ± 5.28	28.00 ± 0.7
Sportsman	1.65 ± 0.78	9.65 ± 0.71	26.00 ± 2.8
Supermatch	1.70 ± 0.67	25.00 ± 0.80	25.00 ± 0.0
Embassy Kings	1.15 ± 0.22	7.80 ± 0.76	20.90 ± 1.9
Rooster	1.75 ± 0.79	12.15 ± 3.07	32.20 ± 5.4
Ten cent	1.00 ± 0.00	7.50 ± 0.612	24.85 ± 4.4
Score	1.10 ± 0.22	9.40 ± 1.52	28.90 ± 4.7
Overall mean	1.434 ±0.30	12.256 ± 5.87	25.231 ± 4.

Table 4.11: Heavy metal content of cigarettes cited in literature (μg/cigarette or μg/g).

COUNTRY	CADMIUM	LEAD	ZINC
Italy[41]	T-0.585μg/g	T-7.395μg/g	0.03-25.74μg/g
W.H.O.[42]	-	10-12μg/cig.	-
Modern Germany[38]	~0.5-1.5μg/cig.	-	-
Romania[39]	1.75μg/cig.	-	-
Switzerland[40]	1.70μg/cig.	-	-
Poland[127] (local)	1.03-3.07 μg/cig.	-	-
Poland[126] (foreign)	1.46-1.83 μg/cig.	-	-

Key : T = Traces.

It is important to note here that for Kenyan cigarette brands, tobacco used for any particular brand does not come from any specific region. The tobacco is blended to produce the desired taste. What is important is the method of curing.

From the two tables, it is observed that cadmium levels in Kenyan cigarettes (1.00-1.75 μg/cigarette) are within the range of those reported in the literature (Traces-3.07 μg/cigarette). Those of lead (7.50-25.00 μg/cigarette), however, seem to be slightly higher than those reported (Traces-12.00 μg/cigarette), though relatively few data were obtained during the literature search. In the case of zinc only one literature value was found during literature search. The mean zinc level in Kenyan cigarettes is above this range (0.03-25.74 μg/g) which if the average weight of a cigarette is ~0.71 g, then the range would be ~0.02-18.27 μg/cigarette. If the values in Table 4.10 were converted to μg/g, then the overall mean levels of Cd, Pb and Zn would be 2.01, 17.26 and 35.54 μg/g respectively.

On comparing these values with the mean levels of these metals in fresh tobacco leaves appearing in Table 4.0 we find that; the Cd (2.01 μg/g) and Pb (17.26 μg/g) levels in cigarettes are much higher than those in fresh tobacco leaves (1.135 and 10.48 μg/g) respectively. On the other hand the mean Zn level is very much higher in fresh tobacco leaves (70.48 μg/g) than in cigarette tobacco (35.54 μg/g). This indicates that during processing of tobacco into cigarettes there is some change in the heavy metal content. The cadmium and lead levels seem to increase probably due to contamination during processing. On the other hand zinc

levels decrease. This could be due to loss during processing.

From the two tables one observes also that Kenyan cigarette consumers are exposed to relatively high levels of lead compared to those in other countries. This is made worse by the fact that a majority of vehicles in Kenya use leaded gasoline. Though the levels of cadmium appear low in Kenyan cigarettes, the fact that it is a cumulative element is reason enough to take precautions to avoid disappointments.

Table 4.12 below gives the mean levels of Cd, Pb and Zn in flue-cured, fire-cured and processed tobacco.

Table 4.12: Mean levels of Cd,Pb and Zn in flue-cured, fire-cured and processed tobacco $\mu g/g$ [mean ± SD]

METAL	FLUE-CURED	FIRE-CURED	PROCESSED
CADMIUM	1.03 ± 0.23	0.88 ± 0.25	1.33 ± 0.34
LEAD	8.50 ± 1.14	10.25 ± 0.65	66.48 ± 1.25
ZINC	44.22 ± 11.15	55.35 ± 0.56	79.89 ± 3.65

In this table processed tobacco refers to snuff and chewing tobacco. On comparing this table with Table 4.10, we find that there is no statistically significant difference between Cd levels in cigarettes and flue-cured, fire-cured or processed tobacco leaves. This suggests that the curing or

processing method does not affect the levels significantly. In the case of Zn levels, there are statistically significant differences at the 99% confidence limit between the levels in cigarettes and those in flue-cured, fire-cured and processed tobacco. The zinc levels in the cured and processed tobacco are much higher than those in cigarettes. This could be due to contamination during curing/processing part of which is lost during cigarette manufacture. The Pb levels in fire- or flue-cured tobacco are however lower than those in cigarettes. This is probably because of surface contamination during cigarette making. But the levels in processed tobacco is much higher than that in cigarettes. This is probably because during their processing other substances are added, for example in snuff making spices and fat are added to improve the taste. This could be an area where contamination comes in. In the chewing tobacco, the curing process may include open air drying subject to surface contamination, hence the high Pb levels.

On comparing the above table with Table 4.0 we find that even though fire cured tobacco was not washed this did not seem to affect the heavy metal levels in these leaves. On the contrary they have Cd levels lower than most of the fresh leaf samples analysed, the Pb and Zn levels fall within the range of fresh leaf samples considered.

4.9: SUMMARY

Cadmium is known to be particularly toxic to almost all organs in the body. It interacts with calcium, zinc, iron, copper, sodium and magnesium by either preventing their absorption through the intestines or by replacing them in enzymes and proteins.

Lead is known to be a protoplasmic poison that acts slowly but subtle. It interacts with calcium, phosphorous, copper and iron by replacing them or interfering with their metabolism. It interferes with copper metabolism which secondarily leads to dysfunction of iron metabolism and haem synthesis[108]. The two most important points of interference in the biosynthesis of haem are in the formation of the enzyme δ-aminolaevulinic acid dehydratase (δ-ALAD) and the insertion of iron into protoporphyrin[108]. Lead interference with the insertion of iron into the protoporphyrin results in the accumulation of the latter in the erythrocytes.

Lead, like cadmium, has an affinity for sulfur. It therefore competes with calcium and zinc for sulfhydryl groups. It normally accumulates in the liver, kidneys and the bones and affects both the hematopoietic and the nervous systems. Cigarette smoking has been associated with increased blood lead levels and increased cadmium levels in the kidney

cortex and blood in both passive and active smokers.

In view of the toxicity of lead and cadmium a study was carried out to find out their levels in tobacco and cigarettes whose consumption has been increasing steadily in the recent past especially among the youth. The same was done for soils and crop plants from the sample areas to see whether the levels in the tobacco compared with those in other crop plants and in soils on which they grow. The soil pH was determined to find out if it had an effect on the metal levels in the tobacco.

The results show that the mean cadmium concentration in Kenyan cigarettes (1.00-1.75 μg/cigarette) and in tobacco leaves (0.5-2.33μg/g) compare well with those reported in the literature (Traces-3.07 μg/cigarette and 0.5-5.00 μg/g respectively). Those of lead (7.50-25.00 μg/cigarette) and zinc (16.00-32.20 μg/cigarette) in cigarettes are on the higher side than literature values (Traces-12.00 μg/cigarette) and (0.02-18.27 μg/cigarette) respectively. In fresh tobacco leaves most of the Pb levels (7.40-20.83 μg/g) fall above the range of plants grown on uncontaminated soils (0.1-10.0 μg/g)[50]. The zinc levels (18.75-169.00 μg/g) in fresh tobacco leaves are also above the normal range (25-250 μg/g)[47] recorded for plants.

The results also indicate that tobacco has higher metal content than some crop plants. For instance it has higher cadmium levels than passion fruit, kale and pumpkin and it has higher zinc levels than pumpkin leaves and sorghum seeds. Some crop plants, however, have higher lead levels than tobacco eg. pumpkin leaves, passion fruit, cowpea seeds and sorghum seeds. Notable is the fact that cadmium and lead levels in the cigarettes are higher than in the fresh tobacco leaves while those of zinc are lower in cigarettes than in the fresh leaves.

The results indicate that the soils from the sample areas were fairly acidic with most of the soils having pH less than 7.0 but greater than 4.0.

CHAPTER 5

5.1: CONCLUSION AND RECOMMENDATIONS

5.1.1: Conclusions.

From this study a number of conclusions may be made. First, tobacco accumulates more cadmium than some crop plants such as pumpkin leaves, kale and sorghum seeds, while some of the crop plants considered accumulate more cadmium than tobacco. The cadmium levels in fresh tobacco leaves ranged from 0.5-2.33 μg/g while those in the other crop plants ranged from 0.17-1.00 μg/g.

Secondly, the Cd levels in the fresh tobacco leaves (0.50-1.37μg/g) and in the cured/processed tobacco (0.688-1.75μg/g) are not very different. The difference in levels of lead in the fresh leaves (7.40-20.83μg/g) and in the cured/processed leaves (7.50-12.50μg/g) respectively, is also small. Since there is very little difference in the levels of cadmium and lead levels between fresh tobacco leaves and the cured or processed tobacco, the curing process does not seem to affect the metal content of the tobacco. This is, however, not the case with zinc. The level in the fresh

leaves is in the range 18.75-90.00μg/g and in the cured/processed leaves is 33.75-102.50μg/g. In this case, curing appears to increase the levels in tobacco leaves.

Thirdly, the cadmium levels in Kenyan cigarette brands range from 1.00 to 1.75μg/cigarette while those reported in literature range from traces to 3.07 μg/cigarette. Therefore these compare well. Those of lead range from 7.5 to 25 μg/cigarette while literature values range from trace-12μg/cigarette. Zinc values in Kenyan cigarettes range from 16-32.2μg/cigarette while literature values range from 0.02-18.27 μg/cigarette. This indicates that Kenyan cigarettes have higher Pb and Zn levels than those reported.

Fourthly, the mean cadmium content of fresh tobacco leaves is 1.135 μg/g while that of cigarettes is 2.01 μg/g. The levels in most of the fresh leaves fall within the range 0.5-1.375 μg/g which is lower than those in cigarettes (1.40-2.46) μg/g. The mean lead content in fresh tobacco leaves is 10.48 (7.40-16.67) μg/g and that in cigarettes is 17.26 (11.56-32.68) μg/g. This indicates that the Cd and Pb levels in Kenyan cigarettes are higher than those present in fresh tobacco leaves. The zinc levels are 70.48 (range 18.75-123.75) and 35.54 (range 22.54-45.35) μg/g respectively. This indicates that zinc levels are higher in fresh

tobacco leaves than in cigarettes.

Finally, the soil pH of all the regions considered in this study was between 4.5 and 7.4. This shows that most of the soils were fairly acidic apart from two. This implies that tobacco thrives well in fairly acidic soils.

5.1.2: Recommendations

The toxicity of heavy metals is governed by such processes as complexation with organic and inorganic metal ligands, and adsorption to organic particulates and hydrous metal oxides. Complexes formed by coordination with weak field monodentate ligands are usually of low stability and are suspected of being more potent toxicants. Strong field monodentate complexes are relatively more stable and are considered to be less potent toxicants than the former. In this study 'total' concentration of the heavy metals Cd, Pb and Zn were determined. The forms in which they occur were not determined and therefore speciation studies need to be carried out to ascertain the chemical forms of these metals in fresh tobacco and its products.

It would be interesting to find out if Kenyan smokers have higher levels of lead and cadmium in their blood and urine than non-smokers.

It is also necessary to determine the amounts of these and other heavy metals present in the fertilizers that are supplied to farmers so that we can advice them accordingly.

It is important also to determine the levels of these metals using other analytical techniques such as polarography and X-ray fluorescence to find out if similar results will be obtained. This is necessary because of AAS is limited by the fact that samples have to be in solution.

Factors that influence the metal uptake by tobacco plant need to be investigated and the forms in which these metals occur in the soil need to be determined.

There is a need to also find out the particle sizes of the metals in cigarette smoke since these might differ from those in ambient air in their availability for absorption.

There is a need to determine levels of other toxic metals in tobacco leaves and cigarettes.

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APPENDIX A

STATISTICAL TREATMENT OF DATA

1. Mean

This gives the average of the values obtained.

$$\bar{x} = \frac{\sum_i^n x_i}{n} \qquad \text{(A-1)}$$

Where x_i is the ith term of the determination and n is the total number of determinations.

2. Standard deviation

This was used to measure the dispersion of the obtained readings about the mean. The following equation (A-2) was used to calculate the standard deviation.

$$s = \sqrt{\frac{\sum_i^n (x_i - \bar{x})^2}{n-1}} \qquad \text{(A-2)}$$

3. Confidence limits

For increasing accuracy confidence limits were used. This is given by equation (A-3) below.

$$C.L = \bar{x} \pm \frac{ts}{\sqrt{n}} \qquad \text{(A-3)}$$

4. Coefficient of correlation

Coefficient of correlation was employed to find out whether the levels from different areas were correlated; whether the levels in fresh tobacco leaves correlated with those in cured or processed tobacco leaves. Also the levels in soil and fresh leaves were correlated and equation (A-4) below was used.

$$r = \frac{\sum_i^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 (y_i - \bar{y})^2}} \qquad \text{(A-4)}$$

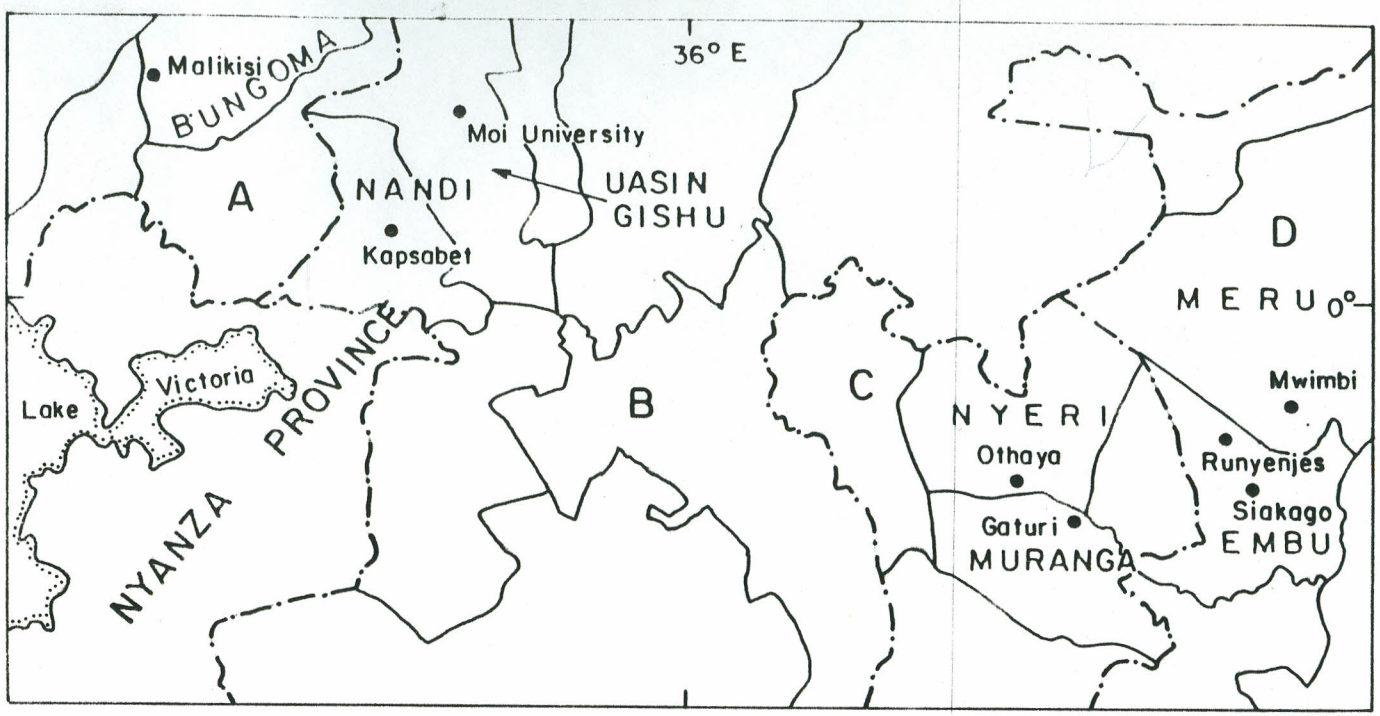
5. Test of significance

It is necessary to use a proper test to see whether a correlation coefficient is indeed significant bearing in mind the number of pairs of points used in the calculation. The t-test was used in this case using the equation (A-5) below.

$$t = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}} \qquad \text{(A-5)}$$

The calculated t value was compared with the tabulated value at the desired significance level, using a two-tailed t-test and n-2 degrees of freedom. If the calculated value of t is greater than the tabulated one we conclude that a significant correlation does exist, otherwise it does not.

APPENDIX B: MAP OF SAMPLING AREAS.



BMK