# ASSESSMENT OF COMMUNITY AWARENESS, PHYSICOCHEMICAL PARAMETERS, ORGANIC CONTAMINANTS AND TOXIC METALS OF ALCOHOLIC BEVERAGES IN MUTHITHI, MURANG'A COUNTY, KENYA

By

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## DECLARATION

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

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## DEDICATION

To my dear parents Elena and Charles Githinji, who passed on the passion for education to me. To my dear wife Millicent, who champions for the cause of education in the family. To my children Brian, Keith and Paige who wait on the promises of education.

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This work has taken time and finances. My wife and children stood by me patiently and prayerfully the whole time. For this encouragement I am forever grateful to them.

Ultimately all glory and honour to God for shepherding me this far.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AAS | Atomic Absorption Spectroscopy |
| ABV | Alcohol by Volume |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ADCB | Alcoholic Drinks Control Bill of 2009 |
| ADH | Alcohol Dehydrogenase |
| AIDS | Acquired Immune Deficiency Syndrome |
| ANOVA | Analysis of Variance |
| ARBD | Alcohol Related Birth Defects |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BDL | Below Detection Limit |
| BEIs | Biological Exposure Indices |
| CDC | Centres for Disease Control and Prevention |
| CNS | Central Nervous System |
| DALYs | Disability Adjusted Life Years |
| DNA | Deoxyribonucleic Acid |
| EHC | Environmental Health Criteria |
| FID | Flame Ionization Detector |
| FIDA | International Federation of Women Lawyers |
| GC | Gas Chromatography |
| HED | Heavy Episodic Drinking |
| HIV | Human Immunodeficiency Virus |

| | |
|---|---|
| HPLC | High Performance Liquid Chromatography |
| IARC | International Agency for Research on Cancer |
| ICP-MS | Inductively Coupled Plasma – Mass Spectrometry |
| ILO | International Labour Organisation |
| IPCS | International Programme on Chemical Safety |
| IRIS | Integrated Risk Information System |
| ITII | International Technical Information Institute (Japan) |
| KDHS | Kenya Demographic and Health Survey |
| KEBS | Kenya Bureau of Standards |
| LD | Lethal Dose |
| MAL | Maximum Allowable Limits |
| MLD | Minimum Lethal Dose |
| MRI | Magnetic Resonance Imaging |
| NACADA | National Authority for Campaign against Alcohol and Drug Abuse |
| NACOSTI | National Council for Science, Technology and Innovation |
| NAS | National Academy of Sciences |
| NCPD | National Coordination Agency for Population and Development |
| NIOSH | National Institute for Occupational Safety and Health |
| NTU | Nephelometry Turbidimetric Units |
| PMTDI | Provisional Maximum Tolerable Daily Intake |
| RDA | Recommended Dietary Allowance |
| SCEs | Sister Chromatid Exchanges |
| SPSS | Statistical Package for the Social Sciences |

| | |
|---|---|
| STD | Sexually Transmitted Diseases |
| TDI | Total Daily Intake |
| TLVs | Threshold Limit Values |
| UNEP | United Nations Environmental Programme |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |

## ABSTRACT

Brewing is an ancient industry that dates back 9000 years and to date more than 100 billion liters of beer are consumed annually. In Kenya alcoholic beverages industry is more liberal with the previously referred to as 'illicit alcoholic beverages' being legalized in November 2010, by the passing of Alcohol Law (Mututho Law). Production and consumption of some alcoholic beverages has been on increase partly due to high cost of commercial alcoholic drinks. People have died, others gone blind or suffered a myriad of other health conditions related to consumption of some alcoholic beverages. Unlicensed alcoholic beverages have no standard procedures of preparation and this may lead to contamination by organic compounds and toxic metals including aluminium (Al), iron (Fe), lead (Pb), copper (Cu), cadmium (Cd), manganese (Mn) and zinc (Zn). Despite the wealth of knowledge on levels of organic compounds and toxic metals in commercial wines, beers, spirits and some indigenous alcoholic beverages from urban settings, little has been reported on the beverages in rural areas where some are prepared and where most Kenyans reside. The objective of this study was to assess the types of alcoholic beverages available in Muthithi location of Murang'a County, determine levels of some organic compounds, toxic metals, turbidity and pH. A questionnaire was used to establish the types of alcoholic beverages available in Muthithi and samples of beverages purchased. Determination of ethanol, methanol, propan-1-ol, propan-2-ol, ethanal, methanal, ethyl ethanoate, amyl alcohols and ethanoic acid was done using gas chromatography, GC, while that of Al, Fe, Pb, Cu, Cd, Mn and Zn was done using atomic absorption spectrometry, AAS. Turbidity and pH were done using a turbidimeter and a pH meter, respectively. The alcoholic beverages available in Muthithi were bottled beers, Keg Beer, Muratina, Miti ni dawa, Changaa, various spirits and Busaa. The social demographic characteristics of study participants who consumed alcoholic beverages included married people (77%) of whom men (79%) were more than women. Most people had secondary level of education (58%) and were salaried (46%). The most popular alcoholic beverage was Keg Beer (45%). Most alcohol consumers took 5-10 glasses (53%) of alcoholic beverage in a sitting and drank 7 days per week (25%). Respondents reported no negative effects (67%) of their drinking, while storage containers were reported as aluminium (39%) and plastic (38%). Most respondents presumed that methanol (84%) was the additive that makes alcoholic drinks stronger and that they did not know of anyone (97%) hospitalized or dead from drinking alcohol at the time. The mean levels of organic compounds ranged from the highest 258190±7942 mg/L for ethanol to the lowest below detection limit (BDL) to 14.6 mg/L for methanol. The mean levels of toxic metals ranged as follows from the highest 3.06±0.48 mg/L for Al to BDL (<0.01 mg/L) for Cd and Cu. Levels of all organic compounds were within safe limits but levels of some toxic metals were above maximum allowable limits (MAL). However, excessive consumption of alcoholic beverages was the main problem. The results of this study will be availed to the relevant authorities for the necessary action.

# CHAPTER ONE
# INTRODUCTION

## 1.1 Background

Beer is the world's most popular beverage after tea. Beer brewing is an ancient art dating back to 7000 BC when small scale brewing in China was being carried out (Roach, 2005). Mass production of beer started in the 18$^{th}$ century with the industrial revolution and today over 100 billion liters of beer are being produced annually (Ian, 2007). In Kenya, the East Africa Breweries Limited is one of the leading beer and spirits producers. Preparation and consumption of unlicensed alcoholic beverages in Kenya has been on the increase in the recent past. The high cost of commercial beers, spirits and wines has become prohibitive to majority of Kenyans, thus they seek cheaper alcoholic beverages. Consumption of these beverages has led to some people losing their lives and others losing their sight among other health problems (Daily Nation, 2010). Beer and wine which are produced by fermentation of sugar or starch containing plant materials have a lower alcohol content compared to spirits which are produced by distillation of the fermented products (Rudgley, 1993).

In the process of making beer, starch is converted to sugary liquid called wort. Fermentation effected by yeast converts wort into an alcoholic beverage called beer. The first step of brewing is where wort is prepared by mixing starch mainly derived from malted barley with hot water in a process called mashing which takes between 1-2 hours (Ian, 2007). The starches are converted to sugars and then the sweet wort is drained off the grains. The grains are then washed in a process called sparging that allows the brewer to collect as much fermentable liquid as possible. The sweet wort collected is boiled in a

kettle for about one hour. Boiling kills the remaining enzymes and concentrates wort by evaporating water. Wort is then cooled and hops are added for flavouring and preservation. The brewer's yeast (*Saccharomyces cerevisae* and *Saccharomyces uvarum*) is then added at 15-24˚C to ferment wort to beer in a process that takes one week to several months (Ian, 2007). Once fermentation is complete, fine particulate matter and yeast settle leaving the beer clear. Clarifying agents are added by some brewers to precipitate protein solids found in trace amounts in the finished product. The beer is then packaged in bottles, aluminium cans or Kegs. Beer colour is determined by the proportion of roasted darker malt or other colourants, such as caramel, added (Ian, 2007). The strength of beers range from less than 3% alcohol by volume (ABV) to around 14% and typically brewing yeast cannot withstand alcohol concentrations above 12% (Ian, 2007).

Locally available beers were bottled lagers, Keg Beer, Miti ni dawa, Muratina and Busaa. Miti ni dawa, and Muratina are homemade fermentation products of sugar with some herbs added for Miti ni dawa and slices of Muratina fruit (*Kigelia africana*) for Muratina (Gitu, 2001). In both alcoholic beverages, honey may be added for flavour. Busaa is also home made where crushed grain which is in plenty is fermented, normally maize or sorghum. Changaa and a wide variety of plastic bottled spirits (Kali) were the common spirits. Changaa is a home distilled spirit obtained from Busaa or sugar fermentation products (Gitu, 2001).

Alcoholic beverages are a mixture of alcohols, esters, aldehydes, acids, carbohydrates, proteins, vitamins, polyphenols, minerals and other substances derived from raw materials, chemical reactions, and preparation processes (Ian, 2007). At concentrations above the maximum allowable levels (MAL), these organic and mineral compounds cause health problems (Boggan, 2007). Ethanol in the body is converted into ethanal which is linked to most of the clinical effects of alcohol; increased the risk of developing cirrhosis of the liver, multiple forms of cancer, and alcoholism (Boggan, 2007). Alcoholism is a leading cause of many social problems including unproductivity, broken marriages and contracting HIV (Puja *et al.*, 2011). Methanol and methanal are found in some alcoholic beverages that cause blindness and many deaths (Daily Nation, 2010).

Among the mineral components found in alcoholic beverages are metals. Iron is required in substantial amounts in the human body and is known as a major mineral element. Zinc, manganese and copper are required in small quantities and are usually referred to as trace elements. Lead, cadmium and aluminium are non-essential elements with no known biological function in the body. When consumed, these metals accumulate in certain body organs where they cause biological as well as physiological disorders including organ damage, nervous disorders, cancers and faulty reproduction (Nordberg, 2010).

## 1.2 Problem statement and justification

There has been a public outcry on harms done by some alcoholic beverages: People have died others gone blind or suffered health conditions related to consumption of some

alcoholic beverages (Daily Nation, 2010). Chronology of some reports related to alcohol poisoning from The Daily Nation newspapers as this research was going on include;

- 19.07.2010: in Kiambu, 5 people die while in Nairobi 4 people die after taking the same illicit brew
- 28.07.2010: in Kibera slums Nairobi, 23 die after taking illicit brews
- 10.08.2010: in Laikipia Central District, 7 die and scores lose sight after taking an illicit brew 'Country Walker'
- 12.06.2011: in Banana Kiambu, 10 die after consuming illicit brews
- 15.09: in Nyahururu, 19 die, 4 die in Ruiru and 4 go blind after taking an illicit brew 'Yokosuna'
- 19.09.2011: EABL advertisement: 'Concerned about rise of illicit brews in Kenya'
- 20.09.2011: in Mucatha village, Banana in Kiambu 4 die after taking a toxic brew
- 21.09.2011: at Loitokitok border point, 7500 litres of methanol in 30 drums from Tanzania siezed
- 16.12.2011: in Singrampur, West Bengal State in Eastern India 155 die after consuming methanol laced liquor
- 10.08.2012: in Mbeere South District, 8 die after consuming a killer brew

Muthithi location in Muranga County Kenya is a low income subsistence farming area where many types of cheap alcoholic beverages are consumed. Trends in Muthithi show increasing consumption of some alcoholic beverages, a decreasing population and

increasing dropout rate in secondary schools (NCPD, 2005). If this trend goes on unchecked, the residents of Muthithi may suffer larger disease burden and a weaker manpower for economic growth. This will increase poverty and thus the cycle of seeking cheaper alcoholic beverages.

With the enactment of Alcohol Bill 2009 in 2010 in Kenya, all alcoholic beverages became legal if their components were declared and their preparation and distribution conformed to the laid down standards (ADCB, 2009). Unlicensed alcoholic beverages have no regular ways of preparation and this may lead to contamination by organic compounds and toxic metals. In addition these facts may be unknown to alcohol consumers who therefore get exposed to harmful effects of these alcoholic beverages. There is need therefore to regularly keep monitoring the alcoholic beverages' levels of organic compounds, toxic metals and physicochemical parameters for example the pH and turbidity to ensure safety of these drinks.

### 1.3 Hypothesis

Alcoholic beverages from Muthithi location of Muranga County in Kenya contain organic contaminants and toxic metals which are beyond the maximum allowable levels (MAL) for human consumption.

## 1.4 Objectives of the study

### 1.4.1 General objective

To assess community awareness, organic contaminants, toxic metals and physicochemical parameters of alcoholic beverages consumed in Muthithi location of Murang'a County.

### 1.4.2 Specific objectives

(i) To determine the alcoholic beverages and social demographic characteristics of persons consuming them in Muthithi location.

(ii) To determine the levels of ethanol, methanol, propan-1-ol, methanal, ethanal, ethyl ethanoate and amyl alcohol in selected alcoholic beverages consumed in Muthithi location of Murang'a County.

(iii) To determine the levels of Al, Fe, Pb, Cu, Cd, Mn and Zn in selected alcoholic beverages consumed in Muthithi location of Murang'a County.

(iv) To determine the pH of selected alcoholic beverages consumed in Muthithi location of Murang'a County.

(v) To determine the turbidity of selected alcoholic beverages consumed in Muthithi location of Murang'a County.

## 1.5 Scope and limitations of the study

The study was done only in Muthithi location of Murang'a County. The study only determined the pH, some organic compounds: Ethanol, methanol, propan-1-ol, methanal, ethanal, ethyl ethanoate, amyl alcohols and some toxic metals: Al, Fe, Cd, Pb, Cu, Zn and Mn in selected alcoholic beverages from Muthithi. Other factors like seasonal variations,

water, soils, raw materials, the origin of the alcoholic beverages, age and the health status of the respondents were not considered.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Introduction

Alcoholic beverages, commonly referred to as alcohol, are drinks that contain ethanol and are generally classified as beers, wines and spirits. All alcoholic beverages are derived from fermentation of carbohydrates, that is, starch and sugars. Distillation beverages derived from potatoes, grains, fruits, sugarcane products and mezcal are vodka, whisky, brandy, rum and tequila respectively (Chester, 1993). Liquors are distilled beverages that have been flavored and sweetened. Fortified beverages have alcohol added during preparation (Chester, 1993).

Production and consumption of alcoholic beverages is as old as mankind since the beverages formed an important part of peoples' cultures. The earliest preparation of alcoholic beverages dates back about nine thousand years with mead, a honey beer, likely to have been the first (Sournia, 1990). Later 5000-6000 years ago, with coming of cultivation, wines were prepared on small scale (Burnett, 1999). In the 2$^{nd}$ century BC viniculture and wine-making was widespread in Roman Empire (Burnett, 1999). During the industrial revolution large quantities of stronger, cheaper and a wide variety of alcoholic beverages became available. Drunkenness became more common, more public and associated with poverty (Barrows and Room, 1991).

Traditionally drinking used to take place during special occasions, such as harvesting, new born celebration, marriage or completion of projects where the custom was to drink until the alcoholic drink ran out. Industrially produced alcoholic beverages provide an

inexhaustible supply and this has brought about a pattern of drinking until money runs out. Developing countries like Kenya are experiencing new patterns of heavy drinking without the traditional social controls. Heavy episodic drinking (HED) is a measure of alcohol consumption pattern defined as drinking of at least 60 g of pure ethanol on at least one occasion in the past 7 days (WHO, 2011). Other patterns in this new culture include drinking games, buying rounds and binge drinking which is associated with low per capita consumption but more health problems (Jernigan, 1997).

## 2.2 Local alcoholic beverages

Locally available alcoholic beverages were bottled lagers, Keg Beer, Miti ni dawa, Muratina, Changaa, a wide variety of bottled spirits and Busaa. Lagers and Keg Beer are prepared in large industries that apply the manufacture practices in section 1.1 while the others could be prepared on a smaller scale.

Muratina (or Karubu) is a traditional alcoholic drink which is sweet-tasting, sweet smelling and slowly intoxicating. It is popular among the Kikuyu people of Central Kenya where Muthithi is located. It is prepared by fermenting sugar and honey mixture in water with some slices of Muratina fruit (*Kigelia africana*) added (Gitu, 2001). The split Muratina fruits have their seeds removed and are then boiled in water about six times while pouring the water each time. The fibrous material left behind is dried in sun for a day or two then dropped into the fermenting mixture to supply yeast, the catalyst. The honey added improves on the smell and the taste of the beverage. Fermentation in a well

stoppered vessel takes two to three days in a warm place after which more sugar is added and fermentation continued for two more days. At times sugarcane juice may be used in place of sugar but when commercial yeast obtained from the shops is used in place of Muratina fruit, the alcoholic beverage name changes to Matinga (Gitu, 2001). Miti ni dawa is prepared by fermenting sugar and yeast mixture just like Matinga but with some herbs added. Miti ni dawa is perceived to be medicinal by its consumers (Gitu, 2001).

Busaa, originally a popular traditional brew among Luhya people of Western Kenya, is basically prepared by fermentation of ground cereals mainly maize or sorghum (Odero, 1998). Refined sugar, molasses or jiggery may be added to the fermenting mixture to increase the alcohol content while yeast from some tree barks is added to speed up the fermentation. The stoppered mixture is kept in a warm place (near fireplace or in cow dung) for about a week or two within which the fermentation process is complete. The mixture is then decanted and the brew is ready for consumption or sale (Odero, 1998).

Changaa is a spirit obtained by distillation of fermentation products mostly Busaa (Mwanasi, 1990). The concentration of ethanol and other organic compounds normally higher in spirits including Changaa and therefore dilution is advisable to their consumers. In distillation of Changaa, the steel drum carrying the pre-fermented mixture is placed in the fire place and connected to the second metallic drum in the river with a pipe. On heating the fumes carrying ethanol and the other organic compounds condense into the second drum in the cooler river water. The distillate, ‘Biko’ which means virgin, is then diluted and is now ready for consumption or sale (Mwanasi, 1990).

Production of various forms of alcohol for domestic consumption is widespread and decentralized in many developing countries (WHO, 2001). Locally, this has seen an introduction of second generation alcoholic beverages characterized by high alcoholic content and low pricing (NACADA, 2011). Examples of these second generation alcoholic beverages are the Keg Beer and a large variety of spirits or liquors popularly called 'Kali' which means bitter or strong in Kiswahili. Availability of alcoholic beverages that are cheaper and of higher alcoholic content could have compounded the alcohol problems cited in section 2.1.

## 2.3 Alcohol control policies

Due to adverse effects of alcohol drinking on productivity, reproductive and family life, alcohol control measures based on practical experience and scientific evidence have been put in place. The most effective of these regulations include; increasing price and taxes of alcoholic beverages, restricting production and consumption through minimum drinking age, production or retailing monopolies, restricting the number, types and opening hours of outlets selling alcohol, drink-drive laws and refusing to serve alcohol to intoxicated persons (Edwards *et al*., 1994). Other measures are control of alcohol advertising, alcoholism treatment and rehabilitation, public education by use of warning labels on alcoholic beverages and restricting illicit production and sale of alcohol.

Alcoholism is a major challenge in our society today and is mainly caused by schizoid qualities, depression, alcohol dependency, hostile and self-destructive impulsivity and

sexual immorality (Berkow *et al.*, 1982). Consumption of alcoholic beverages significantly harms physical, psychological and social health of individuals, families and communities. Alcohol can damage nearly all organs in the body and is a risk factor to global health accounting for 3.5% of the disability adjusted life years (DALYs) (Room *et al*., 2001). Alcohol harm to drinker includes alcoholic psychosis, alcohol dependency syndrome, alcoholic polyneuropathy, alcoholic cardiomyopathy, alcoholic gastritis, alcoholic liver cirrhosis, ethanol toxicity and methanol toxicity (English *et al*., 1995). Nondrinkers may suffer fetal alcohol syndrome prenatally while all suffer consequences of alcohol use such as traffic accidents, drowning, burns and suicides (Baker *et al.*, 1992).

Symptoms of acute intoxication are exhilaration, boastfulness, talkativeness, remorse, belligerency, slowed reaction time, slurred speech, ataxia, diplopia, vertigo, flushing of face, rapid pulse, sweating, nausea and vomiting, incontinence of urine and faeces, drowsiness, stupor and finally coma, with impaired or absent tendon reflexes. Convulsive episodes may indicate hypoglycemia, peripheral vascular collapse, hypotension, tachycardia, cold pale skin, hypothermia, slow stertorous respirations. During convalescence post alcoholic headache, gastritis, delirium tremens, infections such as pneumonia and septicemia may occur. Eventually death from respiratory or circulatory failure or from aspiration pneumonitis may result (Gosselin *et al*., 1984). Many alcoholics have chronic diarrhea as a result of malabsorption in the small intestine, the major symptom being frequent loose stools (Hardman *et al*., 2001).

In analysis of patients with ischemic infarction, it was indicated that intellectual impairment may be the earliest sign of alcohol abuse (Reynolds *et al*., 1982). When alcohol is absorbed into the blood from small intestines depression of the central nervous system (CNS) is its principal effect Berkow *et al.* (1982) as shown in table 2.1.

**Table 2.1: Alcohol level effects on CNS**

| **Alcohol level in blood (mg/100 ml)** | **Effect on CNS** |
|---|---|
| 50 | Sedation/tranquility |
| 50 – 150 | Lack of coordination |
| 150 – 200 | Intoxication/delirium |
| 300 – 400 | Unconsciousness |

Source: Berkow *et al.* (1982).

## 2.4 Unit of alcohol

A unit of alcohol is a measure of the volume of pure alcohol in an alcoholic beverage. Units of alcohol are used in some countries as a guideline for alcohol consumption. One unit of alcohol is defined as 10 mL or as 10 g of pure ethanol contained in the so called a standard drink. In one hour, an average healthy adult can metabolize 75% to 95% of a unit of alcohol. The number of units contained in a typical serving of an alcoholic beverage is publicized and printed on bottles in some countries.

Units of alcohol in a drink can be determined by multiplying the volume of the drink (in litres) by their percentage alcohol by volume (ABV) shown in Eq. 2.1.

1 Unit = ABV (%) * Volume (L) .……..……Eq. 2.1

Units of alcohol contained in a pint of beer of volume 568 mL at 4% ABV are calculated as: 4 * (568/1000) = 2.272 units. Others are as follow:

i. A standard wine bottle of volume 750 mL at 12% ABV contains 9 units.

ii. A half litre of lager at 5% ABV contains 2.5 units.

iii. A medium glass of wine (175 mL) of 12% ABV wine contains about 2 units of alcohol.

iv. A small glass (50 mL) of sherry, fortified wine or cream liquor (≈20% ABV) contains about one unit.

v. A single pub measure (25 mL) of most spirits at 40% ABV contains one unit of alcohol.

vi. A regular bottle (275 mL) of alcopops like Smirnoff Ice at 5.45% ABV contains 1.5 units of alcohol.

Some medical benefits derived from use of limited wine are longevity and reduced incidences of heart disease (Copper *et al.*, 2004). Resveratrol ($C_{14}H_{12}O_3$) found in wine could be responsible for the said protection (Lindsay *et al*., 2002), while increases in high density lipoprotein cholesterol after ethanol ingestion may explain the lower risk of myocardial infarction and death from coronary disease after moderate drinking (Mezey, 1985).

The United Kingdom government had advised that regular consumption of 3–4 units a day for men, or 2–3 units a day for women, would not pose significant health risks, however regular intake of 3 or more alcoholic drinks daily by men and women of different races was associated with raised blood pressure, cancer of the mouth, pharynx,

and esophagus, and primary cancer of the liver (Reynolds and Prasad, 1982; Kabat and Wynder, 1989).

## 2.5 Community awareness of toxic effects of consumption of alcoholic beverages

Alcohol consumption is the world's third largest risk factor for disease and disability; in the developing world it is the greatest risk (WHO, 2011). Alcohol is the causal factor in 60 types of diseases and injury and a component cause in 200 other diseases. Almost 4% of all deaths worldwide are attributed to alcohol, greater than deaths caused by HIV/AIDS, violence or tuberculosis (WHO, 2011). Alcohol is associated with many serious social issues including violence, child neglect and abuse and absenteeism from work place. In 2005, of an average of 6.3 liters of pure alcohol per person consumed globally, 28.6% was homemade, illegally produced or sold outside government controls (WHO, 2011). Heavy episodic (binge) drinking leads to serious health problems especially injuries – this is highest in Mexico and sub–Saharan African states including Kenya (WHO, 2011).

A survey on morbidity, health and social problems from alcohol use in Kenya revealed that most motor accidents in Eldoret involved intoxicated drivers (Odero, 1998). Findings from another research carried out in Nairobi showed that women whose partners were intolerant alcohol drinkers had significantly higher incidences of reporting domestic violence (FIDA, 2002). In November 2000 it was reported that consumption of illegal 'Kumi kumi' brew in Mukuru slums in Nairobi killed 140 people, many others went blind while hundreds were hospitalized (Mureithi, 2002).

## 2.6 Chemical composition of alcoholic beverages

The major organic product of fermentation is ethanol. Other organic products including methanol, propan-1-ol, ethanal, methanal, ethyl ethanoate and amyl alcohols may form during fermentation. The threshold limit values for some organic compounds are given in table 2.2.

**Table 2.2: Threshold limit values (ACGIH-TLVs) for some organic compounds in blood**

| Compound | Amount (mg/L) |
|---|---|
| Ethanol | 1000 |
| Methanol | 200 |
| Propan-1-ol | 200 |
| Ethyl ethanoate | 400 |
| Ethanal | 100 |
| Butan-1-ol | 50 |
| Amyl alcohol | 50 |

Source: Muir (1977)

The organic compounds considered in this study are discussed in the following subsections.

### 2.6.1 Ethanol

Ethanol is a volatile, flammable, colourless liquid. It oxidizes to ethanal then to ethanoic acid. In the human body, these oxidation reactions are catalyzed by the enzyme liver alcohol dehydrogenase (ADH). Ethanal is linked to most of the clinical effects of alcohol; increased the risk of developing cirrhosis of the liver, multiple forms of cancer, and

alcoholism (Boggan, 2007). Ethanol content in alcoholic beverages ranges from 2-6% in beers, 10-20% in wines and 40-50% in spirits (Mara *et al.,* 1993). Bingham *et al.* (2001) reported that ethanol levels of > 400 mg/dL caused coma, respiratory depression, hypotension, hypothermia and death.

The clinical features of ethanol intoxication in a non-tolerant individual were related to blood alcohol levels: at 50 to 150 mg/dL (0.05 to 0.15%), there is mild intoxication including slight impairment of visual acuity, muscular incoordination, slowed reaction time and behavioral changes in mood and personality. At 150 to 300 mg/dL (0.15 to 0.30%) moderate intoxication occurs resulting in visual impairment, sensory loss, muscular incoordination, slowed reaction time and slurred speech. At 300 to 500 mg/dL (0.30 to 0.50%), there is severe intoxication characterized by marked muscular incoordination, blurred or double vision, sometimes stupor and hypothermia, vomiting and nausea, and occasional hypoglycemia and convulsions. Above 400 mg/dL (0.40%), there is coma, respiratory depression, hypotension and hypothermia, and death from respiratory or circulatory failure or as a result of aspiration of stomach contents (Bingham *et al*., 2001).

Ethanol and ethanal inhibit enzymes involved in gonadal testosterone synthesis. Levels of estrogenic steroids increase as a result of clearance of androgens. In human males acute exposure to alcohol primarily affects testicular synthesis and secretion of testosterone. However, women are not sensitive to the direct gonadal effects of alcohol

and are therefore less vulnerable to antifertility effects with chronic alcohol abuse (NRC, 1986).

The relative risk of breast cancer with alcohol consumption was much higher, independent of the levels of alcohol consumed compared to the relative risk in those who do not consume alcohol (Meara *et al*., 1989). An estimated 81% of the esophageal cancers are directly attributed to alcohol use. In a study involving interviews with the next of kin or close friends of black males who had died of esophageal cancer in Washington DC, the major factor responsible for the cancers was reported to be excess alcohol consumption (Pottern *et al*., 1981).

Fetal alcohol syndrome is the collection of characteristic malformations found in the infants and children of mothers who drank alcohol during pregnancy. Cases of eye abnormalities, horizontal shortness of the palpebral fissure, ptosis and strabismus, myopia, amblyopia, pale optic discs, facial anomalies, subnormal weight, delayed growth, and mental retardation have been reported (Grant, 1986; Hardman *et al*., 2001). In another study on effects of alcohol consumption on pregnancy, miscarriages, stillbirths, and neonatal deaths were reported. Women who miscarried drank significantly higher volumes of beer than those with live births or stillbirths (Walpole *et al*., 1990).

Chronic ethanol users develop alcoholic ketoacidosis after a few days of "binge" drinking. They vomit; become acutely starved, nauseated, feel abdominal pains from gastritis, hepatitis or pancreatitis. Sepsis, meningitis, pyelonephritis, or pneumonia may

be present, and delirium tremens may develop (Geokas, 1984; Goldfrank, 2002). Acute alcohol ingestion can lead to alterations of mechanical function or electrophysiological properties of the heart leading to progressive cardiac dysfunction and congestive cardiomyopathy (Segel *et al*., 1984).

Ethanol intoxication causes metabolic acidosis and respiratory depression. Mortality from alcohol poisoning studied by age, sex, marital status and occupation in Finland indicated that most fatal alcohol poisonings were due to ethanol. The males predominated and the mortality was highest among persons aged 45-54 years (Poikolainen and Vuori, 1985). The mean ethanol content of beer in the U.S. was reported to range from 4.33% (by volume) in 1995 to 4.66% in 2000 (Kerr and Greenfield, 2003). In a study of some Ethiopian traditional alcoholic beverages reported levels of ethanol, expressed as percentage alcohol by volume (ABV), as 3.84-39.9% (Tadele *et al.*, 2013). In another study Bahera *et al.* (2006) reported ethanol levels of 6.70-96.61% v/v in country made liquors in India.

### 2.6.2 Methanol

Methanol is volatile, colourless, flammable liquid a distinctive odor that is very similar to ethanol (NIOSH, 2005). Methanol has a high toxicity in humans (WHO, 2005). If ingested, 10 mL can cause permanent blindness while 30 mL is potentially fatal. Methanol is metabolized to methanal by alcohol dehydrogenase in the liver (WHO, 1997). The initial symptoms of methanol intoxication include central nervous system depression, headache, dizziness, nausea, lack of coordination, confusion, and with

sufficiently large doses, unconsciousness and death (WHO, 1997). A study by WHO (2005) reported that oral administration of 1 g methanol/kg to mice increased the incidence of chromosomal aberrations.

The symptoms and signs of methanol poisoning appear after an asymptomatic period. They include visual disturbances ranging from mild photophobia, blurred vision, reduced visual acuity to complete blindness, nausea, abdominal and muscle pain, dizziness, weakness and disturbances of consciousness ranging from coma to chronic seizures and in extreme cases death results. The principal clinical feature is severe metabolic acidosis of the anion-gap type attributed to the formic acid produced when methanol is metabolized (EHC-196, 1997). Methanol has a latency usually of 12-18 hours, during which time the clinical signs are headache, anorexia, weakness, fatigue, leg cramps, vertigo, restlessness nausea, vomiting and diarrhea, violent abdominal pain, back pain, leg pain, delirium then coma. Breathing is rapid and shallow, mild tachycardia is common and death in coma is due to respiratory failure but rarely due to circulatory collapse. Blindness caused by methanol poisoning is usually permanent (Gosselin *et al*., 1984).

In a report by Bingham *et al*. (2001) teacher aides who worked at or near spirit duplicators that used a 99% methanol duplicator fluid whose exposures ranged from 1 hr/day for 1 day/wk to 8 hr/day for 5 days/wk for 3 years experienced headaches, dizziness and eye irritation, blurred vision and nausea while working near the machines.

Nykanen and Soumalainen (1983) reported levels of methanol in spirits ranging from 80-10335 mg/L.

### 2.6.3 Propan-1-ol

It is a volatile, colourless, flammable liquid at room conditions. It has an alcohol-like sweet pleasant odor (Tephyl, 1991). It is completely miscible in water and in most organic solvents. Propanol is not a human carcinogen (ACGIH, 2008). Oral $LD_{50}$ of propanol for rat is 1.87 g/kg (TMI, 1983).

Exposure of propan-1-ol occurs through ingestion of contaminated drinks or foods, skin contact or inhalation. Continuous exposure can lead to loss of sensitivity (Hellman and Small, 1974). Propan-1-ol is metabolized to propanoic acid by alcohol dehydrogenase (ADH) (Wiese *et al.,* 2000). After ingestion propan-1-ol causes headache, drowsiness, abdominal cramps, gastro-intestinal pain, ataxia, nausea and diarrhea. Eye contact produces irritation, while repeated skin contact causes dermatitis (IPCS, 1995). Isopropyl and n-propyl alcohols are about twice as toxic as ethanol (Gosselin *et al*., 1984). The fatal propanol dose by ingestion is 250 ml (Clayton and Clayton, 1982).

Main effect of acute isopropyl or n-propyl alcohol poisoning is CNS depression. Symptoms of propanol inhalation, ingestion, or skin absorption include dizziness, incoordination, headache, confusion, stupor, ataxia, coma, gastroenteritis with vomiting, hematemesis, diarrhoea, hypotension, nausea, vomiting, abdominal pain, areflexia, depressed respirations, diuresis, circulatory collapse and death by respiratory arrest.

Prolonged contact with the skin can cause corrosion (Gosselin *et al*., 1984; Sittig, 1985; Dreisbach, 1987). Exposure to 400 ppm propanol for 3 to 5 minutes produced mild irritation of eyes, nose, and throat (Clayton and Clayton, 1982). High concentrations of propanol can cause CNS depression and are irritating to the eyes, throat and mucous membranes. Contact to the skin of the liquid can cause mild chemical burns (Sullivan and Krieger, 1992).

Alcoholic beverages almost always contain n-propanol as a product of fermentation. Beer contains up to 195 mg/L, wine up to 116 mg/L, various types of spirits up to 3520 mg/L, and neat ethanol up to 2910 mg/L (WHO, 1990). Propan-1-ol is metabolized by the enzyme alcohol dehydrogenase, first to propanoic acid and then to carbon dioxide, water and small amounts of lactic acid (Snyder, 1992). Nykanen and Soumalainen (1983) reported levels of propan-1-ol in alcoholic beverages ranging from 4-225 mg/L.

### 2.6.4 Ethanal

Ethanal occurs widely in nature and can be produced by oxidation of ethanol, reaction being catalyzed by the enzyme liver alcohol dehydrogenase (ADH). Ethanal is a metabolic intermediate in humans that has been identified in food, beverages and cigarette smoke. The main source of exposure to ethanal in the general population is through metabolism of ethanol. Ethanal has been implicated as the toxic metabolite in alcohol associated liver damage, facial flushing and developmental effects. Ethanal increases addiction particularly in adolescents and is believed to be a cause of hangovers (Gosselin *et al*., 1984).

Ethanal vapor irritation of the human eye is detectable at 50 ppm in air and becomes excessive for chronic industrial exposure above 200 ppm. Higher concentrations and extended exposure may injure the corneal epithelium, causing persistent lacrimation, photophobia and foreign body sensation (Grant, 1986). Clinical effects of exposure to ethanal vapors include erythema, coughing, pulmonary edema, and narcosis. At high concentrations, paralysis leading to death can occur (Gosselin *et al*., 1984; ACGIH, 2007). Large doses may cause death by respiratory paralysis. Symptoms of chronic intoxication resemble those of chronic alcoholism (Budavari, 1989). Repeated exposure to ethanal vapours causes dermatitis and conjunctivitis (Dreisbach, 1987).

Ethanal is a probable human carcinogen since incidences of nasal tumors in male and female rats and laryngeal tumors in male and female hamsters increase after inhalation exposure (IRIS, 2000; Nicholas *et al.,* 2004). Most people are exposed to ethanal through consumption of alcoholic beverages (Wiese *et al.,* 2000). In the brain, oxidation of ethanol to ethanal is by enzyme catalase (Hipolito *et al*., 2007). People who have a genetic deficiency for ethanal dehydrogenase may have a greater risk of Alzheimer's disease (Nakamura *et al.,* 2007). Ethanal derived from the consumption of ethanol binds to proteins to form adducts that are linked to organ disease (Nakamura *et al.,* 2007). Ethanal-DNA adducts have been observed in granulocytes and lymphocytes of human alcohol abusers (IARC, 1999).

Chronic alcohol consumption is a major risk factor for upper aero-digestive tract cancers, including cancer of the esophagus since ethanal plays a role in TP53 mutations in esophageal cancers (Paget *et al*., 2012). Hepatocytes from livers of humans with alcoholic hepatitis were more susceptible to cytotoxicity of ethanal than hepatocytes from normal liver of humans that were non-symptomatic or with viral hepatitis, alcoholic fatty liver, or stable alcoholic cirrhosis (Kakuma *et al*., 1981). A study of 818 heavy drinkers reported that those who are exposed to more ethanal than normal through a defect in the gene for ethanal dehydrogenase are at greater risk of developing cancers of the upper gastrointestinal tract and liver (Nils *et al.,* 2002).

Ethanal is genotoxic *in vitro*, inducing gene mutations, clastogenic effects and sister chromatid exchanges (SCEs) in mammalian cells (WHO, 1995). Ethanal plays a major role in the cause of alcohol-related birth defects (ARBD). Exposure of pregnant rats and mice to ethanal induced fetal malformations (Hard *et al*., 2001). Chromosomal aberrations and sister chromatid exchange occurred when human lymphocytes were exposed to ethanal concentrations of 0.02 mg/mL and 0.04 mg/mL. This indicated that ethanal is mutagenic (Badr and Hussain, 1983; Norppa *et al.*, 1985; Lambert, 1990). Ethanal induces DNA cross-links in human cells (Lambert *et al*., 1985). The capacity of human lymphocytes to metabolize ethanal is very low if present at all since they are not able to detoxify ethanal and its potent sister chromatids exchange inducing effect (Lambert and He, 1988). Nykanen and Soumalainen (1983) reported levels of ethanal in alcoholic beverages ranging from 0.9-100 mg/L.

### 2.6.5 Methanal

Methanal is the simplest aldehyde which is a colorless gas with a characteristic pungent odour. Methanal can be toxic, allergenic, and carcinogenic (IARC, 2006). At concentrations above 0.1 ppm in air methanal can irritate the eyes and mucous membranes, causes headaches, a burning sensation in the throat, and difficulty breathing, as well as triggering or aggravating asthma symptoms. Ingestion of methanal has been shown to cause vomiting, abdominal pain, dizziness, and in extreme cases can cause death; (Broder *et al.*, 1991). Methanal has been implicated in spontaneous abortion and abnormal menstrual cycles in women (Dales *et al.*, 2008). Methanal is a suspected human carcinogen (IRIS, 2000; USEPA, 2006; IARC, 2006; ACGIH, 2010). Approximate Minimum Lethal Dose (MLD) of methanal for a 70 kg man is 30 mL (Arena, 1979). Lowest lethal dose for human taking methanal orally was recorded to be 36 mg/kg (Bingham *et al.*, 2001).

Acute effects of airborne methanal exposure are odour detection, 0.05-1.0 ppm; eye irritation, 0.01-2 ppm; upper respiratory tract irritation, 0.10-11 ppm; Lower airway irritation, 5-30 ppm; Pulmonary edema, inflammation, pneumonia, 50-100 ppm; Death >100 ppm (Zenz *et al*., 1994). Local symptoms of methanal poisoning include conjunctivitis, corneal burns, brownish discoloration of skin, dermatitis, urticaria (hives) and pustulovesicular eruption. Inhalation symptoms are rhinitis and anosmia (loss of sense of smell), pharyngitis, laryngospasm, tracheitis and bronchitis, pulmonary edema, cough, constriction in chest, dypsnea (difficult breathing), headache, weakness, palpitation (rapid heartbeat) and gastro enteritis (inflammation of the stomach and

intestines). Ingestion of methanal causes burning in mouth and esophagus, nausea and vomiting, abdominal pain, diarrhea, vertigo (dizziness), unconsciousness, jaundice, albuminuria, hematuria, anuria, acidosis and convulsions (ITII, 1998). Signs and symptoms of acute methanal ingestion are severe abdominal pain accompanied by vomiting and diarrhea, altered mental status and coma. Examination may demonstrate epigastric tenderness, hematemesis, cyanosis, hypotension, tachypnea, hypotension, decreased myocardial contractility, shock and cardiovascular instability. Early endoscopic findings include ulceration, necrosis, perforation and hemorrhage of the stomach (Goldfrank, 2002).

Alteration of tissue proteins by methanal causes local toxicity and promotes allergic reactions and dermatitis (Gilman *et al.*, 1980). In a survey of 57 embalmers who were exposed to atmospheric concentrations below 2 ppm, high incidences of symptoms of irritant effects on the eyes, nose and throat were reported. Other respiratory effects included cough, chest tightness, wheezing and shortness of breath (Plunkett and Barbela, 1977). An investigation of reproductive function in female workers exposed to methanal in the garment industry revealed increased incidence of menstrual disorders, inflammatory disease of the reproductive tract, sterility, anemia, and low birth weights among their offspring (Zenz *et al*., 1994). Jendral *et al.* (2011) reported average levels of methanal of up to 0.27 mg/L in 26% of the samples of Russian alcoholic beverages.

### 2.6.6 Ethyl ethanoate

Ethyl ethanoate is a colorless liquid with a characteristic sweet smell manufactured on a large scale for use as a solvent. Ethyl ethanoate is the most common ester in wine and when in excessive amounts is considered a wine fault **(**Robinson, 2006). Levels of ethyl ethanoate reported in USA lager beer were 25 to 50 ppm (Reed, 1983). Ethyl ethanoate forms from the reaction of acetyl coenzyme-A with ethanol at 20-25˚C (Kirk-Othmer, 1984). The oral $LD_{50}$ for rabbit is 4.94 g/kg (Clayton and Clayton, 1994) while its $LD_{50}$ for rat is 11.3 g/kg, indicating low toxicity (Wilhelm *et al.*, 2005).

A study of 30 workers exposed chronically to 15-50 mg of ethyl ethanoate in addition to 20 to 80 mg of amyl acetate/l of air showed no abnormalities in cornea. Prolonged inhalation is damaging to lung, liver, kidney and the heart (Grant, 1986). The odour of ethyl ethanoate was reported to be objectionably strong at 200 ppm, and produced mild eye, nose, and throat irritation at 400 ppm (ACGIH, 1986). Ethyl ethanoate tested at 10% in petrolatum produced no irritation after a 48-hour closed patch test in 25 human subjects (Opdyke, 1979).

Symptoms of acute intoxication include early emotional liability, sensory disturbances, flushing of face, rapid pulse, sweating, Nausea, eventual incontinence of urine and feces, drowsiness, stupor, coma, shock, hypotension, tachycardia, cold pale skin, hypothermia, slow respiration finally death from respiratory or circulatory failure (Gosselin *et al.*, 1984). Ethyl ethanoate inhalation is moderately toxic, by intraperitoneal and subcutaneous routes is mildly toxic, and by ingestion is highly toxic. Chronic poisoning

produces anemia, leucocytosis and fatty degeneration of the viscera (Lewis, 1996). Pradyot (1992) reported exposures to concentrations of 400-500 ppm of ethyl ethanoate in air produced eye, throat and nose irritation, weakness, drowsiness and unconsciousness.

Ethyl ethanoate is one of the least toxic of the volatile organic solvents (ACGIH, 1991). Ethyl ethanoate vapor is irritating to the eyes and respiratory passages of man at concentrations above 400 ppm. This substance is a defatting agent, and prolonged exposure may cause irritation of the skin (Mackison *et al.*, 1981). Over exposure to ethyl ethanoate may cause irritation of the eyes, nose, and throat. Severe over exposure may cause weakness, drowsiness and unconsciousness (Mackison *et al.*, 1981).

Humans exposed to a concentration of 400 ppm in 1.4 mg/L ethyl ethanoate for a short time were affected by nose and throat irritation (Clayton and Clayton, 1994). Ethyl ethanoate is an irritant of the conjunctiva and mucous membrane of the respiratory tract. At very high concentrations, the ester is a CNS depressant and has lethal effects. At concentrations of 20000 to 43000 ppm, there may be pulmonary edema with hemorrhages, symptoms of CNS depression, secondary anemia and damage of the liver (ILO, 1983). Nykanen and Soumalainen (1983) reported levels of ethyl ethanoate in alcoholic beverages of up to 30 mg/L in beers, 257 mg/L in wines and 1010 mg/L in Irish whiskey.

### 2.6.7 Amyl alcohols

An amyl alcohol is any of 8 alcohols with the formula $C_5H_{11}OH$. Amyl alcohols are used as solvents and in esterification. The most important is 3-methyl-1-butanol being the chief constituent of fermentation. Clayton and Clayton (1982) indicated that inhalation of amyl alcohol vapors by man caused marked irritation of the eyes and respiratory tract, headache, vertigo, dyspnea, cough, vomiting, diarrhea, double vision, deafness, delirium, and occasionally fatal poisoning.

Amyl alcohol is a CNS depressant that irritates the mucous membranes of the eyes, nose, and throat (ILO, 1971). Amyl alcohols are reported to be about four times as toxic as ethyl alcohol (Sax, 1975). All amyl alcohols are irritating to the eyes and can cause corneal opacity. High concentrations cause central nervous system effects and death by respiratory failure (Clayton and Clayton, 1982). All isomers of amyl alcohol are CNS depressants and in high dosage lethal to animals (Browning, 1965). A brewery manager reported to have inhaled the amyl and isoamyl alcohol isomers from fermentation vats exhibited psychic stimulation, insomnia, and chromatopsia while a lacquerer exposed to amyl alcohol and amyl acetate had digestive symptoms and secondary anemia (Clayton and Clayton, 1982). Nykanen and Soumalainen (1983) reported levels of amyl alcohols in alcoholic beverages of 3-72 mg/L.

### 2.6.8 Ethanoic acid

Ethanoic acid is a colourless liquid systematically called ethanoic acid. It is the main component of vinegar formed by oxidation of ethanol by ethanoic acid bacteria. Vinegar

which is 4-18% by mass of ethanoic acid is used as condiment and in pickling of foods and vegetables. Exposure to 60 ppm of ethanoic acid was reported to cause conjunctivitis, bronchitis, pharyngitis, and erosion of exposed teeth (ACGIH, 2001). At higher concentrations, glacial ethanoic acid has resulted in perforation of the esophagus and has caused permanent corneal opacification (Mackison *et al.*, 1982). Nykanen and Soumalainen (1983) reported levels of ethanoic acid in alcoholic beverages up to 11.7 mg/L in spirits, 155 mg/L in ales and 300 mg/L in wines.

## 2.7 Toxic metals

Presence of toxic metals in alcoholic beverages is due to natural sources; the atmospheric deposition of airborne particulate matter on raw materials, from soil through root absorption and contamination during preparation and handling processes (Pyrzynska, 2007). Some of the metal containers may leach metals into the acidic drinks (Laureys and Perinet, 1983). The WHO recommendation for maximum allowable concentrations of various metals in drinking water is shown in table 2.3.

**Table 2.3: Maximum allowed limits of toxic metals (mg/L) in drinking water**

| **Metal** | **Al** | **Cd** | **Cu** | **Fe** | **Mn** | **Pb** | **Zn** |
|---|---|---|---|---|---|---|---|
| **Limit** | 0.2 | 0.005 | 2.0 | 0.3 | 0.1 | 0.1 | 50 |

Source: WHO, 1993; KEBS, 1998

Levels of some toxic metals in alcoholic beverages have been reported by Mwanasi, 1990, Mosha *et al*., 1996, Gitu, 2001 and Woldemariam and Chandravansi, 2011 as shown in tables 2.4-2.7.

**Table 2.4: Total elemental concentrations (mg/L) in some brews**

| Alc. Beverage | Cu | Fe | Pb | Cd | Al |
|---|---|---|---|---|---|
| **Nyuki** | 0.985 | 24.71 | 0.280 | 0.090 | 29.355 |
| **Mnazi** | 0.785 | 4.480 | 0.100 | 0.080 | 1.715 |
| **Chibuku** | 1.16 | 48.26 | 0.225 | 0.090 | 6.225 |
| **Busaa** | 0.745 | 140.7 | 0.215 | 0.085 | 133.590 |
| **Tusker** | 0.13 | 0.740 | 0.290 | 0.075 | 2.165 |

Source: Mwanasi, 1990

**Table 2.5: Heavy metal concentrations (mg/L) in some Tanzania traditional brews**

| Alc. Beverage | Cd | Pb | Cu | Al |
|---|---|---|---|---|
| **Dengelua** | BDL | 0.11 | 0.056 | 0.53 |
| **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** | 0.09 | <0.03 | 0.55 | 3.82 |
| **Mnazi** | 0.11 | 0.15 | 0.46 | 1.43 |
| **Gongo** | 0.04 | <0.0003 | 0.1-31.2 | <0.03 |
| **Safari** | 0.05 | 0.11 | 0.04 | 2.50 |
| **Konyagi** | 0.18 | 0.3 | 0.03 | BDL |

BDL = Below detection limit

Source: Mosha *et al.*, 1996

**Table 2.6: Concentration of metals (mg/L) in illicit alcoholic beverages**

| Alc. Beverage | Al | Cd | Cu | Pb |
|---|---|---|---|---|
| **Viena special** | BDL | BDL | 0.290 | 0.056 |
| **Macore** | 1.803 | BDL | 0.153 | 1.803 |
| **Mangara** | 0.273 | BDL | BDL | 0.273 |
| **Karubu** | 0.093 | BDL | 0.130 | 0.006 |
| **Kibuku** | BDL | BDL | 0.240 | 0.043 |

BDL = Below detection limit

Source: Gitu, 2001

**Table 2.7: Concentrations (mg/L) of metals in Ethiopian wines**

| Metal | Axumite | Gouder | Awash | Kemila |
|---|---|---|---|---|
| **Cd** | BDL | BDL | BDL | BDL |
| **Cu** | 1.5 | 0.55 | 0.61 | 0.5 |
| **Fe** | 3.16 | 1.49 | 1.42 | 2.33 |
| **Mn** | 1.46 | 1.56 | 1.88 | 1.04 |
| **Pb** | 0.25 | 0.16 | 0.31 | 0.14 |
| **Zn** | 2.14 | 2.70 | 2.40 | 1.82 |

BDL = Below detection limit

Source: Woldemariam and Chandravansi, 2011

The metals considered in this study are discussed in the following subsections.

### 2.7.1 Lead

Lead ores make up 0.002% of the earth's crust. These are galena (lead sulphide), anglesite (lead sulphate), cerussite (lead carbonate), mimerite (lead chloroarsenate) and pyromorphite (lead chlorophosphate).

Lead metal has been used for thousands of years and has thus been accumulating in the environment. Lead is used in making lead-acid batteries, solder and alloys. Major sources of lead exposure are; tetraethyl lead is antiknock in petrol, in lead-based paints, ceramic glazes, herbal medicine, lead based industry, drinking water systems with lead solder and lead pipes, lead in e-waste, lead solder in food cans and contaminated soils (WHO, 2003). Amount of lead leached from the plumbing system depends on pH, water hardness and standing time of water with soft, acidic and longest standing water being most plumbosolvent. Lead concentration in drinking water is usually below 5 µg/L, however higher concentrations of 100 µg/L have been reported (WHO, 2009).

Lead is a cumulative poison and causes both chronic and acute intoxication (Demayo, 1992). Lead poisoning accounts for 0.6% of the global burden of disease (WHO, 2009). Long-term exposure to lead causes nephropathy, colic-like abdominal pains, damages the brain and kidneys and causes death. High levels of exposure to lead may cause miscarriage and reduce fertility in males (Golub, 2005). Needleman *et al.* (1990) reported that blood levels ≤5 µg/dL of lead causes injury to the developing human brain. The immune system (Lutz *et al.*, 1999) and reproductive system (Iavicoli *et al.*, 2006) are adversely affected at lower than 10 µg/dL levels of exposure to lead. Due to these health concerns lead water pipes are no longer in use while leaded petrol was phased out by 2010 except in 9 countries (UNEP, 2009). Tables 2.5 and 2.6 show reported values of lead in alcoholic beverages ranging from BDL-1.83 mg/L.

### 2.7.2 Cadmium

Cadmium naturally occurs with lead and zinc in their sulphide ores. It is mainly used in electroplating steel. Its compounds are used in electric batteries, electronic components or as dyes in plastics (Friberg *et al.*, 1986; Ros and Slooff, 1987). Waste water, galvanized pipes, solders, some metal fittings and inorganic phosphate fertilizers are a source of Cadmium in the environment. In water, cadmium concentration is below 1 µg/kg, in vegetables, fruit and meat below 10 µg/kg and in kidneys 100 -1000 µg/kg. In Saudi Arabia, Mustafa *et al.* (1988) reported mean concentrations of 1 – 26 µg/L in potable water, some from private wells with corroded pipes. Estimated daily cadmium intake in

Netherlands is 20 µg/person (IARC, 1976). In contaminated areas in Japan in 1980's daily intakes were 150 – 250 µg (Friberg *et al*., 1986).

Cadmium concentration in the body increase with age since its biological half-life is 10 – 35 years. Cadmium has no known useful role in higher organisms (Hoggan, 2010) and ranks high among the most toxic metals to man with a cumulative toxicity (Manahan, 1992). Acute exposure to cadmium fumes may cause flu like symptoms, pulmonary edema, osteomalacia, osteoporosis, impaired kidney and liver function, acute testicular necrosis, anaemia, and loss of consciousness (Nordberg, 2010).

'Itai itai' disease reported in Japan in 1955 was characterized by osteomalacia and low molecular weight proteinuria which was associated with cadmium poisoning among people living in contaminated areas (Jarup, 1998). Tables 2.5, 2.6 and 2.7 show reported values of cadmium in alcoholic beverages ranging from BDL-0.11 mg/L.

### 2.7.3 Copper

Copper is a stable transition metal and has two forms of cations; $Cu^{+}$ and $Cu^{2+}$. Common copper compounds are $Cu(C_2H_3O_2)_2.H_2O$, $CuCl_2$, $Cu(NO_3)_2.3H_2O$, CuO and $CuSO_4.5H_2O$. Copper metal is malleable and ductile. It is used in electrical wiring, pipes, valves, fittings, coins, cooking utensils and building materials. Copper compounds are used in fungicides, algaecides, insecticides, fertilizers, pharmaceuticals, pyrotechnics, electroplating, wood preservatives, engraving, azo dye manufacture, lithography and petroleum refining (ATSDR, 1990; Lewis, 1993).

In water $Cu^{2+}$ is the common oxidation state of copper and its fate is influenced by pH, dissolved oxygen and the presence of oxidizing and complexing agents (USEPA, 1995). Presence of chelating agents increases solubility of copper in water. Copper levels in unpolluted river Periyar in India ranged from 0.0008 to 0.010 mg/L (WHO, 2004) whereas the highest reported levels in a US survey of 678 ground water supplies was 0.47 mg/L (USEPA, 1991). Food is a major source of copper exposure to humans from organ meats like the liver, sea foods, nuts, and seeds that are good sources of dietary copper (NAS, 1989). Safe limit of copper intake in food and water is estimated at 1-3 mg/day for adults (WHO, 2004).

Copper is an essential dietary element at low concentration but high concentrations it is toxic (Pradyot, 1992). Exposure limit of copper to man in drinking water is 2.0 mg/L (Brewer, 2007). Acute lethal dose to humans is between 4 and 400mg $Cu^{2+}$ per kg body weight (Agarwal *et al.*, 1993). Inhalation of copper causes irritation of the eye, mucous membrane, chills and fever. Skin contact leads to dermatitis (Pradyot, 1992). Gant *et al.* (2007) reported liver cirrhosis in Indian children that was linked to boiling milk in copper cookware. In another study US Centers for Disease Control reported 155 cases of copper poisoning from drinking water that showed the water contained 4.0 – 156 mg/L (USEPA, 1987; CDC, 1993, 1996). Tables 2.6 and 2.7 show reported values of copper in alcoholic beverages ranging from BDL-1.5 mg/L.

### 2.7.4 Zinc

Zinc occurs in small amounts in almost all igneous rocks and its chief compound is ZnS as sphalerite and wurzite. In soils average zinc content is 1 – 300 mg/kg. Major uses of zinc are alloys such as brass and galvanizing steel. Zinc compounds like ZnO is used as white pigment, zinc carbamates are used as insecticides. In natural surface waters the level of zinc is 0.01mg/L, in ground water it is 0.04 mg/L, and much higher in tap water due to leaching zinc from pipes and fittings. Low pH, high carbon dioxide content and low mineral content make the water most corrosive. In a survey of 67% public water in Finland, average zinc content was 1.1 mg/L while the highest was 24 mg/L. Water with zinc levels above 3 mg/L tends to be opalescent, develops a greasy stain when boiled and has a sharp astrigent taste.

Zinc is an essential element in all organisms and has been reported in over 200 enzymes (O'Dell, 1984). Zinc is an essential element and a cofactor in dehydrogenases and DNA polymerases (Schmidt-Nielsen, 1990). In the U.S., the Recommended Dietary Allowance (RDA) is 8 mg/day for women and 11 mg/day for men.

High zinc diet was shown to cause hypocalcaemia and bone resorption. Zinc has antagonistic effects on other toxic metals including cadmium lead and nickel (Reddy *et al.,* 1987). Zinc poisoning has been reported among the drinkers of acidic beverages kept in galvanized containers and symptoms include fever, nausea, vomiting, stomach cramps and diarrhea (Elinder, 2006). Consumption of excess zinc can cause ataxia, lethargy, iron and copper deficiency (Ensminger and Konlande, 1993).

Symptoms of acute zinc toxicity are diarrhea and depression of nervous system while chronic symptoms are growth retardation, faulty reproduction (Pradyot, 1992). Yamaguchi *et al.* (1983), linked high zinc diets to hypocalcaemia and bone resorption while Rath (1990) reported that 1.5 g/L of zinc ethanoate in drinking water increased metastases of cancer in rats. Table 2.7 shows reported values of zinc in alcoholic beverages ranging from 1.82-2.70 mg/L.

### 2.7.5 Manganese

Manganese is one of the most abundant metals in nature occurring with iron. Manganese metal is mainly used in manufacture of steel while manganese compounds are used in batteries, glass, fireworks, fertilizers, animal feeding supplements, fungicides and oxidants. Manganese can exist in 11 oxidation states the most important oxidation states in nature and biology being $Mn^{2+}$, $Mn^{4+}$ and $Mn^{7+}$.

The greatest exposure to manganese is usually food. In fresh water typical levels of manganese are 1 – 200 µg/L and as high as 10 mg/L in acidic groundwater. Guideline value for manganese is 0.4 mg/L, TDI is 0.06 mg/kg of body weight, and limit of detection by AAS is 0.01 µg/L and 0.05µg/L by ICP-MS (ATSDR, 2000). Manganese is an essential trace nutrient in all forms of life (Emsley, 2001). Normal intake of manganese in water is about 20 µg/day for an adult (WHO, 1999).

Chronic manganese toxicity affects the nervous system characterized by liver cirrhosis, neurological and psychological manifestations and Parkison's Disease (Young *et al.,* 1996) associated with mining activities, dry cells and welding. According to results from a study, Baselt (2008) associated higher levels of exposure to manganese in drinking water with reduced intelligence quotients in children. Table 2.7 shows reported values of manganese in alcoholic beverages ranging from 1.04-1.88 mg/L.

### 2.7.6 Iron

Iron is one of the most abundant metals in the earth's crust and reported in natural waters at levels ranging from 0.5 – 50 mg/L. Iron is an essential element and minimum daily requirement is dependent on age, sex, physiological status and iron bioavailability at 10 – 50 mg/day. PMTDI is 2 mg/L in drinking water as a precaution against storage in the body of excessive iron (WHO, 1993). Iron is abundant in biology and in iron-proteins found in all living organisms and often is bound to cofactors, for example in hemes.

Toxic effects begin to occur at doses above 10-20 mg/kg of elemental iron. Ingestion of more than 50 mg/kg of elemental iron is associated with severe toxicity (Nanami *et al.,* 2005). Iron overload disorder, such as hemochromatosis, may present with the following clinical syndromes: Cirrhosis of the liver, diabetes, cardiomyopathy, arthritis and testicular failure. Brar *et al.* (2009) using MRI reported that iron accumulates in the hippocampus of the brains of those with Alzheimer's disease and in the substantia nigra of those with Parkison disease. Table 2.4 shows reported values of iron in alcoholic beverages ranging from 0.74-140.7 mg/L.

### 2.7.7 Aluminium

Aluminium is the most abundant metallic element and constitutes about 8% of the earth's crust. Some of its common compounds include; $Al(OH)_3$, $Al_2O_3$, $Al_2(SO_4)_3$ and $AlCl_3$. Aluminium metal is used in construction, automotive and aircraft industry, production of alloys, electric industry, in cooking utensils and in food packaging. Aluminium compounds are widely used in water treatment as coagulants to reduce organic matter, colour, turbidity and microorganism level (WHO, 1997).

Factors that influence aluminium mobility and subsequent transport within the environment are chemical speciation, hydrological flow paths, soil water interaction and composition of the underlying rock. Acid environments can cause increased aluminium content in surrounding waters (ATSDR, 1992; WHO, 1997). A US survey in treated water using aluminium sulphate indicated average aluminium concentration in water as 0.01 to 0.03 mg/L. Highly acidic waters affected by mine drainage showed concentrations of up to 90 mg/L (ATSDR, 1992; WHO, 1997).

Aluminium intake from foods containing aluminium compounds additives is a major route of aluminium exposure to the public. Use of aluminium cookware, utensils and wrappings can increase the amount of aluminium in food (FAO/WHO, 1989). In a study, it was reported that the level of aluminium in bananas boiled in aluminium saucepans was 42 µg/g while concentration of those boiled in a beaker was 0.2 µg/g (Wanjau, 1991). In another aluminium study in Kenya by Githua (1994), leaves were reported to

have more aluminium than grains. Richardson (1993), showed that beverages packed in aluminium cans contained more aluminium due to leaching. Tables 2.4 and 2.5 show reported values of aluminium in alcoholic beverages ranging from BDL-133.5 mg/L.

Although aluminium is poorly absorbed, the degree of absorption depends on some parameters including; the aluminium salt, pH (for aluminium speciation and solubility), bioavailability and dietary factors. Average aluminium intake for an adult is 5 mg (WHO, 1997). Aluminium has no known function in living cells and presents some toxic effects in elevated concentrations. Symptoms of aluminium toxicity are like those of Alzheimer's disease, osteoporosis, colic, rickets, gastro-intestinal problems, extreme nervousness, anaemia, decreased kidney and liver functions, memory loss, speech problems, bone softening and aching of muscles (Richardson, 1993). Rondeau *et al.* (2008) cited aluminium exposure as a risk factor for Alzheimer's disease.

## 2.8 Turbidity and pH of alcoholic beverages

Turbidity expresses the optical property of the interaction between light and suspended particles in a liquid (Steiner *et al*., 2010). Turbidity measure is an important indicator of quality change in beverages. In alcoholic beverages sources of turbidity are classified into three; from native particles by coagulation and precipitation, from process particles and from foreign particle precipitation (Steiner *et al*., 2010). Turbidity by native particles occurs due to protein-polyphenol haze that tends to limit the shelf life of alcoholic beverages. Maximum haze was reported to occur near pH 4 (Karl *et al*., 1996). In chemistry, pH is a measure of acidity or basicity of a solution. Acidic solutions have pH

values less than 7; alkaline solutions pH values are greater than 7 while neutral solutions have a pH value of 7. Bahera *et al.* (2006) reported pH levels in Indian made liquors as 4.0-8.5 while Tadele *et al*. (2013) reported pH levels of 4.00-4.99 in some Ethiopian traditional alcoholic beverages.

## 2.9 Analytical methods and instrumentation

### 2.9.1 Chromatographic techniques

These methods separate compounds of similar chemical properties. Isolation and analysis of compounds from complex matrices is through different fluorescent properties they possess. As the mobile phase transports the mixture across the stationary phase, the mixture components interact with the stationary phase causing partition due to their differential affinity to the mobile phase. Components with less affinity to stationary phase pass more quickly than the components with greater affinity. As the separated components elute, the detector responds with signal changes that are plotted against time to give a chromatogram (Settle, 1997).

Chromatographic methods include: planar chromatography (thin layer chromatography) (Aitken *et al.*, 2013), liquid chromatography (LC) (Powell and Brown, 2013), gas chromatography (GC), capillary electrophoresis, supercritical fluid chromatography and ion chromatography (Buckee, 2013). Among the most common and widely used are thin layer chromatography, high performance liquid chromatography and gas chromatography (Verzele *et al.*, 2013).

**Gas chromatography (GC)**

Technique separates and identifies all types of volatile compounds and some inorganic gases. It is mostly applied for separation, qualitative and quantitative determination of compounds. The GC partitions gaseous solutes between the inert gas mobile phase and a stationary liquid or solid phase on the column. The major components of a GC instrument shown in figure 2.1 include; the gases, the injection port, the column, the detector and the data acquisition system.

**Gases**

Usually consist of nitrogen, helium, hydrogen or a mixture of argon and methane. Hydrogen and air included as additional gases are associated with flame ionization detector.

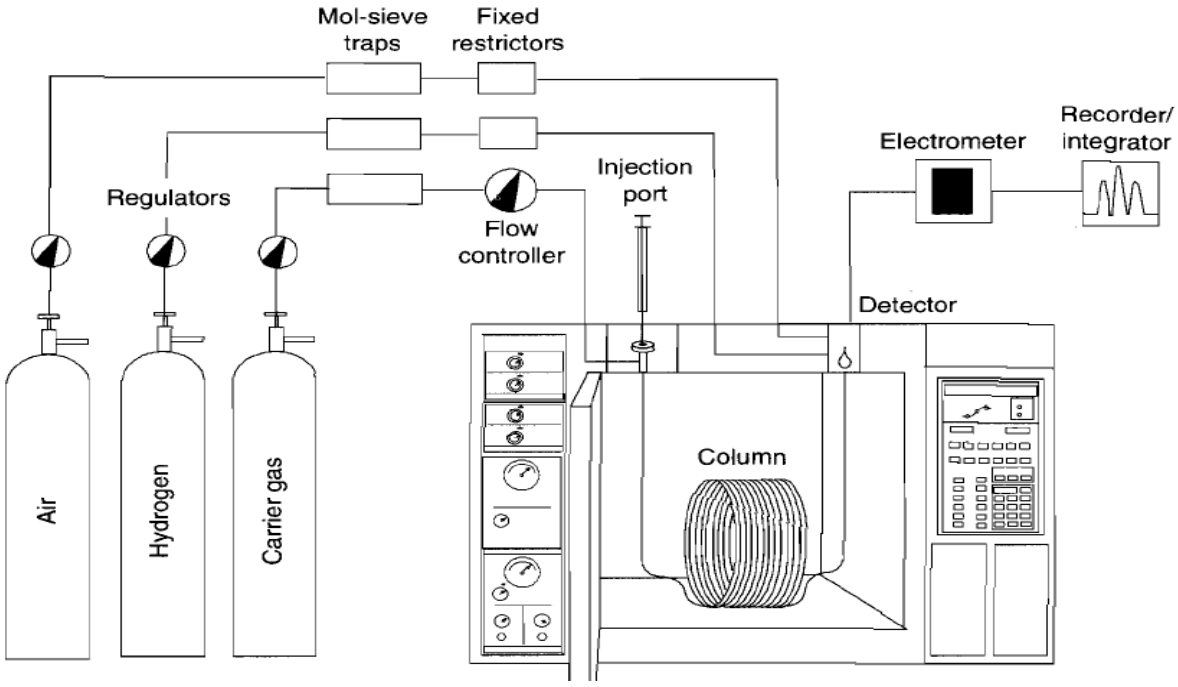



**Figure 2.1: Typical GC (Settle, 1997)**

**Injection port**

There are two types of injection ports; split (Figure 2.2) and a splitless capillary injector system that was used shown in figure 2.3.

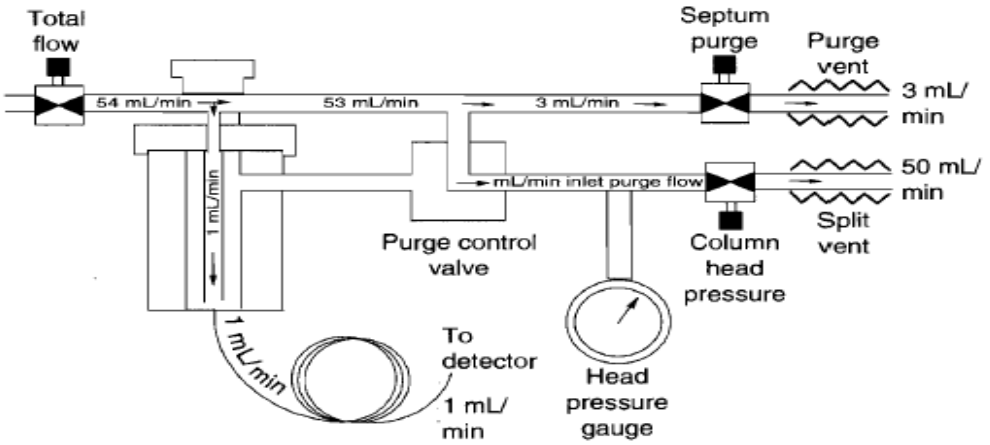


**Figure 2.2: Split inlet of a GC (Settle, 1997)**

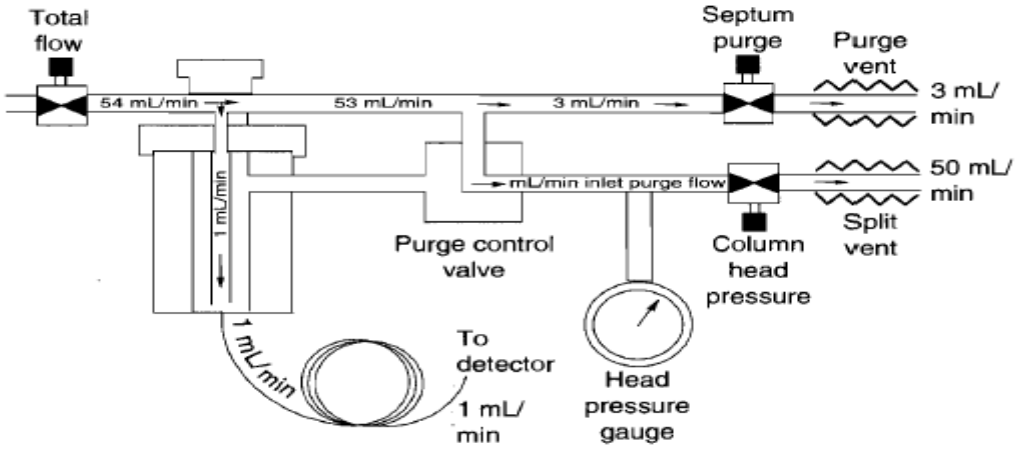



**Figure 2.3: Splitless inlet of a GC (Settle, 1997)**

This inlet introduces the sample into the carrier gas stream. This involves injection of 1 to 3 µL of a liquid sample into a heated inlet manually or by an automated injecting device.

**Column**

There are two types of GC columns; the packed column that was used in this study shown in figure 2.4 and the capillary columns shown in figure 2.5.

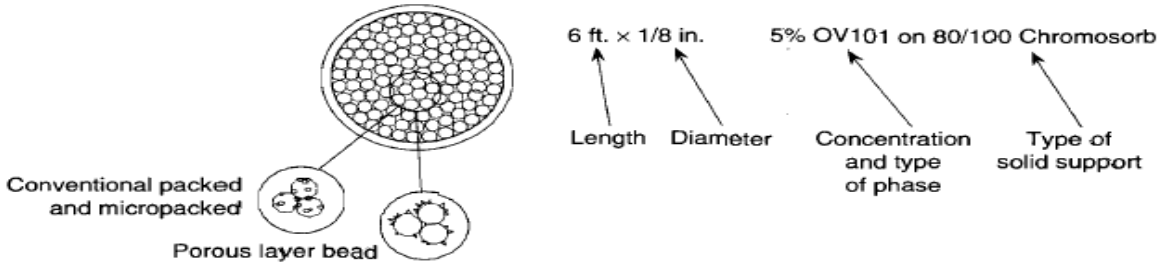



**Figure 2.4: Packed column (Settle, 1997)**

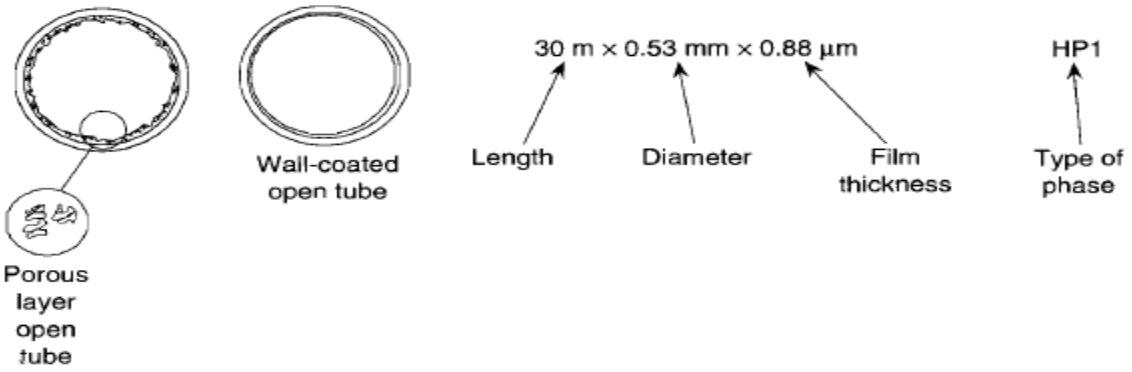



**Figure 2.5: Capillary columns (Settle, 1997)**

Columns are described by; length in meters, diameter in millimeters, stationary phase thickness in micrometers and the type of stationary phase. Columns are responsible for the separation of components of the mixture.

**Detectors**

They sense components different from carrier gas and convert that information to an electric signal. Choice of a detector is based on selectivity and sensitivity required by the analyst. Common GC detectors include: thermoconductivity detector (TCD), flame ionization detector (FID), nitrogen-phosphorous detector (NPD), electron capture detector (ECD), flame photometric detector (FPD), electrolytic conductivity detector (ELCD), photo ionization detector (PID), mass selective detector (MSD), inferred detector (IRD) and atomic emissions detector (AED). The flame ionization detector that was used in this study burns organic components in a flame producing ions that are collected and converted to an electric signal. It has fairly low detection limits of parts per billion (picogram) and responds to any type of hydrocarbon component.

**Data acquisition**

The integrator and the personal computer are the devices used to generate the chromatogram and the report. They translate the electrical signals from the detector into a peak chromatogram. Retention times are used to detect the various components of the mixture and the concentration of a component is given by Eq. 2.2. Area (%) of compound X= (area of compound X / total area of all peaks) * 100……...Eq. 2.2

The GC technique is mainly used in organic analysis with an accuracy range of 0.3 to less than 3% relative standard deviation and sensitivity in parts per trillion to grams per litre depending on the injection process and the type of detector used.

### 2.9.2 Elemental analysis

#### 2.9.2.1 Spectrophotometric methods

Methods are based on interaction of electromagnetic radiations with matter. At one time, electromagnetic radiation is absorbed or emitted. The techniques are mainly used for quantifying particular analytes in the sample. Some commonly used techniques for determining metallic elements include; the energy dispersive X-ray fluorescence spectrometry (EDXRF) (Ambrose and Scoh, 2000), the inductively coupled plasma atomic emission spectrometry (ICP-AES) (Wanjau *et al.*, 2004), the inductively coupled plasma hyphenated with mass spectrometer (ICP-MS) (Conor, 2004) and the atomic absorption spectrometry (AAS) (Gitu, 2001; Taylor *et al*., 2006; Woldemariam and Chandravansi, 2011). In this study AAS was used due to its availability, sensitivity, selectivity, reproducibility and time efficiency.

### 2.9.2.2 Atomic absorption spectrometry (AAS)

A schematic diagram of AAS instrument is shown in figure 2.6.

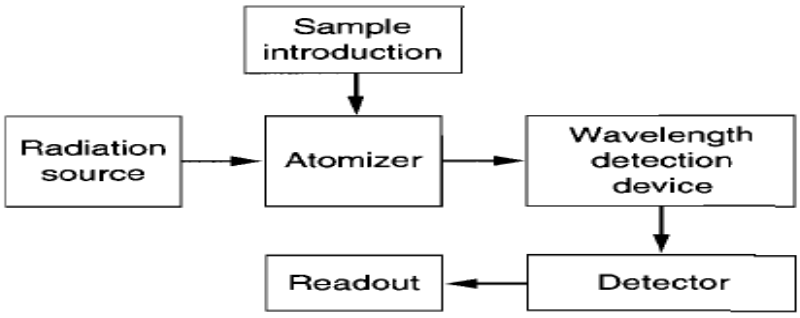



**Figure 2.6: Schematic diagram of AAS**

The AAS is a widely accepted destructive technique that quantifies about 70 metals in any sample at low concentrations of parts per billion. It is used to analyse biological, environmental and food samples among others. The method is easy to use, takes a few seconds per sample at a modest cost per sample.

**Basic principles of AAS**

Light of a specific wavelength is impinged onto ground state atoms. The atoms absorb the light transiting to a higher energy level. The intensity of this transition is related to concentration as shown in Eq. 2.3.

$T = P / P_0$ ..... Eq 2.3

Where;

$T$ = transmittance

$P$ = the power of light source after it passed through the sample

$P_0$ = the power of the light source before it passes through the sample

Transmittance is related to *k* as shown in Eq. 2.4.

$T = P / P_0 = e^{-kb}$ ……Eq. 2.4

Where;

*k* = the absorption coefficient

*b* = the path length

Absorbance *A* is related to transmittance as shown in Eq. 2.5

$A = -\log T = -\log P/P_0 = \log P_0/P = \log 1/T = kb \log e = 0.43\ kb$ ……Eq. 2.5

Beer-Lambert law relates *A* to concentration of element in the cell given by Eq. 2.6.

$A = abc$ or $A = \varepsilon_0 b$ ……Eq. 2.6

Where;

*a* = the absorptivity in g/L-cm

$\varepsilon_0$ = the molar absorptivity in g/mol-cm

*b* = the atom cell width in cm.

AAS involves measurement of the drop in light intensity of $P_0$ to *P*.

**Components of AAS**

**Atomizers**

Pneumatic nebulizers are commonly used to introduce a solution to AAS. Figure 2.7 shows a burner head, expansion chamber and pneumatic nebulizer combined.

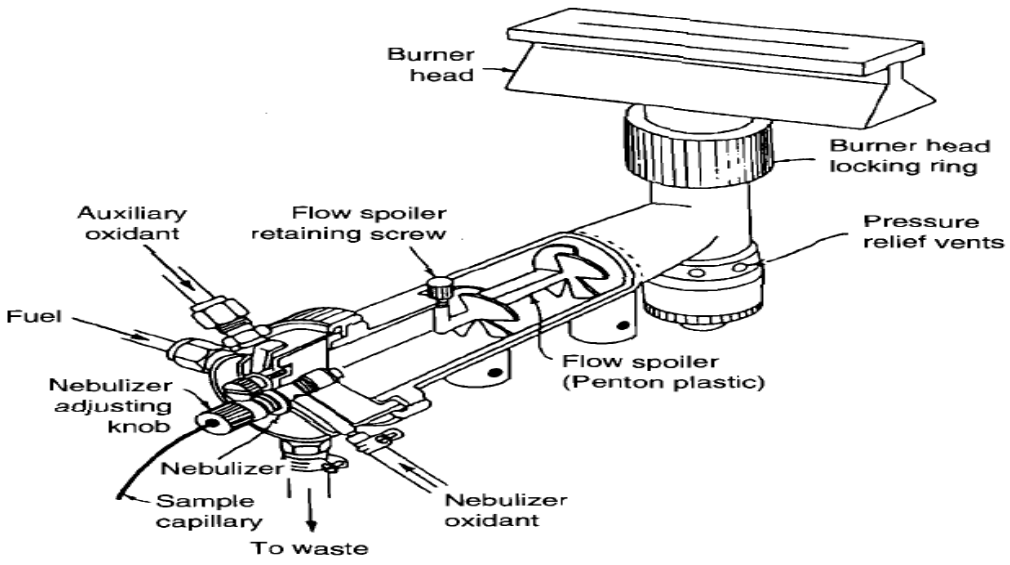



**Figure 2.7: Burner head, expansion chamber and pneumatic nebulizer combined**

A jet of compressed air aspirates and nebulizes the solution when the sample is sucked into the capillary tube. The aerosol sample mixes with oxidant and fuel before it is introduced to the flame. The AAS flame temperatures go up to 3000K, too much heat increases ionization of atoms. Potassium chloride solution may be added to some samples to suppress ionization.

**Radiation sources**

Hollow cathode lamp (HCL) shown in figure 2.8 is the most common line source though radiofrequency electrodeless discharge lamp (EDL) is at times used.

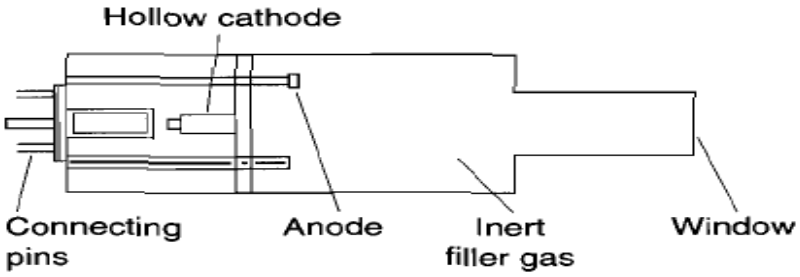



**Figure 2.8: Schematic diagram of a hollow cathode lamp**

Argon filler gas in the hollow cathode lamp ionizes as in Eq. 2.7.

$Ar + e^- \longrightarrow Ar^+ + e^- + e^-$ ……Eq. 2.7

These ions are accelerated to cathode made of metal being analysed. The ions impinge causing metal ions to sputter and ionize due to further collisions with argon ions. These excited metal ions produce a characteristic radiation of metal being analysed as they return to ground state.

**Monochromators**

These components isolate the wavelength of interest from other wavelengths from the radiation source and light emitted by other elements in the flame making AAS a highly selective method with virtually no spectral interferences. Diffraction gratings are preferred to prisms since they offer accuracy over a wide range of wavelengths.

**Detectors**

These components convert radiation energy into electrical signal. Photomultiplier tube (PMT) is the most widely used. It consists of a photo emissive cathode and several

dynodes in a vacuum. Dynodes provide the gain that is, electron multiplication up to $10^7$ useful in detecting low radiation levels.

**Readout devices**

A variety of devices include calibrated meters, external computers, printers and plotters. Computing is nowadays more common making operations like calibration and working out concentration easier.

**Background absorption and correction**

Particulate matter (scatter), that is, the molecular and atomic background, causes major interferences in AAS. Methods of background correction include; continuum source (deuterium arc), Zeeman Effect background correction and Smith-Hieftje background correction**.**

### 2.9.3 The pH measurements

Measurement of pH in an aqueous solution is done with a glass electrode (Figure 2.9) and a pH meter. The definition of pH as adopted in the ion-selective electrodes is shown in Eq. 2.8

$$\mathrm{pH} = -\log_{10}(a_{\mathrm{H}^+}) = \log_{10}\left(\frac{1}{a_{\mathrm{H}^+}}\right) \quad \text{.....Eq. 2.8}$$

Where*;* $a_{\mathrm{H}^+}$ = hydrogen ion activity in a solution.

An ion selective electrode consists of two reference electrodes, whose potentials are constant, separated by a membrane whose potential governs the overall cell potential. The

electrode responds to activity of the ion incorporated in one of the two reference electrodes, such as $H^+$, in the glass pH electrode (Fifield and Kealy, 1995). The Nernst equation for the $H^+$ electrode potential is written in Eq. 2.9.

$$E = E^0 + \frac{RT}{F}\ln(a_{H^+}) = E^0 - \frac{2.303RT}{F}\text{pH} \quad \text{.....Eq. 2.9}$$

Where;

$E$ = the measured potential

$E^0$ = the standard electrode potential

$R$ = the gas constant

$T$ = the absolute temperature (in Kelvin)

$F$ = the Faraday constant

Since in $H^+$ the number of electrons transferred is one, the electrode potential is proportional to pH when pH is defined in terms of activity. The electromotive force (e.m.f.) between a reference electrode and an electrode sensitive to the hydrogen ion activity is measured when they are both immersed in the same aqueous solution. Examples of reference electrodes are the silver chloride and the calomel electrode while the hydrogen-ion selective electrode is the standard hydrogen electrode.

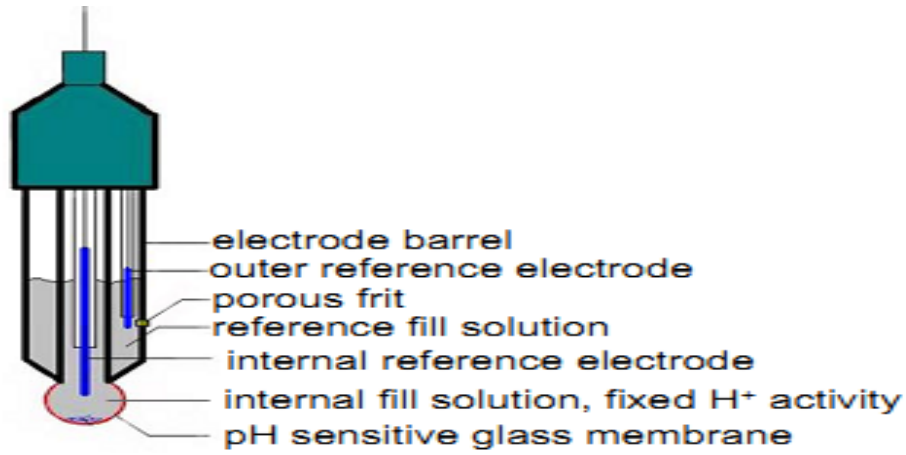



**Figure 2.9: The glass pH electrode (Fifield and Kealy, 1995)**

The galvanic cell formed in the glass pH electrode is represented schematically as shown in figure 2.10

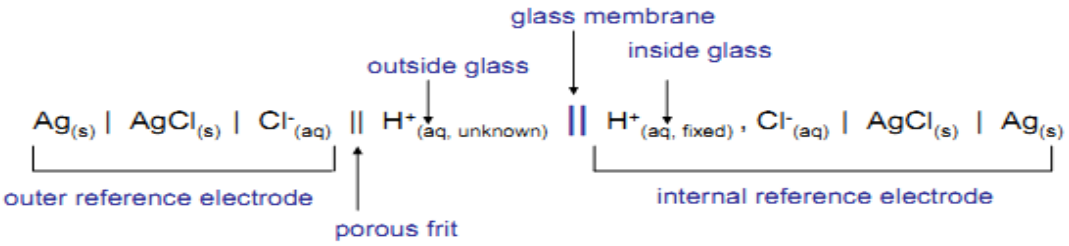



**Figure 2.10: Schematic representation of the glass pH electrode**

The cell is first filled with a solution of known hydrogen ion activity and the emf, $E_S$, is measured. The emf, $E_X$, of the same cell containing the solution of unknown pH is then measured. The equation Eq. 2.10 gives the pH of the unknown solution.

$$\mathrm{pH(X)} = \mathrm{pH(S)} + \frac{E_S - E_X}{z} \quad \text{.....Eq. 2.10}$$

The difference between the two measured emf values is proportional to pH and the proportionality constant (*1/z)* is equal to 1/2.303RT/F (the Nerstian slope).

### 2.9.4 Turbidity measurements

Nephelometer is the instrument that measures turbidity**.** In nephelometry measurement, turbidity is measured by light scattered at an angle of 90˚ from the incident beam as shown in figure 2.11.

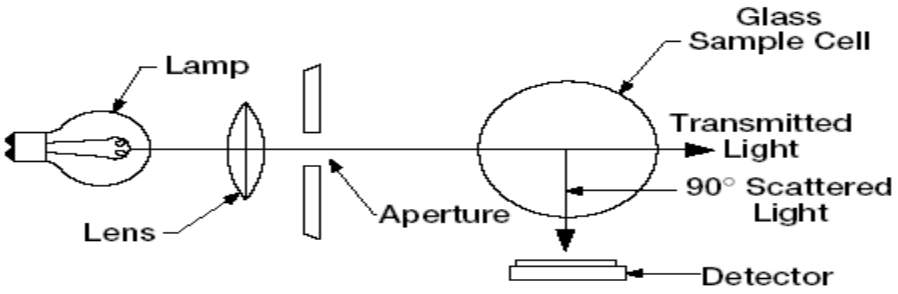
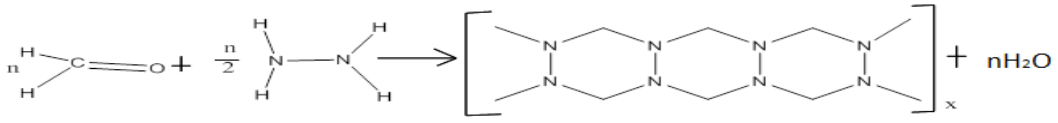



**Figure 2.11: Schematic diagram of turbidity measurement**

The main light source is the tungsten filament bulb and detectors used are the photomultiplier tube, silicon photodiode, vacuum photodiode and cadmium sulphide photoconductor**.** Formazin used to prepare turbidity standards is synthesized as shown in figure 2.12.




Formaldehyde Hydrazine Formazin

**Figure 2.12: Synthesis of formazin**

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 Research designs

One of the designs used in this study was cross sectional-observational: a representative subset of the population was studied at a particular time to provide descriptive information on the entire population under study. Incidences, causes and prognosis of events in chronological order were used to distinguish between cause and effect. The purposive design used in sampling involved the researcher deciding on an information rich sampling unit that facilitated the investigation. This design is effective when used with low population samples to achieve analytical generalizations (Miles and Humberman, 1994). Quantitative and partly qualitative research methods were used.

The study was carried out in two stages: the first stage involved use of a standard questionnaire to assess the demographic characteristics of the people who consumed alcoholic beverages in Muthithi location, the types of alcoholic beverages they took and the likely health risks of consumption of the alcoholic beverages. The second stage of the study was experimental and involved determination of organic compounds using GC instrument, toxic metals using the AAS instrument, pH and turbidity.

## 3.2 Study area

This study was carried out in Muthithi location in Muranga county of Central province, Kenya. Most residents practiced subsistence farming in this coffee growing region. Population density was about 600 persons per square kilometre (NCPD, 2005).

Appendices I and II are maps showing Muranga County and Muthithi location respectively.

## 3.3 Socio-demographic characteristics of persons consuming alcoholic beverages in Muthithi location

### 3.3.1 Recruitment of study participants

Muthithi residents of age 18 years and above who consume alcoholic beverages were recruited from the drinking places in five major markets in Muthithi location including; Kaharati, Heho, Karuri, Muthithi and Ngaburi.

### 3.3.2 Sample size

The sample size was determined using the formula given in Eq. 3.4 by Daniel (1999).

$$n = \frac{Z^2 P(1-P)}{d^2} \quad \text{.....Eq. 3.4}$$

Where;

$n$ = sample size

$Z$ = Statistic for a level of confidence (for the level of confidence of 95 percent, which is conventional, Z value is 1.962)

$P$ = Expected prevalence or proportion (in proportion of one; if 27.8 percent, P=0.278)

$d$ = precision (in proportion of one; if 5.17 percent, d=0.051)

$$n = \frac{1.962 * 0.287(1-0.287)}{(0.0517)^2} \quad \text{.....Eq. 3.5}$$

$n$ = 30

Therefore, from Eq. 3.5 a total of 150 respondents were sampled for the study since five areas were sampled and from each area, 30 respondents were considered.

### 3.3.3 Ethical considerations

Approval for this study was granted by Institute for Research Science and Technology of Kenyatta University (Appendix III), relevant permits and identity card obtained from the National Council for Science, Technology and Innovation (NACOSTI) (Appendices IV and V). Muthithi area administration was informed of the research activity before data collection commenced. On introduction, the reasons for the research and its benefits to the respondent were explained. Confidentiality of the information obtained was assured and that the respondents were not required to give their names as indicated in the Informed Consent (Appendix VI). The interview hours were restricted to early evening from 5.00 pm to 7.00 pm when more people were interviewed successfully before they got drunk.

### 3.3.4 Administration of the questionnaire

A self-administered questionnaire (Appendix VII) was filled by one hundred and fifty subjects (n=150) to find out whether the public was aware of some problems caused by consumption of alcoholic beverages. The questionnaire was used to assess the alcoholic beverages consumed in Muthithi and the social demographic characteristics of the persons who consumed them. Two other questionnaires (Appendices VIII – IX) collaborated with the information from the first one. The questionnaires were used to capture information from the following areas:

i) Respondents background characteristics (age, marital status, education level, and occupation)
ii) Alcoholic beverages in Muthithi location (the commonly available alcoholic drinks, the popular alcoholic drinks and the reason for the drinks' popularity).
iii) Health effects due to consumption of alcoholic beverages.
iv) The types of containers and for alcoholic beverages.
v) Additives to alcoholic beverages.
vi) Reports of any deaths or hospitalization due to consumption of alcoholic beverages then.

### 3.3.5 Sampling of alcoholic beverages

Eight major types of popular alcoholic beverages were randomly sampled 3 times from bars in Heho, Karuri and Muthithi markets in Muthithi location as shown in table 3.1. Purposeful sampling strategy was used in selecting sampling sites and available alcoholic beverages. The alcoholic beverages sampled were common in all markets since they were obtained from certain vendors - there were no reported cases of brewing in homes. Sampling was done between 5.00 pm to 7.00 pm in compliance to Alcohol Law and for security reasons. The samples were transported and stored in clean containers or in original packages. Bulk samples were prepared by mixing 200 mL from each of the three bottles of the same brand, shaken slightly to ensure mixing and preserved in a refrigerator.

**Table 3.1: Types of alcoholic beverages sampled**

| Alcoholic beverage brand | Alcoholic beverage description | Product type | Number of times sampled |
|---|---|---|---|
| **Changaa A** | Homemade spirit ,diluted | 1 | 3 |
| **Changaa B** | Homemade spirit, undiluted | 2 | 3 |
| **Spirit A** | Spirit, colourless, expensive | 3 | 3 |
| **Spirit B** | Spirit, dark, expensive | 3 | 3 |
| **Spirit C** | Spirit, colourless, cheap | 4 | 3 |
| **Spirit D** | Spirit, golden, cheap | 4 | 1 |
| **Bottled beer** | Lager, golden, bottled | 5 | 0 |
| **Keg Beer A** | Lager, golden, in cylinder* | 6 | 3 |
| **Keg Beer B** | Lager, dark, in cylinder | 6 | 1 |
| **Keg Beer C** | Lager, golden, in cylinder** | 6 | 1 |
| **Muratina A** | Homemade beer | 7 | 3 |
| **Muratina B** | Homemade beer, packed | 7 | 3 |
| **Miti ni dawa A** | Homemade beer | 8 | 3 |
| **Miti ni dawa B** | Homemade beer, packed | 8 | 3 |
| **Matinga** | Homemade beer | 9 | 0 |
| **Busaa A** | Porridge beer | 10 | 3 |
| **Busaa B** | Porridge beer, packed | 10 | 1 |

* and ** are from different companies

### 3.4 Reagents, chemicals and solvents

Distilled deionized water was used throughout for rinsing apparatus, sample preparation and dilutions. All reagents that were used in the analyses were analytical grade. Nitric acid ($HNO_3$) (69-70%, Spectrosol, BDH, England) and $H_2O_2$ (30%, BDH, England) were used for digestion of alcoholic beverages.

The standard stock solution for Atomic Absorption Spectrometry (AAS) 1000 mg/L in 2% $HNO_3$ of metals Al, Cd, Cu, Fe, Mn, Pb and Zn were used for preparation of calibration standards and in the spiking experiments. Working standard solutions were obtained by diluting the stock solution.

The standard solvents for Gas Chromatography (GC) grade reagents ethanol, methanol, propan-1-ol, propan-2-ol, butan-1-ol, amyl alcohol, methanal, ethanal, ethanoic acid and ethyl ethanoate were used to prepare standard solutions for GC analysis.

Two pH buffers 4.0 pH and 7.0 pH were used for the calibration of the pH meter while three formazin standards (Stablcal stabilized formazin) range; 0-10, 0-100 and 0-1000 NTU units were used for calibration of the turbidimeter.

### 3.5 Cleaning of apparatus

All sample containers and glassware were cleaned with a mild detergent, rinsed with tap water then soaked overnight in 1% nitric acid, rinsed in distilled de-ionized water then air dried. Sampling containers were stored in plastic bags to prevent contamination.

### 3.6 Instrumentation

Atomic Absorption Spectrometer (AAS) Buckscientific model 210 VGP (East Norwalk, USA) equipped with a deuterium arc background corrector and microprocessor software was used for analysis of metals. The flame used for Al was $N_2O$-acetylene while air-acetylene flame was used for Cd, Cu, Fe, Mn, Pb and Zn.

Gas Chromatograph (Shimadzu model GC-9A), coupled with a flame ionization detector (FID) was used for organic analysis. This was interphased with an integrator model Chrom Jet-CH. A glass packed column (3m x 3mm id) with diethyleneglycol succinate 15% was used.

Hanna Instruments pH meter model pH 211 Microprocessor was used for pH measurements. A Hatch 2100P turbidimeter was used to measure turbidity of samples.

## 3.7 Sample preparation for gas chromatography (GC) analysis

### 3.7.1 Sample distillation

Exact volume of 100 mL of each bulk sample was mixed with about 50 mL distilled water and distilled to produce 100 mL of the distillate. High vacuum grease (Dow Corning, GMBH, USA) and parafilm (Perchiney, 60631, Chicago) were used to ensure that distillation apparatus were airtight and that all vapours were collected. The distillates were collected into 100 mL volumetric flasks under ice. This process got rid of non-volatile solutes and maintained the concentration of volatile organic compounds in the bulk sample and in the distillate constant. The distillate was divided into 10 mL portion in a sealed glass vial (for GC analysis of the organic compounds) and the rest 90 mL in stoppered volumetric flask (for alcoholic content determination) and stored in the fridge.

### 3.7.2 Alcoholic content determination

A pycnometer was used to determine the precise densities of alcoholic beverage distillate samples. Alcoholic distillates and distilled water were separately filled into a dry pycnometer leaving no air bubbles. Masses were recorded in triplicates and subsequently the density of the sample determined at 20 ˚C as shown in Eq. 3.1.

$Sg = W_2\text{-}W_0 / W_1\text{-}W_0$ ……Eq. 3.1

Where;

Sg = density of the distillate

$W_0$ = weight of dry pycnometer

$W_1$ = weight of pycnometer + water

$W_2$ = weight of pycnometer + sample

The alcoholic contents were determined from a table of alcoholic content (%) against densities ($g/cm^3$) (Williams, 1984).

### 3.7.3 Preparation of standards

GC grade reagents; methanol, propan-1-ol, propan-2-ol, butan-1-ol, amyl alcohol, methanal, ethanal, ethanoic acid and ethyl ethanoate of known purity were used. One microlitre (1 µL) of each standard (0.1%), prepared in 10% ethanol, was separately injected into the GC when ready. The retention time (RT) of the major peak was noted.

### 3.7.4 Standard conditions for GC analysis

| | |
|---|---|
| Injection temperature | 220 ˚C |
| Detector temperature | 220 ˚C |
| Column initial temperature | 50 ˚C |
| Column temperature rate | 50 ˚C/minute |
| Column final temperature | 150 ˚C |
| Column final time | 10 minutes |
| Attenuation | 2 (in a scale of 10) |
| Stop time | 32 minutes |
| Chart speed | 0.5 |

### 3.7.5 Analysis of organic compounds by GC

Shimadzu Gas Chromatograph model GC-9A, equipped with a splitless capillary injector system, a flame ionization detector (FID) and a glass packed column with diethyleneglycol succinate 15% (3 m x 3 mm id) was used for the organic analysis. This was interphased with an integrator model Chrom Jet-CH. White spot nitrogen was used as the carrier gas with the head pressure at 37.5 Psi. One microlitre (1 µL) of sample in sealed vial was drawn and injected into the GC. The injection was done in duplicates to check on the instrument's accuracy.

The spectral peaks obtained were compared to those of the standards. The retention times were used to identify the various organic compounds in the samples while the peak area (%), computed by the GC instrument, was used to calculate the concentration of the compounds (ppm) as shown in Eq. 3.2 (Williams, 1984).

$$\text{Conc. (ppm)} = \frac{(\text{peak area of cpd (\%)} * \text{alcohol content (\%)} * \text{density of org. cpd} * 10^6)}{(\text{Total area of peaks (\%)} * 100)} \quad \text{Eq. 3.2}$$

## 3.8 Sample preparation for atomic absorption spectrometry (AAS) and analysis

### 3.8.1 Sample digestion

A volume of 5 mL of each bulk sample of alcoholic beverages were wet ashed (digested) in triplicates to destroy organic matter and give clear solutions according to the procedure described by Lazos and Alexakis (1989), and Sanllorente *et al*. (1998). The procedure was optimized by considering the amount of $HNO_3$ and $H_2O_2$ added the digestion time, optimum temperature and clearness of the digest.

An aliquot of 5 mL was quantitatively transferred to a 250 mL digestion flask and 5 mL of $HNO_3$/ $H_2O_2$ in ratio 2:5 was added. The flask was shaken and placed on the hot plate first at 60 ˚C then slightly increased to 180 ˚C for one hour until the solution became clear. The digest was removed to cool, filtered, transferred quantitatively to 50 mL volumetric flask and topped to the volume with distilled deionized water. The digest was kept in the fridge pending AAS analysis.

### 3.8.2 Digestion of blank samples and detection limit

Six blank samples were prepared the same way as the samples according to a procedure by Galani-Nikolakaki *et al*. (2002) for use in determining method detection limits. This was in order to account for the background effects of the acids and to correct the changes resulting from digestion procedures. Absolute ethanol was added to distilled deionized water to make a 12% alcohol solution similar to that of some alcoholic beverages and then the six blanks were digested as described in section 3.8.1. The absorbancies of the six blanks were recorded, their means and standard deviations were calculated and used for calculating detection limit shown in Eq. 3.3 (Christian, 2005).

$$\text{Detection limit} = \frac{3 * \text{standard deviation of blanks readings}}{(\text{absorbance of standards} - \text{mean absorbance of 6 blanks})} \quad \text{....Eq. 3.3}$$

### 3.8.3 Preparation of standard solutions for AAS

Standard solutions for Al, Cd, Cu, Fe, Mn, Pb and Zn obtained from Buck Scientific USA, were prepared in 2% $HNO_3$ to maintain metals in free ionic form. The 1000 mg/L

stock solution was made by dissolving 1.0g of pure metal in 10 mL of 1:1 HCl:$HNO_3$ then making it to 1L solution (Franson, 1975). Stock standard solutions of Al, Cd, Cu, Fe, Mn, Pb and Zn containing 1000 mg/L in 2% $HNO_3$ were used for calibration and spiking experiments. Four working standards were prepared each day for calibration purposes. All analyses were carried out at specific wavelengths corresponding to each metal.

### 3.8.4 Analysis of metals by atomic absorption spectroscopy (AAS)

Buck Scientific Model 210 VGP AAS was used for the analysis of metals. Absorption measurements for Al, Fe, Cu, Zn, Mn, Pb and Cd were made at the wavelengths 309.3 nm, 248.3 nm, 324.7 nm, 213.9 nm, 279.5 nm, 217.0(or 283.3) nm and 228.8 nm, respectively. Table 3.2 shows the operating parameters for the AAS measurements.

**Table 3.2: The AAS operating parameters**

| Element | Flame type | Wavelength (nm) | Slit width (nm) |
|---|---|---|---|
| Al | $N_2O$/ acetylene | 309.3 | 0.5 |
| Cd | Air/ acetylene | 228.9 | 0.5 |
| Cu | Air/ acetylene | 324.8 | 0.5 |
| Fe | Air/ acetylene | 248.3 | 0.2 |
| Mn | Air/ acetylene | 279.5 | 0.2 |
| Pb | Air/ acetylene | 283.3 | 1.0 |
| Zn | Air/ acetylene | 213.9 | 1.0 |

### 3.9 The pH measurements

The bulk alcoholic beverage samples were removed from the fridge for 4 hours to acquire the room temperature. The pH meter was calibrated using freshly prepared buffers 4.0 pH and 7.0 pH to compensate for any inherent offsets of the sensor. The buffers used had pH values close to those of alcoholic beverage solutions under test for the best calibration accuracy.

### 3.10 The turbidity measurements

The bulk alcoholic beverage samples were removed from the fridge for 4 hours to acquire the room temperature, shaken lightly and put into the instrument's sample bottle and turbidity value measured. Where turbidity was beyond 1000 nephelometry turbidimetric units (NTU), dilution was done and the turbidity value was obtained by multiplying the value displayed in the read out with the dilution factor.

## 3.11 Methods validation

### 3.11.1 The questionnaire

Before administration of the questionnaire, a pre-test questionnaire was given to a few subjects for filling in the neighbouring Kangari location of Muranga County. Adjustments were done to some questions which were not clear to the respondents. The questionnaires were then issued to the subjects in Muthithi location who were able to fill them successfully. Where necessary, the interviewer orally translated the questionnaire to Kiswahili or Kikuyu for the subjects who did not understand English. The questionnaire was therefore reliable for use in assessment of community awareness of the harmful effects of consumption of alcoholic beverages of Muthithi location.

### 3.11.2 The gas chromatography (GC) instrument

Samples and standards were analysed in duplicates in GC instrument to give comparable spectral peaks. Attenuation was increased to make the peaks clearer where necessary. The instrument was optimized as shown in section 3.7.4 to give clear peaks in samples and standards. This ensured reliability of this method in determination of the organic compounds in alcoholic beverages.

### 3.11.3 The atomic absorption spectrometer (AAS)

The AAS instrument was calibrated by aspirating the standards whose absorbancies were used to calculate correlation coefficient (r) values and hence determine the linear ranges for reliably determining toxic metals in alcoholic beverages.

#### 3.11.3.1 Linear ranges for AAS calibration curves

Toxic metals Cd, Cu, Fe, Mn, Pb and Zn were determined using air/acetylene flame while Al required $N_2O$/acetylene flame. Stock standard solutions of Al, Cd, Cu, Fe, Mn, Pb and Zn containing 1000 mg/L in 2% $HNO_3$ were used for calibration. Four working standards within the optimum working ranges were prepared each day from the stock solution for calibration purposes. All analyses were carried out at specific wavelengths corresponding to each metal. Table 3.3 shows levels of standards and the linear ranges of AAS calibration curves.

**Table 3.3: Levels of standards for linear ranges of AAS calibration curves**

| Element | Concentration range of standards (mg/L) |
|---|---|
| **Al** | 0.0 – 40.0 |
| **Cd** | 0.0 – 2.0 |
| **Cu** | 0.0 – 5.0 |
| **Fe** | 0.0 – 5.0 |
| **Mn** | 0.0 – 4.0 |
| **Pb** | 0.0– 3.0 |
| **Zn** | 0.0 – 1.0 |

The results in table 3.3 indicate that the calibration curves obtained were linear over a wide range of concentration. The wide linear range accommodated the elements being determined into the working range and therefore the AAS analysis gave precise and accurate results.

#### 3.11.3.2 The AAS detection limits and calibration curves

The samples were aspired into the air acetylene flame under the standard conditions for each element and the absorbancies measured. The concentrations of the toxic metals in mg/L were obtained by direct comparison of absorbance of the sample and that of the

standards. Regression analysis was used to evaluate the linearity of AAS using the calibration curves obtained. The absorbance readings and concentration of the standards were used to calculate the correlation coefficients (r). The calibration curves shown in Appendices XXVI-XXXII were obtained when absorbance readings were plotted against the corresponding concentration of standards. Detection limit is the lowest concentration that gives an absorbance signal above the instrumental noise while sensitivity is the instruments response to analyte showing concentration of any analyte that absorb ≥1% of the incident radiation. The AAS detection limits, the correlation coefficients, and the equations of the calibration curves for the determination of toxic metals are given in table 3.4.

**Table 3.4: The AAS detection limits, correlation coefficients and equations of the calibration curves**

| Metal | Method detection limit (ppm) | Instrument detection limit (ppm) | Correlation coefficient | Equation for the calibration curve |
|---|---|---|---|---|
| Al | 0.01 | 0.004 | 0.999 | Y=0.006X+0.006 |
| Cd | 0.01 | 0.005 | 0.999 | Y=0.159X+0.014 |
| Cu | 0.03 | 0.020 | 0.999 | Y=0.051X+0.026 |
| Fe | 0.10 | 0.030 | 0.999 | Y=0.021X+0.007 |
| Mn | 0.02 | 0.001 | 0.999 | Y=0.086X+0.089 |
| Pb | 0.10 | 0.100 | 0.999 | Y=0.021X+0.004 |
| Zn | 0.05 | 0.005 | 0.999 | Y=0.230X+0.019 |

The results in table 3.4 indicate that there was a high positive correlation between concentration and absorbance.

### 3.11.4 The physicochemical parameters instruments

The pH meter and turbidimeter were calibrated as shown in sections 3.9 and 3.10 respectively. The readings obtained from the two instruments were reproducible making them reliable for measuring pH and turbidity of the alcoholic beverages.

## 3.12 Data analysis

The data obtained was analysed by one-way ANOVA at 95% confidence level using SPSS (version 18.0). Where statistical comparison was $p>0.05$, it was assumed that there was no significant difference. Whenever significant differences existed, the means were compared at $p=0.05$ which accounted for errors since the sample was representative of the population (Sawyer *et al*., 2004).

# CHAPTER FOUR

# RESULTS AND DISCUSSIONS

## 4.1 The social demographic characteristics of persons consuming alcoholic beverages in Muthithi location

A questionnaire was administered to recruited persons in five villages of Muthithi location namely Kaharati, Heho, Karuri, Muthithi and Ngaburi. It was intended to find out which alcoholic beverages were being consumed in Muthithi location, the residents' drinking patterns and possible health consequences of drinking. The results are presented in the following subsections.

### 4.1.1 The gender of respondents

The gender of the respondents is shown in figure 4.1.

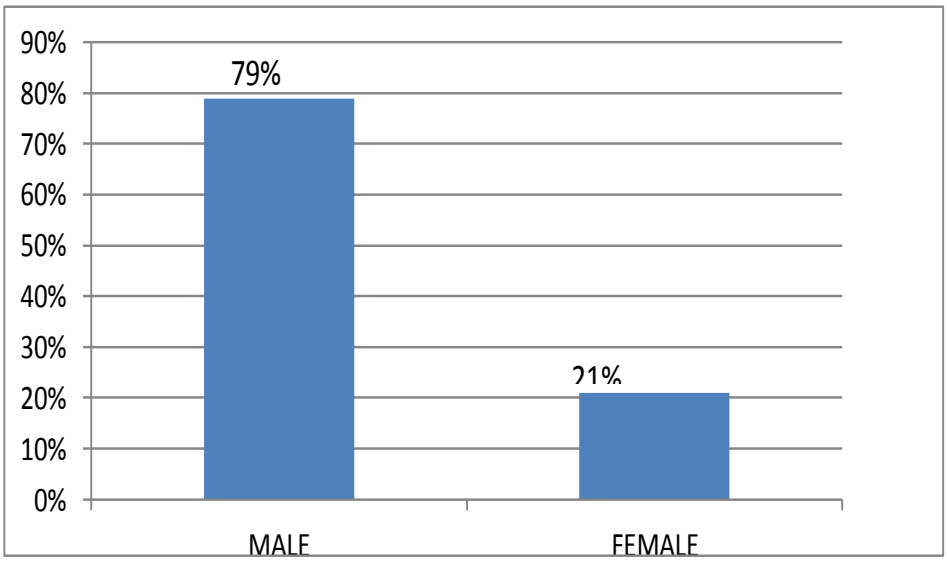



**Figure 4.1: Gender of respondents**

Figure 4.1 indicates that the majority of the respondents were male (79%). Worldwide, more men than women are involved in weekly binge drinking in the ratio of 4:1 (WHO,

2011). Men have higher rate of total disease burden attributed to alcohol compared to women, that is, 7.4% for men compared to 1.4% for women (WHO, 2011). Therefore men were more likely to suffer the negative effects of excessive drinking in Muthithi than women.

### 4.1.2 The marital status of respondents

The marital status of the respondents is shown in table 4.1.

**Table 4.1: Marital status of respondents**

| Marital status | % respondents |
|---|---|
| Married | 77 |
| Separated | 1 |
| Divorced | 10 |
| Single | 6 |
| Widowed | 6 |

Table 4.1 indicates that the majority of the respondents were married (77%). Harmful effects of drinking such as physical and psychological health of the drinker, financial strains, diseases and injuries are consequences of alcohol drinking are felt in the family of the drinker (Laslett, 2010). This has led to domestic problems and therefore public outcry due to excessive drinking by men (Daily Nation, 2010).

### 4.1.3 The education level of respondents

The education level of the respondents is shown in figure 4.2.

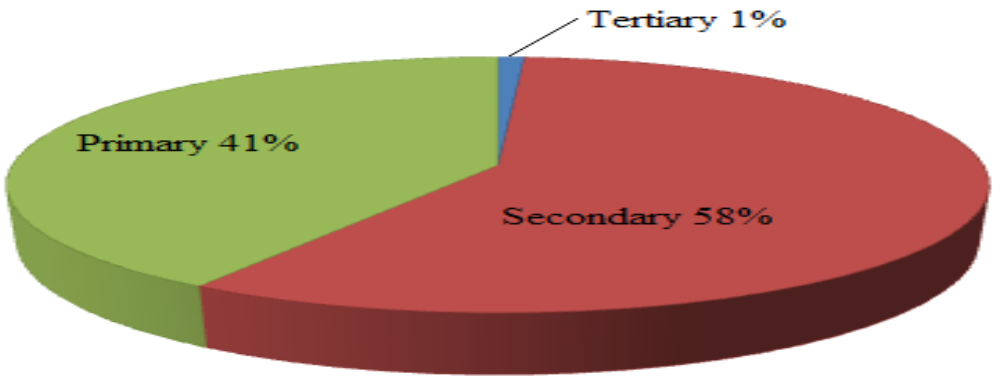



**Figure 4.2: Education level of respondents**

From figure 4.2 it can be seen that majority respondents had secondary level of education (58%) and none was illiterate. In Muranga County, where Muthithi is located, 95.3% of the population is literate while 41.4% had acquired secondary education or higher (KDHS, 2010). The people who consumed alcohol were at various levels of education, those of higher education levels were more probably due to their better financial status.

### 4.1.4 The occupation of respondents

The occupation of the respondents is shown in table 4.2.

**Table 4.2: Occupation of respondents**

| Occupation | % respondents |
|---|---|
| Salaried | 46 |
| Business | 23 |
| Casual | 19 |
| Peasant farmer | 12 |

The results shown in table 4.2 indicate that all respondents were employed, that is, they had worked in the last 7 days (KDHS, 2010). Employment in the professional, technical or managerial sector comprised the 46% salaried workers. Casual (19%) comprised of those employed in the agricultural sector mainly to provide unskilled manual labour. Self-employed were those in small scale businesses (23%) and the peasant farmers (12%). The residents of Muthithi could therefore afford various types of alcoholic beverages.

### 4.1.5 The alcoholic beverages consumed in Muthithi location

Some alcoholic beverages consumed in Muthithi, their consumption and popularity is shown in table 4.3.

**Table 4.3: Consumption and popularity of alcoholic beverages in Muthithi**

| Alcoholic beverage | % consumption | % popularity |
|---|---|---|
| Bottled beer | 29 | 37 |
| Keg Beer | 45 | 36 |
| Fortified wine (Kali) | 21 | 21 |
| Spirits | 4 | 4 |
| Miti ni dawa | 1 | 2 |

The results shown in table 4.3 indicate that the most consumed alcoholic beverage was Keg Beer (45%) although the more prefered were (glass) bottled beers (37%). Consumption of Keg Beers was higher due to their lower cost compared to bottled beers. Fortified wines (Kali) are spirits or liquors of high alcoholic content that were being prepared from various industries and were being sold at low prices. They were more

prefered to spirits since they were strong and cheaper just like Changaa while Miti ni dawa was consumed by those who thought it was medicinal and due to its lower cost.

Reasons for alcoholic drink preference by respondents is shown in figure 4.3.

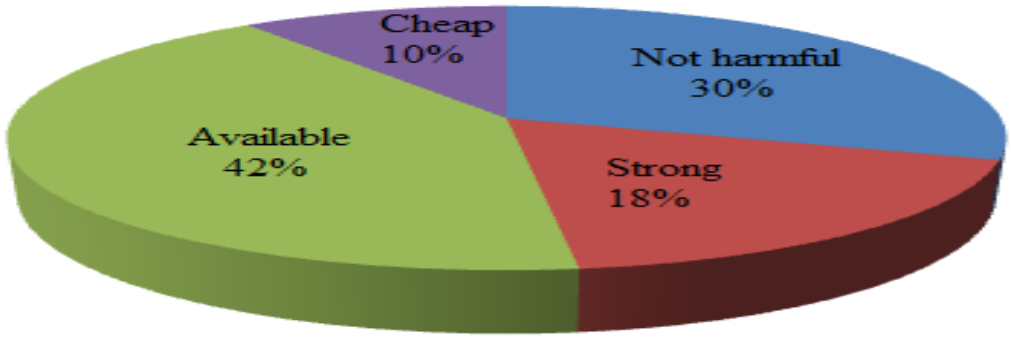



**Figure 4.3: Reason for drink preference**

Figure 4.3 indicates that most respondents prefered the alcoholic beverage they consumed due to its availability (42%) while few for its low cost (10%). Those who prefered strong drinks (those with high alcoholic content) were 18% while those who thought alcoholic beverages were not harmful were 30%.

The number of glasses of alcoholic drink and the equivalent units of alcohol consumed per sitting are shown in table 4.4.

**Table 4.4: Number of drink glasses per sitting and the units of alcohol taken**

| **Drink glasses (200mL) taken per sitting** | **1-2** | **3-5** | **5-10** | **Above 10** |
|---|---|---|---|---|
| % consumption | 3 | 35 | 53 | 9 |
| Units of alcohol taken for 4% ABV beer | 2.4-4.8 | 7.2-12 | 12-24 | Above 24 |
| Units of alcohol taken for 40% ABV spirit | 24-48 | 72-120 | 120-240 | Above 240 |

ABV=alcohol by volume

Results shown in table 4.4 indicate that the average number of 200 mL glasses of alcoholic beverage taken in a sitting was between 5 to 10 (53%) equivalent to 12-24 units of alcohol in 4% alcohol by volume (ABV) beer or 120-240 units of alcohol in 40% ABV spirit. This was beyond the limit set by the United Kingdom of 3-4 units a day for men and 2-3 units a day for women (Sournia, 1990).

The number of drinking days per week is shown in table 4.5.

**Table 4.5: Number of drinking days per week**

| **Drinking days per week** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **% consumption** | 4 | 7 | 11 | 14 | 18 | 21 | 25 |

Results in table 4.5 indicate that most respondents drank everyday (25%), 6 days per week (21%) and 5 days per week (18%). Heavy episodic drinking (HED) also referred to as binge drinking is a measure of alcohol consumption pattern defined as drinking of at least 60 g of pure ethanol on at least one occasion in the past 7 days (WHO, 2011). This pattern of drinking is associated with low per capita consumption but more health problems (Jernigan, 1997). The drunkenness displayed in Muthithi is associated with

poverty (Barrows and Room 1991), and health consequences including raised blood pressure, cancer of the mouth, pharynx, and esophagus, and primary cancer of the liver (Reynolds and Prasad, 1982; Kabat and Wynder, 1989).

### 4.1.6 Perceived health effects on consumption of alcoholic beverages

The negative effects of consumption of alcoholic beverages are shown in table 4.6.

**Table 4.6: Negative effects of drinking alcoholic beverages**

| Negative effects of drinking alcoholic beverages | Immediate (%) | Long term (%) |
|---|---|---|
| None | 67 | 67 |
| Weakness | 15 | 14 |
| Stomach upsets | 8 | 9 |
| Headache | 6 | 7 |
| Lack of appetite | 4 | 3 |

Results in table 4.6 indicate that most of the respondents were not aware of short term or long term harmful effects of drinking (67%), but a few cited weakness (15%) as one of the immediate negative effects. Alcohol consumption is the world's largest risk factor for disease and disability in the developing world (WHO, 2011). It is the causal factor in 60 types of diseases and injury and a component in 200 other diseases (WHO, 2011). Alcohol eventually damages nearly all organs in the body and is a risk factor to global health accounting for 3.5% of the disability adjusted life years (DALYs) (Room *et al*., 2001). Alcohol harm to drinker includes alcoholic psychosis, alcohol dependency syndrome, alcoholic polyneuropathy, alcoholic cardiomyopathy, alcoholic gastritis and alcoholic liver cirrhosis among others. Alcohol drinking also affects productivity, reproductive and family life of the drinker (WHO, 2011).

Most Muthithi residents who consumed alcoholic beverages were either unaware or ignored these facts hence their harmful trend of binge drinking. The respondents cited weakness on drinking with respect to low sexual libido. This affects men due to inhibition of enzymes involved in testicular testosterone synthesis and secretion on acute exposure to ethanol and ethanal in alcoholic beverages (NRC, 1986). Stomach upsets were due to alcoholic gastritis while some contaminants like methanol could have been responsible for the headaches reported (WHO, 1997).

The number of people who died or were hospitalized in the last six months is shown in figure 4.4.

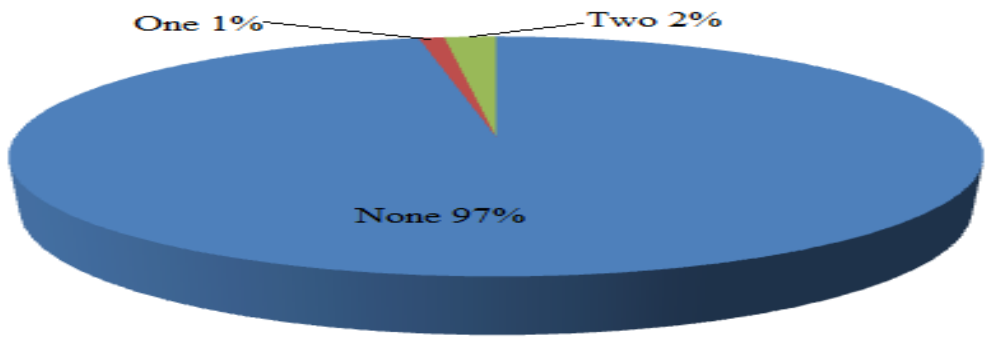



**Figure 4.4: Number of people who died or were hospitalized in the last six months**

Results in figure 4.4 indicate that most respondents reported none (97%) died or was hospitalized for the previous six months due to consumption of local alcoholic beverages. This research was conducted at a time when there were no reported cases of alcohol poisoning around Muthithi area or in Muranga County.

Almost 4% of all deaths worldwide are attributed to alcohol (WHO, 2011). This proportion is greater than deaths caused by HIV/AIDS, violence or tuberculosis (WHO, 2011). Alcohol is associated with many serious social issues including violence, child neglect and abuse and absenteeism from work place. Heavy episodic (binge) drinking leads to serious health problems especially injuries (WHO, 2011).

### 4.1.7 Storage of alcoholic beverages

The containers for storage of alcoholic beverages are shown in figure 4.5.

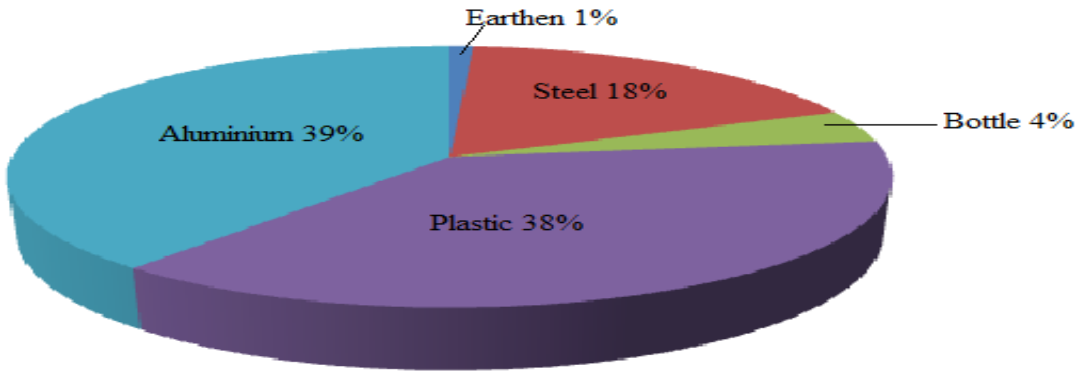



**Figure 4.5: Containers for alcoholic beverages**

Results in figure 4.5 indicate that most respondents reported that alcoholic beverages were stored in aluminium (39%) or plastic containers (38%). All Keg Beers came in aluminium cylinders and were dispensed using a pump. Aluminium is resistant to corrosion but leaches into alcoholic beverages due to their low pH. Similarly iron from steel containers used in preparation and storage of Muratina and Miti ni dawa leaches into these alcoholic beverages due to their high acidity (Richardson, 1993). Most beers from industries were in glass bottles while most fortified wines (Kali) were in plastic bottles which may leach some organic contaminants such as dioxins into alcoholic beverages.

### 4.1.8 Perceived additives to alcoholic beverages

Perceived additives to alcoholic beverages are shown in table 4.7.

**Table 4.7: Perceived additives to alcoholic beverages**

| **Additive** | **% Response** |
|---|---|
| Methanol | 84 |
| Formalin | 6 |
| Pieces of metal | 4 |
| Jik (disinfectant) | 1 |
| Laundry blue | 1 |
| Torch cells | 1 |
| Battery acid | 1 |
| None | 2 |

From table 4.7, it can be seen that most respondents (84%) reported methanol as the additive they thought made alcoholic beverages stronger. This response was due to occasional media reports of deaths caused by methanol contamination in alcoholic beverages cited in section 1.2.

The information obtained from community respondents collaborated well with reponses from opinion leaders (Table 4.8) and health workers (Table 4.9).

**Table 4.8: Opinion leaders' responses (%)**

| | |
|---|---|
| 1 | Average age of people who took alcohol: 18-25years (5%), 25-35years (30%) and 35-45years (65%) |
| 2 | Common alcoholic beverages consumed: Bottled beer, Keg Beer, spirits, fortified wine (kali), Muratina, Changaa, Busaa, Matinga and Miti ni dawa. |
| 3 | Alcoholic beverage people preferred taking: bottled beer, Keg Beer or spirits (75%), fortified wine (kali) (20%) and Miti ni dawa (5%) |
| 4 | Reason for drink preference: Cheap (15%), Available (40%), strong (25%) and not harmful (20%). |
| 5 | Negative effects of drinking alcoholic beverages: None (55%), weakness (25%), stomach upsets (5%), headache (5%), lack of appetite, other (10%) |
| 6 | Number of people who were hospitalized or died from consumption of local alcoholic beverages in the last 6 months: None (80%), 1 (15%), 2 (5%) |
| 7 | Source of local alcoholic beverages: Within (5%), neighbouring villages (10%) and towns (85%). |
| 8 | Perceived additives to the alcoholic beverages: Torch cells (5%), battery acid (5%), formalin (15%), methanol (70%) and pieces of metal (5%). |

**Table 4.9: Health workers' responses (%)**

| | |
|---|---|
| 1 | Average age of people who took alcohol: 25-35years (25%) and 35-45years (75%) |
| 2 | Common alcoholic beverages consumed: Bottled beer, Keg Beer, spirits, fortified wine (kali), Muratina, Changaa, Busaa, Matinga and Miti ni dawa. |
| 3 | Alcoholic beverage people preferred taking: bottled beer, Keg Beer or spirits (58%), fortified wine (kali) (33%) and Miti ni dawa (9%) |
| 4 | Negative effects of drinking alcoholic beverages: None (42%), weakness (33%), stomach upsets (8%), headache (8%) and lack of appetite (8%) |
| 5 | Number of people who were hospitalized or died from consumption of local alcoholic beverages in the last 6 months: None (83%) and 1 (17%) |
| 6 | Perceived additives to the alcoholic beverages: Battery acid (8.5%), formalin (8.5%) and methanol (83%) |

## 4.2 Levels of organic contaminants in alcoholic beverages

Alcoholic beverage samples were analysed for alcoholic content measured in percentage alcohol by volume (% ABV). The alcoholic beverages were then analysed for organic compounds present using a GC instrument. They were found to contain mainly ethanol and various levels of methanol, propan-1-ol, propan-2-ol, 1-butanol, amyl alcohol,

methanal, ethanal and ethanoic acid. The GC spectra for some samples and standards are shown in Appendices X-XXV. The results of alcoholic content and the mean values of organic compounds in the alcoholic beverages are discussed in the following subsections.

### 4.2.1 Alcoholic content of alcoholic beverages

The results of alcoholic content of some alcoholic beverage samples obtained from Muthithi location are as shown in table 4.10.

**Table 4.10: Alcoholic content (%ABV) of some alcoholic beverages**

| Alcoholic beverage | Actual % ABV | % ABV on label | Cost per 200 mL glass (Ksh) |
|---|---|---|---|
| **Changaa A** | 10.25 | - | 20 |
| **Changaa B** | 24.72 | - | 40 |
| **Spirit A** | 42.92 | 40 | 200 |
| **Spirit B** | 33.12 | 40 | 180 |
| **Spirit C** | 47.05 | 40 | 60 |
| **Spirit D** | 11.46 | 40 | 60 |
| **Keg Beer A** | 2.43 | 4.2 | 30 |
| **Keg Beer B** | 2.78 | 4.5 | 13 |
| **Keg Beer C** | 3.56 | 5.0 | 10 |
| **Muratina A** | 2.64 | 5.5 | 13 |
| **Muratina B** | 3.34 | - | 10 |
| **Busaa A** | 3.27 | - | 10 |
| **Busaa B** | 2.50 | - | 13 |
| **Miti ni dawa A** | 3.34 | - | 10 |
| **Miti ni dawa B** | 1.82 | - | 10 |

ABV=Alcohol by volume

From table 4.10, it can be seen that the spirits including Changaa had higher alcohol content with Spirit C having the highest alcoholic content at 47.05% ABV. Miti ni dawa B was reported to have the lowest alcoholic content at 1.82% ABV. Spirits are obtained from distillation of fermented products like beer. Distillation increases the alcoholic

content in spirits since yeast can only withstand an alcohol concentration of up to 12% (Chester, 1993).

The cost of alcoholic beverages was highest for spirit A and B that sold at around Ksh 200 and lowest for Keg Beer C, Muratina B, Busaa A and Miti ni dawa at Ksh 10 per 200 mL glass. The cost of alcoholic beverages was not pegged on % ABV since Spirit C having the highest at 47.05% ABV sold at Ksh 60 while Spirit D at 11.46% ABV sold for the same price.

### 4.2.2 Mean levels of organic compounds in alcoholic beverages

The results of the mean values of organic compounds in alcoholic beverages from Muthithi are compared as shown in table 4.11.

**Table 4.11: Mean levels of organic compounds in alcoholic beverages**

| | Ethanol (mean± SE)mg/L | Methanol (mean ±SE) mg/L | propan-1-ol (mean ±SE) mg/L | propan-2-ol (mean ±SE) mg/L | Butan-1-ol (mean ±SE) mg/L | Amyl alcohol (mean ±SE) mg/L | Methanal (mean ±SE) mg/L | Ethanal (mean ±SE) mg/L | Ethanoic acid (mean ±SE) mg/L |
|---|---|---|---|---|---|---|---|---|---|
| **Changaa A** | 159880 ±41620$^{bc}$ | 7.47 ±5.12 | 90.26 ±25.47$^{c}$ | 99.98 ±27.79$^{c}$ | 5.42 ±0.00 | 283.11 ±59.46$^{d}$ | BDL | 15.67 ±4.91 | 8.86 ±5.84 |
| **Changaa B** | 91570 ±9590$^{ab}$ | BDL | 65.75 ±2.14$^{bc}$ | 61.42 ±5.21$^{b}$ | BDL | 113.74 ±5.61$^{cd}$ | BDL | 4.81 ±1.19 | 15.82 ±2.37 |
| **Spirit A** | 227620 ±73500$^{c}$ | BDL | BDL | BDL | BDL | 3.89 ±0.00$^{a}$ | 3.15 ±0.00 | 0.78 ±0.00 | 2.93 ±1.24 |
| **Spirit B** | 259980 ±30050$^{c}$ | BDL | 0.94 ±0.29$^{a}$ | BDL | BDL | 20.98 ±0.00$^{b}$ | BDL | BDL | BDL |
| **Spirit C** | 240330 ±26520$^{c}$ | BDL | 8.73 ±0.00$^{a}$ | 1.22 ±0.01$^{a}$ | 3.88 ±0.51 | 8.68 ±1.42$^{c}$ | BDL | BDL | BDL |
| **Keg Beer A** | 21720 ±2350$^{a}$ | 10.75 ±9.73 | 9.73 ±3.05$^{a}$ | 4.07 ±0.50$^{a}$ | BDL | 65.81 ±17.85$^{cd}$ | BDL | 2.49 ±0.00 | 10.10 ±6.68 |
| **Muratina A** | 24690 ±2740$^{a}$ | BDL | 19.71 ±3.45$^{ab}$ | 2.89 ±1.60$^{a}$ | BDL | 77.68 ±14.57$^{cd}$ | BDL | 4.97 ±0.00 | 40.18 ±37.03 |
| **Mitinidawa A** | 27330 ±8480$^{a}$ | BDL | 49.01 ±15.13$^{abc}$ | 5.65 ±3.71$^{a}$ | BDL | 169.08 ±25.85$^{d}$ | 128.78 ±0.00 | 8.87 ±2.79$^{a}$ | 11.29 ±0.00 |
| **Mitinidawa B** | 19390 ±3260$^{a}$ | BDL | 34.18 ±6.51$^{ab}$ | 4.89 ±1.27$^{a}$ | BDL | 134.63 ±37.70$^{cd}$ | BDL | 14.05 ±3.02 | BDL |
| **Busaa A** | 22620 ±1750$^{a}$ | BDL | 39.10 ±3.26$^{ab}$ | 3.01 ±1.86$^{a}$ | BDL | 117.54 ±8.45$^{cd}$ | BDL | 7.27 ±1.10 | 229.50 ±215.15 |
| **p-value** | <0.001 | 0.587 | 0.002 | <0.001 | 0.330 | 0.001 | <0.001 | 0.248 | 0.731 |

Mean value followed by the same letter(s) within the same column are not significantly different (SNK test, α=0.05), BDL = Below detection limit

#### 4.2.2.1 Mean levels of ethanol in alcoholic beverages

The results in table 4.11 show the mean levels of ethanol were significantly higher in spirits A, B, C and Changaa A ($p<0.05$). Spirit B had the highest concentration of ethanol at 259980±30050 mg/L while Miti ni dawa B had the lowest at 19390±3260 mg/L. Ethanol is the main organic product of carbohydrate fermentation but other organic compounds may form during fermentation. A healthy adult can metabolize from 75% to 95% of 10 grams of pure ethanol (one unit of alcohol) in one hour. Blood ethanol levels above 400 mg/dL may cause coma, respiratory depression, hypotension, hypothermia and death (Bingham *et al.,* 2001).

#### 4.2.2.2 Mean levels of methanol in alcoholic beverages

The results in table 4.11 indicate that methanol was only detected in Changaa A and Keg Beer A at low levels that were not significantly different ($p>0.05$). Methanol may be produced in small amounts during fermentation of grain or fruits with high levels of pectin (Ian, 2007). Presence of methanol in the alcoholic beverages may have been due to contamination (Daily Nation, 2010). Methanol is slowly metabolized in liver and is highly toxic to humans. Ingestion of 10 mL pure methanol would cause permanent blindness while 30 mL is fatal (WHO, 2005).

#### 4.2.2.3 Mean levels of propan-1-ol in alcoholic beverages

From the results in table 4.11 mean levels of propan-1-ol were significantly different in alcoholic beverages ($p<0.05$). Changaa and Miti ni dawa had significantly higher mean levels of propan-1-ol than other alcoholic beverages. Propan-1-ol was below detection limit (BDL) in Spirit A. Alcoholic beverages almost always contain propan-1-ol as a

byproduct of fermentation. Beers contain up to 195 mg/L while spirits may contain up to 3520 mg/L of propan-1-ol (WHO, 1990). After ingestion propan-1-ol may cause headache, drowsiness, abdominal cramps, nausea and diarrhea. A fatal dose of pure propan-1-ol by ingestion is 250 mL (Clayton and Clayton, 1982).

#### 4.2.2.4 Mean levels of propan-2-ol in alcoholic beverages

The results from table 4.11 show mean levels of propan-2-ol in Changaa A and B were significantly higher than in all other alcoholic beverages ($p<0.05$). Levels of propan-2-ol were highest in Changaa A but were below detection limits in spirits A and B. Propan-2-ol is a less toxic isomer of propan-1-ol and may be produced in small quantities during fermentation and its effects are similar to those of propan-1-ol (Gosselin *et al.*, 1984).

#### 4.2.2.5 Mean levels of amyl alcohol in alcoholic beverages

The results in table 4.11 indicate levels of amyl alcohol were significantly different ($p<0.05$), highest in Changaa A 283.11±59.46 mg/L and lowest in spirit A 3.89±0.00 mg/L. Amyl alcohols are produced in small quantities during fermentation the major component being 3-methyl-1-butanol. Amyl alcohols are CNS depressants and are reported to be about four times as toxic as ethyl alcohol. Lethal dose for a human adult is 50 mL of a tertiary amyl alcohol (Sax, 1975).

#### 4.2.2.6 Mean levels of methanal in alcoholic beverages

The results in table 4.11 indicate that methanal was only detected in Miti ni dawa A and spirit A at 3.15±0.00 mg/L and 128.78±0.00 mg/L repecctively. Methanal contamination

could have been due to oxidation of methanol or deliberate addition as a preservative. Lowest lethal dose for human taking methanal orally is 36 mg/kg (Bingham *et al.*, 2001).

#### 4.2.2.7 Mean levels of ethanal in alcoholic beverages

The results in table 4.11 indicate that ethanal detected in some samples had levels that showed no significant difference ($p>0.05$). The main source of exposure to ethanal to humans is by oxidation of ethanol, reaction being catalyzed by the enzyme liver alcohol dehydrogenase (ADH). Ethanal is the toxic metabolite in alcohol associated with liver damage, facial flushing, hangovers and increases addiction in adolescents (Gosselin *et al*., 1984).

#### 4.2.2.8 Mean levels of ethanoic acid in alcoholic beverages

The results in table 4.11 show that levels of ethanoic acid were not significantly different but were below detection limit (BDL) in spirits B and C and Miti ni dawa B. Consumption of Busaa A that had the mean levels of ethanoic acid at 229.5$\pm$215.15 mg/L, which was above 60 mg/L could have caused conjunctivitis, bronchitis, pharyngitis, perforation of oesophagus and erosion of exposed teeth (ACGIH, 2001). The levels of ethanoic acid in alcoholic beverages from Muthithi was comparable to those in other parts of the world since Nykanen and Soumalainen (1983) reported levels of ethanoic acid from 11.7 mg/L in to 300 mg/L in alcoholic beverages.

### 4.3 Mean levels of toxic metals in alcoholic beverages

Eight varieties of alcoholic beverage samples were analysed for some toxic metals by AAS. They were reported to contain various levels of Al, Fe, Mn, Pb and Zn. Cd and Cu

were below the method detection limit in all the samples. The mean levels of toxic metals in alcoholic beverages are shown in table 4.12.

**Table 4.12: Mean levels of toxic metals in alcoholic beverages (mg/L)**

| Alcoholic beverage | Zn (n=45) (mean±SE) | Pb (n=45) (mean±SE) | Fe (n=45) (mean±SE) | Al (n=35) (mean±SE) | Mn (n=22) (mean±SE) | Cu (n=0) | Cd (n=0) |
|---|---|---|---|---|---|---|---|
| **Changaa A** | 0.15±0.02$^{ab}$ | 0.04±0.00$^{a}$ | 0.21±0.06$^{b}$ | 0.80±0.11$^{a}$ | 0.03±0.00$^{a}$ | BDL | BDL |
| **Spirit A** | 0.18±0.06$^{ab}$ | 0.03±0.01$^{a}$ | 0.11±0.03$^{a}$ | 0.70±0.12$^{a}$ | BDL | BDL | BDL |
| **Spirit B** | 0.12±0.03$^{a}$ | 0.06±0.00$^{b}$ | 0.08±0.01$^{a}$ | 1.05±0.10$^{a}$ | 0.08±0.01$^{a}$ | BDL | BDL |
| **Spirit C** | 0.08±0.01$^{a}$ | 0.12±0.01$^{c}$ | 0.07±0.01$^{a}$ | 3.00±0.37$^{b}$ | 0.63±0.00$^{ab}$ | BDL | BDL |
| **Keg Beer A** | 0.09±0.00$^{a}$ | 0.19±0.00$^{d}$ | 0.08±0.01$^{a}$ | 3.06±0.48$^{b}$ | 0.63±0.28$^{ab}$ | BDL | BDL |
| **Muratina A** | 0.26±0.05$^{b}$ | 0.23±0.01$^{e}$ | 0.26±0.04$^{b}$ | 2.37±0.07$^{b}$ | 1.15±0.20$^{bc}$ | BDL | BDL |
| **Muratina B** | 0.09±0.00$^{a}$ | 0.25±0.01$^{e}$ | 0.34±0.03$^{d}$ | 2.56±0.39$^{b}$ | 1.58±0.23$^{c}$ | BDL | BDL |
| **Miti ni dawa A** | 0.21±0.04$^{ab}$ | 0.25±0.01$^{e}$ | 0.29±0.02$^{c}$ | 2.47±0.15$^{b}$ | 1.69±0.12$^{c}$ | BDL | BDL |
| **p-value** | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | | |

Mean value followed by the same letter(s) within the same column are not significantly different (SNK test, $\alpha$=0.05), BDL=below detection limit.

The results in table 4.12 show that the mean levels of zinc in all beverages were below 50 mg/L, the maximum allowable limit (MAL). The mean level of zinc in Muratina A was significantly higher than the mean levels in spirit B, spirit C, Keg Beer A and Muratina B, but not the mean levels in Changaa A, spirit A and Miti ni dawa A ($p<0.05$). Spirit C, Keg Beer A, Muratina A, Muratina B and Miti ni dawa A had mean levels of lead above MAL (0.1 mg/L); the mean levels of Changaa A, spirits A and spirit B were below MAL.

Mean levels of lead in Muratina A, Muratina B and Miti ni dawa A were significantly higher than the mean levels in the other alcoholic beverages sampled ($p<0.05$). The mean level of iron was beyond MAL (0.3 mg/L) only in Muratina B and was significantly higher than the mean levels in the other alcoholic beverages sampled ($p<0.05$). The mean levels of aluminium were reported to be beyond MAL (0.2 mg/L) in all alcoholic beverages sampled and were significantly higher in spirit C, Keg Beer A, Muratina A, Muratina B and Miti ni dawa A ($p<0.05$). The mean level of manganese was below the limit of detection in spirit A. The mean levels of manganese in Changaa A and spirit B were below MAL (0.1 mg/L) but above MAL in Muratina A, Muratina B and Miti ni dawa A. The mean levels of manganese in Muratina A were significantly higher than those of Changaa A and spirit B but not those in spirit C and Keg Beer A ($p<0.05$).

Consumption of all the sampled beverages exposed the Muthithi residents to aluminium toxicity which is associated with Alzheimer's disease (Rondeau *et al.,* 2008) but not to zinc, copper or cadmium toxicity. High levels of aluminium may be attributed to storage of some beverages like Keg Beer A in aluminium containers and the high acidity of these beverages (ATSDR, 1992; WHO, 1997).

Consumption of spirit C, Keg Beer A, Muratina A, Muratina B and Miti ni dawa A exposed the residents of Muthithi to lead poisoning. Lead is a cumulative poison and accounts for 0.6% of the global burden of disease (WHO, 2009). Long-term exposure damages all organs, destroys immune system (Lutz *et al.,* 1999) and reproductive system (Iavicoli *et al.,* 2006) Lead contamination could have been due to unhygienic preparation,

storage and dispensing of alcoholic beverages. The contamination could have been by lead-based paints, ceramic glazes, drinking water systems with lead solder and lead pipes, lead in e-waste, lead solder in containers or contaminated soils (WHO, 2003). The low pH and long standing times in lead environments made the alcoholic beverages highly plumbosolvent (WHO, 2009).

Consumption of Muratina B exposed the residents to iron toxicity which is associated with Cirrhosis of the liver, diabetes, cardiomyopathy, arthritis and testicular failure (Brar *et al.,* 2009). Preparation and storage of Muratina in steel drums, contamination and its low pH could have led to high levels of iron in the alcoholic beverage (Woldemariam and Chandravansi, 2011).

Consumption of Muratina A, Muratina B and Miti ni dawa A exposed the residents to manganese toxicity. Differences in mean levels of manganese could have been due to contamination when handling the alcoholic beverage. The likely sources of manganese were dry cells, fertilizers, fungicides or steel containers (Woldemariam and Chandravansi, 2011). Chronic manganese toxicity is characterized by liver cirrhosis, neurological and psychological manifestations and Parkison's Disease (Young *et al.,* 1996).

## 4.4 The pH of alcoholic beverages

The mean levels of toxic metals were correlated to pH in table 4.13

**Table 4.13: Correlation of pH with mean levels of toxic metals in alcoholic beverages**

| Toxic metal | Zn | Pb | Fe | Al | Mn |
|---|---|---|---|---|---|
| **Pearson's correlation** | -0.343 | -0.309 | -0.433 | -0.28 | -0.294 |
| **p-value** | 0.033 | 0.055 | 0.006 | 0.121 | 0.163 |

Table 4.13 indicates that the mean values for the toxic metals showed negative correlation with the pH which was significant with zinc and iron. Negative correlation in this case showed that the lower the pH, the higher the levels of toxic metals in the alcoholic beverages. Raw materials, preparation and handling introduce toxic metals into alcoholic beverages. Decreased pH of alcoholic beverages increased leaching of some metals into the acidic drinks (Laureys and Perinet, 1983).

## 4.5 The turbidity of alcoholic beverages

The mean levels of toxic metals were correlated to turbidity in table 4.14.

**Table 4.14: Correlation of turbidity with mean levels of toxic metals in alcoholic beverages**

| Toxic metal | Zn | Pb | Fe | Al | Mn |
|---|---|---|---|---|---|
| **Pearson's correlation** | 0.099 | 0.622 | 0.563 | 0.296 | 0.567 |
| **p-value** | 0.551 | 0 | 0 | 0.1 | 0.004 |

Table 4.14 indicates that the mean levels of the toxic metals showed positive correlation with the turbidity which was significant with lead, iron and manganese. Positive correlation in this case showed that the higher the turbidity, the higher the levels of toxic metals in the alcoholic beverages. Increase in turbidity indicated increase in number of suspended particles in alcoholic beverages and consequently increase in levels of toxic metals (Steiner, 2010).

# CHAPTER FIVE
# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Introduction

The conclusions made in this section are based on the results obtained from information obtained from the questionnaires, pH measurements, AAS analysis and GC analysis data. The objectives of this study were to determine the types of alcoholic beverages that are popularly taken in Muthithi, levels of toxic metals (Al, Cd, Cu, Fe, Mn, Pb and Zn), levels of organic contaminants (methanol, ethanol, propanol, amyl alcohol, methanal, ethanal, and ethyl ethanoate), turbidity and the pH of these alcoholic beverages. The following conclusions were made:

(i) There were many types of alcoholic beverages in Muthithi location including beers (branded and unbranded), spirits (branded and unbranded) and wines. The most popular alcoholic beverage among them was Keg Beer.

(ii) All alcoholic beverages contained varying levels of organic compounds which were within acceptable limits.

(iii) All alcoholic beverages had one or more toxic metal level beyond maximum allowable levels (MAL).

(iv) The alcoholic beverages had varying pH values below 7 that showed negative correlation with levels of toxic metals.

(v) The alcoholic beverages had varying turbidity values that showed positive correlation with levels of toxic metals.

## 5.2 Recommendations from this study

(i) Preparation, storage and serving of alcoholic beverages should be done hygienically using recommended materials and procedures to avoid contamination.

(ii) All alcoholic beverages should be packed and their labels to bear information on levels of various organic compounds, toxic metals, pH, turbidity and the actual alcoholic strength.

(iii) Residents of Muthithi should be sensitized on the levels of organic contaminants and toxic metals in alcoholic beverages.

(iv) Residents of Muthithi should also reduce quantities of alcoholic beverage taken per sitting and number of drinking days per week for better health and productivity.

## 5.3 Recommendations for further work

(i) More types of alcoholic and other types of beverages from various regions should be analysed for pH, toxic metals, organic contaminants and turbidity.

(ii) The levels of toxic metals and organic compounds in containers used during preparation, storage and serving of alcoholic beverages need to be determined.

(iii) There is need to assess the effect of seasonal variations on level of pH, toxic metals, organic contaminants and turbidity.

(iv) Determination of biological toxins such as mycotoxins and immunoassays on undistilled alcoholic beverages need to be carried out.

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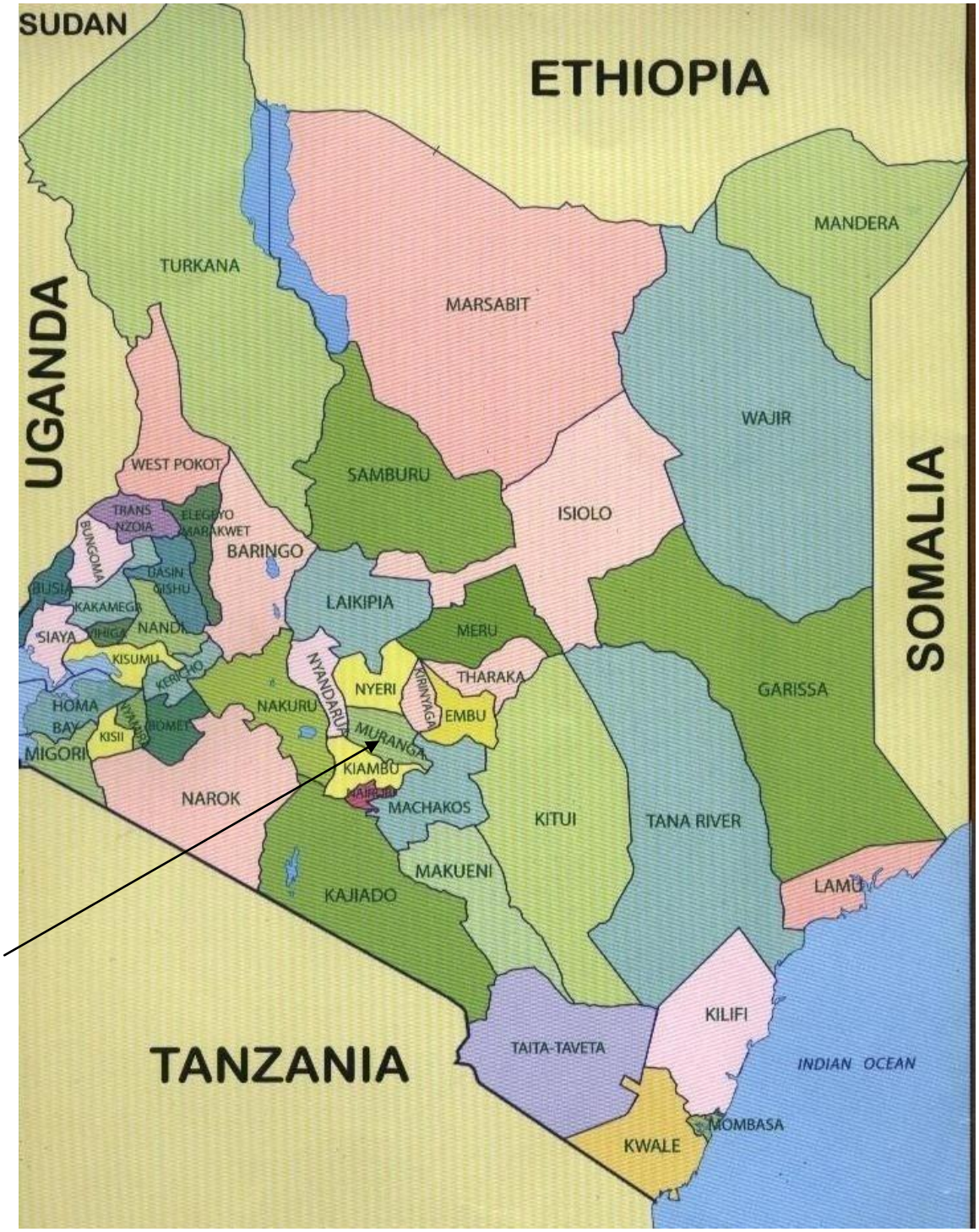
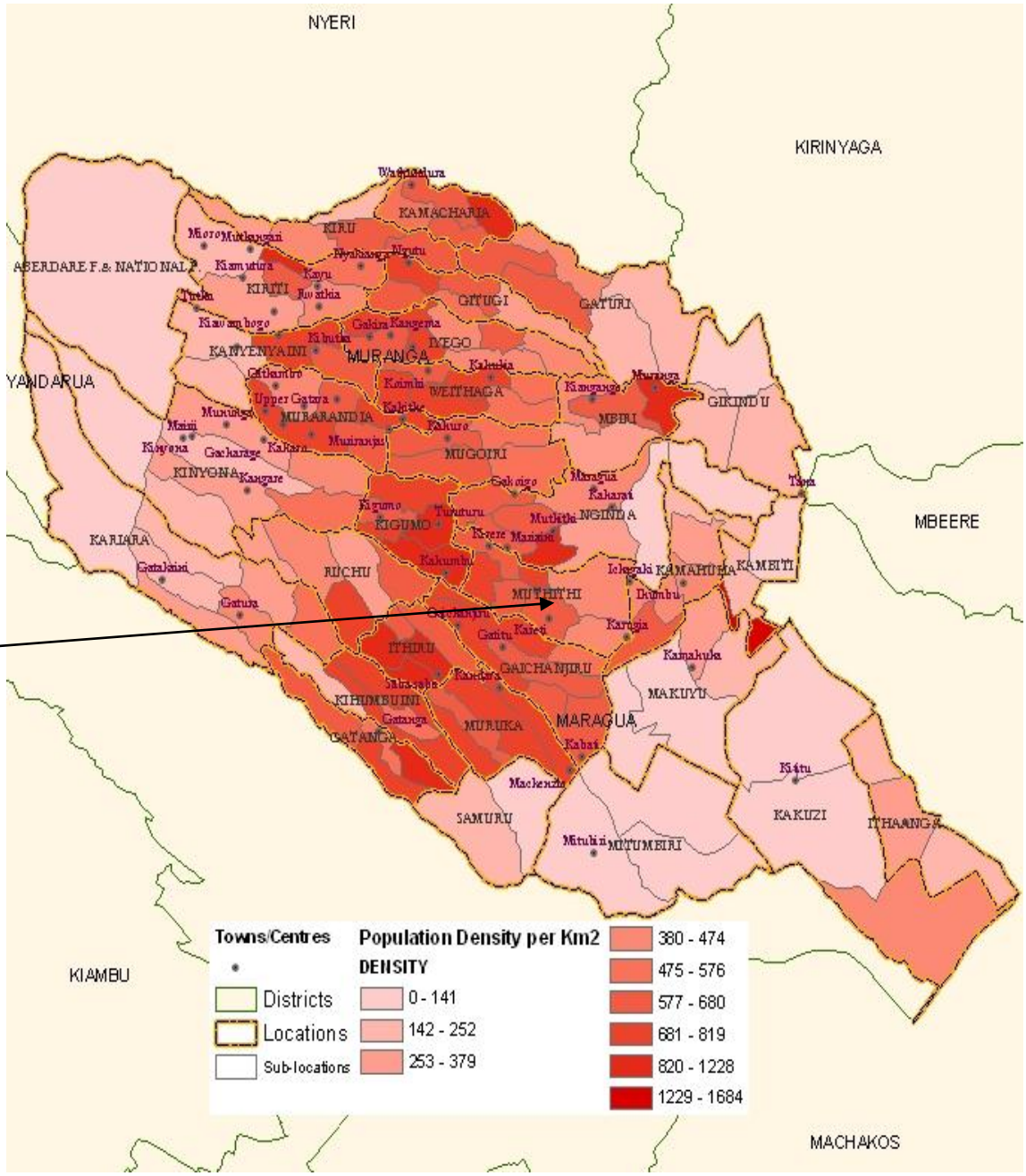
# APPENDICES

## Appendix I: Map of Kenya showing Muranga County



**Appendix II: Map of Muranga County showing Muthithi location**



**Appendix III: Research approval from Institute for Research Science and Technology of Kenyatta University**

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

**FROM:** Dean, Graduate School **DATE:** 3rd October, 2011

**TO:** **Githinji Peter Kaguru**
**C/o Chemistry Dept.** **REF:** 156/CE/15492/08

**SUBJECT: APPROVAL OF RESEARCH PROPOSAL**

This is to inform you that Graduate School Board, at its meeting of **28th September 2011**, approved your Research Proposal for the M.SC Degree.

You may now proceed with your data collection.

Thank you.

**JOHN M. ODONGI**
**FOR: DEAN, GRADUATE SCHOOL**

c.c. Chairman, Chemistry Department

Supervisors:

1. Dr. Ruth Wanjau
   C/o Department of Chemistry

2. Dr. Nicholas Gikonyo
   C/o Department of Pharmacy and Complementary/Alternative Medicine

JMO/bwk

*Committed to Creativity, Excellence & Self-Reliance*

**Appendix IV: Research permit from National Council for Science Technology and Innovation (NACOSTI)**

REPUBLIC OF KENYA

**NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY**

Telegrams:"SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123.
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RRI/12/1/BS-011/94/4**

Date: **21st November, 2011**

Peter Kaguru Githinji
Kenyatta University
P. O. Box 43844
NAIROBI

**RE: RESEARCH AUTHORIZATION**

Following your application for authority to carry out research on "***Determination of some organic contaminants & toxic metals in selected alcoholic beverages brewed & consumed in Muthithi Location Muranga County Kenya***" I am pleased to inform you that you have been authorized to undertake research **in Kigumo District** for a period ending ***31st August 2012.***

You are advised to report to **the District Commissioner & the District Education Officer, Kigumo District** before embarking on the research project.

On completion of the research, you are expected to submit **one hard copy and one soft copy** of the research report/thesis to our office.

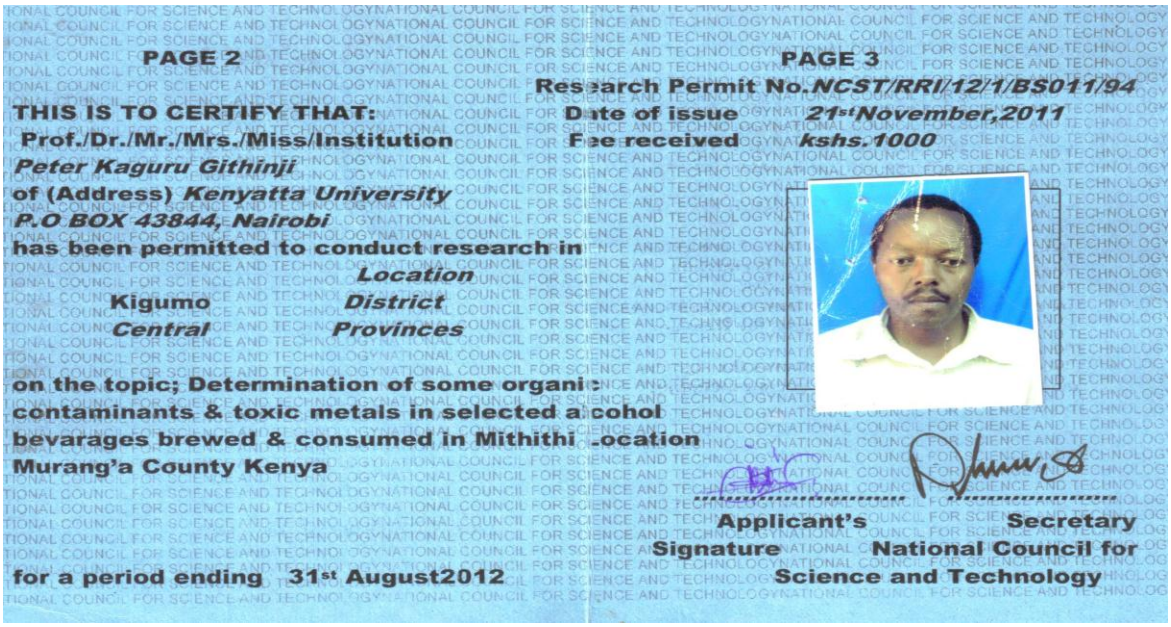
**P.N. NYAKUNDI**
**FOR: SECRETARY/CEO**

Copy to:

The District Commissioner
Kigumo District

The District Education Officer
Kigumo District

**Appendix V: Researcher's identification card (ID) from National Council for Science Technology and Innovation (NACOSTI)**



PAGE 2

THIS IS TO CERTIFY THAT:

Prof./Dr./Mr./Mrs./Miss/Institution *Peter Kaguru Githinji*

of (Address) *Kenyatta University P.O BOX 43844, Nairobi*

has been permitted to conduct research in *Location* Kigumo *District* *Central* *Provinces*

on the topic; Determination of some organi: contaminants & toxic metals in selected a cohol bevarages brewed & consumed in Mithithi .ocation Murang'a County Kenya

for a period ending 31st August2012

PAGE 3

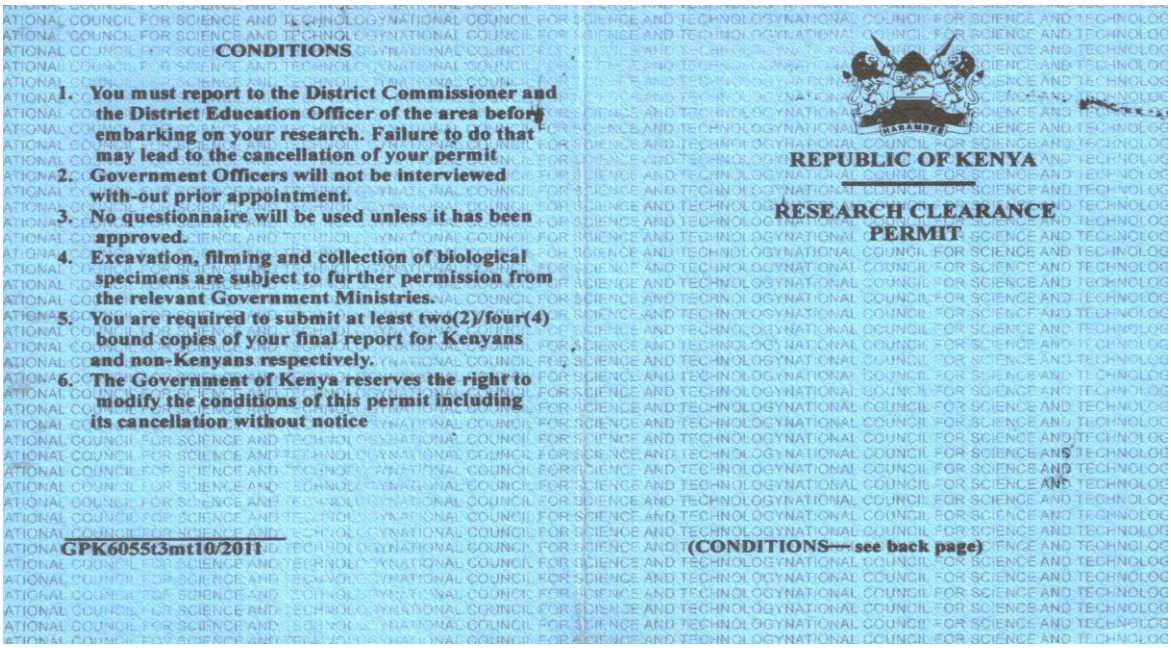
Research Permit No. *NCST/RRI/12/1/BS011/94*

Date of issue *21st November,2011*

Fee received *kshs.1000*

Applicant's Signature

Secretary National Council for Science and Technology



CONDITIONS

1. You must report to the District Commissioner and the District Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed with-out prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2)/four(4) bound copies of your final report for Kenyans and non-Kenyans respectively.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

GPK6055t3mt10/2011

REPUBLIC OF KENYA

RESEARCH CLEARANCE PERMIT

(CONDITIONS— see back page)

**Appendix VI: Informed consent**

## INFORMED CONSENT

I am **Peter Kaguru Githinji**, a Kenyatta University masters student in Applied Analytical Chemistry carrying out a research in a study - **Determination of pH, selected organic contaminants and toxic metals in some alcoholic beverages consumed in Muthithi, Muranga, Kenya.** I wish to recruit you to participate in answering a few questions by filling a questionnaire on alcoholic beverages consumed in Muthithi location of Muranga County.

This study will benefit you by finding out if the alcoholic beverages being consumed here are safe.

Your participation poses no risks to you or others since the information given will be held in strict confidence. One may withdraw from the interview any time or withhold their names if they so wish.

In case of any question(s) please contact my supervisors:

i) Dr. Wanjau RN 0722423183

ii) Prof. Gikonyo NK 0722763186

Participant's name....…….…….. Sign…….…… Date……..

Researcher's name....…….…….. Sign…....…… Date……..

Tel 0723431626

**Appendix VII: Interview guide for community respondents**

**Instructions**

- Respondents will be residents of Muthithi location aged 18 years and above.
- Respondents shall be assured of confidentiality of information they give and will not be required to give their names.
- Respondents will give information willingly and will have a choice to opt out

| A1 Area code | A2 Number of interview | A3 Date of interview | A4 Initials of interviewer | A5 Initials of supervisor |
|---|---|---|---|---|
| | | | | |

1. Age of Respondent 1. Male 2. Female
2. Marital Status 1. Married 2. Divorced 3. Widowed 4. Separated 5.single
3. Education level 1. No formal education 2. Primary 3. Secondary 4. Tertiary
4. Occupation 1. Salaried 2. Business 3. Casual 4. Peasant farmer
5. Which are the common alcoholic beverages consumed in your area?
   1. Bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa
6. Which of the above drinks do you often take? 1. bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa
7. Why do you prefer taking the chosen drink named above? 1. It is cheap 2. It is available 3. It is strong 4. It is not harmful
8. How many drinks do you take in a sitting? 1. 1-2 2. 3-5 3. 5-10 4. Above 10
9. What are the immediate bad effects of drinking on you? 1. None 2. Weakness 3. Stomach upsets 4. Headache 5. Lack of appetite
10. What long term bad health effects has drinking had on you? Please give an example
    1. 2. 3. 4. 5. 6.

11. Which types of containers are used in preparation of the local alcoholic beverages? 1. Steel 2. Alluminium 3. Plastic 4. Earthen 5. Guards
12. What is added to the alcoholic beverages to make them good (strong) drinks?
    1. Torch cells 2. Battery acid 3. Formalin 4. Methanol 5. Pieces of metal 6. Inorganic fertilizer 7. Jik (household bleach) 8. Laundry blue 9. Any other? Please name
13. How many people have died or hospitalized in the last 6 months from consumption of local alcoholic beverages in this area? (please state when, how they were affected).

**Appendix VIII: Interview guide for opinion leaders**

**Instructions**

- Respondents will be leaders of Muthithi location (administrators, community leaders, religious leaders, serving or retired officers).
- Respondents shall be assured of confidentiality of information they give and will not be required to give their names.
- Respondents will give information willingly and will have a choice to opt out

1 What is the average age of people who drink alcohol in this area 1. 15-17years 2. 18-25years 3. 25-35years 4. 35-45years 5. 46years and above

2 Which are the common alcoholic beverages consumed in this area? 1. bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa 8. Other

3 Which of the above drinks do people prefer taking in this area? 1. bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa 8.other

4 Why do they prefer taking the drink named above? 1. It is cheap 2. It is available 3. It is strong 4. It is not harmful 5. Other

5 What are the immediate bad health effects of drinking on the people who take local alcoholic beverages? 1. None 2. Weakness 3. Stomach upsets 4. Headache 5. Lack of appetite 6. Other

6 What long term bad health effects have local alcoholic beverages drinking had on the residents? Please give an example 1. 2. 3. 4. 5.

7 How many people have been hospitalized or died from consumption of local alcoholic beverages in the last 6 months in this area?

8 Where do local alcoholic beverages being consumed in this area come from? 1. Within 2. Neighbouring villages 3. Towns

9 What do brewers or traders add to the local alcoholic beverages to make them good (strong) drinks? 1. Torch cells 2. Battery acid 3. Formalin 4. Methanol 5. Pieces of metal 6. Inorganic fertilizer 7. Jik (household bleach) 8. Laundry blue 9. Any other? Please name

**Appendix IX: Interview guide for health workers**

**Instructions**

- Respondents will be health personnel serving residents of Muthithi location.
- Respondents shall be assured of confidentiality of information they give and will not be required to give their names
- Respondents will give information willingly and will have a choice to opt out

1 What is the average age of people who drink alcohol in this area 1. 15-17years 2. 18-25years 3. 25-35years 4. 35-45years 5. 46years and above

2 Which are the common alcoholic beverages consumed in this area? 1. bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa 8. Other

3 Which of the above drinks do people prefer taking in this area? 1. bottled beer, Keg or spirits 2. Fortified wine (kali) 3. Muratina 4.Changaa 5. Busaa 6. Matinga 7. Miti ni dawa 8.other

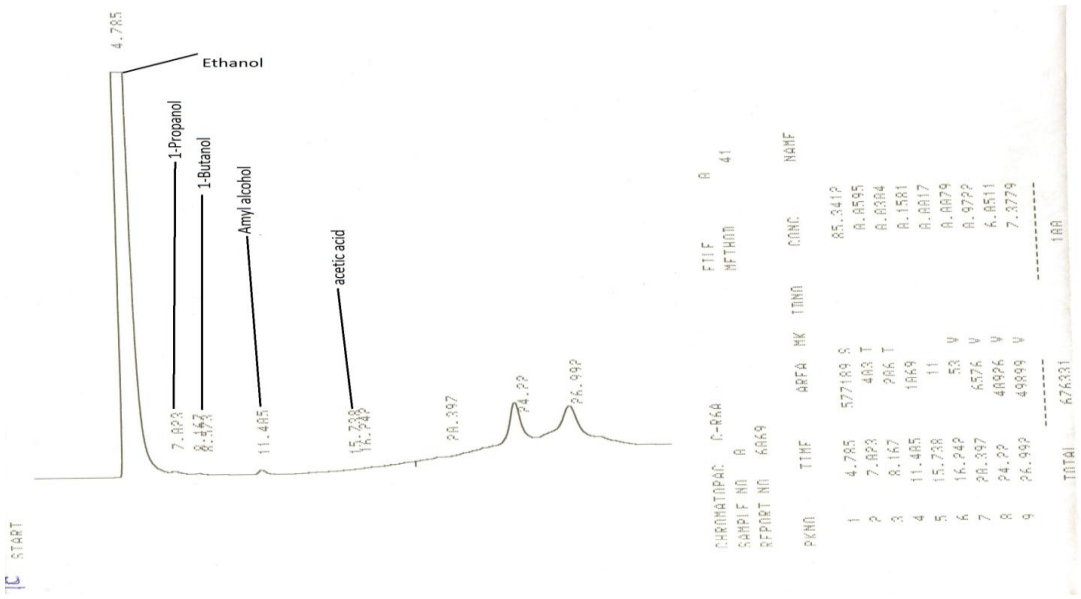
4 What are the immediate bad health effects of drinking on the people who take the preferred brew? 1. None 2. Weakness 3. Stomach upsets 4. Headache 5. Lack of appetite 6. other

5 What long term bad health effects have local alcoholic beverages drinking had on the residents? Please give an example 1. 2. 3. 4. 5.

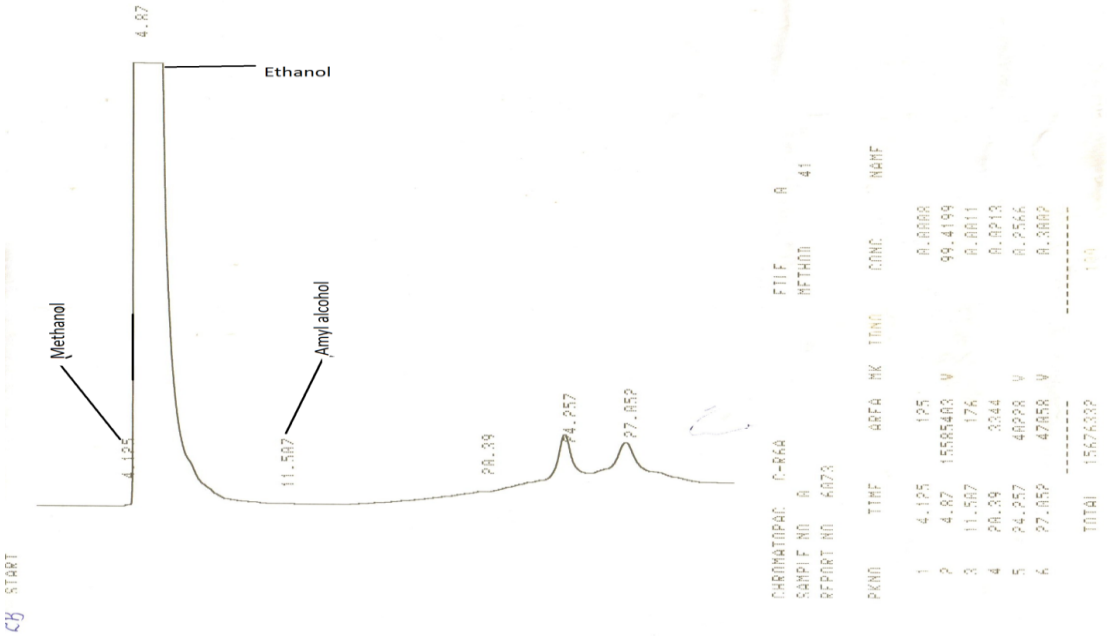
6 How many people have been hospitalized or died from consumption of local alcoholic beverages in the last 6 months in this area?

7 What do brewers or traders add to the local alcoholic beverages to make them good (strong) drinks? 1. Torch cells 2. Battery acid 3. Formalin 4. Methanol 5. Pieces of metal 6. Inorganic fertilizer 7. Jik (household bleach) 8. Laundry blue 9. Any other? Please name

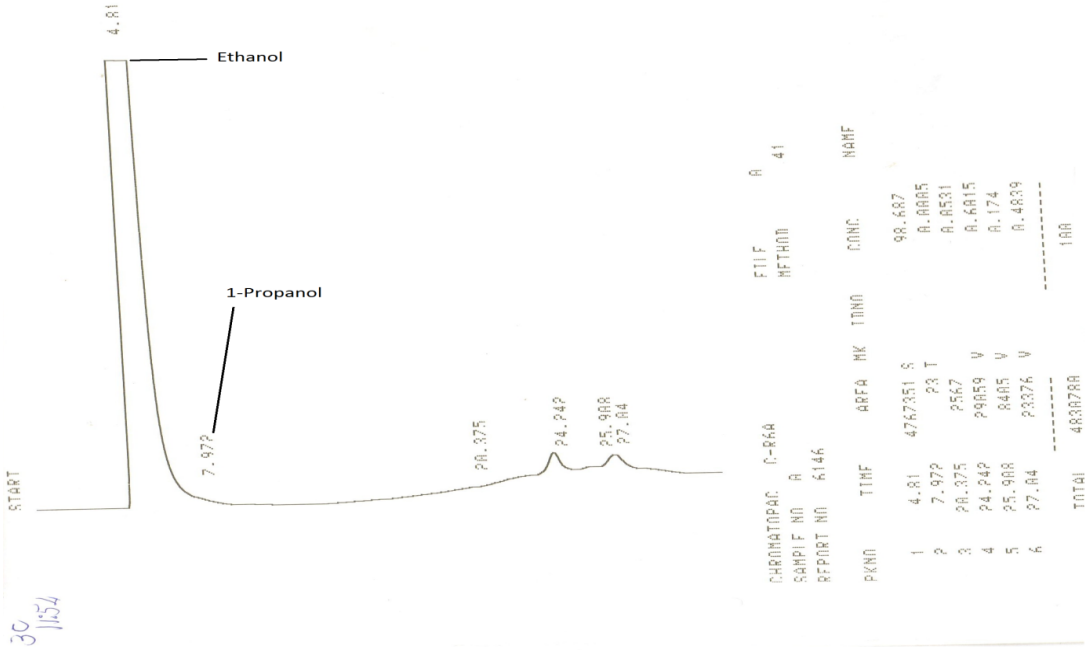
## Appendix X: The GC spectrum for Changaa B



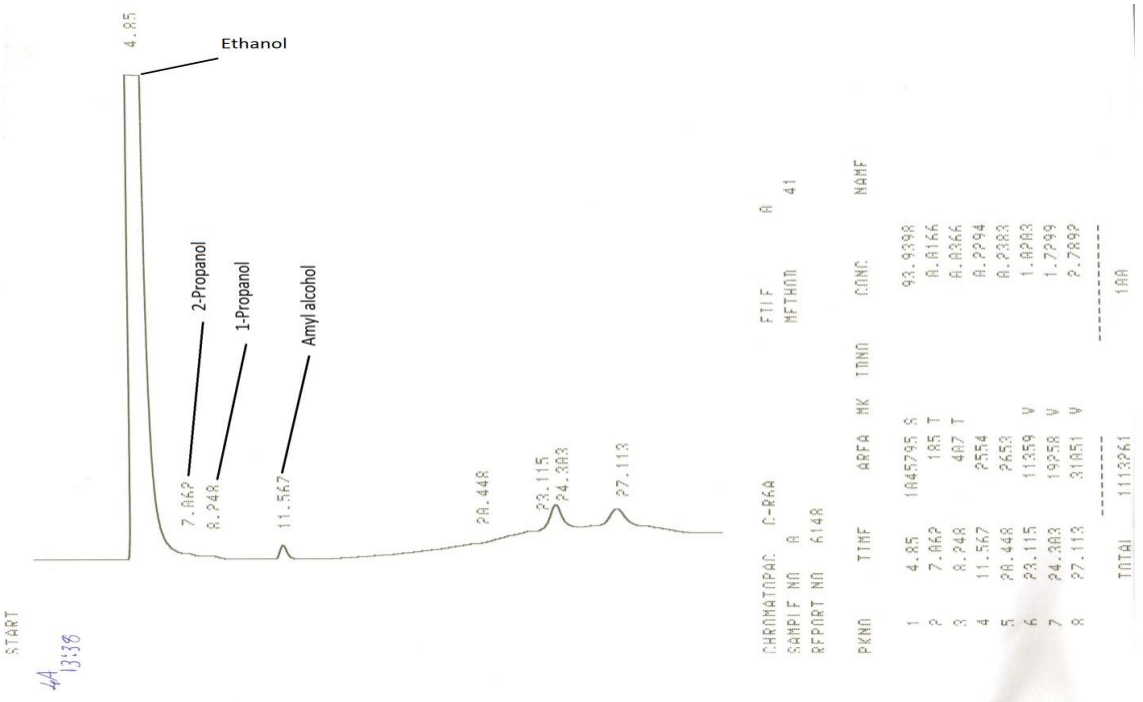

## Appendix XI: The GC spectrum for Spirit A



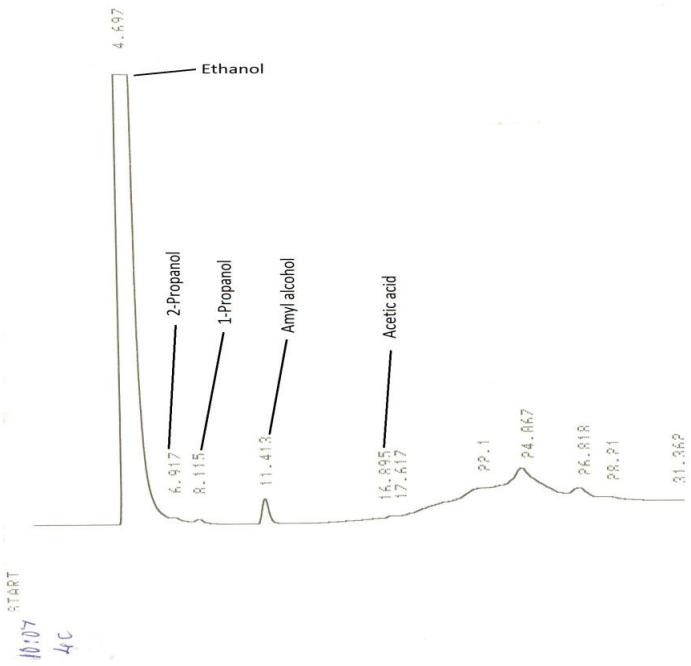
## Appendix XII: The GC spectrum for Spirit C




## Appendix XIII: The GC spectrum for Keg Beer A



## Appendix XIV: The GC spectrum for Keg Beer C

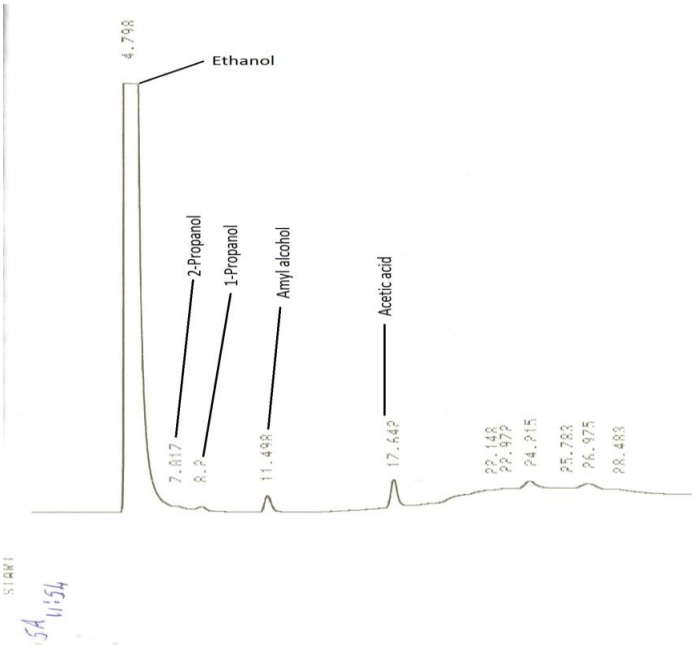



CHROMATOPAC C-R6A
SAMPLE NO A
REPORT NO 6153

FILE A
METHOD 41

| PKNO | TIME | AREA | MK | TYPE | CONC | NAME |
|---|---|---|---|---|---|---|
| 1 | 4.697 | 1358935 | S | | 98.83 | |
| 2 | 6.917 | 214 | T | | 0.0147 | |
| 3 | 8.115 | 807 | T | | 0.0555 | |
| 4 | 11.413 | 5026 | | | 0.3455 | |
| 5 | 16.895 | 78 | | | 0.0054 | |
| 6 | 17.617 | 654 | V | | 0.0449 | |
| 7 | 22.1 | 26988 | V | | 1.8549 | |
| 8 | 24.867 | 50893 | V | | 3.5815 | |
| 9 | 26.818 | 11642 | V | | 0.8004 | |
| 10 | 28.21 | 5989 | V | | 0.4063 | |
| 11 | 31.362 | 888 | V | | 0.061 | |
| TOTAL | | 1454525 | | | 100 | |

## Appendix XV: The GC spectrum for Muratina A

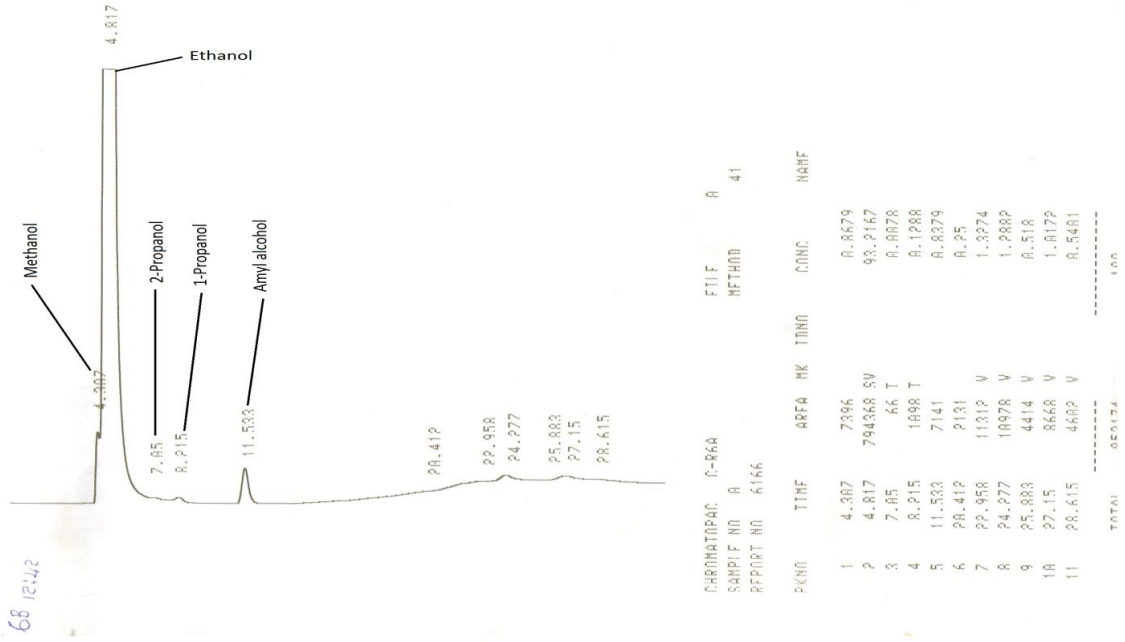



CHROMATOPAC C-R6A
SAMPLE NO A
REPORT NO 6155

FILE A
METHOD 41

| PKNO | TIME | AREA | MK | TYPE | CONC | NAME |
|---|---|---|---|---|---|---|
| 1 | 4.798 | 1179528 | S | | 95.2607 | |
| 2 | 7.017 | 96 | T | | 0.0091 | |
| 3 | 8.9 | 949 | T | | 0.0766 | |
| 4 | 11.498 | 3419 | | | 0.2761 | |
| 5 | 17.642 | 5101 | | | 0.412 | |
| 6 | 22.148 | 10169 | | | 0.8212 | |
| 7 | 22.972 | 6524 | V | | 0.5269 | |
| 8 | 24.915 | 13158 | V | | 1.0626 | |
| 9 | 25.783 | 5051 | V | | 0.4079 | |
| 10 | 26.975 | 10462 | V | | 0.8449 | |
| 11 | 28.483 | 3827 | V | | 0.3091 | |
| TOTAL | | 1238525 | | | 100 | |

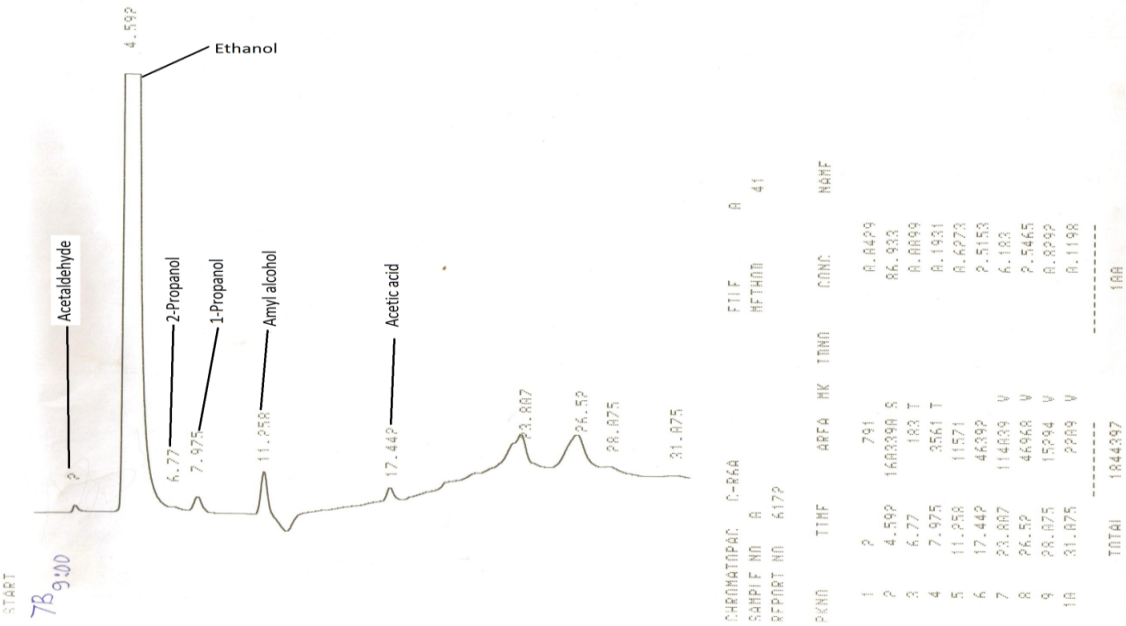
## Appendix XVI: The GC spectrum for Miti ni dawa A




CHROMATOPAC C-R6A FTIF A
SAMPLE NO A METHOD 41
REPORT NO 6166

| PKNO | TIME | AREA | MK | TYPE | CONC. | NAME |
|---|---|---|---|---|---|---|
| 1 | 4.807 | 7396 | | | 0.8679 | |
| 2 | 4.817 | 794368 | SV | | 93.2167 | |
| 3 | 7.85 | 66 | T | | 0.0078 | |
| 4 | 8.215 | 1098 | T | | 0.1288 | |
| 5 | 11.533 | 7141 | | | 0.8379 | |
| 6 | 28.419 | 2131 | | | 0.25 | |
| 7 | 29.958 | 11319 | V | | 1.3279 | |
| 8 | 24.277 | 10978 | V | | 1.2880 | |
| 9 | 25.883 | 4414 | V | | 0.518 | |
| 10 | 27.15 | 8668 | V | | 1.0172 | |
| 11 | 28.615 | 4602 | V | | 0.5401 | |
| TOTAL | | 852474 | | | 100 | |

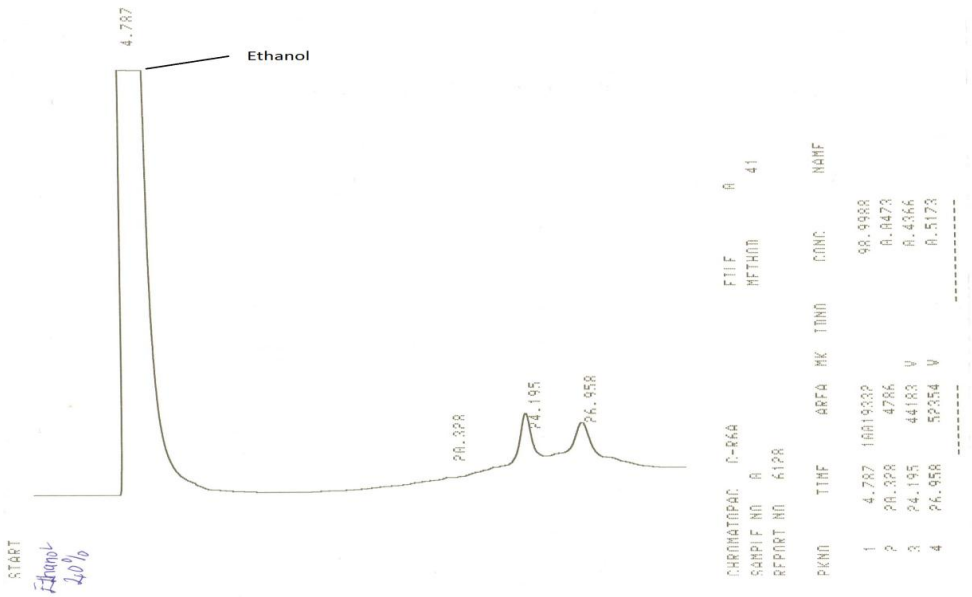
## Appendix XVII: The GC spectrum for Busaa A



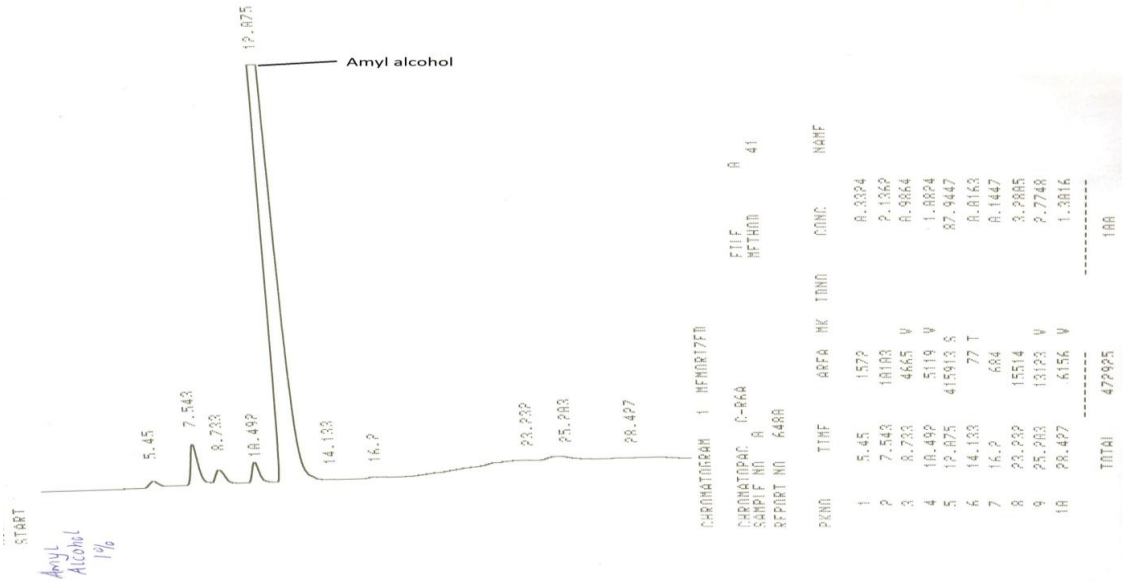

CHROMATOPAC C-R6A FTIF A
SAMPLE NO A METHOD 41
REPORT NO 6172

| PKNO | TIME | AREA | MK | TYPE | CONC. | NAME |
|---|---|---|---|---|---|---|
| 1 | P | 791 | | | 0.0429 | |
| 2 | 4.592 | 1683390 | S | | 86.933 | |
| 3 | 6.77 | 183 | T | | 0.0099 | |
| 4 | 7.975 | 3561 | T | | 0.1931 | |
| 5 | 11.958 | 11571 | | | 0.6273 | |
| 6 | 17.442 | 46398 | | | 2.5153 | |
| 7 | 23.807 | 114039 | V | | 6.183 | |
| 8 | 26.52 | 46968 | V | | 2.5465 | |
| 9 | 28.875 | 15294 | V | | 0.8292 | |
| 10 | 31.875 | 2989 | V | | 0.1198 | |
| TOTAL | | 1844397 | | | 100 | |

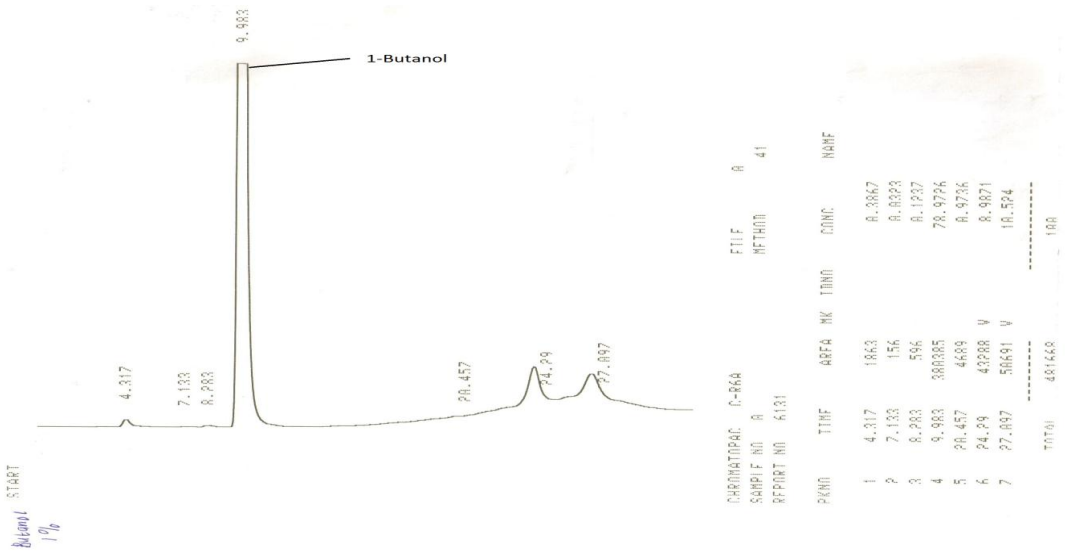
**Appendix XVIII: The GC spectrum for ethanol**



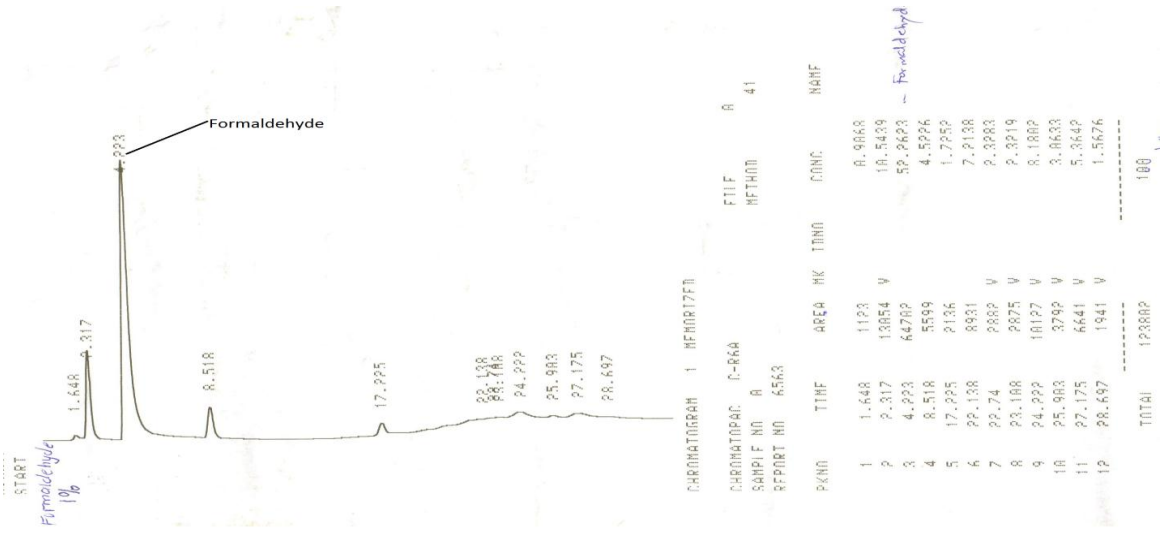

**Appendix XIX: The GC spectrum for amyl alcohol**



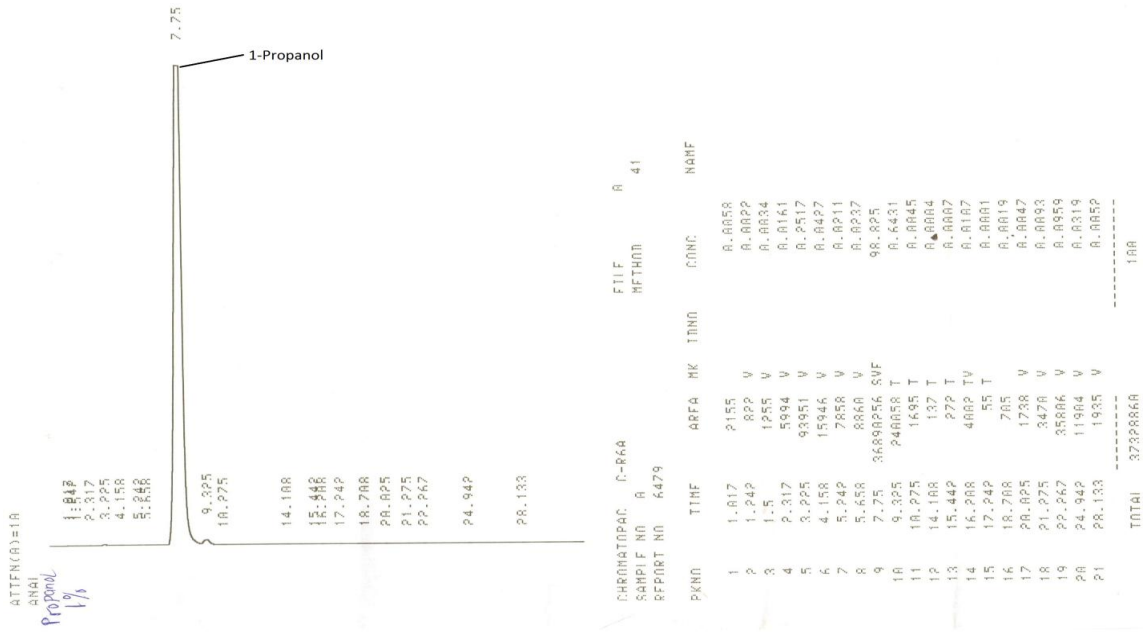
**Appendix XX: The GC spectrum for 1-butanol**



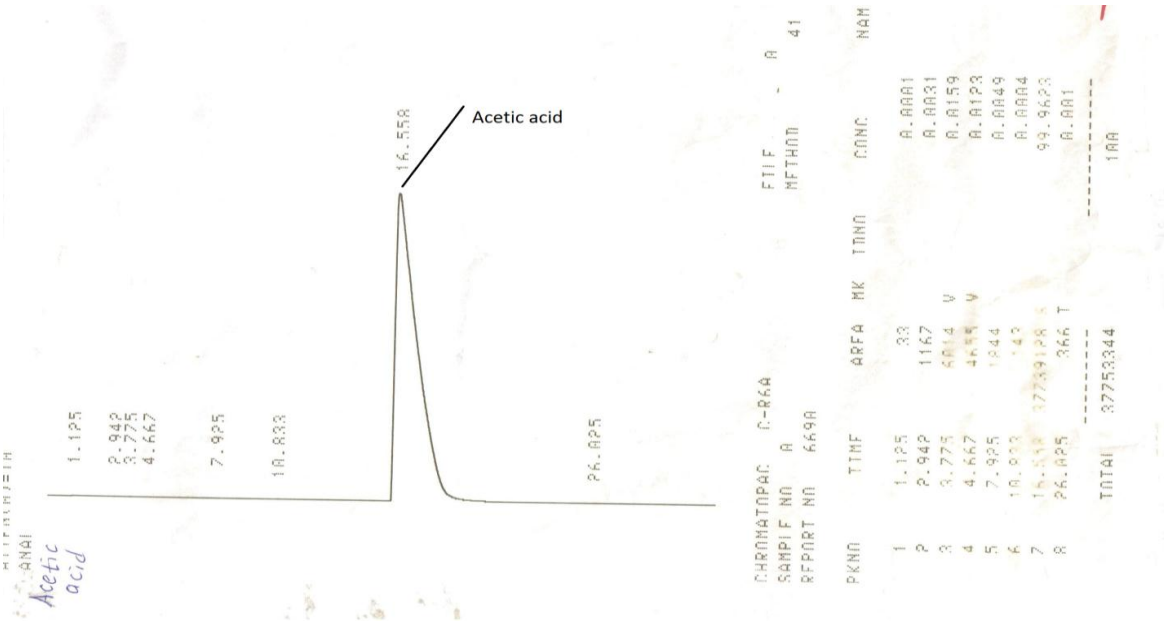

**Appendix XXI: The GC spectrum for methanal**



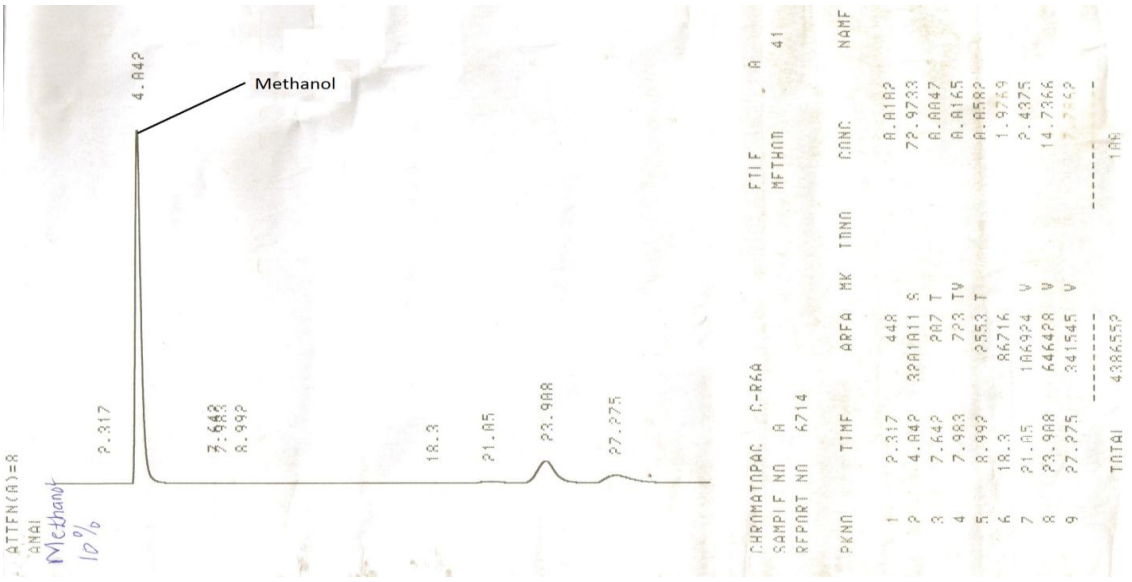
**Appendix XXII: The GC spectrum for propan-1-ol**



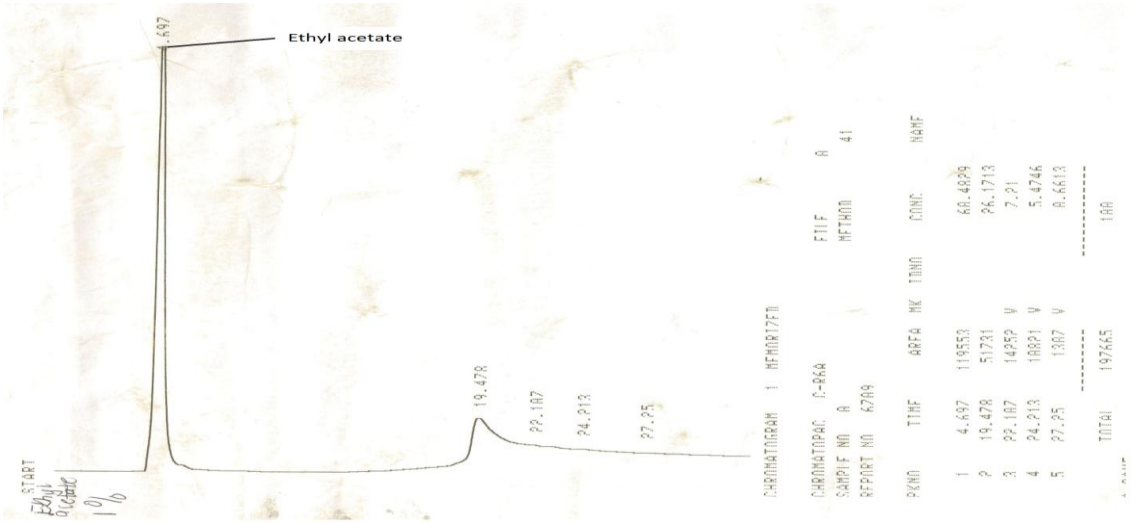

**Appendix XXIII: The GC spectrum for ethanoic acid**



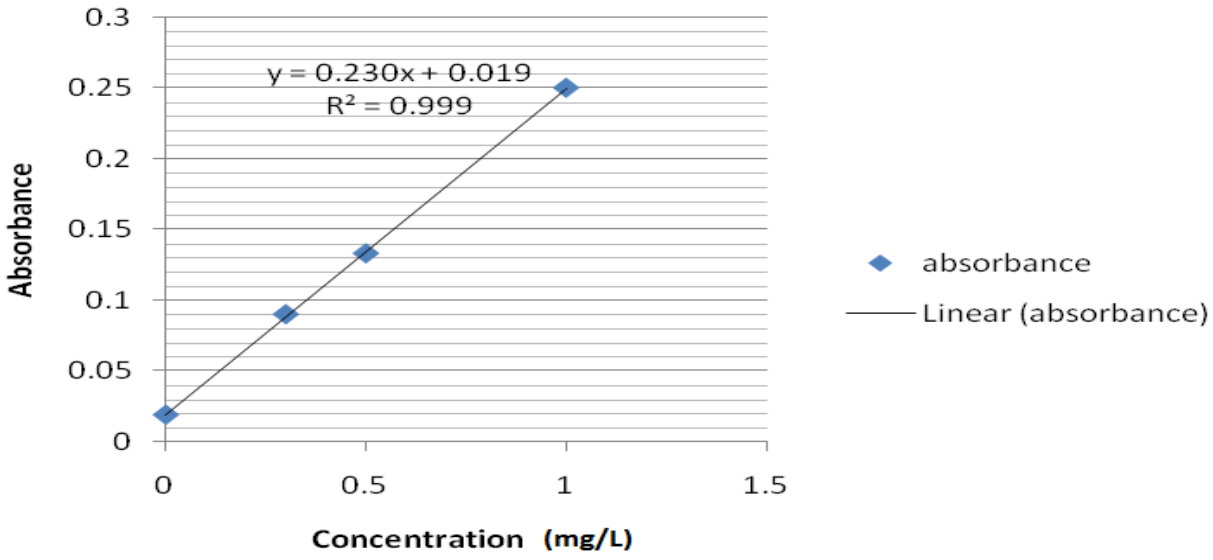
**Appendix XXIV: The GC spectrum for methanol**



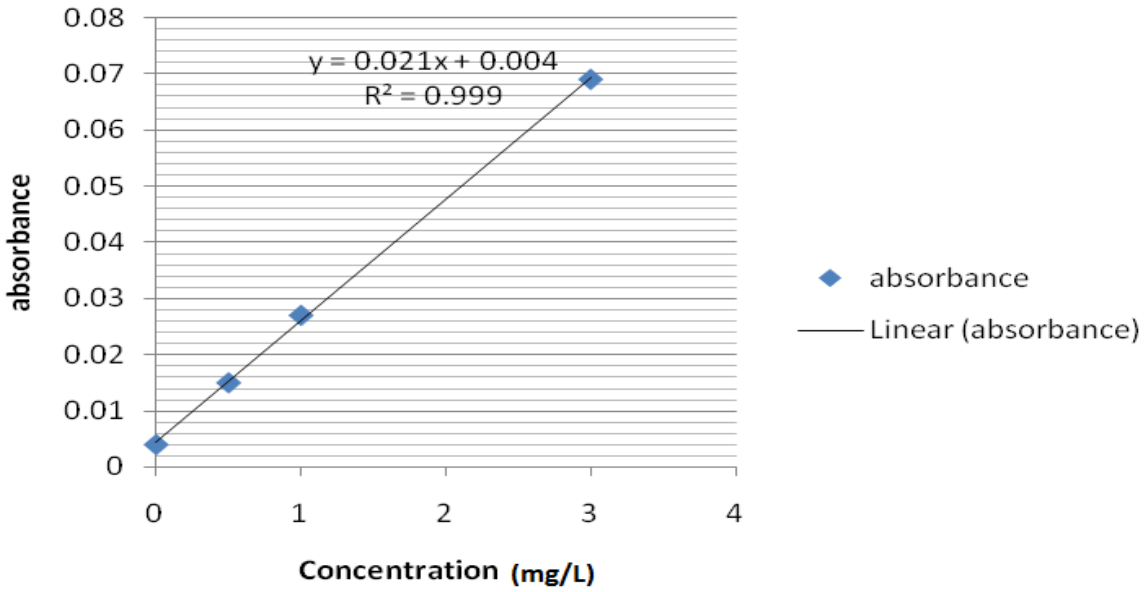

**Appendix XXV: The GC spectrum for ethyl ethanoate**



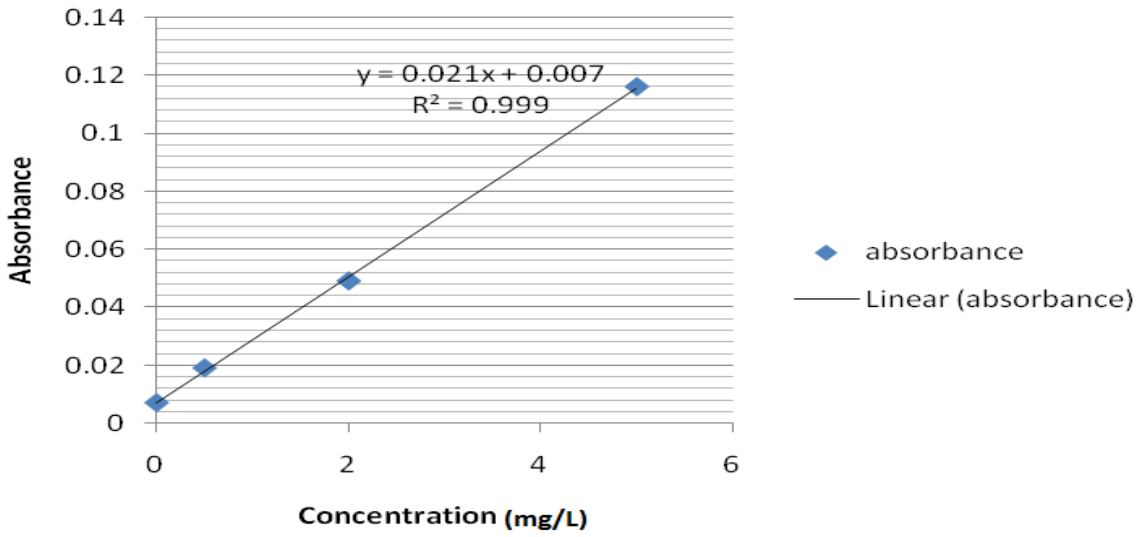
**Appendix XXVI: Calibration curve for Zn**



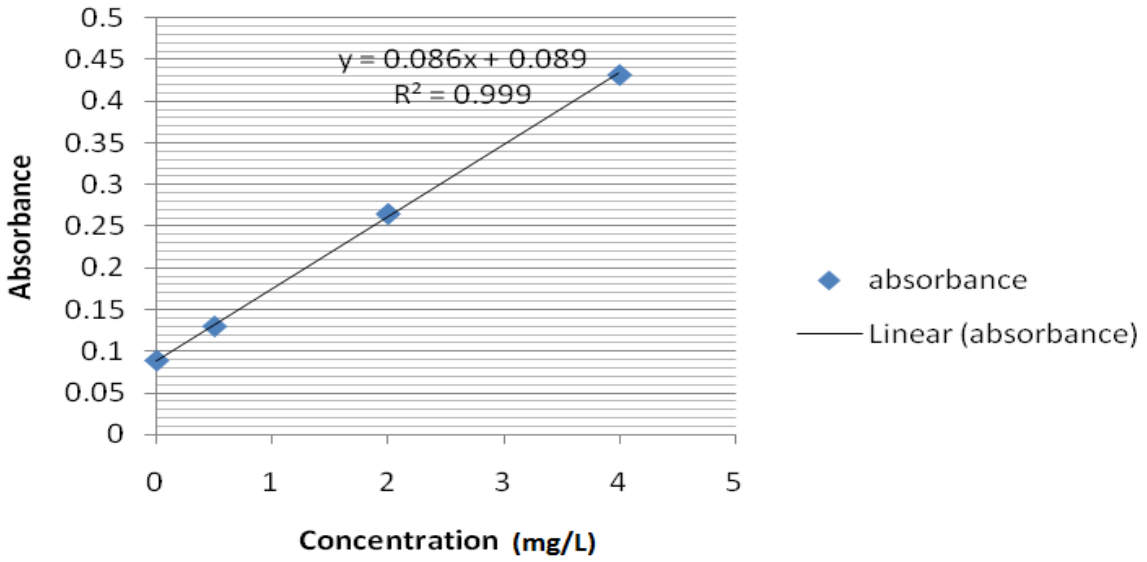

**Appendix XXVII: Calibration curve for Pb**



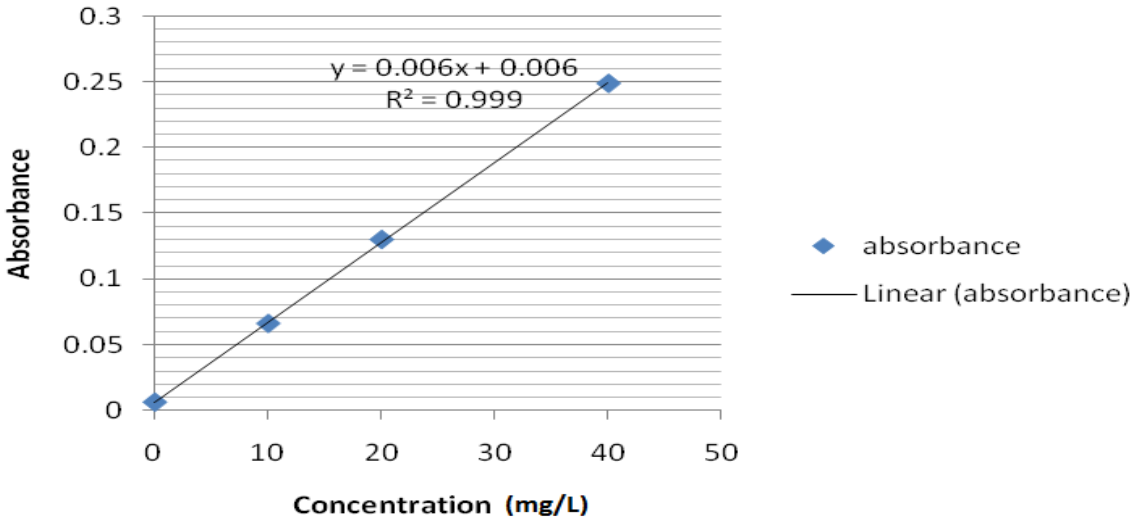
**Appendix XXVIII: Calibration curve for Fe**



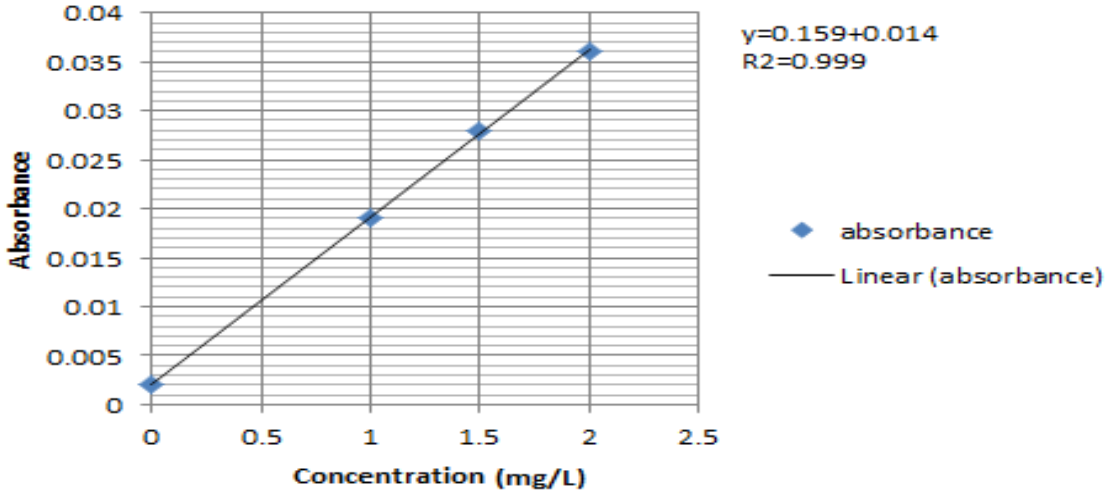

**Appendix XXIX: Calibration curve for Mn**



**Appendix XXX: Calibration curve for Al**




**Appendix XXXI: Calibration curve for Cd**



**Appendix XXXII: Calibration curve for Cu**