ANALYSIS OF TRACE ELEMENTS IN KENYAN ALCOHOLIC BEVERAGES AND THEIR SOURCE RAW MATERIALS

BY

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DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in any university.

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DEDICATION

To the glory of God and to my mother Mrs. Jacinta Waceke Karanja.

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LIST OF ABBREVIATIONS

AAS	ATOMIC ABSORPTION MASS SPECTROSCOPY.
ACS	AMERICAN CHEMICAL SOCIETY.
AIDS	ACQUIRED IMMUNO-DEFFICIENCY SYNDROME.
APDC	AMMONIUM PYRROLIDINE DITHIOCARBAMATE.
APHA	
AWWA	
WPCF	AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION AND WATER POLLUTION CONTROL FEDERATION.
DC	DIRECT CURRENT.
DPASV	DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRY.
FAO	FOOD AND AGRICULTURE ORGANISATION.
FDA	FOOD AND DRUGS ADMINISTRATION.
FSC	FOOD STANDARDS COMMITTEE.
HMDE	HANGING MERCURY DROP ELECTRODE.
IARC	INTERNATIONAL AGENCY FOR RESEARCH ON CANCER.
ICAP	INDUCTIVELY COUPLED ARGON PLASMA.
ICPMS	INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY.
ILO	INTERNATIONAL LABOUR ORGANISATION.

IPCS	INTERNODAL PROGRAM ON CHEMICAL SAFETY.
ISO	INTERNATIONAL STANDARD ORGANISATION.
KEBS	KENYA BUREAU OF STANDARDS.
KM- KILOMITA MOJA	A SLUM AREA ONE KILOMETRE FROM KENYATTA UNIVERSITY GATE.
MIBK	METHYL ISOBUTYL KETONE.
NAS	NATIONAL ACADEMY OF SCIENCES.
PTWT	PROVISIONAL WEEKLY TOLERABLE INTAKE.
PVPP	POLYVINYLPOLYPYRROLIDINE.
SCE	SATURATED CALOMEL ELECTRODE.
TFME	THIN FILM MERCURY ELECTRODE.
UNEP	UNITED NATIONS ENVIRONMENTAL PROGRAMME.
UNEPIE	UNITED NATIONS ENVIRONMENTAL PROGRAMME IN INDUSTRY AND ENVIRONMENT.
USEPA	UNITED STATES ENVIRONMENTAL PROTECTION AGENCY.
WHO	WORLD HEALTH ORGANISATION.
XRMF	X-RAY MICROFLUORESCENCE.

ABSTRACT

One hundred and twelve (112) alcoholic beverage and twenty six (26) raw material samples were analysed for lead, cadmium, copper and aluminium by atomic absorption spectroscopy (AAS). A few of these samples were analysed by differential pulse anodic stripping voltammetry (DPASV) and inductively coupled plasma mass spectrometry (ICPMS). Some samples contained trace elements above limits set by Kenya Bureau of Standards (KEBS) and World Health Organization (WHO). Concentrations of lead ranged from 0.003-0.466 mg/l, copper: 0.013-2.363 mg/l, cadmium: ND-0.05 mg/l and aluminium: 0.043-56.906 mg/l. Only one (1) sample contained copper above 2.0 mg/l. The limits set by KEBS and WHO are as follows: lead, 0.1 mg/l; copper, 2.0 mg/l; aluminium, 0.2 mg/l; cadmium, 0.005 mg/l and arsenic; 0.5 mg/l. Of great concern is the fact that aluminium was found in over half of the brews. This is a serious health threat as aluminium is associated with Alzheimer's disease. Speciation studies were carried out on samples that had high levels of cadmium, copper and lead. It was established that most of these metals are "bound", as most of the samples did not produce peaks upon analysis. However, some samples contained free lead or cadmium or both. Free metals are considered to be more dangerous than complexed ones. The results obtained by AAS and DPASV were correlated for each of the metals analysed and there was no significant difference in the concentrations obtained by both methods. Arsenic was determined by ICPMS and concentration ranges were 1.467-3.758 mg/l. This study has highlighted the presence of some trace elements in alcoholic beverages. The findings are useful in that they can provide a justified investment by the state in fighting the brewing of illicit alcoholic beverages.

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND AND FOCUS OF THE STUDY.

Consumption of illicit (unlicensed) brews has been on the increase in recent years. Although people have lost lives after taking these brews, this has had little effect on the trends in preparation and consumption of these brews. There has been proliferation of companies doing licensed brewing business as well as unabated commercial illegal brewing. There was need to analyse these beverages in light of the health problems associated with them. Production and consumption of illicit brews is probably due to the high cost of commercial beers, spirits and wines.

1.1.1. THE BREWING PROCESS.

Brewing is the oldest of all applications of biotechnology and was practiced albeit in a rudimentary way in ancient Egypt. The processes then used had in common with more modern counterparts only in the exploitation of active yeast, to convert fermentable carbohydrates into ethanol. Brewing is a mature industry with a global capacity to produce 100 billion litres of beer annually (Ian, 1992). Beer is a mixture of alcohols, esters, aldehydes, acids, carbohydrates, proteins, vitamins, polyphenols and a plethora of other substances. All these make a contribution, however subtle, to the product quality. These substances are derived either directly from the raw materials or by chemical and biochemical evolution during the brewing process (Ian, 1992).

A comprehensive review of the many different variations of the brewing process whereby standard raw materials can be converted into a range of different beer products (e.g. lager, ale and stout) is available in the literature (Briggs et al, 1981, Hough et al, 1982 and Pollock J.R.A., 1979, 1981, and 1987). During these processes contamination of the beverages with trace elements is possible.

Traditionally the brewing process has consisted of a linear sequence of processing event. Malted barley is the ingredient of the brewing process, from which is obtained the fermentable sugars and all the other requirements for the growth of yeast. Grains of barley (*Hordeum vulgare*) contain about 40-65% of their dry weight as starch, the main carbohydrate reserve material, but unmodified starch is not fermentable by yeasts. Barley grain is converted into malt thus, dried barley grains are steeped in water for about two days, during which time their water content increases by about four times to 40-45%. The process of germination is then initiated under conditions of controlled aeration and temperature. The enzymes carbohydrases and proteases are formed. The germination process is terminated prematurely after about five days and the grains dried in a kiln. Before the barley is malted it must be cleaned. Dust, larger foreign objects, and impurities are removed by screening. A magnetic separator removes iron and steel particles. Foreign objects and impurities of the same size as the barley grains, are removed by means of separators. These foreign materials may contain toxic metals (UNEPIE, 1988).

The malted grains are extracted with hot water to yield a nutritionally rich liquid termed the "sweet wort". Sweet worts are boiled in specialized kettles or coppers for 1-2 hours. Female flowers of the hop plant (*Humulus lupulus*) are added to the kettle

before boiling commences to bitter the wort. Solids are allowed to settle from the suspension. The clarified (clear) and sterilized hopped wort is then removed from the kettles.

Hopped worts are cooled to a temperature of between 8 °C and 18 °C suited to the growth of the yeast strain in use. Typically, brewing yeasts are strains of *Saccharomyces uvarum* (formerly *S. Carlsbergensis*). The yeast is added to the hopped wort in the fermenter. Settlement, centrifuging and filtration separates the yeast from the fermented wort.

A controlled secondary fermentation and storage follows in a vat. A small amount of fresh yeast and unfermented wort are added to a much larger volume of the fermented wort and held at between –2°C and 18°C. This is called lagering i.e. breaking down of flavour-active substances at very low temperature and improves the colloidal stability of the product.

Stabilization involves addition of preservatives and substances that prevent haze formation e.g. powdered silica gel and polyvinylpolypyrrolidine (PVPP) which absorb high molecular weight proteins and polyphenols which are the principal components of beer haze. Fermented wort may be distilled to form whisky. It is worth noting that not all alcoholic beverages are based on barley or cereals in general. For instance distillation of fermented mollases yields rum while brandy is obtained by distillation of wine (WHO, 1983).

Unlicensed brews have no standard way of preparation and various additives add to possibilities of contamination. Busaa is prepared by soaking a mixture of maize and sorghum flour in warm water for three days. Filtration by squeezing the flour on sisal sacks follows. The flour is then sun dried and half-baked on large pans. Baked flour is then fermented to yield busaa. Distillation of busaa yields chang'aa. Most of the other brews are based on molasses (e.g. kangara), sugarcane (e.g. muratina), and fruits such as pineapples (e.g. pineapple wine). In all these cases the sugars present are fermented by use of yeast to produce the desired brew. The water, additives and containers used in brewing determine the level of trace elements in the beverages. Therefore the production of illicit brews differs from conventional methods in that no care is taken to prevent contamination and no tests are carried out during the production process to ensure good quality.

Since the conditions under which the illicit brews are prepared are unhygienic, brews are usually contaminated with toxic substances, which include trace elements. Contamination with trace elements may come from water used in brewing, contaminated raw materials or leaching of metals from containers. Aerial depositions as well as use of additives in brews are other factors that can influence the levels of these elements in the brews.

Trace elements play a role in human toxicity and most of them are cumulative toxins. Since the body has inadequate mechanisms for their excretion, chronic low-level intakes can accumulate to toxic proportions. Treatment has been relatively unsuccessful because no effective means have been discovered to increase their excretion (U.S. Council on Environmental Quality, 1980). Therefore there is need for

continuous monitoring of substances consumed by the public to make sure that they are not exposed to dangerous levels of these toxic metals.

1.2. OBJECTIVES OF THE STUDY

1. To determine the concentration of toxic metals such as cadmium, lead, copper and aluminium in both locally manufactured and imported alcoholic beverages.
2. To determine the concentration of the above metals in the raw materials used in the manufacture of alcoholic beverages.
3. To compare the sensitivity of atomic absorption spectroscopy (AAS) and differential pulse anodic stripping voltammetry (DPASV) as methods of analysis of the heavy metals.
4. Determine the extent of free and associated metals in the brews.

1.3. JUSTIFICATION.

In Kenya people have died after taking illicit alcoholic beverages. Some have lost their eyesight. These are events that have lead to a crackdown by the administration on the brewers of these illicit brews. Despite the known hazards of these metals, little care is taken to ensure their absence in many illicit brews. Although studies on trace elements in alcoholic beverages have been carried out, there is need for regular monitoring especially of new brands.

This study was carried out in order to establish whether these beverages contain high levels of these toxic metals and whether these metals in the beverages are as a result of

contaminated raw materials or the processes involved in their preparation. Some of these beverages are made in containers that may not be safe and since some beverages are sometimes acidic, metals could be leached from containers depending on what the containers are made of (Lauwerys and Perinet, 1993). Some studies have indicated that some raw materials such as millet, finger millet and sorghum tend to accumulate these metals. The public outcry (in the print and electronic media) over the effects of these beverages call for studies in all chemical aspects of these beverages so that the public can be warned of possible dangers of drinking these beverages.

Beer is widely consumed both locally and internationally. Much research work has been done on beer and whisky i.e. purified spirits but data on trace metals in illicit brews is lacking. There was need to ascertain the presence of these metals and their concentrations.

Although other metals (e.g. zinc and mercury) are important in human toxicity, they were not analysed due to the following reasons. Firstly, the method for analysing mercury (cold vapour atomic absorption spectrometer) requires specialized equipment, which was not installed. Secondly, zinc toxicity limits are so high (50 mg/l) and therefore is not a serious threat to health. Lastly, although aluminium, cadmium and lead are not nutrients, they are indispensable in industrialization and therefore end up in the environment. They are very toxic and cause a host of ailments.

CHAPTER TWO

LITERATURE REVIEW

2.1. TRACE ELEMENTS IN THE ENVIRONMENT.

2.1.1 Lead.

The principal lead mineral is the sulphide, galena, but the element is also found as cercussite, $PbCO_3$, anglesite $PbSO_4$, phyromorphite $PbCl_2 \cdot 3Pb_3 \cdot (PO_4)_2$ and other minerals. It is also found in association with molybdenum, vanadium, arsenic, copper, bisthmuth, antimony and silver (Young, 1992).

The principal use of lead is in the lead acid accumulator, tetramethyllead and tetraethyl lead in petrol, roofing, flashing and sound proofing in building industry, cable sheathing, ammunition, solder (67% Pb and 33% Sn), heavy duty bearing metal and as a PVC stabiliser (Fergusson, 1990).

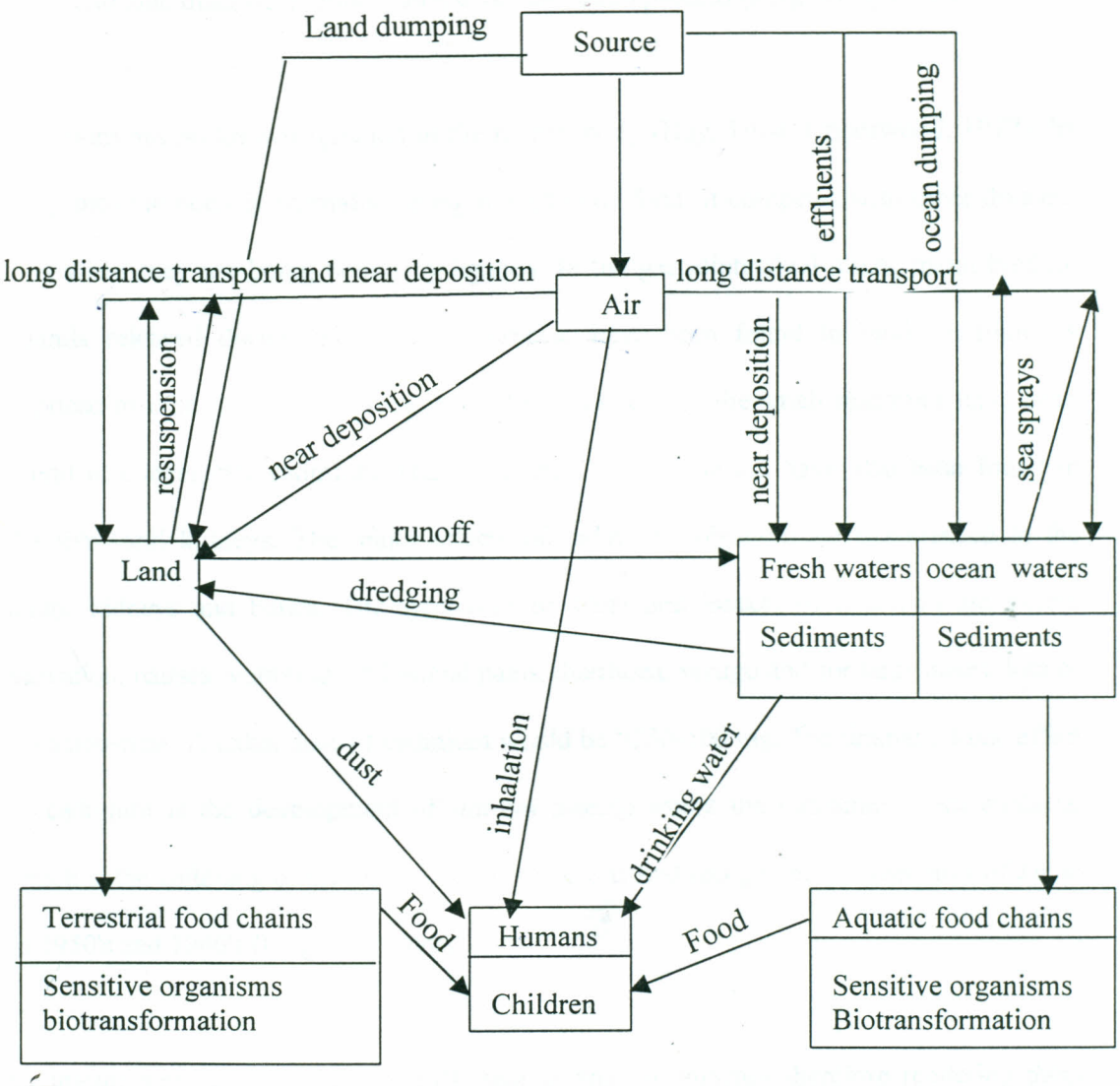
The main routes of entry for lead compounds in the both are gastrointestinal tract and the lungs. Up to 65% of ingested lead can be absorbed in the bloodstream (Lane, 1981). Lead contamination of alcoholic beverages can be through the use of lead compounds in the manufacture, storage, transportation of the drink. It can also result from foodstuffs prepared in cookware containing leachable lead as well as fruits sprayed with lead-based pesticides. Aside from its use in plumbing, lead became a potentially serious contaminant when it began to be used along with arsenic many years ago as a pesticide (Ian, 1992). For a long time lead arsenate was the principal spray used on apples and pears to control

insect infestation. Another direct source of lead contamination is the high-lead solder used on the side seams of tin cans. Studies have shown that the amount of lead so contributed is usually not great, but it is often detectable, as in the case of evaporated milk or canned tomato juice (Eschnauer, 1982).

Lead has no known function in the body. It is a potential protein inhibitor (Hay, 1984) because it binds the sulphydryl (SH) group. Lead inhibits the biosynthesis of heme (the iron porphyrin component of haemoglobin) and utilization in the body (Luckey and Venugopal, 1977). Chronic exposure to lead results in its mineralization in the bones from where it can be released following metabolic disturbances (Winter, 1990). This is a potential source of lead for other tissues (Schroder and Nason, 1971).

Acute effects of lead poisoning are nausea, vomiting, abdominal pains, anorexia, constipation, insomnia, anaemia, irritability, mood disturbance and co-ordination loss (Fergusson, 1990). In more severe situations neurological effects such as restlessness, hyperactivity, confusion and impairment of memory can result as well as coma and death (Grandjean and Nielson, 1979). High lead levels in the human body affect the haemopoietic system, leading to anaemia. In serious cases the nervous system is affected leading to irreversible brain damage i.e. acute encephalopathy, and the renal system. In mild form, lead affects the central nervous system manifested in hypertension and impaired motor skills. Even low levels of lead affect the human biochemistry and so lead cannot be taken lightly (Fergusson, 1990).

FIGURE 1. Bio-geochemical cycle of lead and routes of human exposure (Fergusson, 1990).



2.1.2. Cadmium

Cadmium is used in electroplating, anticorrosive coating, pigments, stabilizer in polyvinyl chloride, batteries and in alloys. Cadmium is associated with zinc in many of its ores and the Cd/Zn ratio for a number of ores is around 0.0042:1 (Fergusson, 1990). Cadmium is always in association with zinc as an impurity in the metal and its compounds and is obtained as a by product of zinc manufacture. For example rubber

tyres can contain 20-90 μg g^{-1} of cadmium due to the use of zinc compounds such as zinc oxide and zinc dialkylcarbamates in the vulcanization process (Fergusson, 1990).

Cadmium has no known function in the human body (Hay, 1984, Underwood, 1977). Its entry into the body is normally during ingestion of food. It competes with other divalent ions when they are being bound by ligands. In the gastrointestinal tracts, metal binding ligands released during the digestive process have been found to bind cadmium as opposed to other divalent ions e.g. zinc. Mucosal cells of the small intestines have been found to concentrate cadmium. High concentrations of the ion have also been found in the liver and kidneys. The major effects of cadmium poisoning are experienced in the lungs, kidneys and bones. The symptoms of acute oral intakes of cadmium are excess salivation, nausea, vomiting, abdominal pains, diarrhoea, vertigo and for large doses, loss of consciousness. A lethal dose of cadmium would be >350-500 mg. The dramatic toxic effect of cadmium is the development of *Itai-itai* disease where the outcome is osteomalacia, which is the softening of the bones. This disease was first recognised in Jinstu area of Japan in 1950's and 1960's (Ferguson, 1990).

Cadmium replaces essential elements such as zinc in enzymes therefore rendering them biologically inactive. It also causes anaemia probably due to competition with iron. Cadmium induces the synthesis of metallothionein in the liver. The metallothionein-cadmium complex is transferred to the liver and when the capacity to bind cadmium is exceeded, cadmium appears in urine. Therapy for cadmium poisoning is not readily available. Cadmium also interferes with Ca^{2+}, PO_4^{3-} and vitamin D metabolism hence these appear in urine indicating cadmium poisoning. Cadmium is also believed to play a role in cardiovascular diseases especially hypertension (Ferguson, 1990).

Cadmium is a very cumulative toxin with a biological half-life of over 10 years in man (a half-life of over 30 years has been shown in the muscles). In blood over 70% of the cadmium is bound to red blood cells (Lauwerys, 1978). The kidney is the critical organ of intoxication after long-term exposure to cadmium. One of the initial signs of renal dysfunction is an increased urinary excretion of proteins. Cadmium-induced protenuria is generally considered to be characterized by the excretion of low molecular weight proteins particularly α_2, β_2, and γ_2-globulins (Hutton, 1987). Glomerular type of dysfunction may also be an early effect of cadmium exposure, as evidenced by an increased excretion of high molecular weight proteins. Later effects on renal function are manifested by aminoaciduria, phosphaturia and glycosuria (Frieberg *et al.*, 1974).

2.1.3. Copper.

Copper is widely distributed in nature in free state, in sulfides, arsenides, chlorides and carbonates. Copper is used in making electrical wires and alloys used in various industrial applications. Copper enters the aquatic environment from soils and mineral deposits by erosional action of water. Tubes made of copper and copper alloys are widely and increasingly used for domestic plumbing and heating systems, air conditioning, refrigeration and industrial applications. In agriculture, copper compounds, especially copper sulphate is used as fungicides, pesticides, algaecides, nutritional supplements in animal feeds and fertilizers. It is also used in growth promoters and disease control in livestock and poultry (WHO, IPCS, 1998).

Copper is a definite constituent of several enzymes catalysing oxidation-reduction reactions (oxidases) in which the activity is believed to be due to the shifting of copper between the

+1 and +2 oxidation states. The human adult requirement is 2 mg/day and the adult body contains 100-150 mg of copper, the greatest concentration existing in the liver and bones (Considine, 1989).

In non-occupationally exposed persons, gastrointestinal absorption is the main route of copper entry to the body (Lauwerys and Perrinet, 1993). It is highly toxic at elevated levels of exposure, causing Wilson's disease which is characterised by an accumulation of potentially toxic levels of copper in both the brain and the liver (NAS, 1977). An important part of its toxicity is its combination with thiol groups in proteins thus inactivating them. Effects of chronic exposure are cirrhosis of the liver, failure of growth and jaundice (Considine, 1989).

Copper is an essential element and adverse health effects are related to deficiency as well as excess. Excess copper causes epigastric pain, headache, nausea, dizziness, vomiting and diarrhoea, tachycardia, respiratory difficulty, haemolytic anaemia, haematuria massive gastrointestinal bleeding, liver and kidney failure and death. Chronic ingestion of copper leads to liver failure (WHO, IPCS, 1998). In a family in Vermont, U.S.A. living at the end of a copper main water pipe there were recurrent episodes of gastrointestinal illness. There were no symptoms in two other families of similar age and sex distribution on the same street. The symptoms ceased with a change of water source, confirming that copper was being leached from the mains water supply (WHO, IPCS, 1998).

Five investigations of gastrointestinal upsets associated with ingestion of copper-contaminated water have also been carried out (Knobeloch *et al,* 1994). Data were obtained from questionnaire on age, weight, water use habits, duration of exposure and

symptoms. There was generally a higher incidence of intermittent or constant symptoms of diarrhoea, abdominal cramps or nausea in those who consumed first-draw water, in infants and young children and among residents of newly constructed or renovated houses. In one study, gastrointestinal symptoms occurred in 8 out of 14 people ingesting 0.6-3.8 mg Cu/day from drinking fountains (1.6-7.77 ppm Cu) compared with 3 out 26 people ingesting ≤ 0.55 mg Cu/day from drinking water.

2.1.4. Aluminium.

Aluminium is the third most abundant element in the earth's crust after oxygen and silicon. Aluminium finds its use in the building and construction industry, for transportation equipment (e.g. automobiles airplanes) and for electrical goods including long distance power transmission lines. It is also used for machinery equipment, packaging of consumer goods (beverage cans), cooking pots and other applications. Aluminium concentrations in the soils range from 10 to 30 g/Kg (Jones, 1986). Consumption of drinking water directly or through food preparation will lead to ingestion of natural aluminium.

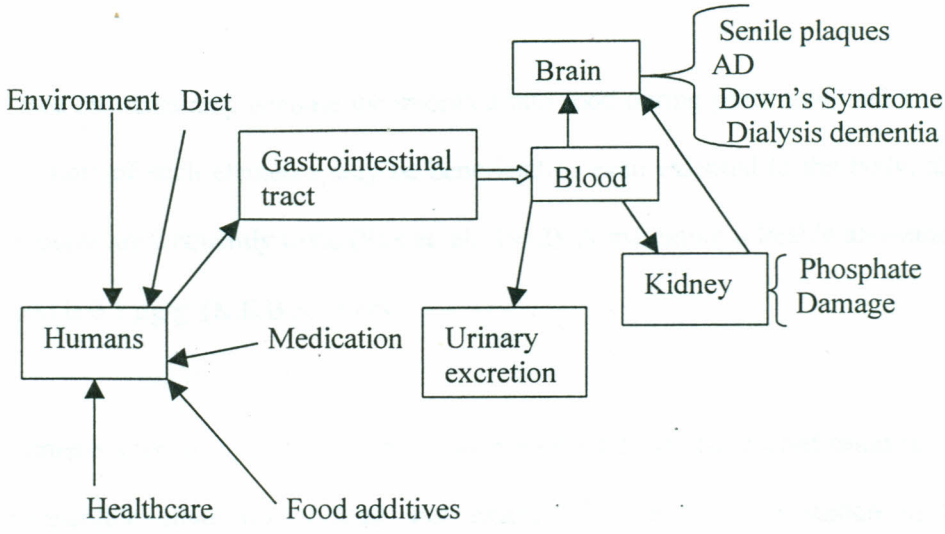
Aluminuim compounds are widely used for industrial, medical, domestic and agricultural applications. Aluminium sulphate, for example, is widely used to improve the clarity and purity of drinking water. Various aluminium compounds are used in the processing, packaging and preservation of food, while others are used to prevent hyperphosphataemia in patients suffering from renal failure, and as antacids, antiperspirants and adjuvants for vaccines and toxoids (Jones, 1986)

Aluminium is available to humans through uptake in the gut. The major factor influencing aluminium uptake through the gut is its solubility, which in turn must be, affected by both pH and complexing agents. The pH of the gastric juice is 2 and this increases aluminium bioavailability in man. Aluminium has been implicated in Alzheimer's disease, which is characterized by elevated concentrations of aluminium in some neurones (Massey and Taylor, 1988). Neurological disorders like parkinsonism-dementia, speech disturbance, tremors, psychosis and personality changes have been associated with toxicity to aluminium (Richardson, 1993). Aluminium also causes proximal myopathy (Ellis, 1984).

The major concern on aluminium toxicity is however on development of Alzheimer's disease. In the United States, Alzheimer's disease affects 1.5 million people and costs the US Government 30 billion dollars every year in health care. There is a possibility that the number of those affected will double in the next 40 years (Nicholas, 1999). In the United Kingdom an estimated 500,000 people are affected while in Britain one person in 20 over the age of 60 is affected, (Nicholas, 1999).

Alzheimer's disease is a progressive brain disease of unknown aetiology, characterised by development of large numbers of neurofibrillary tangles and senile plaques in the brain. Typical early symptoms of the disease are reduced short-time memory, bad time orientation and speech defect. Later on long-time memory will also become worse. The disease mostly affects those above 70 (Thompson, 1989). Aluminium poisoning is also known to cause diarrhoea, skin rashes, sore throats and ulcers but on short-term basis (Prescot, 1989). The gastrointestinal absorption is considered to be the main entry route leading to systemic toxicity (Randal, 1983). Aluminium forms weak complexes with fluoride and the fluoride in food and water may modulate aluminium absorption (Spencer et. al. 1980).

Figure 2. Pathways of aluminium in humans, (Duffield and Williams, 1988).



Exposure to trace elements occurs primarily through inhalation of air and ingestion of food and water. Since there are many sources of trace elements that enter the food chain through food, water, drugs, etc., this study was undertaken to highlight the contribution of these beverages to accumulation of these trace metals in the body.

It is also important to study these elements as emphasis has been on organic compounds as the cause of problems with the killer brews ignoring the health risk posed by trace elements. Therefore there is need for continued analysis of the levels of these toxic metals in brews as a way as a way of determining their safety and the contribution to the body's burden of these metals as a result of drinking.

2.2. TRACE ELEMENTS STUDIES IN FOODS.

Trace elements may become incorporated into food during processing and although minute amounts of such elements may be beneficial or even essential to the body, slightly larger amounts are frequently toxic (Fox et. al., 1972). A maximum tolerable allowance for lead in food is 0.1 μg/g (K.E.B.S., 1998).

Numerous analyses of both fresh and preserved foods have confirmed the presence of measurable quantities of lead. For example, the experiment station of the Instituto Superiore di Sanita in Italy has reported an average of slightly more than 0.1 mg/l in a large number of samples of the fresh juice of oranges, lemons, pears, apples, and grapes, (Nicholas, 1999). In each of the corresponding canned juices, it reported higher levels with the average lead content of 15 samples of pears being 0.9 mg/l. One series of American studies reported 0.14 mg/l of lead in unprocessed tomato juice and slightly less than 0.4 mg/l in the canned product (Nicholas, 1999). In the U.K., analysis of some 3,000 samples of a wide variety of foods revealed a weighted average of 0.13 μg/g of lead in the diet as a whole, the highest figure for 65 samples of canned baby foods being 1.1 μg/g (FAO/WHO, 1976). Numerous similar analysis are available in the literature, showing measurable amounts of the fresh product and appreciably more in the processed product (FAO/WHO, 1976).

A case study of lead in Mexican seasoning and medicinal herbs was carried out at the US-Mexican border area of Elpaso-Juarez (Nicholas, 1999). The role of the diet as a source of exposure to toxic metals was investigated using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). Lead levels in the 10-50 μg/g range were detected in several

samples of Oregano and Ruda, traditional and widely used home brewed medicinal herbs. The worst contaminated, Ruda, was not packaged or processed, only dried, sold in the market and placed in a paper bag. This suggests uptake from the soil or airborne contamination during growth. Elevated levels of lead were also reported in Oregano, also used in local Mexican-style cooking (Nicholas, 1999).

A study of leachable lead in ceramic food vessels was also carried out. It is known that lead is a major component of ceramic glazed vessels because it is an efficient flux (temperature lowering) for the silica which gives the vitreous appearance and properties to glazes. Lead was detected in 19 out of 42 ceramics screened by X-Ray Micro Fluorescence (XRMF). Samples had lead in excess of the Food and Drug Administration (FDA) limits. Some bowls tested lead levels 1000 times the FDA limits (Nicholas, 1999). In about 15 products, levels of one or more specific elements (Cr, Pb, As, Sn, Cd etc) were sufficiently high to warrant further investigations and testing. The results were compared to published levels of toxicity concern (Smith, 1988; Harrison, 1993; and Seiler *et al*, 1994) to determine whether the concentrations were a potential health threat or merely elevated compared to other food products. Lead and arsenic levels in several products proved sufficiently high to warrant intensive case study.

Diet is always a potential source of toxic metals and elements. Research data suggests a relationship between prolonged low-dose exposure and cancer, hypersensitivity, chronic fatigue, birth defects, and neurological deficits ranging from mental retardation to attention deficit disorder. In July 1992, Food and Drugs Administration (FDA) Department in the US warned people on purchasing Jumex brand of fruit juices and nectors. In this particular case elevated levels of blood lead were detected in a US toddler through a health screening. The

source was traced in Jumex manufactured in Mexico. The acidic juices had leached lead from the solder forming the seams of the cans (Nicholas, 1999).

Prepared food contains small but significant amounts of lead. The lead content is increased when the water used for cooking or the cooking utensils contain lead, or the food, especially if acidic, has been stored in lead-ceramic pottery ware or lead soldered cans. In some countries dietary intakes of as high as 500µg/day have been reported (Galal-Gorchev, 1991).

In another study, analysis of the total diets served daily for one year in institutions, which included expensive boarding schools, as well as financially linked orphanages in 28 US cities was done. Average cadmium content in the diets of the various institutions ranged from 0.027 µg/g to 0.064 µg/g. This was attributed to cadmium present in the seams of food containers as well as its uptake from the soil ending up in the processed products (FAO/WHO, 1976).

The concentrations of aluminium in food vary widely depending on the nature of the foodstuffs. Studies suggest that aluminium leached from tea leaves can make a significant contribution to dietary intake (Fairweather-Tait *et al,* 1987). In addition to its presence in food, aluminium leaching from cooking utensils may represent a potential source of dietary exposure (Vozam, 1962 and Trapp and Canon, 1981). Use of aluminium by the food industry in containers and packaging constitutes another dietary source (Gregor, 1985).

A study on aluminium in Kenyan tea and foods has also been carried out (Wanjau, 1991). Concentrations in various foods are as shown in Table 1.

Table 1. Aluminium concentration in foods (Wanjau, 1991).

Food	Aluminium concentration (μg/g)
Maize	0.042
Whole grain maize flour	0.5
Sifted maize flour	ND
Finger millet	36.2
Sorghum	76.2
Millet	141.5

Bananas boiled in a beaker had 0.2 μg/g aluminium while those boiled in an aluminium saucepan contained 42 μg/g aluminium. This shows how leaching from containers can affect levels of aluminium in foods and by extension beverages (Wanjau, 1991). Finger millet, sorghum and millet are the principal ingredients in the making of the majority of cereal-based illicit alcoholic beverages.

Another study on aluminium in Kenyan tea and other plants by Githua, 1994 showed that grains accumulate very little aluminium although the leaves contain a lot of it. Hence the probable source of aluminium in alcoholic beverages would be through leaching from containers especially when the beverages are acidic. No aluminium was detected in maize grains or bananas.

Yulita, (1996) carried out an analysis of lead, cadmium and zinc on cigarettes, tobacco leaves and inter-cropped plants. cadmium and lead concentrations in sorghum seeds from Nandi district were 0.13 μg/g and 8.13 μg/g respectively. Sorghum seeds from Siakago in Embu district had cadmium and lead concentrations of 0.25 μg/g and 263.33 μg/g respectively.

Food is the main source of cadmium intake for non-occupationally exposed persons. Crops grown in polluted soils or irrigated with polluted water may contain increased concentrations of cadmium (IARC, 1979). It is worth noting that sewage sludge is used as manure for growing sorghum and millet in some slum areas of Nairobi especially along river banks of Nairobi, Kamiti, Pangani, Mathare, Kasarani, Githurai and Mbagathi rivers. These are used for brewing the alcoholic beverages. Sewage sludge and the water used are usually likely to be contaminated with toxic metals.

Kaara, (1992) reported low levels of copper in sewage effluent at Kariobangi the range being 0.02-0.12 mg/l. concentration of lead was as high as 0.16 mg/l while cadmium concentration was 0.02 mg/l. this study also reported an increasing trend in concentration of trace elements from semi urban to urban areas as shown in Table 2.

Table 2. Concentrations of heavy metals in soil samples (µg/g) (Kaara, 1992)

Metal / Collection Site		Copper	Lead	Cadmium
Ngong, Kiserian	Mean	43.4	45.4	3.2
	Range	12.6-69.5	24.0-55.5	2.5-3.7
Limuru Town	Mean	26.8	78	7.36
	Range	18.0-36.5	27.5-158.0	2.5-12.8
Kiambu Town	Mean	32.6	83.8	4.97
	Range	19.0-148.0	61.0-128.0	1.5-12.0
Nairobi, Kariobangi	Mean	128.4	263.8	17.0
	Range	40.0-220.0	75-508.0	0-50

The normal levels of these metals in soils are: copper; 50-100 (µg/g), lead; 15-25 (µg/g) and cadmium 0.07-1.1 (µg/g). These metals are washed into rivers from which the brewers draw water for brewing. This study also established that plants such as kales, spinach and *amaranthus* accumulate heavy metals. Finger millet, sorghum and other cereals grown on sewage sludge or sewage amended soils and later used for brewing could contaminate the brews.

2.3. TRACE ELEMENTS STUDIES IN DRINKS.

Traces of lead are liable to be present in beer if it comes into contact with lead metal used in making capsules. Pearson (1962) notes that The Food Standards Committee's 1954 report on lead, recommended that the use of lead pipes and lead containers should be discontinued in breweries, bottling plants and licensed premises, (Pearson, 1962). A maximum of 0.1 mg/l is recommended in beer (K.E.B.S., 1998). The WHO, 1996 guideline for lead in drinking water is now set at 0.01 mg/l. Lead contamination of alcoholic beverages may occur in several ways: from lead solder used to repair casks or kegs, lead capsules used as seals or from residues of lead arsenate pesticides in soils now used to grow grapes. These beverages tend to be acidic hence there is the possibility that large amounts of lead can be dissolved during preparation, storage or serving (Wai *et. al.*, 1979). Published reports on lead levels in wine show that important variations occur from sample to sample (Jorhem *et. al.*, 1988).

Sherlock *et. al.*, (1986) found that the majority of canned and bottled beer (90% and 86% respectively) contained less than 0.01 mg/l lead. Draught beers typically contained higher lead concentrations with 55% having lead concentrations greater than 0.01 mg/l, 16% with

over 0.02 mg/l and 4% with concentrations of over 0.1 mg/l. The higher lead concentration in the draught beers are considered most likely due to the draught dispensing equipment which sometimes contains brass or gun metal, both of which contain low but significant amounts of lead. The natural lead content of German wines has been reported to be 0.01-0.03 mg/l (Eschnauer, 1982). The results of analysis carried out by the US Treasury Department on some 432 different brands of wines showed various concentrations of lead as shown in Table 3.

Table 3. Distribution of lead in table wines in USA

Range (mg/l)	Number of samples studied
0-0.01	36
0.011-0.025	62
0.026-0.05	105
0.051-0.1	144
0.101-0.25	64
0.251-0.5	12
0.501-0.673	9

Source: The US Department of Treasury (1992)

Most of the published literature on trace elements in alcoholic beverages concerns wine (IARC, 1988). Trace elements from grapes are transferred during crushing into the must and eventually into wine (Eschnauer, 1982). The total concentration of mineral constituents e.g. sodium, potassium, lead, cadmium, aluminium and others, in wine, may be as high as 1000 mg/l and more (Eschnauer, 1967). The regular consumption of wine can result in a significant increase in lead intake; an average level of 0.073 mg/l has been reported (Elinder, 1988).

A concentration of 0.0002-0.003 mg/l cadmium has been reported in European wines, the majority of levels being in the range 0.0002-0.0015 mg/l (Golimowski et. al., 1979). Kenya Bureau of Standards sets the maximum tolerable limits for cadmium and lead in alcoholic beverages at 0.005 mg/l and 0.1 mg/l respectively. Cadmium intake from beverages is trivial compared with dietary intakes. Plant-based foodstuffs represent the largest source of dietary cadmium in most populations and thus changes in crop cadmium levels may have a marked impact on cadmium exposure. The FAO/WHO, (1976) tolerable intake for cadmium is 0.070 μg/g/day (Hutchinson and Meema, 1987) while the limit in drinking water in U.S. is 0.01 mg/l (U.S. Council on Environmental Quality, 1984).

Diet studies in the US have been carried out (FAO/WHO, 1976). Out of 360 samples 213 samples had cadmium, the highest concentration being 0.2 μg/g. Examination of 373 samples showed 139 with less than 0.01 μg/g with an overall average of 0.019 μg/g cadmium.

A growing interest in trace metal toxicity and health effects of alcoholic beverages has led to a number of studies. Muchai, 1985, studied presence of trace elements (copper, cadmium and lead) in beers, soft drinks and miraa by differential pulse anodic stripping voltammetry. Table 4 shows the levels of lead present in some of the samples analysed.

Table 4. Levels of lead in miraa and beverages (Muchai, 1985).

Sample analysed	Concentration (mg/l)
Tusker export	0.4
White cap export	0.36
Bond 7	0.4
Smirnoff Vodka	0.17
Papaya Wine	0.3
Pineapple Jelly crystals	0.81 μg/g
Miraa	3.97 μg/g
Coke	0.42

Pineapple jelly crystals contained 4.02 μg/g copper and 0.4 μg/g cadmium while Bond 7 and miraa contained 0.4 mg/l 0.06 μg/g cadmium respectively. Lead could have been leached from the plumbing system especially in Tusker export due to the old lead pipes used in water supply. Water used in brewing is normal tap water and is not purified in any way. Lead present in coke is a result of leaching from lead capsules in the corks. Miraa may have had high concentrations of lead due to uptake from the soils as well as handling especially during loading and unloading during transportation. Urban soils are highly contaminated and more so at bus terminuses. These are the loading and offloading sites of miraa. It could have resulted from air-borne lead being deposited on the twigs on display. Pineapple jelly crystals had the highest concentration of lead. This is attributed to use of lead based pesticides in pest control in pineapple farms. The residues end up in the processed product.

A study of trace metals in alcoholic beverages was carried out on Nyuki, Mnazi, Chibuku, Busaa and Tusker export (Mwanasi, 1990). Nyuki is a honey-based licensed alcoholic beverage. Busaa is a cereal-based beer which is porridge-like it is unlicensed. Chibuku is the licensed version of Busaa. It is more homogenous and packed in attractive containers. Mnazi is a brew based on coconut juice. This contains wild yeast which ferments the sugars to ethánol. The beverages were found to have high levels of cadmium, copper, lead, and aluminium, (Table 5). Tusker and Busaa had the highest levels of lead (0.29 mg/l) and 133.59 mg/l aluminium respectively. All the brews analysed had high levels of cadmium.

Table 5. Total concentrations in some brews (Mwanasi, 1990).

Brew	Elemental concentration(mg/l)					
	Cu	Co	Fe	Pb	Cd	Al
Nyuki	0.985	0.375	24.71	0.280	0.090	29.355
Mnazi	0.785	0.275	4.480	0.100	0.080	1.715
Chibuk	1.16	0.205	48.26	0.225	0.090	6.225
Busaa	0.745	0.860	140.7	0.215	0.085	133.590
Tusker	0.13	0.265	0.740	0.290	0.075	2.165

A study by Mosha et al. in 1996 on traditional alcoholic beverages in Tanzania indicates that there is no link between trace element concentrations in water and brews. Trace elements in water were below the WHO standards. The results obtained indicate contamination from lead, cadmium, copper and aluminium (Table 6).

Table 6. Heavy metal content in traditional brews in Tanzania, (Mosha et al., 1996) (values in mg/l).

Name of brew	Cd	Pb	Cu	Al
Dengelua	n.d.	0.11	0.056	1.53
Kangara	0.02	0.253	1.276	2.39
Kabungusi	0.003	0.101	0.084	1.344
Kibuku-mtwara	n.d.	0.224	0.134	0.878
Uraka	0.005 (<0.001)	0.141 (0.041)	0.620 (0.009)	7.79 (n.d.)
Dadii	0.01 (<0.001)	0.4 (0.27)	0.63 (0.009)	1.50 (n.d.)
Ulanzi	0.08	0.82	0.40	0.40
Komoni	0.04	0.12	0.37	4.14
Wanzuki	<0.001	0.17	0.1	4.38
Mbege	0.009	<0.03	0.55	3.82
Mnazi	0.11 (0.003)	0.15 (0.04)	0.46 (0.035)	1.43 (n.d.)
Gongo	0.04	<0.003	0.1-31.2	<0.03
Safari	0.05	0.11	0.04	2.50
Kibuku	0.001	0.4	0.20	4.10
Konyagi	0.18	0.3	0.03	n.d.
City water supply	0.0003	0.0006	n.d.	n.d.
WHO limits	0.005	0.10	1.5	0.2

KEY:

The values in parenthesis are for processing water sampled near the collection sites.

n.d.-not detected.

Lead is present in tap water to some extent as a result of its dissolution from natural sources but primarily from household plumbing systems in which the pipes, solder, fittings or service connections to homes contain lead. PVC pipes also contain lead compounds that can be leached from them and result in very high lead concentrations in drinking water. The amount of lead dissolved from the plumbing systems depends on several factors including the presence of chlorides and dissolved oxygen, pH, temperature, water hardness and standing time of the water, soft, acidic water being the most plumbosolvent (Shock, 1989

and 1990). Soldered connections in recently built homes fitted with copper piping can release enough lead (0.2-0.37 mg/l) and cause intoxication in children. The lead levels in drinking water may be reduced by corrosion control measures such as the addition of lime and the adjustment of pH in the distribution system from less than 7 to 8-9 (Moore, 1981 and Sherlock *et al*, 1984). Across Canada and US lead levels in drinking water are between 0.0011-0.037 mg/l (Department of National Health and Welfare, 1992).

Another study on licensed and unlicensed alcoholic beverages was done to determine the ethanol content, suspended solids, specific gravity and acidity (Macharia, 1996). Nyuki had a pH of 3 compared to a literature value of 4.14. Sorghum beers had 16-18% ethanol content compared to 5-7% found in malt beers. Wines had an average specific gravity of 0.990. Malt beers had 1.98-3.53% solids compared to 1.0-1.46% in sorghum-based beers. It is possible that these solids could contain heavy metals.

Aluminium is usually present in treated drinking water in the form of reactive species of low relative molecular mass and in natural waters, it is usually associated with particulate matter or organic complexes of high molecular mass (Gardner and Gunn, 1991). It may be present in natural waters as a consequence of leaching from soil and rock. In a survey of aluminium in raw waters in the US, ranges between 0.014-0.28 mg/l in ground water and 0.016- 1.17 mg/l in surface water were reported (Miller et al, 1984). In the United Kingdom, concentrations of 0.2-0.3 mg/l were associated with low pH levels and 0.4-0.6 mg/l with an afforested catchment (Bull and Hull, 1986).

Alcoholic beverages packed in aluminium cans can be contaminated as some aluminium has been shown to be leached into the contents (Richardson, 1993). The WHO Drinking water

guideline has been set at 0.2 mg/l. This can be extended to alcoholic beverages. A provisional tolerable weekly intake (PTWT) of 7.0 mg/kg body weight has also been recommended (WHO, IPCS, 1997). Most of these traditional beverages are prepared in aluminium containers and thus aluminium is liable to be present in these beverages.

Cadmium contamination of drinking water may occur as a result of its presence in the zinc galvansed pipes or cadmium-containing fittings, water heaters, water coolers and taps. Drinking water from shallow wells in areas of Sweden where the soil has been acidified contained concentrations of cadmium close to 0.005 mg/l (Friberg *et al,* 1987). In Saudi Arabia, mean concentrations of 0.001-0.026 mg/l were found in samples of portable waters, some of which were taken from private wells or old corroded pipes (Mustafa *et al,* 1986). Levels of cadmium could be higher in areas supplied with soft water of low pH, as this would tend to be more corrosive in plumbing systems containing cadmium. In the Netherlands, a survey of 256 drinking water plants showed presence of cadmium (0.0001-0.0002 mg/l) in only 1% of the drinking water samples (Ros and Slooff, 1987).

The limit of toxicological tolerance for copper is so high that no problems arise in its use for parasite control in crops (Conciel International Pour le Development du Cuivre, 1979). Kenya Bureau of Standards sets the maximum tolerable limit for copper in all alcoholic beverages at 2.0 mg/l (K.E.B.S., 1998). A limit of 1 mg/l has been set for drinking water in the U.S (U.S. Council on Environmental Quality, 1984).

Natural copper concentrations in drinking water are around a few micrograms per litre (Slooff *et al,* 1989). Depending on such properties as hardness, pH, anion concentration, oxygen concentration, temperature, and the technical conditions of the pipe system. Water from copper pipes may contain several milligrams of copper per litre (Slooff *et al,* 1989). In

a sample of water for human consumption which had remained stagnant for 12 hours, an extreme level of 22 mg/l copper was found (WHO, 1996).

Table 7. Maximum allowed limits of trace elements in alcoholic beverages, raw materials and drinking water (WHO, 1996 and KEBS, 1998).

Element	Limit (mg/l)
Cadmiun	0.005
Lead	0.05 (drinking water) and 0.1(beer and raw materials)
Zinc	50 (beer and raw materials and drinking water)
Chromium	0.05 (drinking water)
Arsenic	0.05 (drinking water) and 0.1 (beer and raw materials)
Iron	0.3 (drinking water)
Manganese	0.1 (drinking water)
Sodium	200 (drinking water)
Aluminium	0.2 (drinking water)

2.4. ANALYTICAL METHODS AND NSTRUMENTATION.

The most common methods that have been used for the determination of heavy metals in foods and beverages are spectrophotometric methods, neutron activation analysis, X-ray fluorescence, spectroscopic methods and polarography.

2.4.1. CRITERIA FOR INSTRUMENTAL ANALYSIS.

Brewing is a business, and so, for any instrumental method to take a role in the brewing chemist's analysis, it must be judged by how well it serves an identified need, and at what cost. A typical hierarchy of criteria for the endorsement of an analysis procedure within a commercially oriented laboratory is as follows: -

1) Rapidity, reliability and versatility.
2) Minimal manual preparation.
3) Minimal sample preparation.
4) Unattended operations capability.
5) Minimal use of expensive consumables.
6) Low capital investment.
7) Computer-interface capability.

In this section a review of the analytical methods (in heavy metal analysis in foods and beverages and soft drinks) is given. Polarography and Atomic Absorption Spectroscopy have been discussed at length, as they were the main methods, which were used in this work.

2.4.1.1 SPECTROPHOTOMETRIC METHODS

In spectrophotometric determinations, the quantity of an element (or ion) is estimated from the intensity of the colour of the solution due to the presence of a coloured compound of that element. The more intense the colour the higher the concentration of that element (or ion) in the solution. If two solutions under identical conditions and containing the same coloured compound have colour of the same intensity, then the concentration of the given element (or ion) in them are also equal. Therefore from a given known standard one can obtain a calibration curve from which the concentration of the unknown substance can be obtained. Spectrophotometric methods are still of great interest to many workers as they require relatively simple and cheap equipment and are therefore attractive to small laboratories. However there are doubts about their specificity although reasonable sensitivities are observed.

2.4.1.2. NEUTRON ACTIVATION ANALYSIS

In this analytical method the sample is bombarded by neutrons so that some of the atoms in the sample are changed into isotopes with a relative atomic mass one unit higher and in addition γ-radiation is emitted. Many of the isotopes produced are initially unstable, resulting in further emission of radiation. The energy of the emitted radiation is characteristic of the element and hence examination by a gamma spectrometer can identify the elements present in the original sample.

This technique is one of the most sensitive methods for the determination of trace amounts of metals in biological materials. The method is ideally suited to multi-element determination and for small amounts.

2.4.1.3. X-RAY FLUORESCENCE SPECTROMETRY

For quantitative analysis of mixtures by x-ray spectrometry the relationship between the intensity and element sought must be established. The usual procedure is to set up a calibration curve for intensity vs. amount of element present from known standard. In most cases the ratio of the intensity of a characteristic line (such as K_α) for the element being analyzed to that of a line in the same range of wavelengths for a known element serving as an internal standard is plotted as a function of composition from standard homogenous samples of known concentrations.

X-ray fluorescence is applicable to all elements with an atomic number greater than 11. The method has the advantage of only requiring limited sample preparation, which only involves freeze-drying, powdering under liquid nitrogen and pelleting in vacuum.

2.5. ATOMIC ABSORPTION SPECTROMETRY (AAS)

The Beer-Lambert law, which linearly relates the concentration of a substance in solution to an absorbance of a beam of monochromatic light, is one of the most powerful and widely used relationships used by analytical chemists.

The principle behind Atomic Absorption Spectroscopy is that the concentration of an element is measured by the absorption of radiation with a characteristic frequency by free atoms of an element. The strength of this method is that atoms absorb only a very narrow range of wavelengths as compared to molecular species. The radiation source is a hollow cathode lamp, with a cathode made of the element to be determined which emits a specific sharp resonance line. Atomization of the element can be achieved by introducing a fine spray of the test solution through a nebuliser into an air/acetylene or nitrous oxide/acetylene flame. When very high sensitivity is required, an alternative atomization system with an electrically heated graphite furnace is used.

Whatever sample dissociation device is used, a monochrometer is needed to isolate the resonance line in the transmitted light, which is then detected by a photomultiplier tube coupled with electronic circuitry to enable high-speed detection limits and sensitivity. Diffraction gratings are preferred over prisms as they offer accuracy over a wide range of wavelengths.

Transitions from the ground electronic state to the first excited state take place when radiation of frequency exactly equal to the resonance frequency passes through the flame gases into which the analyte has been transported as an aerosol. A part of the radiant

energy of the incident light beam, I_0 will be absorbed by analyte atoms. The transmitted intensity, I, may be written as

$$I = I_0 \exp(-K_v d)$$

Where K_V is the absorption coefficient and d is the average thickness of the absorbing medium, that is, the path length of the flame horizontally (Willard et al, 1986). Atomic absorption spectroscopy is an important method of analysis as evidenced by the numerous publications where the instrument has been used. The versatility of the instrument is such that over 70 elements can be analyzed using the instrument. The use of electrothermal atomizers greatly increases the detection limit of the instrument. Currently the instrument is used in the trace analysis of water and effluent, marine chemistry, foodstuffs, geochemistry, petrochemicals, clinical chemistry, and also in the brewing industry. The general concepts and instrumental techniques in atomic absorption spectroscopy have been discussed in details in the literature (Jumba, 1980, Willard et al, 1986, Van Loon, 1980).

2.5.1. INSTRUMENTAL PARAMETERS.

The atomic absorption spectrometer can be subdivided into five parts: -

i) The lamp system.

ii) The nebulizer system.

iii) The burner.

iv) The detectors.

v) The read out system.

2.5.1.1. THE LAMP SYSTEM.

The hollow cathode lamp is used to produce the desired radiation. The cathode of the lamp is made up of the metal under analyses.

2.5.1.2. THE NEBULIZER SYSTEM.

The solution under analysis is aspirated into the nebulizer converting it into a mist. The sample aerosol is mixed with the fuel and oxidant in the nebulizer chamber before introduction into the flame. Here, the metal ions are converted into gaseous atoms and subsequently absorption of the relevant energy occurs. The magnitude of absorption of the energy will be proportional to the concentration of the metal atoms in the flame and hence the concentration in the solution (Willard *et al*, 1986).

2.5.1.3. THE BURNER SYSTEM.

The flame temperature should be in the excess of 2000 K but not too high as to cause the ionization of atoms. However for analysis of metals that form refractory compounds e.g. aluminum and titanium, high flame temperatures are used. Some reagents e.g. potassium chloride may be added to samples to suppress ionization of metals of interest.

2.5.1.4. THE READ OUT SYSTEMS

Nowadays readout systems are digital and are coupled with microprocessors that allow the programming of various aspects bringing simplicity to operation of certain procedures e.g. calibration and calculation of concentration.

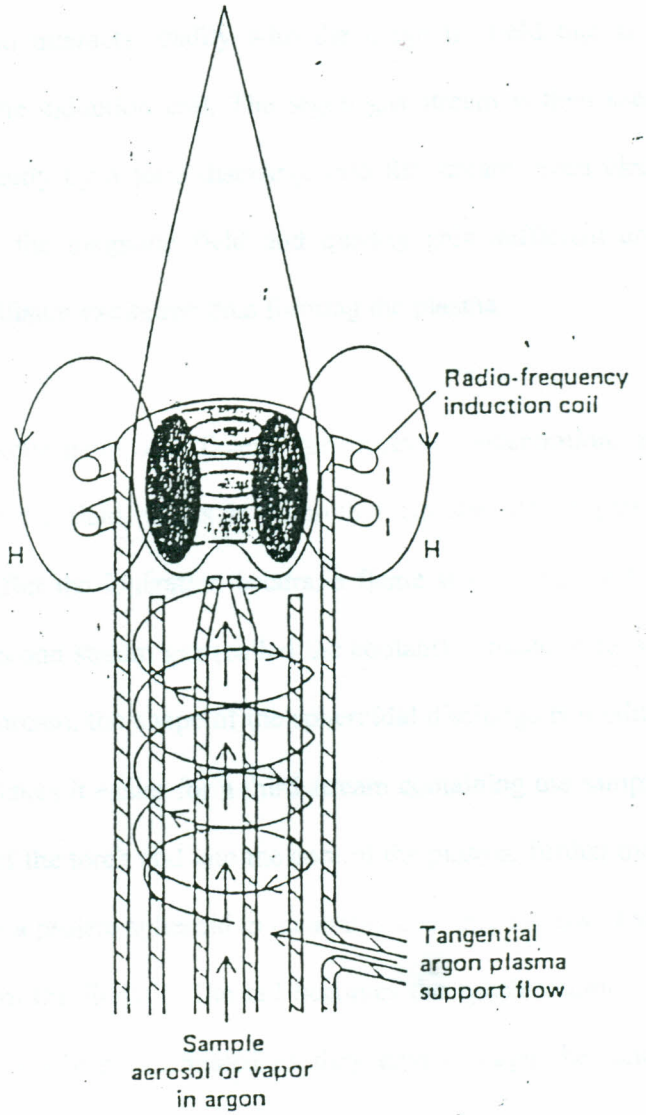
2.6. THE 210 VGP BUCK SCIENTIFIC MODEL ATOMIC ABSORPTION SPECTOMETER

In this study the above instrument, which is microprocessor based, was used in carrying out the analysis. The program software AANALYZE™ was used in data collection. The program allows for convenient calibration, taking multiple readings and some statistical treatment of data. It gives statistical features such as the y-intercept, coefficient of correlation, line of best fit and standard deviation.

2.7. INDUCTIVELY COUPLED ARGON PLASMA (ICAP)

The inductively coupled argon plasma is the current state-of-the-art multi-element method of analysis of trace elements in samples of any of matrix. The plasma derives its sustaining power by induction from a high frequency magnetic field. The plasma, actually a partially ionized gas, is formed electromagnetically by radio-frequency induction coupling of argon gas.

Figure 3. The Torch Configuration (Willard *et al* 1986).



Two concentric silica tubes, the inner tube stopping below the induction coil, contain the argon plasma, while a third tube is inserted into the centre to inject an aerosal through the plasma after it has been formed. The work coil (water cooled) of a high frequency generator is positioned around the top of the outer silica tube (20mm in diameter).

To operate the torch argon gas is fed tangentially into the inner silica tube and is ionized by the magnetic field produced by the induction coil (up to 2 kW of energy at 27.14

MHz). Since the argon plasma has a high density of free electrons, it is a good electrical conductor and interacts readily with the magnetic field that is created once power is supplied to the induction coil. The argon gas stream is then seeded with free electrons provided directly by a tesla discharge into the stream. Seed electrons in the coil space interact with the magnetic field and quickly gain sufficient energy to ionize the gas stream by collision excitation thus forming the plasma.

Once the plasma gains a sufficient-free electron concentration, an eddy current, induced by the magnetic field, flows in azimuthal circular closed paths around the discharge periphery. After the ionization occurs, a flame shaped plasma forms near the top of the torch. If a second stream gas (called the coolant) is made to flow tangentially around the plasma gas stream, the shape of the spheroidal discharge is modified by a flattening of its base. This makes it easier for a third stream containing the sample aerosal, to be injected up the axis of the torch and into the core of the plasma, further modifying the shape of the fireball from a prolate spheroid to an annulus. A long narrow, well-defined tail flame now emerges from the fireball. The tail becomes the spectroscopic source; it contains all the analyte atoms which are heated as they pass through the tunnel in the centre of the annulus. The optical window used for analysis falls just above the apex of the primary plasma cone and just under the flame-like afterglow. In this optical window the high background or current carrying region of the plasma is excluded from the spectrometer. Typical argon flow rates are 1 litre/min in the carrier, 0-1 litre/min in the auxiliary plasma and 15 litre/min in the coolant plasma.

There's no electrode contact in the ICAP source. Therefore, excitation and emission zones are resolved spatially, producing a relatively clean background spectrum, which

consists of argon lines and some weak emissions from OH, NO, NH and CN molecules. This low background combined with a high signal-to-noise ratio of the analyte emission produces low detection limits, typically in the parts per billion ranges. It is the geometry of the ICAP source that renders it particularly useful. The analyte is carried directly through a narrow channel in the centre of the plasma discharge where it experiences high excitation temperature and long residence times (approximately 2msec). The definitive boundary that exists between the analyte column and the current carrying part of the inert plasma is one of the main reasons inter-element and matrix effects are minimal. The higher temperatures of the radiation zone ensure the complete breakdown of the chemical compounds and impede the formation of other interfering compounds. For many analyte species, ion line emission is considerably more intense than neutral atom line emission in the ICAP source. ICAP is also given detailed attention in the literature (Fassel and Kniseley, 1976, Greenfield, 1977, Greenfield *et al*, 1964 and Ward, 1978).

2.8. PRINCIPLES OF VOLTAMMETRIC ANALYSIS

Voltammetry is an electrochemical technique in which the current-potential behaviour at an electrode surface is measured. The potential is varied in some systematic manner to cause electroactive chemical species to be reduced or oxidised at the electrode. The resultant current is proportional to the concentration of the chemical species. Electrochemical principles are well discussed in details in the literature (Heyrovsky and Zuman, 1968, Kolthhoff and Lingane, 1952, Meites, 1965, Bond, 1980, and EG & G, 1980).

Stripping voltammetry is a two-step technique in which the first step consists of the electrolytic deposition of a chemical species into an inert electrode surface at a constant potential. This pre-concentration step can either involve an anodic or cathodic process. The second step consists of the application of a voltage scan to the electrode which causes the electrolytic dissolution, or stripping, of the various species in the amalgam back into solution at characteristic potentials. The remarkable sensitivity of stripping voltammetry is attributable to the pre-concentration that takes place during deposition. For pre-concentration to take place, the deposited material must of necessity adhere to the electrode surface. Although there are exceptions, mercury is generally the electrode of choice. Stripping voltammetry can be used to determine those chemical species that will be retained by the mercury, by formation of either an amalgam or an insoluble mercurous salt.

The polarographic method of analysis is based on the current voltage curves arising at a microelectrode when diffusion is the rate-determining step in the electrochemical reaction. Polarography allows selectivity through control of electrode potential. It has utility for finger print purposes as well as analytical ones. Polarography provides sensitivity to the parts-per-billion level for many electroactive substances.

2.8.1. THEORY OF POLAROGRAPHY

Polarography was introduced as an electroanalytical technique by Heyrovsky (Heyrovsky, 1965). Applications of polarographic techniques range from the simple classical (DC) polarography to the more complex differential pulse anodic stripping voltammetry (DPASV) (Willard *et al* 1986).

Polarographic analysis is based on the measurement of the half-wave potentials ($E_{1/2}$). $E_{1/2}$ corresponds to the inflection point of the polarographic waves, where the current is half the limiting current. The value of $E_{1/2}$ is characteristic of the substance under analysis and this can be compared to literature values hence enabling qualitative analysis to be carried out. This value may be a function of the conditions of the solution under analysis i.e. supporting electrolyte, pH, solvent system and reference electrode. Quantitative analysis is carried out by comparing the wave heights corresponding to the diffusion limiting currents of redox with those of standards or comparing them to those of spiked standards.

Reduction reaction follows the following process

$$M^{n+}{}_{(aq)} + ne \longrightarrow M_{(Hg)} \qquad (1)$$

Where n = Number of electrons involved.

The equilibrium potential, E, of the amalgam electrode is dependent on the ratio of the concentration of the metal ions M^{n+} in solution to the concentration of the metal in the amalgam and is represented by the Nernst Equation

$$E = E^0 + \frac{RT}{nF} \ln \frac{C_{M^{n+}(aq)}}{C_{M(Hg)}} \qquad (2)$$

Where

E^O = Standard Equilibrium Potential

R =Gas constant

ln = Natural log

F = Faraday constant

$C_{M^{n+}(aq)}$ = Concentration of the metal ion in solution

$C_{M(Hg)}$ =Concentration of the metal in amalgam

Since an amalgam is formed when the metal ions discharge at the surface of the electrode, it follows that the concentration in the amalgam must be proportional to the current so produced i.e.

$$C_{M(Hg)} = K'I \qquad (3)$$

Where K' = Proportionality constant

I = Current

The concentration of substances is determined in a solution containing an excess of an indifferent electrolyte referred to as the supporting electrolyte. The supporting electrolyte suppresses the migration of the ions to be determined, thus ions are transported to the surface of the mercury electrode exclusively by diffusion. Under these conditions, limiting current, I_d (maximum diffusion current) is proportional to the concentration of the reducible species in the test solution

$$I_d = K'' C^0{}_{M^{n+}(aq)} \qquad (4)$$

Where K'' = Proportionality Constant

$C^0{}_{M^{n+}}$ = Concentration of reducible species in the test solution

The limiting current corresponds to the complete depletion of ions in the solution to be determined near the drop surface. At any given time after the reaction has started, the current is I lower than I_d, so that the concentration of the metal ions is different from zero and corresponds to a value $C^0{}_{M^{n+}}$, which determines according to equation (1), the electrode potential. Under these conditions, the diffusion current is equal to

$$I = K''(C^O{}_{M^{n+}(aq)} - C_{M^{n+}(aq)}) \qquad (5)$$

Combining (4) and (5) yields

$$C_{M^{n+}(aq)} = \frac{(I_d - I)}{K''} \qquad (6)$$

Substituting the values of $C_{M(Hg)}$ and $C_{M^{n+}(aq)}$ from equations (3) and (6) respectively into equation (1) we have

$$E = E^0 - \frac{RT}{nF} \ln K'K'' - \frac{RT}{nF} \ln \frac{I}{I_d - I} \qquad (7)$$

If $I = \frac{1}{2} I_d$ then,

$$E = E^0 - \frac{RT}{nF} \ln(K'K''E_{\frac{1}{2}}) \qquad (8)$$

Substituting in equation (8) into equation (7) equation (8) simplifies to

$$E = E_{\frac{1}{2}} - \frac{RT}{nF} \ln \frac{I}{I_d - I} \qquad (9)$$

Equation (9) forms the basis of polarographic waves. Three modes of transport are responsible for the migration of ions in the solutions under analysis. First, migration due to the existence of an electric field. Its effect is eliminated by the presence, in the supporting electrolyte, ions in concentrations of about 50 to 80 times the ions under investigation. The second, convection, caused by mechanical agitation of the solution is reduced to negligible proportions by keeping the solution still. The third, diffusion, caused by a concentration gradient between the electrode surface and bulk of the solution, is the mode that is of interest in polarographic analysis.

2.8.2. FUNDAMENTALS OF STRIPPING VOLTAMMETRY.

The demand for the detection and quantitation of trace components in complex samples has come from the public and private sector alike (ACS, 1978) and (Batley and Florence, 1979). Heightened awareness of the often detrimental effects of trace elements in media such as foodstuffs, drinking water and commercial waste-water effluents has led to stringent public legislation and industry-wide quality assurance programs which have been directed toward monitoring components of a sample at sub-ppm levels.

Trace technique of stripping voltammetry has been used in trace analysis with relative ease and success in a variety of analytical applications. With minimal sample preparations, this electrochemical technique is routinely capable of identifying and quantifying trace components from 10^{-5} mol/dm^3 to 10^{-9} mol/dm^3 with excellent sensitivity (Batley and Florence, 1978).

2.8.3. ELECTRODES FOR STRIPPING VOLTAMMETRY.

The ideal working electrode must be stationary, have a reproducible surface area and a low residual current. Solid electrodes such as gold, platinum, glassy carbon, wax-impregnated graphite and carbon paste demonstrate such qualities and have been used successfully. Although solid electrodes give a sensitive response they generally can be used for the analysis of only one species. When a solid electrode is employed in the analysis of several species, it is nearly impossible to obtain the required homogenity of the deposited materials prior to the stripping step.

The most practical electrode for stripping voltammetry employs mercury as the electrode surface. Because of their general versatility and convenience, the hanging mercury drop electrode (HMDE) and the thin film mercury electrode (TFME) are used.

2.8.3.1. HANGING MERCURY DROP ELECTRODE

The hanging mercury drop electrode is the best working electrode for stripping voltammetry because of its extremely reproducible surface. All the characteristics of the dropping mercury electrode, (Heyrovsky and Zuman, 1968), which make it the most suitable electrode for routine analytical determinations, also apply to the hanging mercury drop electrode. The entire stripping voltammetry experiment is performed on one mercury drop. The drop is then dislodged and a new drop is dispensed for the next experiment. Because the electrode is "replaced" for each experiment, the condition of the electrode is not a variable in the analysis. This is not true for solid electrodes.

It is imperative that the hanging mercury drop electrode used in stripping voltammetry should be able to dispense a drop with an area that is reproducible to within 1%. The measured current in an electrochemical experiment is proportional to the electrode area. Since the current from a standard is compared to the current from the sample, an error in the surface area of the drop will lead directly to an error in the calculated sample concentration. Stripping voltammetry with a hanging mercury drop electrode is a much more convenient technique to implement since hanging mercury drop electrodes are automatically dispensed with the push of a button.

The perennial problem of the hanging mercury drop electrodes is in maintaining the drop on the end of the capillary. The mercury drop can fall off in which case the experiment must be aborted. The ability to hold the drop is a function of the mechanical construction of the electrode. The performance characteristics of the hanging mercury drop electrodes can often be improved by siliconizing the interior bore of the capillary. Siliconizing is performed by coating the bore with a material such as dimethyldichlorosilane. Siliconizing enhances the hydrophobic nature of the capillary and minimizes the deleterious effects of minor imperfections in the surface of the glass.

2.8.3.2. THIN FILM MERCURY ELECTRODE.

A thin film mercury electrode is prepared by depositing a film of mercury onto a glassy carbon electrode. Although other electrode may be used, glassy carbon usually gives excellent results. The thin film mercury electrode is generally used only for anodic stripping voltammetry. Such electrodes are most useful when maximum sensitivity is required. The thin film mercury electrode exhibits high sensitivity because only an extremely small amount of mercury is incorporated into the film, resulting in the formation of a very concentrated amalgam during the deposition step. The stripping peaks that are obtained with a thin film mercury electrode tend to be sharper than those observed with hanging mercury drop electrode. The thin film mercury electrode can be prepared by placing the glassy carbon electrode in a well-stirred solution of 2.5 mg/l reagent grade mercuric nitrate made slightly acidic with nitric acid at –0.4 V vs. SCE for 5 minutes. Once the thin film mercury electrode is generated it must be protected from oxygen to prevent oxidation of the film. Also, because the layer of the deposited mercury

is extremely thin, the use of thin film mercury electrode should be limited to analyte concentrations of less than 10^{-7} mol/dm^3.

The thin film mercury electrode can also be prepared in situ by adding 2-5 mg/l Hg^{2+} directly to the sample solution and depositing mercury and the analyte simultaneously (Florence, 1970). The experiment is begun with a completely clean electrode, usually glassy carbon. The mercury and the deposited analyte are removed from the surface either mechanically or electrolytically following the completion of experiment.

Because the same electrode surface is used for repetitive analysis, the condition of the surface is a major consideration. Steps must be taken to ensure that the surface of the thin film mercury electrode is as reproducible as possible prior to each analysis. Failure to guarantee a consistent surface may give rise to irreproducible results, since the current due to a particular analyte concentration is dependent upon a reproducible electrode surface. This problem however, is not a consideration with a hanging mercury drop electrode since a new mercury drop is used for each determination.

The thin film mercury electrode is recommended only when maximum sensitivity is required. Because of the care required to obtain consistent results, the thin film mercury electrode cannot be considered appropriate for routine analytical purposes. It can be used to analyse metals in water at concentrations on the order of 1 part per trillion (Nurnberg *et al,* 1976).

2.8.4. ANODIC STRIPPING VOLTAMMETRY

Anodic stripping voltammetry is used to determine the concentration of trace metals. It consists of a deposition potential that is more negative than the half-wave potentials of the metals to be determined and an anodic (positive going) scan to oxidise the reduced metals back into solution. During deposition, the elemental metal and the mercury on the electrode form an amalgam. Anodic stripping voltammetry can only be used to determine those metals that exhibit appreciable solubility in mercury. These are Antimony, Arsenic, Bisthmuth, Cadmium, Copper, Gallium, Germanium, Gold, Indium, Lead, Silver, Thallium, Tin and Zinc.

Deposition: Applied potential more negative than $E_{1/2}$ of M^{n+}

$$M^{n+}{}_{(aq)} + ne^- \longrightarrow M_{(Hg)}$$

Stripping: Scan in the positive direction, peak current is proportional to the concentration of M.

$$M_{(Hg)} \longrightarrow M^{n+}{}_{(aq)} + ne^-$$

It is worth noting that concentrations of ions exceeding 10 mg/l can cause inter-metallic formation in a hanging mercury drop electrode and in such cases differential pulse polarography is applied instead of differential pulse anodic stripping voltammetry. The hanging mercury drop electrode used with the differential pulse waveform is the most versatile electrode technique combination. Not only is the sensitivity high for trace

determinations, but also the tendency for inter-metallic formation is minimised in all but the most concentrated solutions.

Stripping voltammetry is an indispensable technique in trace metal analysis. It has received great attention in the literature (Vydra et al, 1973, Ellis, 1973, Brezinski, 1976, Nurnberg, 1977, Siegerman and O'Dom, 1972, Osteryoung and Osteryoung, 1972, Cope and Skogerboe, 1974).

2.8.5. STEPS IN STRIPPING VOLTAMMETRY.

The technique of stripping analysis involves two main steps: A concentration or pre-electrolysis step in which the desired component is deposited cathodically and anodically followed by a reverse electrolysis in which the component is determined. In the anodic form of stripping voltammetry, the metal (or metals) concerned is reduced at a controlled potential for a definite time under fixed conditions of geometry and stirring. The final anodic dissolution, or stripping process, involves a linear anodic scan in which the metal is oxidised. The resulting stripping voltammograms shows peaks, their heights (or areas) of which are generally proportional to the concentrations of the electroactive metal ion, and the potentials of which have the same qualitative interpretations as their half-wave potentials in polarography.

2.8.5.1. GAS SCRUBBING.

Aqueous solutions exposed to air contain concentrations of dissolved gaseous oxygen as high as 10^{-3} mol dm^{-3} at room temperature and pressure. Dissolved oxygen interferes in stripping analysis as it does in classical polarography (Glen, 1969). Depending upon the pH, oxygen undergoes reduction in three steps: -

$$O_{2(g)} + 2H^{+}_{(aq)} + 2e^{-} \longrightarrow H_2O_{2(g)} \text{ ---------- } (I)$$

$$O_{2(g)} + 2H_2O_{(l)} + 2e^{-} \longrightarrow H_2O_{2(g)} + 2OH^{-}_{(aq)} \text{ ----} (II)$$

$$O_{2(aq)} + 4H^{+}_{(aq)} + 4e^{-} \longrightarrow 2H_2O_{(l)} \text{ ---------- } (III)$$

Equations II and I occur at an $E_{1/2}$ of between –0.5 ± 0.05V and 1.3 ± 0.005V vs. saturated caromel electrode (SCE). This is also the same range of potential that most reductions of metals occur and hence oxygen may bring about the overlapping of peaks in addition to new peaks. The formation of hydrogen peroxide ion in (I) and (II) is an invidious reaction to other electroactive species since it can function as both an oxidizing agent or a reducing agent. The presence of the peroxide ion may also cause pH changes within the vicinity of the hanging mercury drop electrode increasing the chances of precipitation of heavy metals in turn diminishing the diffusion currents. As a result of the foregoing reactions, it was necessary to ensure that all traces of oxygen were removed. This was done using the vanadous chloride scrubbing system.

2.8.5.2. VANADOUS CHLORIDE SCRUBBING SYSTEM

Nitrogen used in deaeration needed to be of high purity. Although specially manufactured nitrogen was used, it was necessary to further clean it to remove any traces of oxygen. In this project vanadous chloride was used as the scrubbing agent. It was prepared by boiling 2 g of ammonium metavanadate with 25 ml of concentrated hydrochloric acid and diluting with distilled deionised water to 250 ml. The solution so produced was pale-green and contained vanadium in low oxidation states. It was then transferred into the glass washing tower and amalgamated zinc added to reduce it to the +2 oxidation state. The amalgamated zinc was prepared by placing 13 g of powdered zinc in a beaker, covering it with distilled deionised water and adding three drops of concentrated hydrochloric acid. Amalgamation was achieved by the addition of mercury. In addition to this basic scrubbing system, a washtower containing 0.2 mol dm^{-3} ammonium citrate at pH 3 was attached. Its purpose was to ensure that the moisture content of the nitrogen gas bubbling through the analyte was of the same concentration as the supporting electrolyte and to remove any traces of vanadous chloride solution picked up by the gas. Nitrogen to be scrubbed was first bubbled through the vanadous chloride solution, then through the ammonium citrate solution. Exhaustion of the scrubbing gas was detected when the characteristic bluish-green colour changed to a violet colour. Regeneration was achieved by either adding a few mililitres of concentrated hydrochloric acid, more amalgamated zinc or both reagents.

With the scrubbing tower ready white spot nitrogen gas supplied by BOC Kenya Limited was connected to the tower input, while the output of this tower was connected to the input of a second tower containing the same electrolyte as that in the analysis cell i.e. 0.2 mol dm^{-3} ammonium citrate solution buffer. The output of the second tower was

connected to the nitrogen input part of the model 303A. Saturation of the gas with supporting electrolyte prevents sample concentration changes because of evaporation. Traces of vanadous chloride solution in the gas may also contaminate the sample.

2.8.5.3. CONDITIONING.

Conditioning is a term that denotes electrolytic cleaning of the electrode surface. A specified potential is applied to the electrode for a controlled time in order to remove contaminants or materials not removed during the stripping step from the electrode surface. Conditioning is not required with a hanging mercury drop electrode because a new drop is used for each determination. On the other hand, conditioning is a necessity with a thin film mercury electrode because the same electrode surface will be used in subsequent determinations. When the thin film mercury electrode is used to determine metals, the conditioning potential should be positive with respect to the half-wave potentials of the analyte to ensure the oxidation of the metals back into solution. If the thin film mercury is being formed in situ, the conditioning potential may be set positive of the oxidation potential of mercury to provide a clean electrode surface for the deposition step. The solution is stirred during conditioning. A typical conditioning time is 60-120 seconds.

2.8.5.4. DEPOSITION.

The deposition potential is applied to the working electrode to cause the materials of interest to be deposited onto the surface of the working electrode. The solution is generally stirred during deposition to maximise analyte-electrode contact. The selection of the deposition step depends upon whether the material to be determined is to be oxidised or reduced. For a reducible metal, the deposition potential should be negative with respect to the half-wave potential of the metal but not so negative such that the decomposition of the electrolyte is encountered. For oxidisable materials the deposition potential should be selected so that it is positive with respect to the half-wave potential. The deposition time must be carefully controlled. It is an important experimental parameter that is unique to stripping voltammetry. If more sensitivity is required, the analyst simply increases the deposition time. This increases the degree of pre-concentration, making a greater amount of deposited analyte available at the electrode during the stripping step.

2.8.5.5. EQUILIBRATION

During equilibration, the deposition potential is applied to the working electrode but stirring is halted. This allows convection current from the stirring to decrease to a negligible level and also allows time for the amalgam to stabilize.

2.8.5.6. STRIPPING

An excitation waveform is applied from the voltammetric analyser which electrolyses the deposited material back into solution. The current is measured vs. the applied potential. The deposited materials will strip at potentials very close to their half-wave potentials. The measured current at these potentials is proportional to the concentration of the analyte in the original sample. Either a dc or differential pulse waveform may be used during the stripping step.

2.8.6. ANODIC STRIPPING VOLTAMMETRY IN METAL SPECIATION ANALYSIS.

Considerable recent research in the fields of biological and environmental science has focussed on the subject of chemical speciation. It is increasingly realised that the distribution, mobility and biological availability of chemical elements depends not simply on their concentrations but critically, on the chemical and physical associations which they undergo in natural systems. Changes in the environmental conditions whether natural or anthropogenic, can strongly influence the behaviour of both essential and toxic elements by altering the forms in which they occur. Some of the more important controlling factors include pH, redox potential and availability of reactive species, such as complexing ligands (both organic and inorganic) particle surfaces for adsorption, and colloidal matter (Ure and Davidson, 1995).

Metal speciation analysis involves the fractionation of total metal concentration by physical-chemical methods (Florence, 1986). The fractionation of total metal species is

recognised as an essential step in the assessment of the potential biological uptake and toxicity in a liquid sample. As a consequence total metal concentrations are being replaced in water quality standards by an assessment of the bio-available metal fraction.

Differential Pulse Anodic Stripping Voltammetry is sufficiently sensitive, with a typical detection limit of about 10^{-7} mol/ dm^3, for the direct determination of heavy metals in natural waters (Florence, 1982a). This analytical technique can distinguish between the electrochemically available fraction, which may be toxic and the bound or electrochemically inert fraction, which is less likely to demonstrate toxic properties.

Differential pulse anodic stripping voltammetry has commonly been applied to the primary distinction between "labile" and "bound" metals in filtered water samples (Chau and Lum-Shue-Chan, 1974). The normal procedure for estimating the fraction of labile or electrochemically available metal involves a standard addition analysis of an untreated sample and is therefore dependent on the kinetics of the reactions controlling the assimilation of the metal spike (Whitfield and Turner, 1979). However, Florence (1986) avoids metal spike complexation by calibrating using a blank solution containing standards. Labile metal, as defined by the experimental conditions, includes ionic as well as some weakly complexed metal. Bound metal is identifiable as the non-labile fraction and is typically associated with a variety of organic and inorganic colloidal materials (Batley and Florence, 1979).

In this study metal speciation analysis was carried out in untreated samples. Stripping peaks of the samples were compared with those of the standards. No attempt was made to obtain the forms of the metal complexes as this was not an objective of the study. The elemental concentrations were classified as "labile" or "bound" depending on the stripping peaks obtained.

Due to increased pollution there was need to assess the extent of contamination of the brews by toxic metals. Scanty information is available in trace elements in local alcoholic beverages. Increased level of poverty among the general population has led to increased production of these illicit brews hence the need to monitor them. Other brewing companies have been licensed. This commercialisation calls for quality control checks in order to enhance safety.

CHAPTER THREE

EXPERIMENTAL TECHNIQUES

3.1. SAMPLING

Samples of alcoholic beverages and raw materials were randomly sampled in Nairobi, Thika, Kiambu, Embu, Machakos and Kisii districts. Beverages were collected from bars and the raw materials from brewers themselves. The raw materials were put in self-sealing plastic bags and preserved in a deep freezer.

Beverage samples were each divided into two. To one portion, concentrated nitric acid was added to a pH of 3 or less to prevent adsorption of metals on the walls of the container. The other portion was preserved in a deep freezer without any prior treatment. These untreated samples were used for speciation studies.

3.2. CLEANING OF GLASSWARE AND PLASTIC CONTAINERS.

All glassware were cleaned using liquid detergent and rinsed with tap water. Plastic containers purchased from Blowplast Limited, Nairobi, were cleaned in a similar manner and soaked in 1:1 v/v analytical grade nitric acid. All glassware was soaked in freshly prepared concentrated chromic acid overnight. The plastic containers were rinsed with distilled de-

ionized water to remove the acids. The glassware from the acid were rinsed with distilled de-ionized water and dried in an electric oven at 130^0C.

All the solutions used in this analysis were stored in plastic bottles. After the completion of each analysis, the glassware was decontaminated by immersing them in 50% v/v analytical grade nitric acid followed by rinsing with plenty of distilled de-ionized water. Voltammetric cells were soaked in 50% v/v analytical grade nitric acid overnight prior to analysis. They were then rinsed several times with distilled deionized water.

3.3. CHEMICALS.

All the reagents used in this project were of analytical grade. They included cadmium metal, lead nitrate, sulphuric acid, hydrochloric acid, citric acid, ammonium hydroxide, perchloric acid, potassium dichromate, potassium chloride and mercury. They were purchased from Sigma-Aldrich Company, London.

3.3.1. PREPARATION OF STANDARD SOLUTIONS

Lead, copper and aluminium standards were obtained from Buck scientific company, USA. They were prepared in 1 % nitric acid to keep the metal in free ionic state. Their concentration was 1000 µg/ml. However, the standards can be prepared by dissolving 1 g of the pure metal in 10 ml of 1:1 hydrochloric acid. When all the metal has dissolved, the solution is made to one litre and contains 1000 µg/ml of metal ions (APHA-AWWA-WPCF, 1975).

Cadmium standard solution was prepared by weighing 0.5 g of analytical grade cadmium metal and dissolving it in 10 ml of 1:1 analytical grade hydrochloric acid. The reaction was left overnight. After complete dissolution, the volume was made to 50 0ml. This solution contained 1000µg/ml Cd^{2+} (APHA-AWWA-WPCF, 1975).

3.3.2. PREPARATION OF ULTRA PURE AMMONIUM CITRATE BUFFER (SUPPORTING ELECTRLYTE).

42.5 g of citric acid (monohydrate) was dissolved in 750 ml distilled de-ionized water. The pH was adjusted to 3.0 with hydrochloric acid. The solution was diluted to one litre with distilled de-ionized water. This solution could still contain trace amounts of heavy metals. For it to be used as a supporting electrolyte it was necessary to remove these trace amounts. This was achieved by controlled potential electrolysis in a cell made of platinum anode and mercury pool as cathode. 100 ml of supporting electrolyte was introduced in the electrolysis cell and electrolysed for 24 hours at a current of 0.2 mA. During this period, the solution was constantly kept stirred by bubbling nitrogen gas through it. Metals dissolved in the solution were deposited in the mercury pool. At the end of the electrolysis period, the electrolysed solution was extracted from the cell using a pipette and stored. The extraction was carried out with the cell still on to avoid redissolving of the metals which may occur if the current is switched off with the solution still in contact with the mercury (EG&G, Princeton Applied Research, 1980).

3.3.3. PREPARATION OF ULTRA PURE HYDROCHOLIC ACID AND AMMONIA

Ultra pure hydrochloric acid and ammonia were required for adjusting the pH of sample solutions. The high level of purity was a prerequisite in order to avoid introducing contaminants into the solution under analysis. Purification by isothermal distillation at room temperature was used in preparation of pure solutions. 500 ml of concentrated hydrochloric acid and concentrated ammonia to be purified were separately placed in a dessicator in which there was 200 ml of distilled deionized water. They were then left to equilibrate. After a period of 72 hours, the beakers, which originally contained distilled deionized water were each found to have solutions of approximately 1 mol dm^{-3} of the respective solutions (EG&G, Princeton Applied Research, 1980).

3.4. ANALYTICAL PROCEDURES

3.4.1. DIGESTION OF ALCOHOLIC BEVERAGES

Alcoholic beverage samples were digested according to the procedure described by Katz and Jenniss, (1983). 100 ml of the acidified brew was placed in a 150 ml beaker and 3 ml of concentrated nitric acid added. This was heated to near dryness. An additional 3 ml of concentrated nitric acid was added and the beaker covered with a watch glass. The contents were heated gently to complete digestion. The solution was then evaporated to near dryness and 5 ml of 1:1 v/v hydrochloric acid added followed by further heating to dissolve the insoluble material.

The contents were filtered into a 100 ml volumetric flask using a Whatman No. 40 filter paper. The residue was washed several times with distilled deionised water and the filtrate was made to the mark with distilled deionised water. The digested samples were transferred to plastic bottles ready for both polarographic and atomic absorption analysis.

3.4.2. DIGESTION OF RAW MATERIALS.

The solid raw materials were digested according to the procedure described by Van Loon, (1980). The digestion was carried out using a temperature-time programmed Tecator Foss Digester. This digestor allows simultaneous digestion of 12 samples. The range within which temperature should rise and the time this should take is selected by keying in the commands using the controls.

1g of each sample was weighed accurately and put in the digestion tube. A 15 ml mixture of nitric and perchloric acids in the ratio of 2:1 was then added with precaution behind a protective screen due to the explosive nature of perchloric acid. The mixture was placed under low heat under a fume hood. The temperature was slowly brought to 120^0C. Heating was interrupted in when foaming occurred. Within 15 minutes interval the temperature was brought to 140^0C. The temperature rose rapidly to between 200^0C and 203^0C. This is the boiling point of water-perchloric acid azeotropic mixture (72.5% perchloric). Foaming occurred frequently and heating was interrupted. The temperature was maintained at 203^0C for 30 minutes, then put off and the tubes allowed to cool to room temperature. The samples were filtered in a Whatman No. 40 filter paper into 50ml volumetric flasks. The filter papers were washed and the washings added to the filtrate. The filtrate was made to the mark using distilled deionised water. The filtrates were now ready for analysis.

3.5. STRIPPING VOLTAMMETRIC WORK PREPERATIONS.

Each component of the stripping experiment needs to perform its job as required in order to get voltammograms that can be interpreted without doubt of errors. It was therefore necessary to prepare the physical and chemical components of the analysis to get reliable results.

3.5.1. HANGING MERCURY DROP ELECTRODE (HMDE) PREPARATION.

The HMDE was then thoroughly cleaned before the analysis. The capillary was first discharged of mercury into a reservoir. 1 mol dm^{-3} nitric acid was then sucked through the capillary. This was done by plunging the one end of the capillary into a rubber sucker. Distilled deionized water then sucked through the capillary for another a few minutes.

3.5.2. SILICONIZING OF THE CAPILLARY

Siliconizing the capillary was necessary to ensure that the test solution does not penetrate the capillary. Also it makes the interior bore of the capillary hydrophobic and thus the capillary was able to hold the drop and prevent it from falling off. The capillary was rinsed with methanol and air dried at 65^0C for one and a half hours. Siliconizing was achieved by placing the tip opposite the ferrule in a fresh vial of siliconizing fluid. The excess siliconizing fluid from the capillary was removed from the opposite end of the ferrule. This process was completed by air drying the capillary in an oven at 65^0C.

3.5.3. FILLING THE CAPILLARY.

Filling the capillary was done as recommended in the manufacturer's manual (EG & G Princeton Applied Research Corporation (1980). Care was taken in order not to have air trapped in the capillary. This was evidenced by a considerable retraction of the mercury thread when a drop was dislodged. A properly filled capillary will exhibit a suck buck not exceeding 5mm. This was achieved before the analysis was begun.

3.5.4. REFERENCE ELECTRODE

The reference electrode was a simple silver/silver chloride electrode that made contact with the analyte via a porous vycor frit. It was necessary to replace the frit from time to time due to either its contamination after prolonged use, or when the frit material thinned or had a visible crack. The glass sleeve of the reference electrode was filled with the filling solution (saturated with AgCl) making sure that it was free of bubbles. This was done from time to time since when the electrodes are stored in water for days, shifting of peak potentials occur due to dilution of the filling solution by water diffusing into the reference electrode.

3.5.5. COUNTER ELECTRODE

The counter electrode for the model 303A was a teflon sheathed platinum wire installed at the factory, and did not require maintenance. However this was washed frequently with analytical grade nitric acid followed by rinsing with large amounts of distilled deionized water.

3.6. ANALYSIS OF METALS BY DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRY (DPASV).

The analysis of liquid samples using polarography is well documented (Muchai, 1985; American Public Health Association, 1975, and Batley and Gardner, 1987). The following method was adopted for analysis of beverage samples. The best pH for the analysis of samples was first determined by analysing a given sample at different pH's and observing at which pH the instrument was most sensitive to the sample thus giving the highest peak (Wandiga and Jumba, 1982).

9 ml of the digested sample and 1 ml of ultra pure ammonium citrate solution were put in the analysis cell. The pH was then adjusted to 5.8 after which the solution was degassed by bubbling nitrogen for 5 minutes while stirring. After this period the solution was blanketed with nitrogen gas and deposition step of ions carried out for 2 minutes. The stirrer was then switched off to allow the convection currents to cease after which stripping was carried out. The procedure was repeated with cumulative addition of 10 µl of a mixed standard consisting 10 mg/l of each of the ions Cd^{2+}, Cu^{2+} and Pb^{2+} until a total of 100 µl had been added. The calibration curves for both standard method addition and direct calibration are given in appendix 1. The samples were analysed under the following instrumental conditions: -

Working electrode:	HMDE
Drop size:	Medium
Mode of measurement:	Differential pulse
Modulation amplitude:	25 mV

Purge time:	5 minutes
Initial/Deposition potential:	-1.20V vs. SCE
Measurement/Drop time:	0.5 sec.
Current range:	5 μA to 200 μA as appropriate.
Deposition time:	120 seconds (stirring) and as appropriate
Equilibration time:	30 seconds
Scan direction:	"+"
Scan rate:	20 mV/sec

The model 303A of polarographic instrument was then connected to the polarographic analyser/stripping voltameter model 264A which is an electric polarographic instrument capable of performing dc, sampled dc, normal pulse, cyclic voltammetry and differential pulse polarographic analysis, as well as dc and differential pulse stripping analysis.

The voltameter was then interfaced with an X-Y plotter, Phyne LY 16100-11. It was also interfaced with a personal computer (a Tatung Model No. CM 14 5BS) for recording. The software program, POLR4 was used to do the voltammetric recordings. The X-Y plotter gave less noisy voltamograms as compared to the computer print-outs and was adopted.

Sufficient standard additions were made to each sample so as to increase the sample stripping peaks by 50-500%. This was necessary so as to quantitate the metal concentrations accurately. Spikes of 100 μl of standards were carried out for each sample.

For speciation studies, the same procedure was used but the samples were not digested. A 10 ml aliquot was taken and run in a similar way as a digested sample. This was followed by

spiking of a standard of the metal. The standard which gave a stripping peak of above 100% was used in calculating the concentration of the free metal ion in the sample. It is worth noting that the speciation studies were not aimed at determining the forms in which the metals existed in the aqueous media, but whether they were free or bound. Bound metals were not expected to give peaks at the same positions as free metal ions.

Figure 4. Schematic diagram of polarographic analysis system

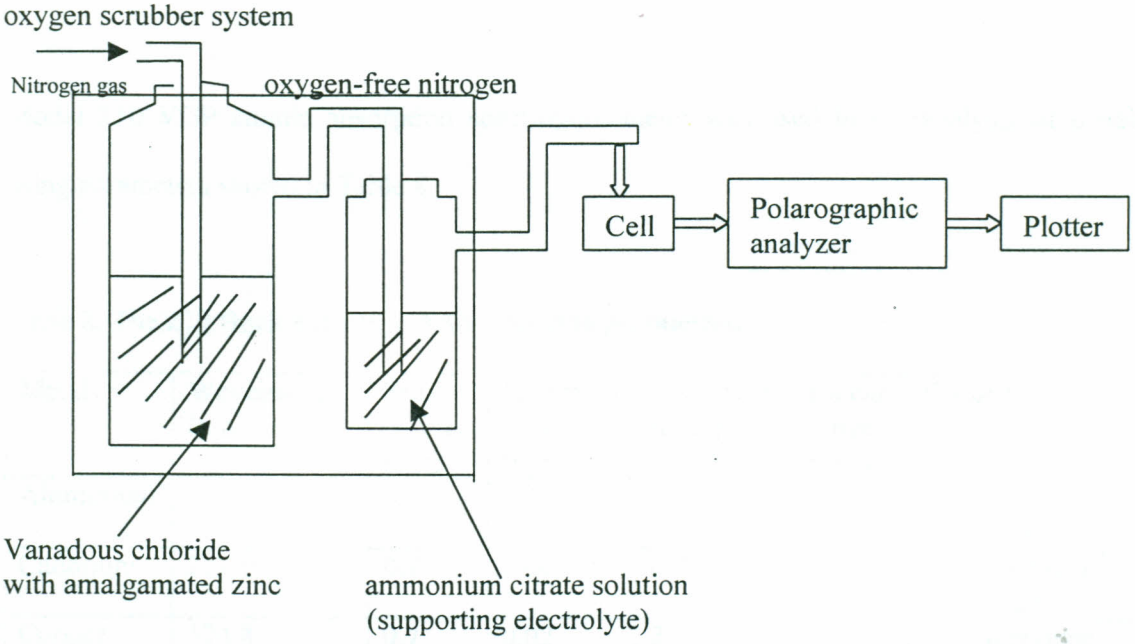
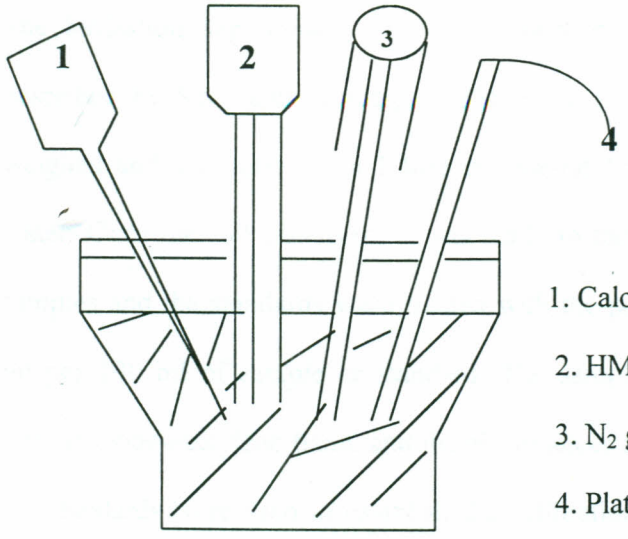


Figure 5.The polarographic cell and its three-electrode system



1. Calomel electrode (Ag/AgCl electrode).
2. HMDE.
3. N_2 gas control tap.
4. Platinum electrode.

3.7. ANALYSIS OF METALS BY ATOMIC ABSORPTION SPECTROSCOPY.

Model 210 VGP atomic absorption spectrophotometer was used in the analysis of metals using parameters shown in Table 8.

Table 8. The 210 Buck Scientific AAS operating parameters.

Metal	Wavelength (nm)	Slit width (nm)	Detection limit (ppm)	Sensitivity (ppm)	Linear range (ppm)	Flame type
Aluminium	309.3	0.7	0.1	25	37.5	Nitrous oxide-Acetylene Rich/red
Cadmium	228.9	0.7	0.005	0.75	1.12	Air-Acetylene Lean/blue
Copper	324.8	0.7	0.02	2	3.0	Air-Acetylene Lean/blue
Lead	283.3	0.7	0.1	10	15	Air-Acetylene Lean/blue

The ionization suppressant used was potassium chloride. This solution was prepared as described by Katz and Jenniss, (1983). 95 g of analytical grade potassium chloride was weighed and transferred to a 1 litre volumetric flask and dissolved with distilled deionised water. Once the salt dissolved, it was made to the mark with distilled deionised water. The samples and the standards were treated with the potassium chloride solution at the rate of 2 ml per 100 ml of sample or standard. The samples and standards were aspirated into the nitrous oxide-acetylene flame and the absorbances at 309.3 nm measured. The absorbances of the standards were used to establish the calibration curve and the aluminium content of the samples determined by direct comparison.

Cadmium and copper were analysed by air-acetylene flame at 228.8 nm and 324.7 nm wavelengths respectively. Samples and standards were aspirated into the air-acetylene flame under identical conditions and absorbances measured. The calibration curves were obtained from the absorbances of the standards. Cadmium and copper content of the samples was obtained by direct comparison.

For lead analysis prepared samples and standards in 1 % nitric acid were aspirated into the air-acetylene flame and the absorbances measured at 283.3 nm wavelength. The calibration curve was obtained from the absorbances of the standards and the lead levels of the samples were determined by direct comparison. The calibration curves for all the elements are shown in figures 8, 9, 10 and 11 in appendix 1

3.7.1. STANDARD CONDITIONS FOR RUNNING A.A.S.

Table 8 is a guide to the sensitivity and performance using flame techniques. The detection limits are defined as the lowest concentration that can give an absorbance detectable above the noise range. Sensitivity is a measure of the instrument response to the analyte, and by convention, shows the concentration of each element required to absorb 1% of the incident light energy. This corresponds to an absorbance value of 0.0044. Elements with greater sensitivity will have the lowest concentration values in that category. The values of sensitivity in Table 7 are the amounts in ppm required to give an absorbance reading of 0.200.

The linear range is the amount of analyte in μg/g, which will produce an absorbance of approximately 0.300 and safely keep the analysis in the linear part of the calibration curve.

CHAPTER FOUR

RESULTS AND DISCUSSION.

One hundred and twelve (112) alcoholic beverage samples were analysed for lead, copper, cadmium and aluminium using both AAS and DPASV. The concentrations obtained by AAS are shown in Tables 9a, 9c, 9d, 9e, 9f and 9g.

Table 9a. Concentration of lead, copper, cadmium and aluminium in various illicit alcoholic beverages by AAS (mg/l).

Sample name	Collection site	Lead	Copper	Cadmium	Aluminium
Mugaca	Kiwanja (KM)	0.146±0.011	0.323±0.005	ND	0.443±0.037
Mugaca	Githurai	0.070±0.010	0.340±0.010	ND	0.043±0.005
Mugaca	Mathare	0.060±0.001	0.686±0.005	ND	ND
Mugaca	Korogocho	0.146±0.005	0.280±0.01	0.020±0.001	22.84±0.400
Afriwine	Githunguri	ND	0.116±0.005	0.023±0.005	ND
Kulta Special	Githunguri	0.040±0.001	0.116±0.005	ND	ND
Kulta Special	Mathare	0.036±0.005	0.223±0.005	ND	3.273±0.037
Kibuku Beer	Maragua	0.043±0.005	0.293±0.005	ND	ND
Kibuku Beer	Zimmermann	0.116±0.005	0.593±0.005	0.020±0.001	9.320±0.0520
Kibuku Beer	Githunguri	0.130±0.005	0.186±0.005	ND	ND
Medusa	Zimmermann	0.033±0.005	0.243±0.005	ND	3.156±0.015
Medusa	Maragua	0.086±0.050	0.220±0.010	ND	3.163±0.011
Medusa	Githunguri	0.086±0.050	0.110±0.001	ND	3.140±0.020
Kibuku Tarzan	Karatina	0.003±0.005	0.216±0.005	ND	ND
Viena wine	Maragua	0.156±0.005	0.263±0.005	ND	ND
Simba Wine	Maragua	0.003±0.005	0.023±0.001	ND	0.306±0.005
Sunwine	Githunguri	0.020±0.010	0.226±0.005	ND	4.446±0.023
Toivo	Mathare	0.016±0.005	0.390±0.001	ND	0.343±0.025
Toivo	Kibera	0.286±0.023	ND	ND	0.586±0.025
Pineapple wine	Githunguri	0.070±0.010	0.133±0.011	ND	ND
Kangara	Witeithie	0.070±0.010	0.496±0.005	0.040±0.001	15.393±0.075
Kangara	Kiandutu, Thika	0.350±0.010	0.713±0.005	0.050±0.001	5.710±0.070
Kangara	Githurai	ND	ND	0.040±0.001	40.340±0.655
Kangara	Ruiru	0.466±0.005	1.093±0.005	0.040±0.001	45.440±0.458

Table 9a Contd/.. Concentration of lead, copper, cadmium and aluminium in various illicit alcoholic beverages by AAS (mg/l).

Sample name	Collection site	Lead	Copper	Cadmium	Aluminium
Viena Special	Murang'a	0.056±0.005	0.290±0.010	ND	ND
Viena Special	Maragua	0.096±0.005	0.226±0.005	ND	ND
Macore	Embu	0.063±0.005	0.153±0.005	ND	1.803±0.030
Mangara	Kibera	0.023±0.011	ND	ND	0.273±0.005
Karubu	Tala	0.070±0.010	0.340±0.010	ND	0.113±0.011
Karubu	Githurai	0.043±0.005	0.213±0.005	ND	0.150±0.001
Karubu	Mathare	0.05±0.005	0.753±0.005	ND	0.343±0.025
Karubu	Embu	0.006±0.005	0.130±0.010	ND	0.093±0.011
Kibuku sorghum root	Karatina	0.043±0.005	0.240±0.001	ND	ND

Out of the one hundred and twelve (112) alcoholic beverages analysed, 16 contained lead above the allowed limit of 0.1 mg/l. Concentrations ranged from 0.103 mg/l to 0.466 mg/l. These are high levels which can pose great risk to the health of consumers. The source of lead can be attributed to general pollution in the areas where the brews are prepared. For instance some brews such as chang'aa and busaa are prepared by the riverbanks to facilitate cooling. Water from these rivers is used in brewing and since it is normally polluted with industrial wastes, this end up in the brews. Oyaro, (2000) has shown that plants such as nappier grass and amaranthus concentrate trace elements from the soils. Plants from heavily polluted areas such as the city centre had higher levels of lead and copper as compared to those from the outskirts. The soils on which the plants were growing had high levels of lead and copper. Some of the results are shown in Table 9b.

Table 9b Mean concentrations of lead and copper in amaranthus and respective soils (μg/g) (Oyaro, 2000).

Metal	Lead		Copper	
Plants/soil / Site	Amaranthus	Soil	Amaranthus	Soil
Outscasts e.g. Juja	35.3	82.3	7.5	36.9
Residential	64.9	117.8	17.3	23.5
City Centre	147.2	143.7	25.9	104.0

The study also included black jack. A similar trend in concentrations was also established (Oyaro, 2000).

Table 9c. Concentration of lead, copper, cadmium and aluminium in Miti ni dawa (mg/l).

Collection site	Lead	Copper	Cadmium	Aluminium
Kiganjo, Thika	0.043±0.005	0.293±0.005	ND	2.040±0.113
Tala	0.066±0.005	0.210±0.010	ND	ND
Kibera	0.046±0.005	0.283±0.005	ND	0.300±0.010
Mathare	0.070±0.010	0.176±0.005	ND	0.853±0.041
Kiwanja (KM)	0.020±0.010	0.260±0.001	ND	ND
Githurai	0.070±0.001	0.310±0.017	ND	0.470±0.026
Kibera	0.283±0.005	0.243±0.005	ND	1.266±0.005
Korogocho	0.080±0.001	0.536±0.015	ND	1.840±0.017
Juja	0.026±0.005	ND	ND	3.053±0.015
Githunguri	0.033±0.005	ND	ND	1.790±0.010
Huruma	ND	ND	ND	ND
Ruiru	0.130±0.010	ND	ND	0.466±0.005
Thika*	0.216±0.015	0.040±0.010	0.020±0.001	2.036±0.041
Makongeni-Thika	0.026±0.011	ND	ND	ND

Miti ni dawa seems to be free from cadmium poisoning apart from one sample from Thika. Brewing of miti ni dawa utilises herbs, twigs and honey. The cadmium in one sample could have been as a result of formaldehyde

("chemical") addition to the brew mixture. Lead above the allowed limit is found in only three samples pointing to contamination by additives. Aluminium is above the allowed limit in almost all the samples. It could originate from the herbs, twigs or leaching from cookware. Copper was in very low concentrations probably due to the fact that miti ni dawa is not distilled. Distillation is usually done using copper pipes.

Table 9d. Concentration of lead, copper, cadmium and aluminium in chang'aa (mg/l).

Collection site	Lead	Copper	Cadmium	Aluminium
Kibera	0.003±0.005	0.153±0.005	ND	0.123±0.030
Zimmermann	ND	1.743±0.005	ND	56.906±0.351
Kisii	0.020±0.001	0.203±0.005	ND	ND
Kiwanja (KM)	0.043±0.011	0.113±0.005	ND	ND
Mathare	0.013±0.005	0.150±0.010	ND	ND
Witeithie	0.050±0.001	0.230±0.010	ND	ND
Kiandutu, Thika*	0.020±0.001	0.243±0.005	ND	ND
University of Nairobi	0.023±0.005	0.156±0.005	ND	ND
Kariobangi	0.023±0.005	ND	ND	ND
Juja	ND	2.363±0.037	ND	ND
Gikomba	0.056±0.015	ND	ND	ND
Mathare*	ND	ND	ND	0.323±0.032
Huruma	ND	ND	ND	0.540±0.020
Githunguri	0.003±0.005	ND	ND	ND
Ruai	ND	ND	ND	0.140±0.017
Thika	ND	ND	ND	0.713±0.020
Thika*	ND	ND	0.020±0.001	ND
Kiambu	0.023±0.005	ND	ND	ND

No sample of chang'aa contained lead above the allowed limit. This is largely attributed to the fact that chang'aa is distilled and therefore only the alcohol is obtained. Two samples contained copper in substantially high concentrations. It may have resulted from leaching of copper pipes used in the distillation process. Cadmium was present in only one sample. It may have resulted from an additive after brewing or from a volatile cadmium compound in the brewing mixture. A few sample had aluminium. One sample from Zimmermann had a very high concentration. This may be attributed to storage methods whereby the chang'aa stored in aluminium containers will have more aluminium than that stored in plastic containers.

Table 9e. Concentration of lead, copper, cadmium and aluminium in busaa (mg/l).

Collection site	Lead	Copper	Cadmium	Aluminium
Kiganjo, Thika	0.133±0.005	0.473±0.011	0.020±0.001	8.876±0.046
Mathare	0.163±0.005	0.946±0.005	ND	4.836±0.041
Kibera	0.083±0.005	0.623±0.011	ND	9.170±0.026
Kiwanja (KM)	0.190±0.017	0.543±0.005	ND	20.400±0.200
Kibera	0.200±0.010	0.200±0.010	ND	12.336±0.090
Kisii	0.073±0.005	0.686±0.005	ND	1.986±0.055
Githurai	0.083±0.005	0.443±0.005	0.020±0.001	4.686±0.005
Witeithie	0.103±0.005	0.440±0.010	ND	10.600±0.043
Kangemi	0.096±0.005	0.320±0.010	0.020±0.001	15.966±0.115

Lead above 0.1 mg/l was found in half of the samples analysed. This may have resulted from the water used and additives like solder added to aid fermentation. Copper was in very low concentrations which pose no health risk. Cadmium was present is three out of the nine samples analysed. This may have resulted from

addition of formaldehyde during fermentation. Aluminium was present above the allowed limit in all the busaa samples. This was due the frying of the flour using aluminium pans and storing the brew in aluminium containers.

Table 9f. Concentration of lead, copper, cadmium and aluminium in muratina (mg/l).

Collection site	Lead	Copper	Cadmium	Aluminium
Kibera	0.073±0.005	0.240±0.001	ND	ND
Zimmermann	0.043±0.005	0.206±0.005	ND	1.806±0.025
Kiganjo, Thika	0.003±0.005	0.390±0.010	ND	0.686±0.005
Kibera	0.006±0.005	0.263±0.015	ND	0.196±0.015
Zimmermann*	0.043±0.005	0.440±0.001	ND	2.840±0.020
Witeithie	0.060±0.010	0.316±0.005	ND	0.453±0.020
Kiwanja (KM)	0.043±0.005	0.156±0.005	ND	0.103±0.005
Korogocho	0.023±0.005	0.146±0.005	ND	0.533±0.015
Kangemi	0.090±0.010	0.480±0.010	ND	0.233±0.015
Kariobangi	0.053±0.005	0.013±0.005	ND	1.290±0.001
Juja	0.053±0.005	0.013±0.005	ND	1.076±0.015
Ruiru	0.200±0.010	0.626±0.005	ND	0.386±0.005
Dagoreti	ND	ND	ND	0.433±0.015
Ruai	0.033±0.020	0.120±0.000	ND	0.353±0.005
Thika	0.053±0.005	0.383±0.005	ND	0.400±0.010

Fifteen samples of muratina were analysed for lead. Only one sample had lead concentration above 0.1 mg/l. This indicates how adulteration can lead to presence of trace elements in these brews. No cadmium was found on any of the muratina samples while copper concentrations were negligible compared to the limit of 2.0 mg/l. Aluminium was present in concentrations above 0.2 mg/l in all but two samples. This is attributed to leaching from containers or using brown sugar as an additive.

Table 9g. Concentration of lead, copper, cadmium and aluminium in various licensed alcoholic beverages by AAS (mg/l).

Sample name	Collection site	Lead	Copper	Cadmium	Aluminium
Tusker lager	Nairobi	0.010±0.010	0.180±0.001	ND	ND
Castle lager	Thika	0.076±0.005	0.183±0.011	ND	ND
Nyuki liquor	Githunguri	0.060±0.001	0.216±0.011	ND	0.406±0.005
Nyuki liquor	Thika	ND	ND	ND	ND
Castle Milk Stout	Thika	0.043±0.011	0.253±0.005	ND	0.196±0.015
Nepoleon Brandy	Nairobi	0.030±0.010	0.240±0.001	ND	ND
Safari Cane	Nairobi	0.003±0.005	ND	ND	ND
Ranger lager	Nairobi	0.033±0.005	0.093±0.005	ND	ND
Hansa Pilsner	Nairobi	0.026±0.005	ND	ND	ND
Citizen Special	Nairobi	0.023±0.005	ND	ND	ND
Meakins Rum xxx	Nairobi	0.006±0.005	ND	ND	ND
MeakinsVodka	Nairobi	0.046±0.011	ND	ND	ND
Meakins dry gin	Nairobi	0.043±0.005	ND	ND	ND
Richot brandy	Nairobi	0.023±0.005	ND	ND	ND
Popov Vodka	Nairobi	0.006±0.015	ND	ND	0.01±0.001
Guiness	Nairobi	0.030±0.010	ND	ND	ND
High Life Fortified wine	Nairobi	0.056±0.005	0.296±0.015	ND	0.066±0.020
Pilsner	Nairobi	0.050±0.001	ND	ND	0.076±0.028

KEY: -KM-Kilomita moja (a slum bordering Kenyatta University).

*sampled twice.

ND- not detected

None of the licensed alcoholic beverages had prohibited levels of lead or copper. Cadmium was completely absent in all. Only nyuki liquor had slightly more than the recommended limit of aluminium. This indicates that the licensed brewers ensure quality of their products by using the right raw materials and equipment.

Table 10. Trace element concentration (μg/g) in raw materials.

Sample name	Collection site	Lead	Copper	Cadmium	Aluminium
Finger millet	Githurai	0.023±0.005	0.060±0.005	ND	2.593±0.025
Kimera	Kibera	0.016±0.015	0.026±0.005	ND	15.720±0.132
Kimera*	Kibera*	0.063±0.005	0.060±0.005	ND	24.303±0.005
Kimera	Thika*	0.016±0.015	0.013±0.005	ND	2.626±0.015
Fried flour	Githurai	0.046±0.015	ND	ND	4.983±0.040
Fried flour	Witeithie	ND	0.003±0.005	ND	ND
Fried flour	Kibera	0.040±0.010	0.010±0.005	ND	0.763±0.005
Fried Kimera	Kibera	0.050±0.010	0.010±0.005	ND	ND
Maize flour	Kibera	ND	0.030±0.005	ND	0.816±0.020
Fermented maize flour	Kibera	0.080±0.010	0.070±0.005	ND	ND
Fermented maize flour*	Kibera*	0.020±0.010	0.010±0.005	ND	ND
Sugar	Kiwanja (KM)	ND	ND	ND	ND
Sugar*	Kiwanja (KM)*	ND	ND	ND	ND
Brown Sugar	Kiwanja (KM)	0.030±0.015	0.013±0.005	ND	0.023±0.005
Brown Sugar	Githurai	ND	ND	ND	ND
Brown Sugar	Kangemi	0.020±0.010	ND	ND	ND
Brown Sugar*	Kiwanja (KM)*	0.046±0.011	ND	ND	8.590±0.017
Formalin	Witeithie	0.020±0.017	0.050±0.005	ND	ND
Formalin	Kiwanja (KM)	0.046±0.015	0.043±0.005	ND	0.063±0.011
Formalin	Witeithie	0.110±0.010	0.010±0.005	ND	ND
Formalin	Kiwanja (KM)*	0.136±0.005	0.050±0.005	0.070±0.005	0.383±0.015
Yeast	Kiwanja (KM)	ND	0.010±0.005	ND	ND
Yeast	Thika*	0.076±0.005	0.030±0.005	ND	ND
Yeast	Kiwanja (KM)	ND	0.050±0.005	ND	ND
Barley	Thika	0.023±0.005	ND	ND	0.436±0.005
Wort	Thika	0.026±0.015	ND	ND	ND

Twenty six (26) raw materials were analysed for cadmium, lead, aluminium and copper. Two samples of formaldehyde, commonly known as 'chemical', contained lead above the allowed limit of 0.1 mg/l. They were from Witeithie (0.11 mg/l) and KM (0.136 mg/l). The formaldehyde commonly referred to as

'chemical' and used in illicit brews is actually a soapy substance used in gas welding mixed with formaldehyde. The soldering bait is dipped into this substance before welding commences. This substance contains cadmium and lead and is probably the sole source of cadmium in the formaldehyde. Contamination is another source of cadmium in the raw materials and water. Other samples which had lead in high concentrations were yeast, 0.076 mg/l, kimera, 0.063 mg/l, and fermented maize flour 0.08 mg/l.

Cadmium was found in formaldehyde from KM at a concentration of 0.07 mg/l, which is 14 times the allowed limit of 0.005 mg/l. This could have contributed to some of the beverages having cadmium above the allowed limits. When asked why they use formaldehyde, the brewers say it quickens the fermentation process and customers get drunk faster. There is a possibility that formaldehyde is reduced by acids to produce methanol, which has been responsible for a number of deaths and loss of eyesight. No raw material sample contained copper above the allowed limit of 2.0 mg/l.

Aluminium concentrations were above 0.2 mg/l in eleven (11) out of twenty six (26) raw materials analysed. It was noted that fermented mixture of maize flour and finger millet, commonly known as kimera, had the highest aluminium concentrations. The process of fermentation could have produced acids which leach aluminium from the containers. Fermentation is done by steeping the flour in water for about three days after which it is removed dried and fried. The fried flour forms the basis of brews like busaa and kibuku beer. Concentrations above 0.2 mg/l ranged from 0.383 mg/l in formaldehyde to

47.02 mg/l in fermented maize flour. These are shown in figure 20a and 20b in appendix 4.

Raw materials used in the preparation of these brews could have contributed to the high levels of lead. Molasses used in the preparation of kangara may be contaminated especially in storage or as a result of lead-based pesticides used in sugarcane farming. Water used in brewing may also be a source of contamination. Contaminated dust falling on brews kept in open containers may also contribute to high lead levels in the alcoholic beverages

Also notable was the fact that lead was missing (in most samples) in the raw materials. This suggests that lead present in the alcoholic beverages originate from the processing events. Water may contain lead if it is drawn from rivers in the urban environment. Containers used to fetch and store water may also contribute to presence of lead in the brews. It is worth noting that these beverages are prepared in the backyard areas of slum areas where garbage disposal is prevalent. Disposal of old lead acid accumulator batteries in the urban environment could also lead to high lead levels in the alcoholic beverages. This is especially so if surface runoffs from garbage sites get into rivers from which brewing water is drawn. Brews are stored in plastic drums made of pvc. Lead can be leached from these containers. Metal containers used for petroleum oil are also used for storing these brews. The seams of these containers are soldered with lead which can be leached into the acidic brews. Concentrations of lead above 0.1 mg/l are shown in figure 18 in appendix 4.

Cadmium was found in formaldehyde from KM at a concentration of 0.07 mg/l, which is 14 times the allowed limit of 0.005 mg/l. This could have contributed to some of the beverages having cadmium above the allowed limits. No raw material sample contained copper above the allowed limit of 2.0 mg/l.

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Copper above the allowed limit of 2.0 mg/l was found in only one alcoholic beverage, chang'aa. Chang'aa is prepared by adding molasses to busaa followed by distillation. Copper pipes from car engines are used in cooling the vapour to form liquid chang'aa. Since chang'aa is highly acidic, leaching of the copper from the pipes could have taken place leading to high levels in the brew. Other brews which had substantially high copper levels are busaa from Mathare, 0.946 mg/l, kangara from Ruiru, 1.093 mg/l and karubu from Mathare, 0.75 mg/l.

These levels indicate increased risk of copper contamination in these beverages. Another possible source is the use of ten-cent copper coins in the brewing process. These are put in the barley or cereal mash to 'activate' the process of fermentation or to make what the brewers call 'tough brew'. After distillation the mash is squeezed and the filtrate added to the distilled product to ensure no loss in the process and also increasing the volume of the final product. This increases the amount of copper in the final product.

Cadmium was present in twelve (12) out of the one hundred and twelve (112) alcoholic beverages samples analysed. Dust is the most probable source as well as contaminated water through poor dry cells disposal. It is also possible that dry cells are used in the brewing process in what is termed as 'activation' to make the brew bitter. If this is the case, then, this could be the sole source of cadmium in the brews. It is also noted that most of the brews containing cadmium were sampled from urban and industrial areas indicating pollution as a major contributor to presence of cadmium in these beverages. As with lead, contaminated water and raw materials can contribute to high levels of cadmium in these brews. Lead was also prevalent in high concentrations in brews that were sampled from urban areas thus pointing at pollution as the main source of these trace elements. Sorghum and millet grown on sewage sludge is used for brewing. This sludge is known to contain cadmium and bioaccumulation in the plants leads to its presence in the alcoholic beverages. Concentrations of cadmium above 0.005 mg/l are shown in figure 19 in appendix 4.

These results compare well with results obtained in a similar study in Tanzania (Mosha, 1996). In particular, kangara in Tanzania had similar levels of cadmium and lead with the kangara from Kenya.

Out of the one hundred and twelve (112) alcoholic beverages samples analysed, fifty eight (58) were found to contain aluminium above the allowed limit of 0.2 mg/l. The concentrations ranged from 0.233 mg/l in muratina to 56.906 mg/l in popov vodka. These high concentrations in popov vodka can be attributed to leaching from distillation equipment. Most of the containers used for the preparation of these beverages are made of aluminium and the fact that the beverages are usually acidic, leaching of the aluminium from these containers is likely to happen and lead to high levels of the same in the alcoholic beverages. As seen in various studies (Wanjau, 1991, Githua, 1994 and Gardner and Gunn, 1991), plants are known to accumulate aluminium from the soils. In particular, Wanjau, (1991) reported high levels of aluminium in finger millet, sorghum and millet (Table 1). These cereals are used to make alcoholic beverages and aluminium is therefore liable to be present in them. Levels of aluminium above 0.02 mg/l are shown in figures 21a, 21b and 21c in appendix 4.

A few samples were analysed using differential pulse anodic stripping voltammetry. The following equation was used in calculating the concentration of each element in the brew and raw material samples : -

$$C_u = \frac{i_1 v C_s}{i_2 v + (i_2 - i_1) V}$$

Where

i_1 = *Sample peak height*

i_2 = *S* tan *dard addition peak height*

v = *Volume of s* tan *dard solution added*

V = *Volume of original Sample*

Cs = *Concentration of s* tan *dard solution*

Cu = *Concentration of original sample*

However, because the volume of the spiking solution added was very small i.e. 100µl, the above equation was simplified to: -

$$Cu = \frac{i_1 v C_s}{(i_2 - i_1)V}$$

This latter equation was used to calculate the concentration of metals in solution. It is valid for 10 ml of sample and 10-100 µl standard additions.

Table 11. Concentration of cadmium, lead and copper in various alcoholic beverages by differential pulse anodic stripping voltammetry (DPASV).

Sample name	Collection site	Concentration (mg/l)		
		Lead	Cadmium	Copper
Mugaca	Kiwanja (KM)	0.139±0.017	ND	NA
Busaa	Kiganjo,Thika	0.133±0.007	0.021±0.001	NA
Kibuku	Zimmermann	0.112±0.014	0.021±0.001	NA
Viena wine	Maragua	0.150±0.008	ND	NA
Busaa	Mathare	0.166±0.009	ND	NA
Busaa	Kiwanja (KM)	0.193±0.011	ND	NA
Filtered Busaa	Kibera	0.193±0.011	ND	NA
Busaa	Witeithie	0.102±0.007	ND	NA
Kangara	Kiandutu	0.354±0.017	0.050±0.002	NA
Miti ni dawa	Kibera	0.267±0.017	ND	NA
Mugaca	Korogoco	0.139±0.0170	0.022±0.003	NA
Toivo	Kibera	0.267±0.017	ND	NA
Muratina	Ruiru	0.193±0.011	ND	NA
Kangara	Ruiru	0.458±0.015	0.063±0.004	NA
Miti ni dawa	Thika	0.209±0.010	0.020±0.001	NA
Popov vodka	Nairobi	0.102±0.007	ND	NA
Afriwine	Githunguri	ND	0.024±0.004	NA
Filtered Busaa	Githurai	ND	0.021±0.001	NA
Busaa	Kangemi	ND	0.022±0.005	NA
Kangara	Githurai	ND	0.039±0.002	NA
Kangara	Witeithie	ND	0.041±0.003	NA
Chang'aa	Makongeni	ND	0.022±0.002	NA
Pilsner	Nairobi	ND	ND	NA
Chang'aa	Zimmermann	ND	ND	1.843±0.114
Chang'a	Juja	ND	ND	2.249±0.118

Table 12. Comparison of concentrations of cadmium, lead and copper in various alcoholic beverages by (AAS) and (DPASV).

Sample name	Collection site	LEAD		CADMIUM		COPPER	
		AAS	DPASV	AAS	DPASV	AAS	DPASV
Mugaca	Kiwanja (KM)	0.146±0.011	0.139±0.017	ND	ND	0.323±0.005	NA
Busaa	Kiganjo,Thika	0.133±0.005	0.133±0.007	0.020±0.005	0.021±0.001	0.473±0.011	NA
Kibuku	Zimmermann	0.116±0.005	0.112±0.014	0.020±0.003	0.021±0.001	0.243±0.005	NA
Viena wine	Maragua	0.156±0.005	0.150±0.008	ND	ND	0.323±0.005	NA
Busaa	Mathare	0.163±0.005	0.166±0.009	ND	ND	0.946±0.005	NA
Busaa	Kiwanja (KM)	0.190±0.017	0.193±0.011	ND	ND	0.543±0.005	NA
Filtered Busaa	Kibera	0.200±0.010	0.193±0.011	ND	ND	0.200±0.010	NA
Busaa	Witeithie	0.103±0.005	0.102±0.007	ND	ND	0.440±0.010	NA
Kangara	Kiandutu	0.350±0.010	0.354±0.004	0.050±0.006	0.050±0.002	0.323±0.005	NA
Miti ni dawa	Kibera	0.283±0.005	0.267±0.017	ND	ND	0.713±0.005	NA
Mugaca	Korogoco	0.146±0.005	0.139±0.017	0.020±0.003	0.022±0.000	0.280±0.010	NA
Toivo	Kibera	0.286±0.023	0.267±0.017	ND	ND	ND	NA
Muratina	Ruiru	0.200±0.010	0.193±0.011	ND	ND	0.626±0.005	NA
Kangara	Ruiru	0.466±0.005	0.458±0.015	0.040±0.006	0.063±0.004	1.093±0.005	NA
Miti ni dawa	Thika	0.216±0.015	0.209±0.010	0.020±0.003	0.020±0.001	0.293±0.005	NA
Popov vodka	Nairobi	0.096±0.015	0.102±0.007	ND	ND	ND	NA
Afriwine	Githunguri	ND	ND	0.023±0.005	0.024±0.004	0.116±0.005	NA
Filtered Busaa	Githurai	ND	ND	0.020±0.002	0.021±0.001	0.443±0.005	NA
Busaa	Kangemi	ND	ND	0.020±0.002	0.022±0.002	0.320±0.010	NA
Kangara	Githurai	ND	ND	0.040±0.003	0.039±0.002	0.323±0.005	NA
Kangara	Witeithie	0.070±0.010	ND	0.040±0.002	0.041±0.003	0.496±0.005	NA
Chang'aa	Makongeni	ND	ND	0.020±0.003	0.022±0.002	0.123±0.005	NA
Pilsner	Nairobi	ND	ND	ND	ND	ND	NA
Chang'aa	Zimmermann	ND	ND	ND	ND	1.743±0.005	1.843±0.114
Chang'aa	Juja	ND	ND	ND	ND	2.363±0.037	2.249±0.118

KEY: -KM-Kilomita moja (a slum bordering Kenyatta University).

ND- not detected. NA- not analysed

The trends of concentrations of trace elements determined using both methods were similar with higher values for DPASV than with AAS. A two-tailed student t-test carried out on the two sets of results at a 95% confidence limit showed no significant difference between the two methods. The calculated student t-test values were lower than the expected values and are given in Table 25 Appendix 2.

The results obtained by the two methods were correlated for each metal analysed. The correlation curves, coefficients and inferences are shown below.

Figure 6. Correlation curve for lead in alcoholic beverages.

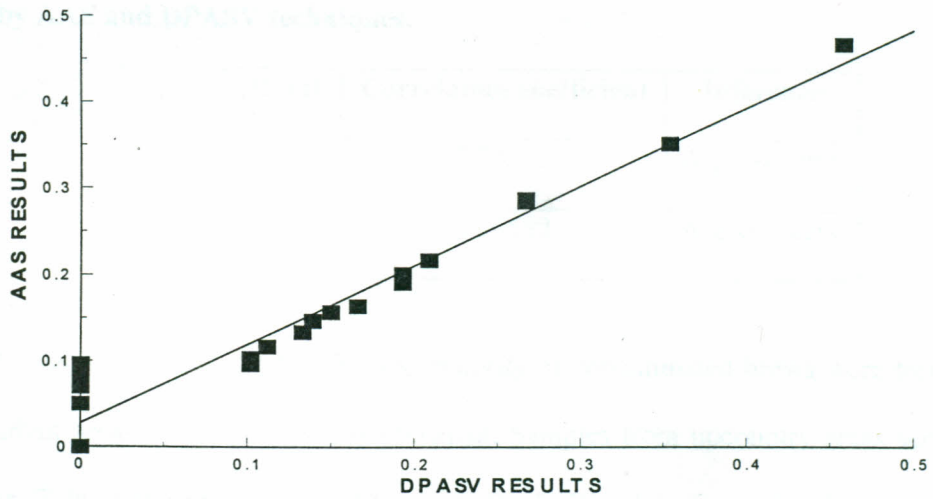

Figure 7. Correlation curve for cadmium in alcoholic beverages.

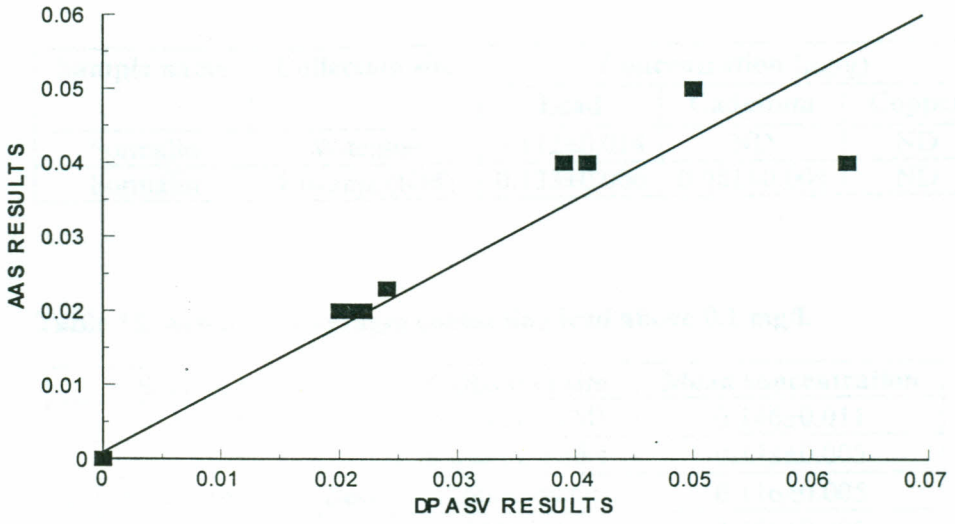

Table 13. Correlation coefficient of trace elements in alcoholic beverages by AAS and DPASV techniques.

Metal	Correlation coefficient	Inference
Pb	0.94822	A good curve
Cd	0.94692	A good curve

It was particularly notable that the majority of contaminated brews were from urban areas where pollution is prevalent. Samples from upcountry areas such as Tala, Githunguri, Kisii, Maragua, Ruai, Kiambu, Gatundu, Embu and Murang'a had the least concentrations of trace elements. In particular, karubu from Embu had 0.006 mg/l lead, 0.130 mg/l copper, no cadmium and 0.093 mg/l aluminium. This is a clear indication that pollution in major towns may be a major contributing factor to the presence of trace elements in these alcoholic beverages.

Table 14. Concentration of lead, copper and cadmium in some raw materials by DPASV.

Sample name	Collection site	Concentration (μg/g)		
		Lead	Cadmium	Copper
Formalin	Witeithie	0.112±0.014	ND	ND
Formalin	Kiwanja (KM)	0.133±0.006	0.081±0.004	ND

Table 15. Acoholic beverages containing lead above 0.1 mg/l.

Sample name	Collection site	Mean concentration
Mugaca	Kiwanja (KM)	0.146±0.011
Busaa	Kiganjo, Thika	0.133±0.005
Kibuku International Beer	Zimmermann	0.116±0.005
Viena wine	Maragua	0.156±0.005
Busaa	Mathare	0.163±0.005
Busaa	Kiwanja (KM)	0.190±0.017
Filtered Busaa	Kibera	0.200±0.010
Busaa	Witeithie	0.103±0.005
Kangara	Kiandutu, Thika	0.350±0.010
Miti ni dawa	Kibera	0.283±0.005
Mugaca	Korogocho	0.146±0.005
Toivo	Kibera	0.286±0.023
Busaa	Kangemi	0.096±0.005
Muratina	Ruiru	0.200±0.010
Miti ni dawa	Thika*	0.216±0.015
Popov Vodka	Nairobi	0.096±0.015
Kangara	Ruiru	0.466±0.005

Profiles of the above concentrations are shown in figures 18 in appendix 4.

Table 16. Acoholic beverages containing cadmium above 0.005 mg/l.

Name	Collection site	Mean concentration
Afriwine	Githunguri	0.023±0.005
Busaa	Kiganjo, Thika	0.020±0.005
Kibuku Beer	Zimmermann	0.020±0.005
Viena wine	Maragua	0.016±0.005
Filtered Busaa	Githurai	0.020±0.005
Busaa	Kangemi	0.020±0.005
Miti ni dawa	Githunguri	0.016±0.005
Kangara	Githurai	0.040±0.005
Miti ni dawa	Thika	0.020±0.005
Kangara	Witeithie	0.040±0.005
Busaa	Witeithie	0.016±0.005
Kangara	Kiandutu, Thika	0.050±0.005
Mugaca	Korogocho	0.020±0.005
Chang'aa	Thika*	0.020±0.005
Pilsner	Nairobi	0.020±0.003
Kangara	Ruiru	0.040±0.003

Profiles on these concentrations are shown in figure 19 in appendix 4.

Aluminium was present in many alcoholic beverages. These were mugaca (Korogocho) ; 22.84 mg/l, busaa: (KM); 20.4 mg/l, Kibera ; 12.336, Witeithie; 10.6 mg/l. Kangemi; 15.966 mg/l, chang'aa: Zimmermann; 56.908 mg/l, kangara: witeithie; 15.393 mg/l, Githurai; 40.34 mg/l, Ruiru; 45.44 mg/l. Muratina and miti ni dawa had the lowest aluminium concentrations although these concentrations were above the required limit.

Raw materials had varying concentrations of aluminium. Unfermented maize flour, formaldehyde and barley had low aluminium levels but above the allowed limit of 0.2 μg/g. Fermented maize flour, finger millet and kimera had the highest levels of aluminium. The levels varied probably due to differences in the

levels of aluminium in the soils on which the cereals were grown and the type of containers in which fermentation occurs i.e. plastic or aluminium pots.

Raw materials had significant levels of aluminium. These could have contributed to high levels in the brews. Levels ranged between 0.383 mg/l in formaldehyde ('chemical') to 47.02 mg/l in fermented maize flour from Kibera. Others were two samples of kimera from Kibera with concentrations of 15.72 μg/g and 24.303 μg/g. Profiles of aluminium concentrations in alcoholic beverages and raw materials are shown in figures 20a, 20b, 21a, 21b, and 21c in appendix 4. A sample of chang'aa from Juja had 2.363 mg/l of copper. This is above the recommended limit of 2.0 mg/l.

Results obtained were compared to studies carried out by Muchai (1985) and Mwanasi (1990). The levels of lead obtained by Muchai, especially on tusker (0.4 mg/l) were extremely high compared to what was obtained in this study on the same brew (0.01 mg/l). This indicates good improvement on quality assurance. The same relationship was shown in Mwanasi's (1990) analysis. However aluminium levels for similar brews were the same. Mwanasi's (1990) reported 6.225 mg/l aluminium in kibuku beer while this study reported 9.32 mg/l and 15.966 mg/l for the same brew.

Speciation studies for lead, cadmium and copper were extremely difficult because of the varying matrices of the different alcoholic beverages. It was only possible to analyse clear beverages which were foamless. Under these circumstances ionic lead, (Pb^{2+}), was found above 0.1 mg/l in kangara (Kiandutu,

Thika), miti ni dawa (Kibera), Toivo (Kibera, kangara (Ruiru) and miti ni dawa (Thika). Cadmium above 0.005 mg/l was found in four samples of kangara from Thika, Ruiru, Githurai and Witeithie. Copper above 2.0 mg/l was found in only one sample of chang'aa from Juja.

Table 17. Results on speciation studies with DPASV.

Sample name	Collection site	Concentration (mg/l)		
		Lead	Cadmium	Copper
Kangara	Kiandutu, Thika	0.102±0.007	0.046±0.002	NA
Mugaca	Kiwanja (KM)	0.066±0.005	NA	NA
Miti ni dawa	Kibera	0.144±0.011	NA	NA
Mugaca	Korogoco	0.070±0.004	NA	NA
Toivo	Kibera	0.138±0.017	NA	NA
Kangara	Ruiru	0.335±0.034	0.045±0.003	NA
Miti ni dawa	Thika	0.102±0.007	NA	NA
Kangara	Githurai	NA	0.031±0.003	NA
Kangara	Witeithie	NA	0.032±0.006	NA
Chang'aa	Zimmermann	NA	NA	1.250±0.076
Chang'aa	Juja	NA	NA	2.113±0.045

Table 18. Comparison of complexed and non-complexed lead in alcoholic beverages by differential pulse anodic stripping voltammetry (mg/l).

Sample name	Collection site	Lead		
		Total	Non-complexed	% Complexed
Mugaca	Kiwanja (KM)	0.139	0.066	52.51
Kangara	Kiandutu	0.354	0.102	71.18
Miti ni dawa	Kibera	0.267	0.144	46.06
Mugaca	Korogocho	0.139	0.070	49.64
Toivo	Kibera	0.267	0.138	48.31
Kangara	Ruiru	0.458	0.335	26.85
Miti ni dawa	Thika	0.209	0.102	51.19

Table 19. Comparison of complexed and non-complexed cadmium in brews by DPASV.

Sample name	Collection site	Cadmium		
		Total	Non-complexed	% Complexed
Kangara	Kiandutu	0.050	0.046	8.0
Kangara	Ruiru	0.063	0.045	28.57
Kangara	Githurai	0.039	0.031	20.51
Kangara	Witeithie	0.041	0.032	21.95

Table 20. Comparison of complexed and non-complexed copper in alcoholic beverages by DPASV (mg/l).

Sample Name	Collection site	Copper		
		Free	Complexed	% Complexed
Chang'aa	Zimmermann	1.843	0.593	32.17
Chang'aa	Juja	2.249	0.136	6.0

From the results in tables 18, 19 and 20, it is evident that about 50 % of the lead present in alcoholic beverages is complexed and may be considered harmless to the consumers. However, the free lead concentrations in the beverages is above the KEBS/WHO limits of 0.1 mg/l. This means that the brews are still dangerous with regard to lead. Cadmium is found free in a majority of alcoholic beverages analysed. The complexed fraction is less than 30% of the total metal content. This means that all the beverages that contain cadmium are dangerous to the consumers as they have cadmium content above the KEBS/WHO limit of 0.005 mg/l. For the sample that had copper above the KEBS/WHO limits, it was found that copper was free to an extent of 94%. This free fraction was still above the 2.0 mg/l limit. However, copper is not a real problem as far as alcoholic beverages are concerned.

The concentrations of the free metals in digested and undigested samples analysed by DPASV were correlated for both lead and cadmium. The trends of concentrations of trace elements determined using both digested and undigested samples were compared. A two-tailed student t-test carried out on the two sets of results at a 95% confidence limit showed no significant difference between the two concentrations. The calculated student t-test values were lower than the expected values and are given in table 26 appendix 3. However, correlation coefficients calculated for both concentrations showed poor correlation between concentration in digested and undigested samples. This was due to the large deviations of metal concentrations in the digested and undigested samples. Correlation curves obtained were poor hence were not shown. The correlation coefficients are shown in the table 21 below. From the correlation values obtained it is evident that many factors determine the amounts of free and complexed metal in the beverages. These could be acidity of

the brews, complexing ligands present or general matrix composition of the brews. However, it is notable that lead has the highest tendency of being complexed than copper and cadmium as shown by the percentage of the complexed fractions of each.

Table 21. Correlation coefficient of trace elements in digested and undigested alcoholic beverages by DPASV.

Element	Correlation coefficient	Inference
Lead	0.8506	A poor curve
Cadmium	0.8482	A poor curve

Four samples were analysed by inductively coupled argon plasma mass spectrometry (ICPMS) technique. The instrument used was ICPMS Optima 3000. Kangara from Githurai contained 0.03 mg/l cadmium which is six times the recommended limit. "Miti ni dawa" from Ruiru contained 0.125 mg/l lead. Chang'aa from Zimmermann and Juja contained 1.414 mg/l and 2.116 mg/l copper respectively. For the samples analysed by inductively coupled plasma mass spectroscopy (ICPMS) no sample exceeded the recommended limit for zinc i.e. 50 mg/l. Arsenic and silicon were found in very high concentrations. These are very dangerous and should be investigated further. The concentrations of arsenic were; Kangara (Githurai), 1.467 mg/l and miti ni dawa (Ruiru), 3.758mg/l. The concentrations of silicon were; Kangara (Githurai, 289.5 mg/l and miti ni dawa (Ruiru), 126.98 mg/l.

Aluminium results were not reported as the samples were covered with an aluminium foil hence contaminating them. These results compare well with those obtained by AAS analysis. ICPMS is capable of doing multielement analysis and therefore other

elements, which were not part of the study, were analysed. The results are shown in Table 22.

Table 22 Concentration of trace elements by ICPMS analysis (mg/l).

Sample name	Chang'aa	Chang'aa	Kangara	Miti ni dawa
Collection site	Zimmermann	Juja	Githurai	Ruiru
Cd	ND	ND	0.030	ND
Pb	ND	ND	ND	0.125
Cu	1.414	2.116	ND	ND
Zn	ND	ND	ND	ND
Cr	ND	ND	0.038	0.108
Ni	ND	ND	0.433	0.370
As	ND	ND	1.467	3.758
Fe	ND	ND	254.310	222.010
Mn	0.040	0.090	24.680	31.650
Ca	6.640	1.320	2048.150	1484.650
Mg	4.560	0.790	551.870	553.370
P	8.220	ND	154.150	362.500
Na	ND	ND	405.630	175.130
Si	ND	ND	289.500	126.980

Table 23. Mean concentrations of trace elements in alcoholic beverages by AAS and DPASV in mg/l.

Element / **Method**	**Lead**	**Copper**	**Cadmium**	**Aluminium**
AAS	0.071±0.076	0.344±0.345	0.028±0.011	6.499±15.579
DPASV	0.198±0.097	2.046±0.203	0.0305±0.014	------

Table 24. Mean concentrations of trace elements in popular alcoholic beverages by AAS in mg/l.

Alcoholic beverage	Lead	Copper	Cadmium	Aluminium
Miti ni dawa	0.079±0.080	0.168±0.166	0.001±0.005	1.008±0.994
Busaa	0.125±0.049	0.519±0.217	0.0066±0.010	9.873±5.815
Chang'aa	0.014±0.018	0.249±0.598	0.0009±0.004	2.67±12.115
Muratina	0.0517±0.048	0.253±0.186	ND	0.719±0.759

From table 24, it is evident that busaa is the most contaminated of the popular alcoholic beverages in all the four metals. However, the limit of copper is not exceeded by any of the beverages. Aluminium was contained in all the four popular alcoholic beverages in levels above the allowed limit of 0.2 mg/l. Busaa contained the highest levels of aluminium.

Although the study covered four elements, it was considered worthwhile to include the results of other elements (silicon and arsenic) obtained by ICPMS in two samples of kangara and miti ni dawa. These results were encountered due to the ability of ICPMS to carry out multielement analysis. It was felt that such important information could not be left out. The levels of arsenic and silicon were high implying that the brews are very poisonous as far as these elements are concerned.

4.1. CONCLUSION

From the results obtained it is evident that alcoholic beverages are potential sources of exposure to toxic elements for humans. Some brews and raw materials contained trace elements above the allowed limits. Cadmium in "kangara brew" was nine times the allowed limit.

Lead was present above the allowed limit in 16 alcoholic beverages the highest being 0.466 mg/l in kangara from Ruiru. This is over four times the allowed limit of 0.1 mg/l.

It can therefore be concluded that illicit alcoholic beverages contain heavy metal elements. However muratina brand seemed to be safe from lead, copper and cadmium poisoning. If it were brewed in steel containers it would be free from aluminium poisoning.

However, trace elements in these beverages are slow killers indirectly as they cause malfunctioning of body organs such as the brain, kidney and the liver.

Kangara brew was the most contaminated of all the alcoholic beverages analysed. It contained at least three of the four elements analysed in levels above the allowed limits.

4.2. RECOMMENDATIONS AND AREAS OF FURTHER RESEARCH

Speciation studies should be done to establish the chemical forms of these elements in the alcoholic beverages so as to establish their bioavailability. It is also important to analyse aluminium by another method e.g. colourimetry to compare the results. Although AAS is a standard method, there exists some drawbacks such as interferences (spectral, matrix etc.) hence the need to use other methods.

This study covered only four elements. In the course of analysis, a dangerous element, arsenic, was found in two brews in very high concentrations. Although this was not part of the study, it is felt that other toxic elements e.g. arsenic, should be analysed. The maximum allowed limit for arsenic in alcoholic beverages is 0.1 mg/l. The brews that had arsenic above this limit were kangara (Githurai), 1.467 mg/l and chang'aa (Gikomba), 3.758 mg/l. These concentrations are over 37 and 14 times the recommended maximum allowable limits respectively. Kangara and chang'aa are brewed from molasses. Some lead arsenate pesticides are used in pest control in sugarcane plantations and these could end up in the brews. This is a very great health risk to consumers. Arsenic can be analysed using the hydride generation technique.

Due to the high increases in beer prices and the fact that majority of Kenyans cannot afford to buy beer. Tax cuts on beers, wines and spirits should be put in place to make them affordable.

Community awareness about heath concerns in brews should be done to sensitize people on the dangers of consuming these illicit brews.

A further study should be carried out on brews from other areas of the country outside the most polluted areas like Thika and Nairobi. These results can be used to compare the brews in the rural areas with those in urban areas.

A process of preconcentration can be carried out before analysis. This is especially necessary when the concentration of the metal is not sufficiently high to determine directly or when considerable amounts of solids are present in the sample. Some of the metals may be chelated and extracted with organic solvents. This approach serves to increase the concentration of the analyte in the prepared sample and simultaneously remove matrix interferences. Ammonium pyrrolidine dithiocabarmate (APDC) in methyl isobutyl ketone (MIBK) is widely used for this purpose and increases direct analysis signals by about 100 times.

Water analysis for heavy metals should be done in order to establish the main source of contamination.

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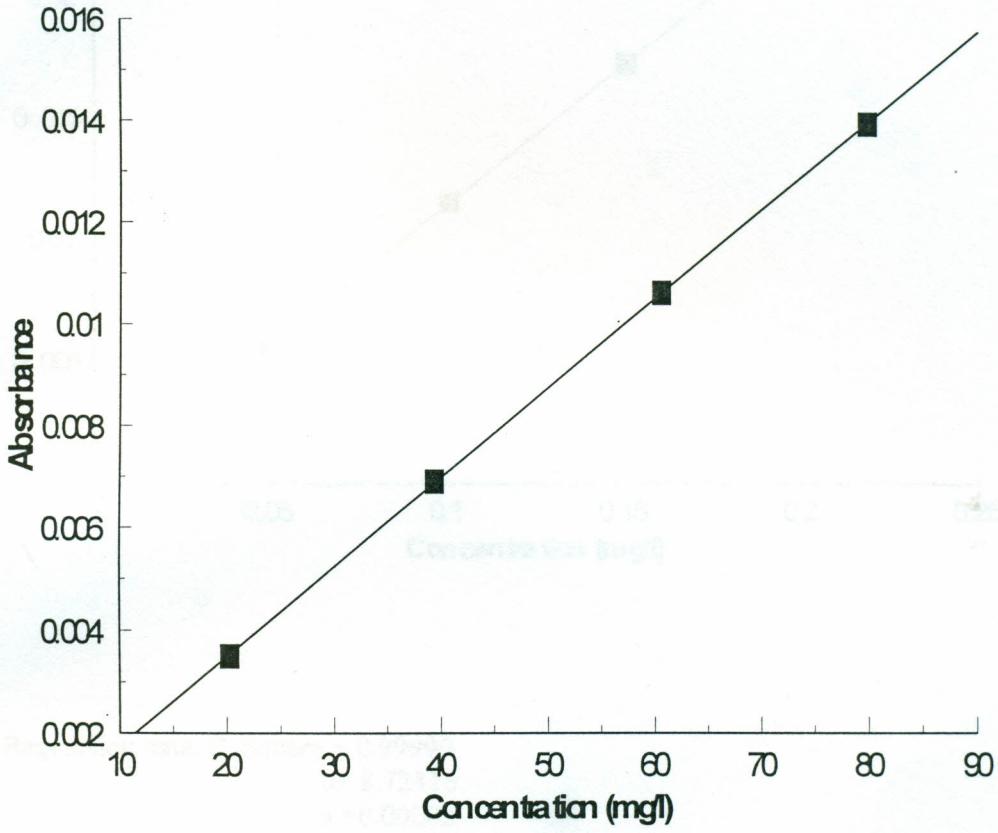
APPENDIX

Appendix 1

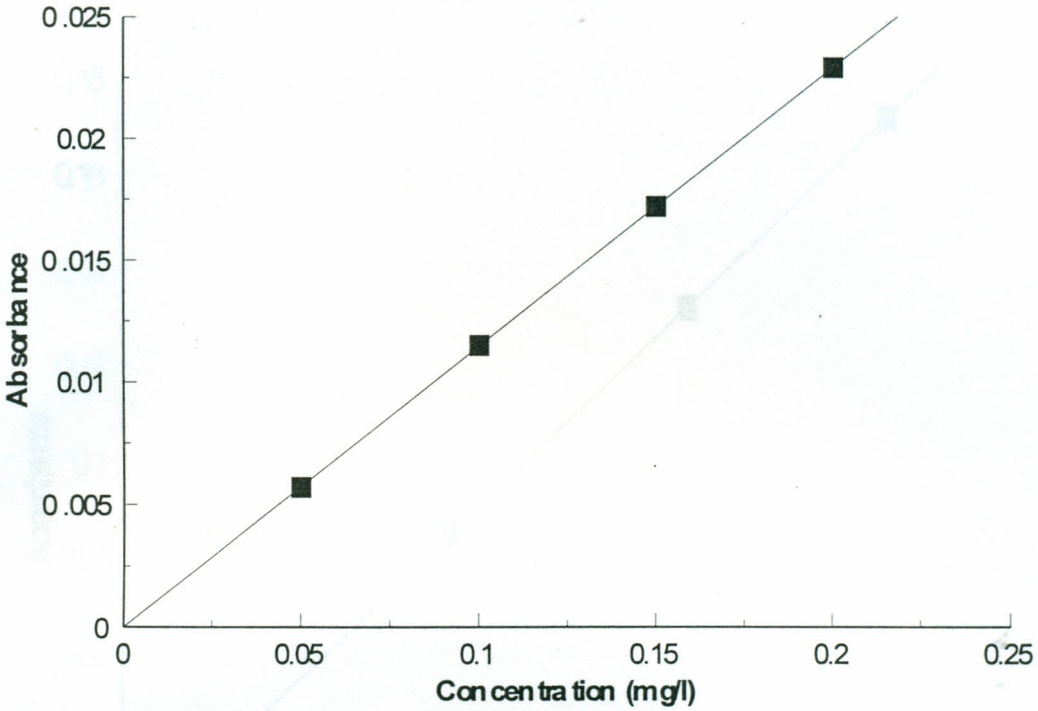
Figure 8. AAS Aluminium calibration curve.



Regression data: R-Square =0.99995
b=0.00017
a = -7.5×10^{-6}

Regression equation: Absorbance = 0.00017 (Concentration, (mg/l)) -7.5×10^{-6}.

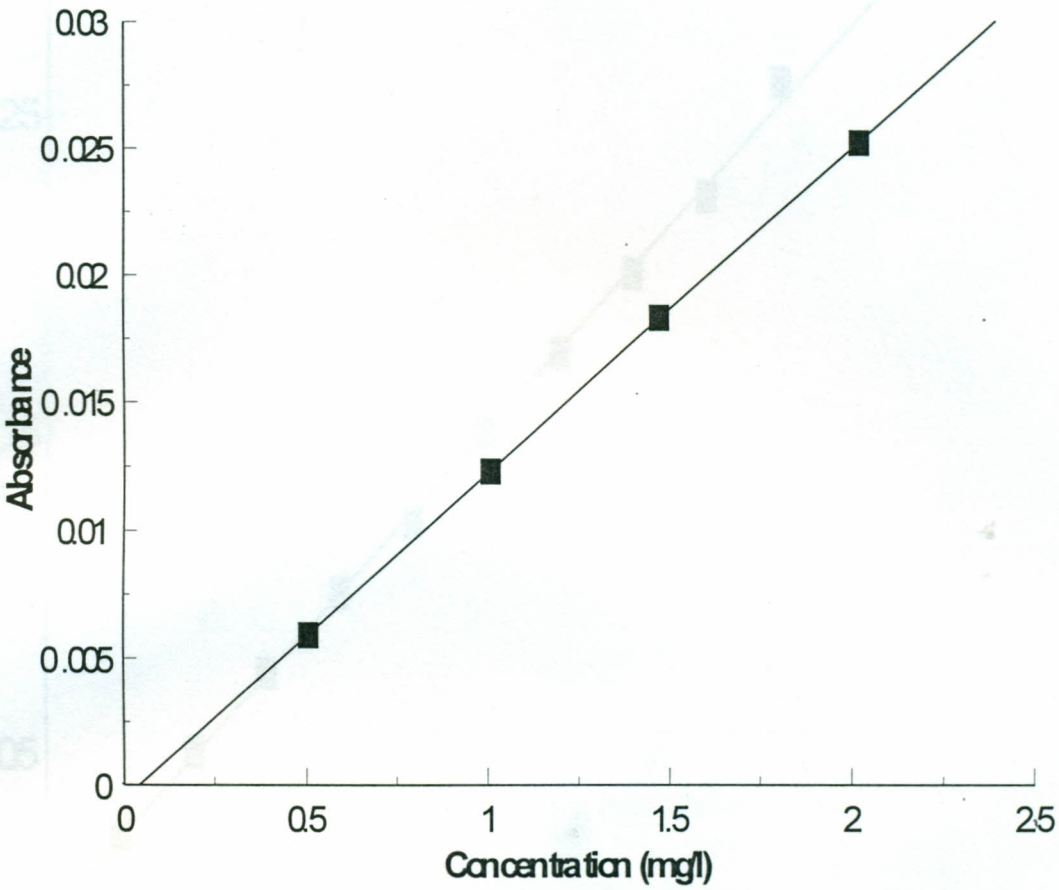
Figure 9. AAS Cadmium calibration curve.



Regression data: R-Square = 0.99999
b= 8.72416
a =0.00002

Regression equation: Absorbance = 8.72416 (Concentration, (mg/l)) + 0.00002

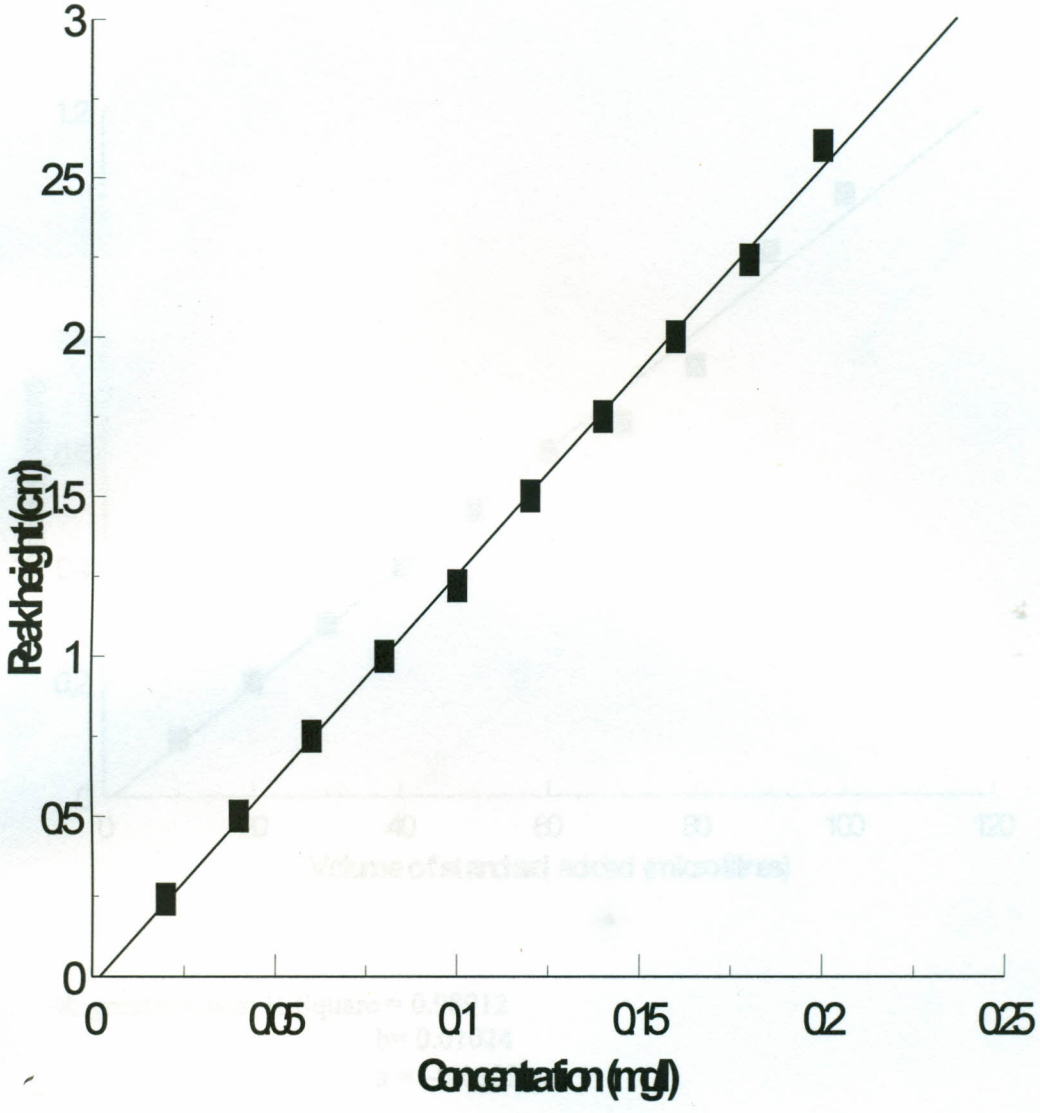
Figure 11. AAS Lead calibration curve.



Regression data: R-Square = 0.99999
b= 0.1276
a = –0.00054

Regression equation: Absorbance = 0.1276 (Concentration, (mg/l)) –0.00054

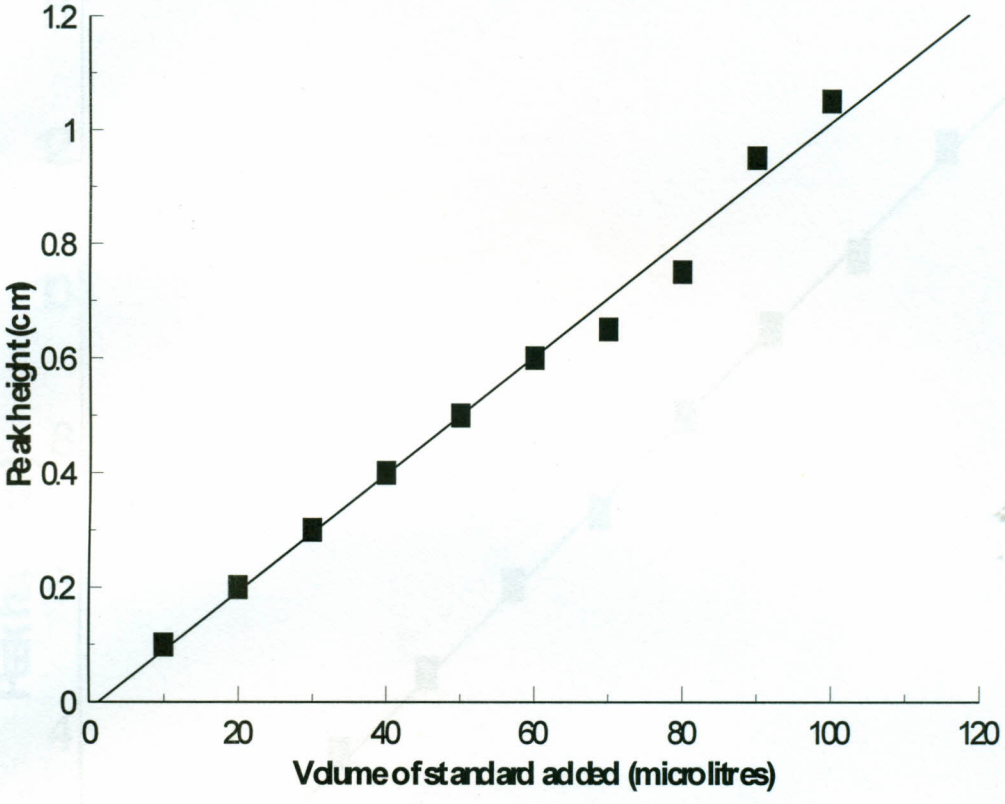
Figure 12. DPASV Calibration curve for cadmium.



Regression data: R-Square = 0.99848
b= 12.7878
a = –0.02666

Regression equation: Absorbance = 12.7878 (Concentration, (mg/l)) –0.02666

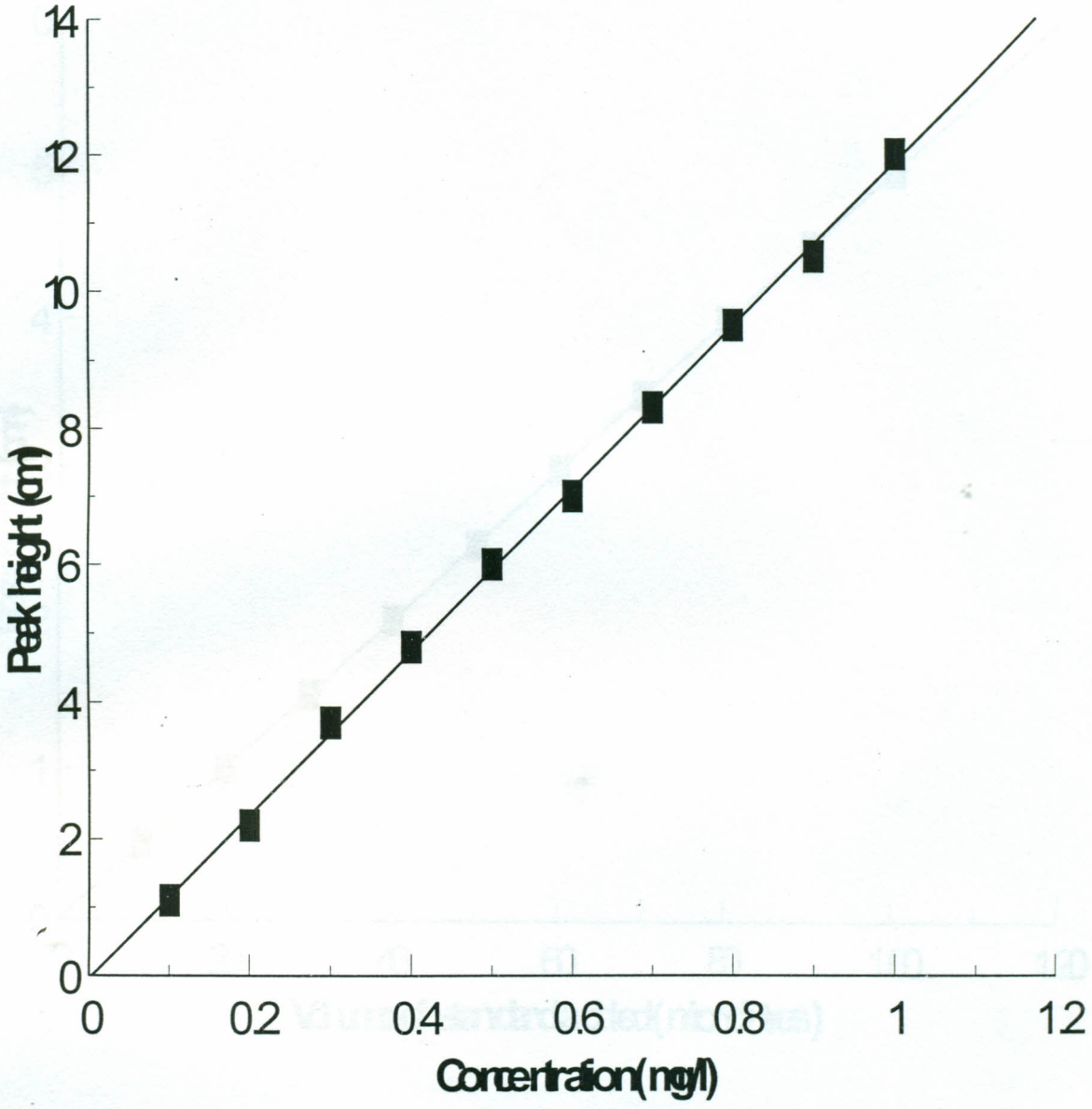
Figure 13. DPASV Standard addition calibration curve for cadmium.



Regression data: R-Square = 0.98912
b= 0.01024
a = –0.01333

Regression equation: Absorbance =0.01024 (Volume, (µl)) –0.01333

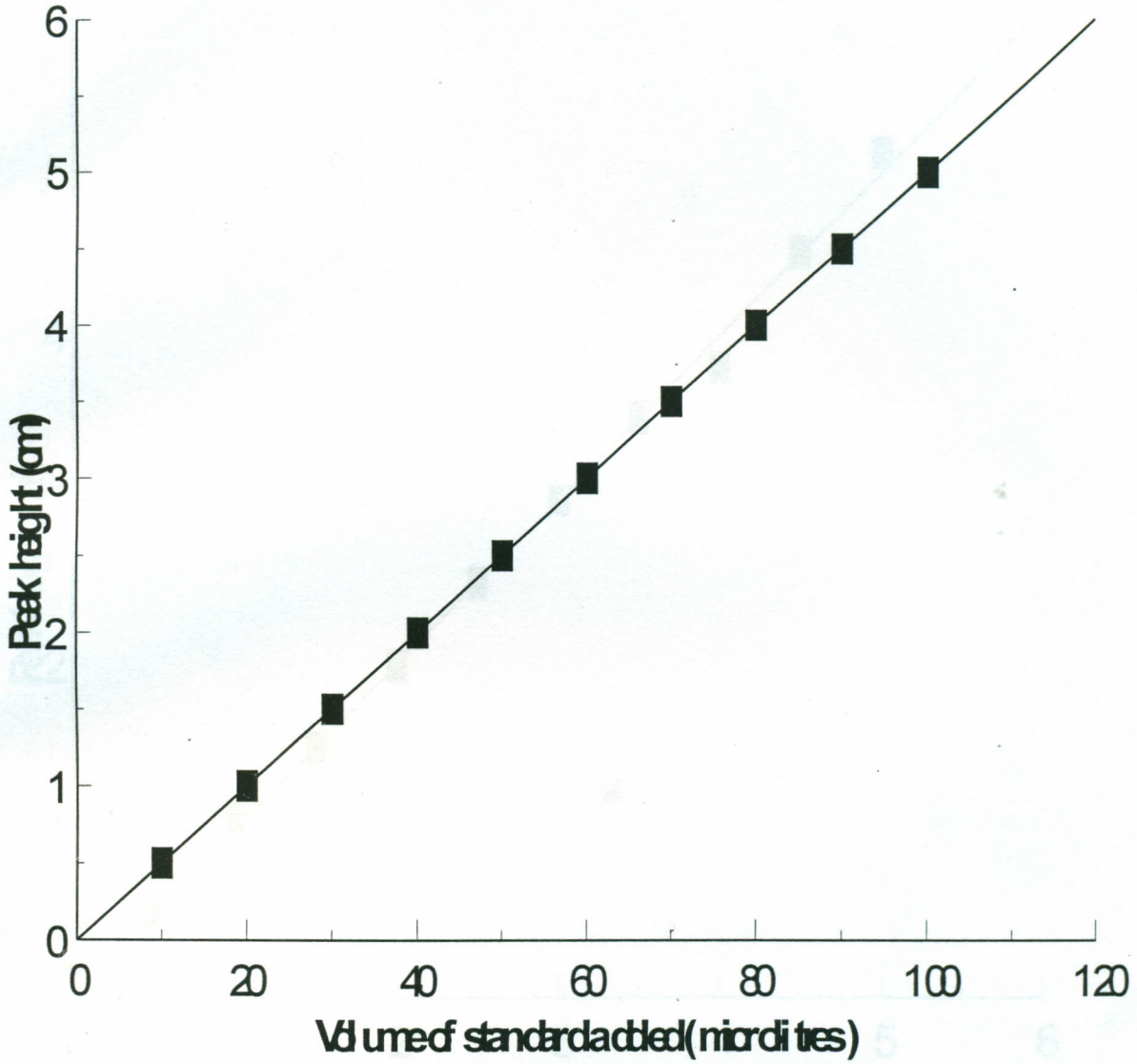
Figure 14. DPASV Calibration curve for lead.



Regression data: R-Square = 0.99894
b= 11.9212
a = –0.04666

Regression equation: Absorbance =11.9212 (Concentration, (mg/l)) –0.04666

Figure 15. DPASV Standard addition calibration curve for lead.



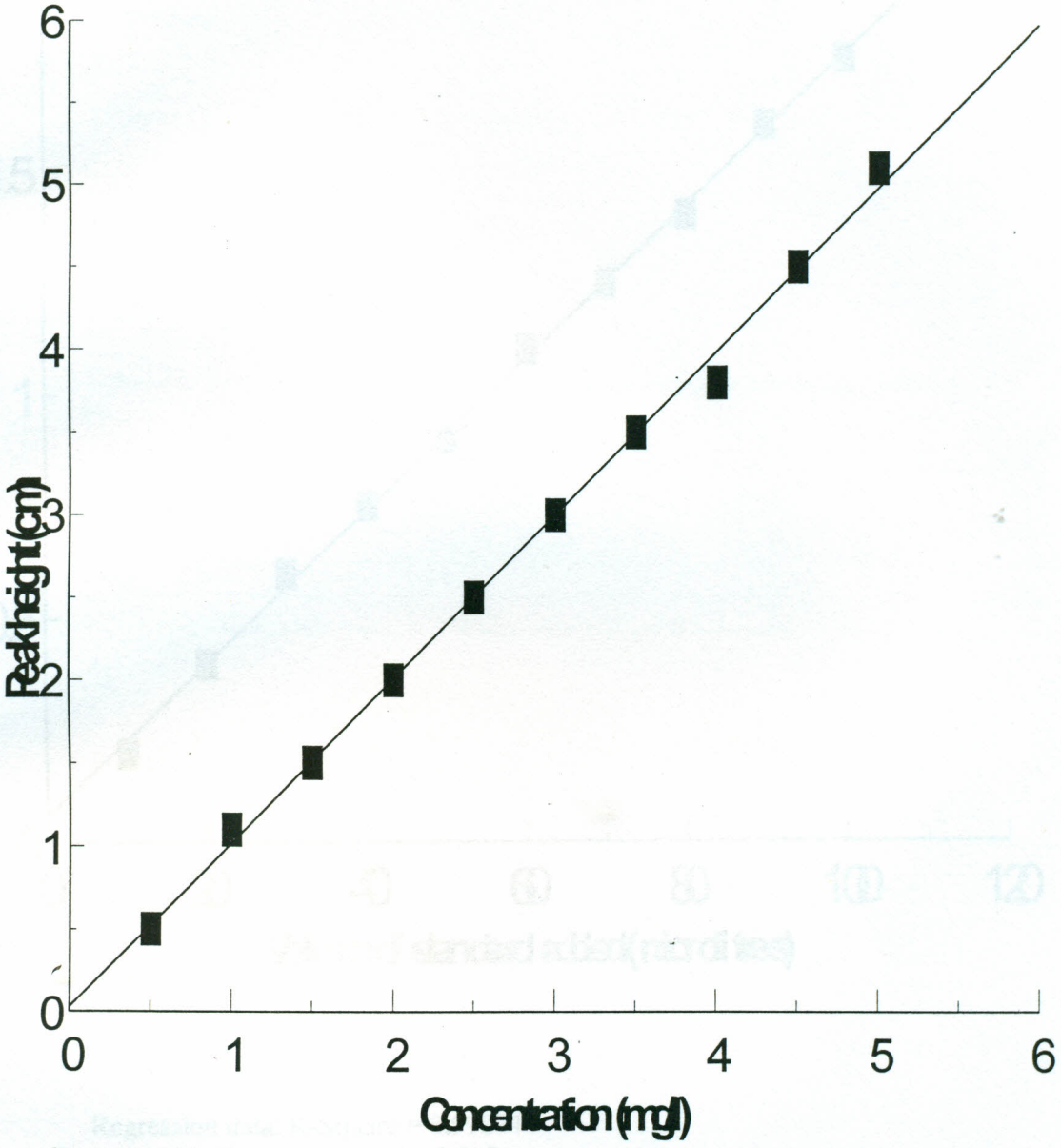
Regression data: R-Square = 1

b= 0.05

$a = -1.5 \times 10^{-16}$

Regression equation: Absorbance =0.05 (Volume, (μl)) -1.5×10^{-16}

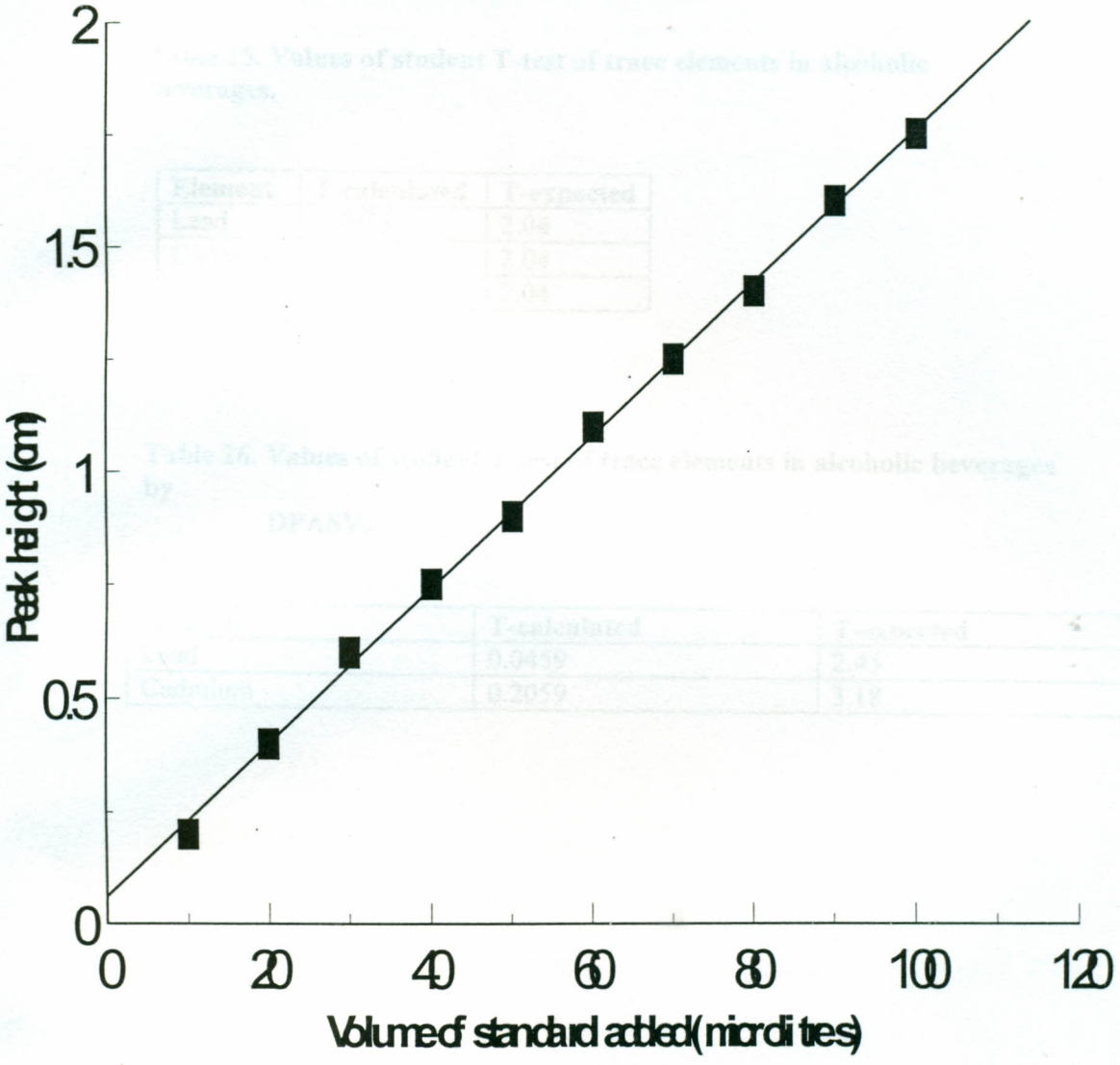
Figure 16. DPASV Calibration curve for copper.



Regression data: R-Square = 0.99713
b= 0.99030
a = 0.02666

Regression equation: Absorbance = 0.99030 (Concentration, (mg/l)) +0.02666

Figure 17. DPASV Standard addition calibration curve for copper.



Regression data: R-Square = 0.99874
b= 0.017
a = 0.05999

Regression equation: Absorbance = 0.017 (volume, (μl)) +0.05999

Appendix 2

Table 25. Values of student T-test of trace elements in alcoholic beverages.

Element	T-calculated	T-expected
Lead	0.7023	2.04
Cadmium	0.6629	2.04
Copper	0.0441	2.04

Table 26. Values of student T-test of trace elements in alcoholic beverages by DPASV.

Element	T-calculated	T-expected
Lead	0.0459	2.45
Cadmium	0.2059	3.18

Appendix 3

Statistical treatment of data.

1. **Mean.**

The mean concentrations of each of each sample were calculated for triplicate determinations using equation 1 below.

$$\bar{x} = \frac{\sum_{i=1}^{i=n}(x_i - \bar{x})}{n} \quad \text{.....1}$$

Where x_i is the i^{th} term of the determination or the set of data and n is the number of determinations or data.

2. Standard deviation, S.

This was used to measure dispersion of values about the mean. Equation 2 was used to calculate the standard deviation.

$$S = \sqrt{\frac{\sum_{i=1}^{i=n}\left(x_i - \bar{x}\right)^2}{n-1}} \quad \text{.....2}$$

3. Student t-Test.

Used to determine whether two sets of data from two methods of analysis differ significantly. The t value obtained is compared to the critical value.

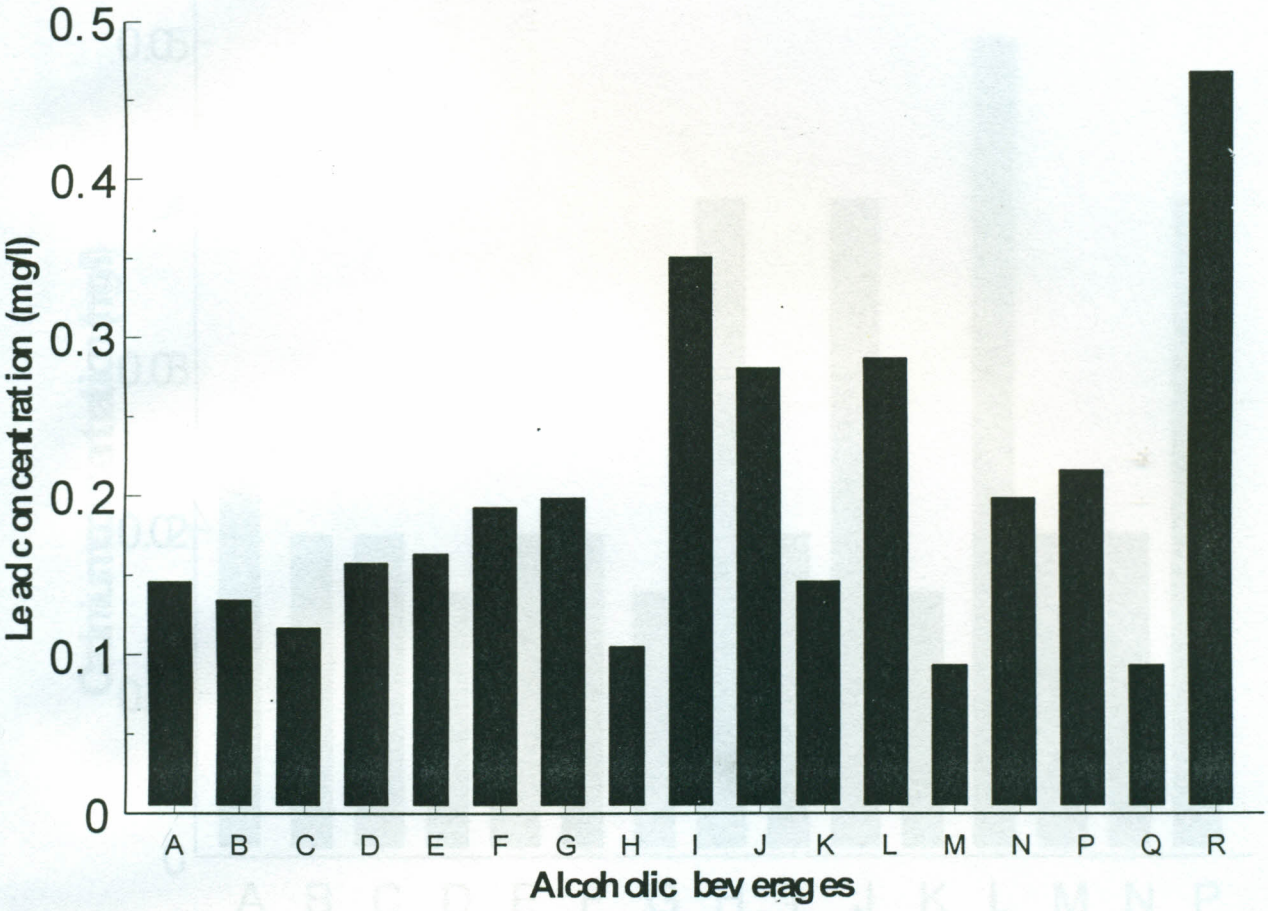
$$t = \frac{|r|\sqrt{(n-2)}}{\sqrt{(1-r^2)}}$$

Where r is the correlation coefficient given by

$$r = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\left\{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]\right\}}$$

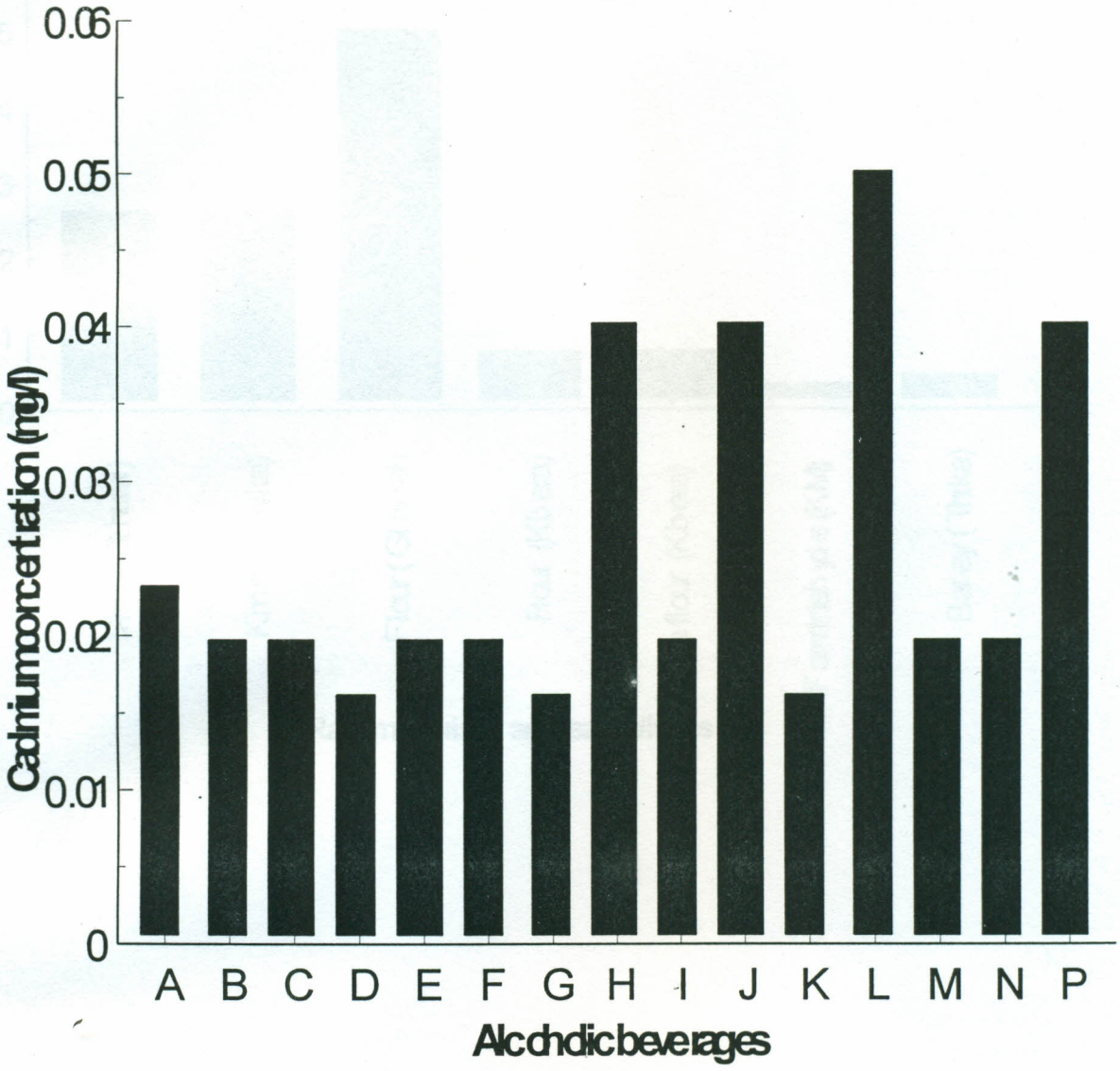
Appendix 4

Figure 18. Alcoholic beverages with lead concentrations above 0.1 mg/l.



KEY: **A**-Mugaca, KM. **B**-Busaa, Kiganjo, Thika. **C**-Kibuku international beer, Zimmermann. **D**-Viena wine, Maragua. **E**-Busaa, Mathare. **F**-Busaa, KM **G**-Filtered busaa, Kibera. **H**-Busaa, Witeithie. **I**-Kangara, Kiandutu, Thika. **J**-Miti ni dawa, Kibera. **K**-Mugaca, Korogocho. **L**-Toivo, Kibera. **M**-Busaa, Kangemi. **N**-Muratina, Ruiru. **P**-Miti ni dawa, Thika. **Q**-Popov vodka, Nairobi. **R**-Kangara, Ruiru.

Figure 19. Alcoholic beverages with cadmium concentrations above 0.005 mg/l.



KEY: **A**-Afriwine, Githunguri. **B**-Busaa, Kiganjo, Thika. **C**-Kibuku international beer. **D**-Viena wine, Maragua. Filtered. **E**-Filtered busaa, Githurai. **F**-Busaa, Kangemi. **G**-Miti ni dawa, Githunguri. **H**-Kangara, Githurai. **I**-Miti ni dawa, Thika.

J-Kangara, Witeithie. **K**-Busaa, Witeithie. **L**-Kangara, Kiandutu, Thika. **M**-Mugaca, Korogocho. **N**-Chang'aa, Makongeni, Thika. **P**-Kangara, Ruiru.

Figure 20a. Raw materials with aluminium concentrations above 0.2 mg/l.

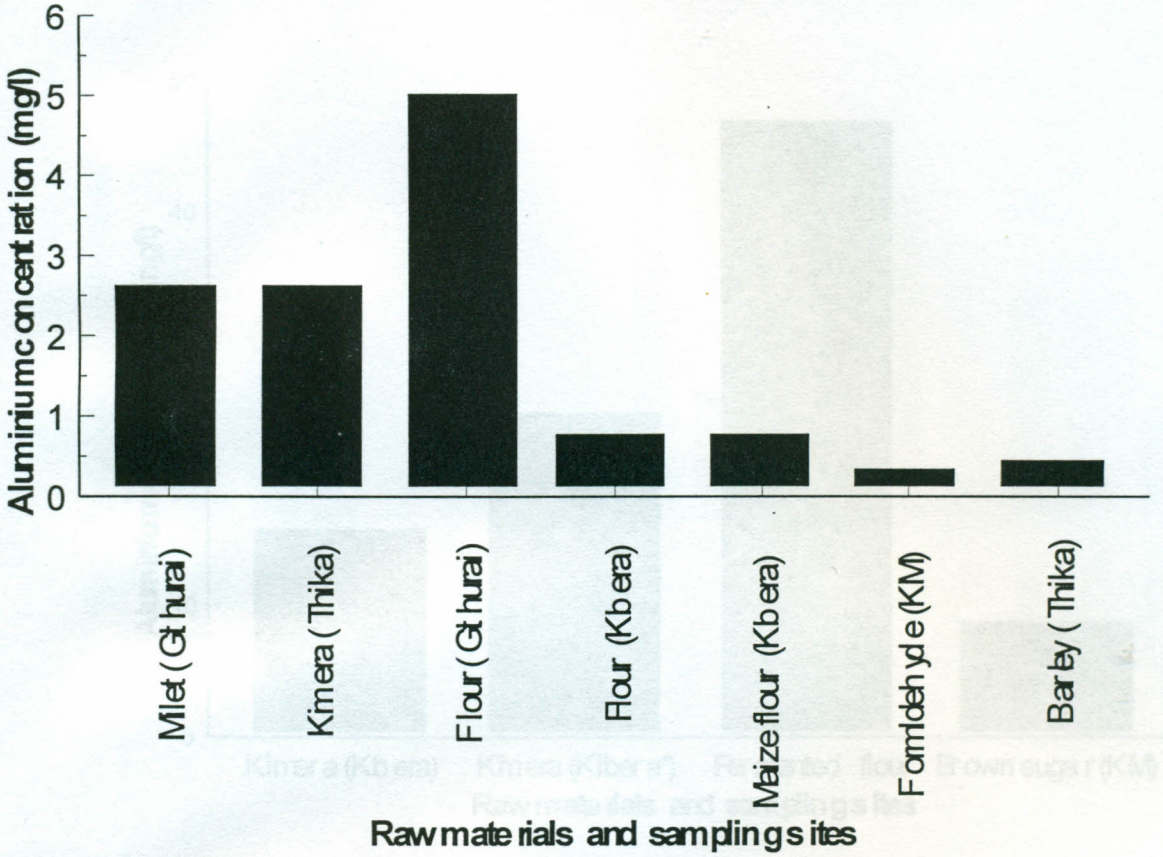


Figure 20b. Raw materials with aluminium concentrations above 0.2 mg/l.

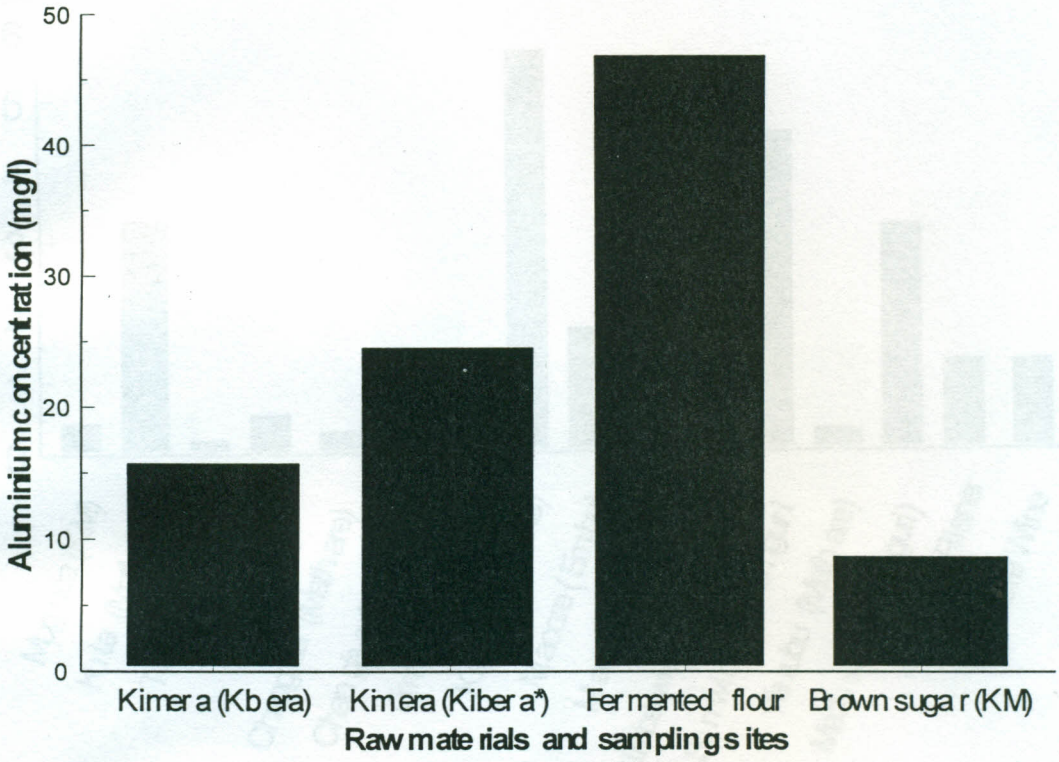


Figure 21a. Alcoholic beverages with aluminium concentration above 0.2 mg/l.

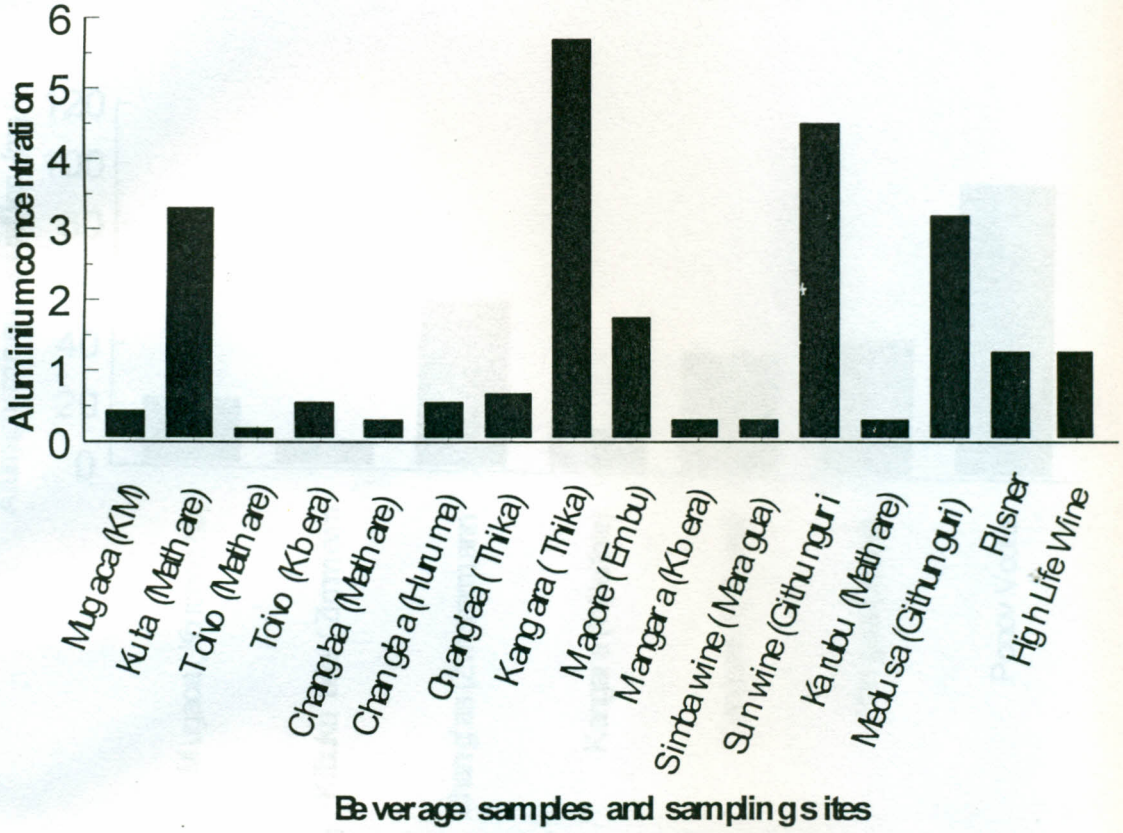


Figure 21b. Alcoholic beverages with aluminium concentration above 0.2 mg/l.

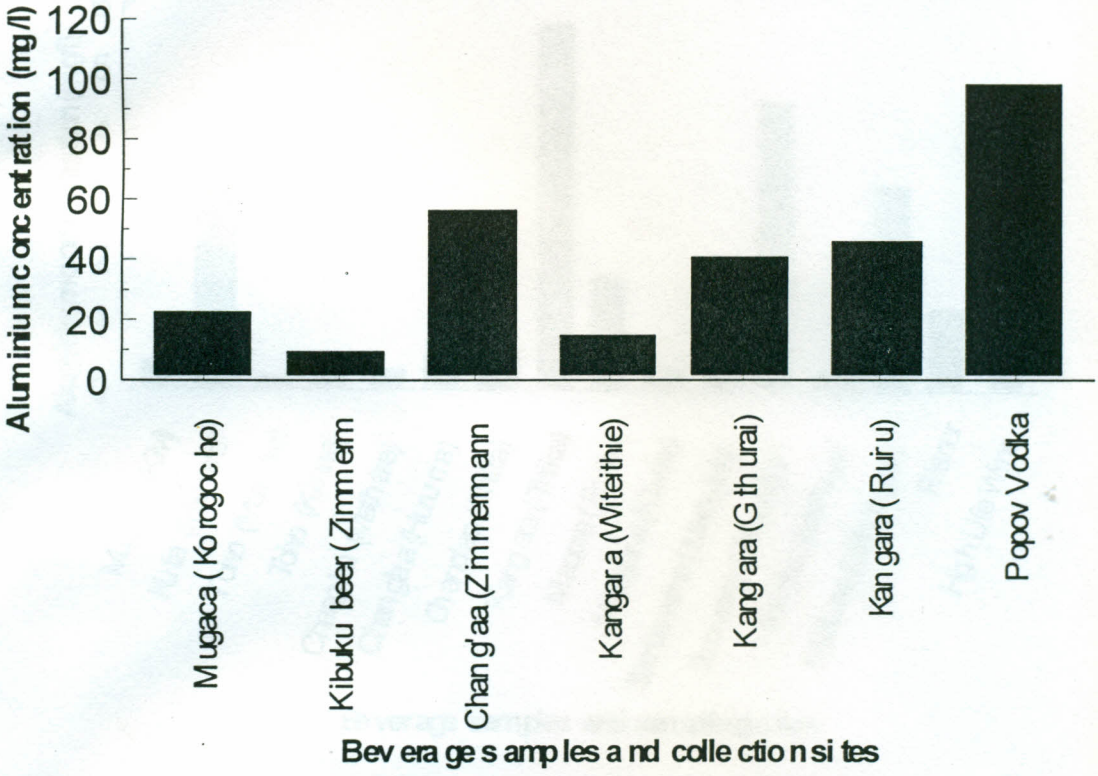


Figure 21c. Alcoholic beverages with aluminium concentration above 0.2 mg/l.