MICROBIAL AND HEAVY METAL CONTAMINATION IN HERBAL MEDICINES IN UGANDA.

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DECLARATION

I declare that this thesis is my own original work and that it has never been submitted to any University or Institution of higher learning for any academic award. Where other people's work has been used, this has properly been acknowledged and referenced in accordance to Kyambogo University requirement

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APPROVAL

This is to certify that this thesis was under my supervision and is now ready for submission to the faculty of Science of Kyambogo University with my approval.

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DEDICATION

My gratitude is expressed to my wife Musoke Immaculate for her continued encouragement, support, and sacrifices throughout my educational pursuits and daily life.

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ABSTRACT

The use of herbal medicines in various countries has been on the increase over the years for primary health care since they are cheap and easily accessible. However, herbal medicines present safety concerns which are continuously undermined by practitioners who use unhygienic methods of preparation. This subjects them to relatively high risks of contamination by pathogenic microbes, organic and inorganic pollutants, mycotoxins, endotoxins, and agrochemical residues.

This research focused on heavy metal and microbial contamination in different herbal medicines sold in Kampala Capital City markets. Five different herbal medicines used for treatment of ulcers, cough, malaria, and diabetes were randomly selected from different markets in Katwe, Nateete and Mengo markets within Kampala Capital City Authority. All the herbal medicines were analysed for microbial contamination and only 14 herbal medicines were tested for heavy metal toxicity. Bacterial isolates were identified basing on morphological, cultural and biochemical tests. Heavy metals were quantitatively determined using atomic absorption spectrophotometry.

The study focused on contamination with most prevalent micro-organisms found in unhygienic and polluted environments of *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus* and heavy metals of Nickel, Chromium, lead, and cadmium and their concentrations in ready to use herbal medicines of cough, malaria, ulcer and diabetes.

Approximately 90% of the herbal medicines analysed were found contaminated with pathogenic bacteria, of which *Staphylococcus aureus*, *Escherichia coli,* were the most prevalent micro-organisms. Herbal medicines used for the treatment of ulcer and cough were found having the highest contamination by microbes of about 65%, diabetes and malaria with 25% contamination. Heavy metal contamination was about 85% of which lead and Nickel were abundant in most herbal medicines analysed in the ranges of 0.0120 to 2.0808

for lead and 0.0120 to 0.9548 for Nickel. Cadmium and Chromium were not detected. About 40% of the ulcer and malaria herbal medicines analysed showed high Nickel concentration to about 60%, while lead contamination predominated to about 75% in most therapies. The concentrations of lead and Nickel were higher than the maximum permissible daily limits in 60% of all herbal medicines analysed. Tests for specific micro-organisms were carried out and samples showed contamination by *Escherichia Coli, Staphylococcus, aureus,* fungus and moulds but none of the samples were contaminated by Salmonella species. Contamination with toxic heavy metals may be as a result of polluted environment, soils and methods used for preparation.

CHAPTER ONE

1.0 INTRODUCTION

1.1Background

The use of herbal medicines is an old tradition which still makes an important part in traditional medicines even in the 21st century due to their being cheap with a variety of biological roles and public perception as being safe with less side effects compared to western medicines. Herbal medicines are plant materials used as alternative medicines for treatment of many infections, injuries, and wounds. Traditional medicines have been used by the world population as they are accessible, affordable and easily administered compared to western medicines. Herbal medicines are prepared from leaves, roots, tree back, fruits, soil, animal products, in form of infusions, decoctions, tinctures, and water baths. (Barnes, 2003, Alqosouimi, 2006; Zaleska, 2008).

In Africa, it's estimated that over 70% of the population use alternative medicines for treatment of diseases such as malaria, cough, asthma, diabetes, ulcers, and other complications. A variety of herbal medicines are increasingly sold in Kampala markets and about 80% of Ugandans rely on these therapies.

The continuous demand of these herbal medicines has led to exposure of these therapies to different market environments which have high pollution rates that subject these alternative medicines to a number of risks. As a result of this rate of pollution in the environment heavy metal contamination has been reported in our soils, urban areas, swamps and waters (Muwanga 1997; Zvinowanda *et al.*, 2009).

Unhygienic storage facilities, improper handling and sales infrastructure within the market are possible sources of microbial contaminants of these herbal medicines (Agency for Toxic Substances and Disease Registry, 2008).

A number of agro-chemicals and fertilizers applied by farmers have been reported to contain heavy metals such as cadmium and lead that finds their way in soils through drainage and erosion (Barnes, 2003). As a result, the plants and waters which are the key materials used in herbal preparations are likely to be contaminated.

The environment from which these raw materials are obtained is highly polluted with industrial fumes, microbes such as fungi and bacteria, sewage, dust and smoke (Rao *et al.,* 2009). Heavy metals exist naturally in the earth's crust, polluted air and these metals through a series of food chains enter into plants from where they are taken into the human body through plant extracts which are used as herbal medicines (Marina *et al* 2009). Accumulation of heavy metals in the body cause health risks such as cancer, hypertension, kidney and liver dysfunction (Robert *et al*., 2008, Martena &Annet 2010). These bio accumulate in the body when ingested through fluids and their rate of storage in the body is higher than that at which they are excreted (Philips W, Balge P 2007; Zaleska, 2008). Many studies have reported microbial contaminants with *Escherichia coli, Staphylococcus aureus* being most prevalent and contents of heavy metals in herbal medicines. Herbal medicines require vigorous scrutiny in terms of contaminants such as heavy metals and microbial contaminants. These are likely to result into a number of side effects rendering these therapies unsafe for consumption (Annan *et al.,*2006) hence a need for an investigative study on the safety of these herbal medicines.

1.2 Statement of the Problem

Herbal medicines are used in Uganda for treatment of various diseases. Due to the increasing rate of pollution in the environment, heavy metal and microbial contamination has been reported in herbal medicines globally. However, there is little information on microbial and heavy metal contamination of herbal medicines in Uganda. These contaminants are likely to be introduced into herbal medicines during preparation, use of untreated water, poor hygiene, poor storage, hence creating a threat to the users of these herbal medicines.

These contaminants cause health risks such as cancer, hypertension, kidney and liver dysfunction. This study aimed at assessing the bio safety of ready to use herbal medicines in terms of microbial and heavy metal contents.

1.3 Justification of the Study

Herbal medicines are used in Uganda for treatment of various diseases. Microbial and heavy metal contamination has been reported in our environment where materials used for herbal medicine mixtures are obtained. Herbal medicines have been used without considerations of bio safety in terms of contaminants such as microbial and heavy metals. Therefore, this research study concentrated on the analysis of contaminants from herbal medicines of cough, malaria, ulcers and diabetes collected from Katwe, Mengo and Nateete markets.

1.4 Objectives of the Study

1.4.1 General objective

To assess the safety of herbal medicines due to heavy metals and microbial contaminants in selected ready to use herbal medicines sold around Kampala Capital City markets.

1.4.2 Specific Objectives

The specific objectives of the study were:

1) To culture and identify microbial contaminants in ready to use cough, malaria, ulcer and diabetes herbal medicines sold in Kampala markets.
2) To isolate micro-organisms in ready to use cough, malaria, ulcer and diabetes herbal medicines sold on Kampala markets.
3) To determine heavy metals concentration in herbal medicines used for treatment of cough, malaria, ulcer and diabetes in Kampala, Uganda.

1.5 Scope of the Study

1.5.1 Geographical Scope

The study was carried out on cough, malaria, ulcers and diabetes herbal medicine collected from Katwe, Mengo and Nateete Kampala Capital City markets.

1.5.2 Content Scope

The focus was on the bio safety of herbal medicines in terms of heavy metals, Ni, Cd, Cr, Pb and microbial contaminants; *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes* and fungus. Herbal medicines of cough, malaria, ulcer and diabetes were collected randomly from herbal clinics with in the markets and a total of twenty samples were subjected for analysis in this study.

1.5.3 Time Scope

The study was conducted in the months of December 2018 and March 2019

1.5.4 Significance of the Study

The results of this study would benefit to the following:

The result of this study will be used to create awareness of safety and health risks associated with the use of herbal medicines sold in Uganda. The research will be a basis for further researchers and scholars who would be interested in carrying out more research in a similar related field.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Herbal Medicine

Herbal medicines are alternative forms of medicines extracted from plants. They contain either raw or processed ingredients from one or more plants. They can be from a different part of the plant such as roots, leaves, bark, fruits and sometimes from animal products including honey and soil. Among the herbal medicines sold in Ugandan markets includes; Mumbwa, Kisakyamuzadde, Kabuti, and are usually administered orally and sometimes topical.

2.1.1 Preparation of herbal medicines

Herbal medicines are prepared in different ways and the method of preparation depends on the plant material to be used and the condition to be treated. They include; Infusions, decoctions, tinctures and macerations, bath remedies, poultices and Compresses**.**

2.1.2 Infusions

These are hot teas which are usually prepared from fresh plants and tender leaves and the method of preparation involves heating water to boiling and then added on to the herbs, covered and allowed to settle for a period of 15 minutes. The amount of water to be added in the herbal preparation depends on the plant used and the form in which the herb is to be used. Preparations of infusions are best when required and are advantageous in that they can be taken when hot, warm and cold.

Ceramic pots are the best to be used for preparing infusions rather than metal containers since metal containers pause a risk of poisoning the herbal remedy (Taylor *et al.*, 2004)

2.1.3 Decoctions

These are boiled teas obtained from tough fibrous plants, barks and roots. The preparation involves boiling the plant material thoroughly well so that the hard-woody plant material is softened. This strong boiling serves to release active compounds. Ceramic pots are used for preparing decoctions and the amount of herb required during preparation is measured. Then, an appropriate amount of cold water added on to the herb according to the quantity of the remedy to be prepared. The cut pieces of the plant material are boiled, stirred thoroughly and pressed so as to get a good amount of the decoction. When the plant material used is powdered, the powder should be allowed to settle at the bottom of the pot and then decant off the decoction (Taylor *et al.*, 2004).

2.1.4 Tinctures

These are mixtures of water and alcohol used when the plant material to be used contains active ingredients which do not dissolve readily in water and when the herbal remedy is to be preserved for some time. The composition of water and alcohol in the different herbs varies since some active compounds dissolve more readily in alcohol than in water. During preparation of tinctures, the cut pieces of the plant material are put into a glass container and a mixture of alcohol and water added.

The container is properly sealed and stored in a dark place at temperatures of 27^0C for a period of two weeks with daily shaking of the container in order to soak the extract. After this period, the tincture is filtered in a clean container to separate the plant material from the extract. This method of preparation is advantageous in that it concentrates the active chemicals through using alcohol since the method uses a high plant to liquid ratio (Manabe *et al.*, 2011).

2.1.5 Macerations

These are commonly called cold-soakings and are prepared by covering the plant material in cold water and soaked overnight, later, the herbal remedy is filtered and ready for use. This method uses flesh plant materials and plants with chemicals which cannot withstand heating and strong alcohol (Watanabe *et al.*, 2011). During preparation, the plant material is crushed into powder, then put in a container and cold water added with stirring followed by filtering to obtain a clean liquid.

2.1.6 Bath remedies

During this preparation, the herbal plant material is crushed and soaked in cold water and the patient uses the medicine as a bath. As the patient bathes the herbal medicine, the active plant chemicals are absorbed by the skin, and to the fat tissue under the skin and later to the blood stream where it's transported to the body parts (Watanabe *et al.*, 2011).

2.1.7 Poultices and Compresses.

Poultices are usually applied directly on the skin on rashes and wounds topically as pain killers. These herbal medicines are obtained from flesh leaves and roots and during preparation hot water is added on the plant material so that it softens. The moist herbal remedy is placed directly on the skin or between a cloth and covered on the affected part of the skin. Compresses involve soaking a piece of cloth or bandage in prepared infusion, tincture or decoction and then laid onto the affected area of the skin (Watanabe *et al*., 2011).

2.2 Biological Contaminants in Herbal Medicines

2.2.1 Microbiological contaminants

Herbs and herbal materials normally carry a large number of bacteria and moulds, often originating in soil or derived from manure (Xu & Chan, 1994). Although a wide range of bacteria and fungi from the naturally occurring micro flora of medicinal plants, aerobic spore-forming bacteria frequently predominate (Wu, 1992; Wong *et al*., 1993). Practices of harvesting, production, transportation and storage may cause additional contamination and microbial growth (Ochieng *et al*., 2009).

The Proliferation of microorganisms may result from failure to control the moisture levels of herbal medicines during transportation and storage, as well as from the failure to control the temperatures of liquid forms and already manufactured herbal products (Xu & Chan, 1994).

The presence of *Escherichia coli*, *Salmonella* spp. and moulds are indicators of poor quality of production and harvesting practices. Microbial contamination also is likely

to enter herbal medicines through poor handling by personnel who are infected with pathogenic bacteria during harvest/collection, post-harvest processing and the manufacturing process.

2.3 Health effects of Micro-Organisms

When exposed to open air, these remedies contaminated with microbes which poses serious effects on patients and when taken in can interfere with the various immune responses and reactions of the lungs causing a threat to human health (Gerberick 1984). During the development and growth process of bacteria, they usually inhabit plants and it has been reported that these pathogenic bacteria are a source of various plant diseases (Vidhyasekaran 2002). Microorganisms find their way into herbal medicines from raw materials used in preparation and sometimes use of contaminated equipment during processing (Esimone *et al*., 2002).

Microorganisms degrade the raw materials used in preparations of herbal medicines before harvesting and also during long time storage and changing climatic conditions rendering these plant and animal products harmful for human consumption (Vidhyasekaran 2002).

The components and constituents of these herbal medicines are sometimes substituted either by error or unscrupulous practice hence a need of quality control measures to detect these contaminants and this can be affected using application of standards (Leung *et al*., 2006). The total number of bacterial contaminants in herbal medicines can be estimated by plate counting of bacterial colonies.

Contamination of herbal medicines with micro-organisms such as *Escherichia coli, Enterobacter aerogenes* has been reported and these are known pathogens, and common causative agents of childhood diarrhoea of bacterial origin (Bonkoungou *et al*., 2013).

2.3.1 Escherichia coli

Escherichia coli well known for causing severe stomach cramps, diarrhoea which is often bloody and vomiting and perhaps slight fever (Bonkoungou *et al*.,2013). They produce toxins that can attack the body organs such as the gut causing bloody diarrhoea, the kidneys resulting into kidney failure and sometimes the nervous system. Several causes of diarrhoea have been attributed to *Escherichia coli* especially after consuming food which has been contaminated due to poor hygiene or contamination as a result of the application of animal manure to land on which crops are grown which are the raw materials for making these herbal medicines (Okunlola *et al*., 2007)

Escherichia Coli contamination may also result into pneumonia especially in patients with compromised immune system and those with conditions such as diabetes mellitus, alcoholism, and chronic obstructive pulmonary disease (Karen *et al*., 2013). *Escherichia Coli* is also known to cause urinary tract infections most especially in females. This is because their ureter is shorter and nearer to the anus and is associated with pregnancy and childbirth related constraints. People develop intestinal *Escherichia Coli* infections by eating contaminated food, touching infected animals, or swallowing contaminated water (Nordmann& Naas, 2009).

Some strains of *Escherichia coli* reside in the digestive tract and some strains have acquired genes that cause infections in the digestive tract and in other parts of the body, most commonly the urinary tract. These bacteria can also cause infections outside the intestine in case of damage for example, by an injury or a disorder, such as inflammatory bowel disease.

Then, the bacteria may leave the intestine and spread to nearby structures that lacking defence against them thus may enter the bloodstream (Bonkoungou *et al*., 2013).

2.3.2 Fungi and Moulds

Fungi take the form of spores, mycelia and hyphae fragments. When inhaled, they contribute to adverse health effects in individuals with respiratory conditions that are susceptible to irritant effects of exposure, and immune compromised patients who are susceptible to infections.

Fungi and moulds can produce allergic reactions usually involving inhalation of mould particles. Such complications are introduced into individuals by ingestion of mould-contaminated foods. Fungi and fungal particles can induce allergic responses in susceptible individuals and with symptoms such as wheezing, cough, itchy nose, sore throat, sinus congestion (Bonkoungou *et al*., 2013).

2.3.3 Parasitic Contamination

Parasites such as protozoa and nematode, and their ova, may be introduced during cultivation and may cause zoonosis, especially if un-composted animal excreta are used. Contamination with parasites may also arise during processing and manufacturing if the person carrying out these processes has not taken appropriate personal hygiene measures (Anyanwu, 2010).

2.4 Mycotoxins

The presence of mycotoxins in plant material can pose both acute and chronic risks to health. Mycotoxins are usually secondary metabolic products which are non-volatile, have a relatively low molecular weight, and may be secreted onto or into the medicinal plant material (Elgorashi, 2004).

They are thought to play two roles, in eliminating other microorganisms competing in the same environment and secondly, helping parasitic fungi to invade host tissues. Mycotoxins produced by species of fungi including *Aspergillus*, *Fusarium* and *Penicillium* are the most commonly reported in herbal medicines.

Mycotoxins consist of three main groups: aflatoxins, ochratoxins, fumonisins and all of which have toxic effects (Elgorashi, 2004). Aflatoxins have trichothecenes, been extensively studied and are classified as major human carcinogens by the International Agency for Research on Cancer. According to Zaleska (2008), endotoxins are found mainly in the outer membranes of certain Gram-negative bacteria and are released only when the cells are disrupted or destroyed. They are complex lipopolysaccharide molecules that elicit an antigenic response, cause altered resistance to bacterial infections and have other serious effects (Gerberick, 1984).

Thus tests for their presence in herbal medicines should be performed in dosage forms for parental use, in compliance with the requirements of national, regional or international pharmacopoeias.

2.5 Chemical Contaminants in Herbal Medicines

For years it has been discovered that herbal medicines contain toxic heavy metals such as lead, manganese, chromium, Iron and cadmium (Martena &Annet, 2010). According to a report by Harvard Medical School (2004), it was observed that 14 out of 70 herbal medicine products analysed in Pakistan contained lead, mercury and cadmium with concentrations above regulatory standards. 70% of the manufactured herbal medicines in both US and India contained lead, mercury or Arsenic (Garvey *et al.,* 2002).

Various studies on herbal medicines sold in different countries such as India, china, Nigeria and others reported presence of heavy metals such as lead, cadmium, arsenic, Nickel in herbal medicines sold in markets (Balge& Phillips, 2007; Zaleska,2008). High exposures of heavy metals such as arsenic is lethal and can cause cancer of the skin, bladder, Liver, lungs and kidneys (Delaware Health &Social services, 2009). Cadmium has an effect on lungs, Kidneys and competes with calcium for binding sites on enzymes (Agency for toxic substances, 2008). A number of agro-chemicals have been reported to contain cadmium, lead that find their way in soils due to application of fertilizers.

Lead is a known human carcinogen and cause serious damage to the nervous system and brain of unborn child (Agency for toxic substances and disease registry, 2007). Mercury can damage the brain, DNA, cancer, allergic reactions, birth defects, miscarriages and memory loss (Agency for toxic substance and disease registry, 1999.

A research carried in US and India on the manufactured herbal medicines for heavy metals such as lead, mercury and Arsenic showed the variation of various heavy metals and their comparison. The concentrations of heavy metals varied from plant to plant from which these medicines are obtained (Garvey *et al.,* 2002). Lead was found to be most common in plants from heavy traffic areas.

A study in India on market samples of herbal medicines carried out indicated that the market samples were contaminated with heavy metals (Robert *et al.,* 2008). Traditional Chinese medicine and other Asian herbal medicines were found to contain some amounts of mercury and lead. It has been shown that herbal medicine preparations in Asia for various purposes caused complications to users (Martena, *et al.*, 2010; Robert *et al.*, 2008).

Ocimumcanum, a medicinal plant has been reported to contain cadmium and Apocynaceae family has been reported to contain high levels of iron which cause toxicity (Annan, *et al*., 2010). Metal such as lead, mercury and Cadmium are cumulative poisons that are associated to some environmental problems .Heavy metals have a tendency to accumulate in the food chain (Barnes, 2003).

Heavy metals have low excretion rates through the kidney which results in damaging effects on humans even at very low concentrations (Wong *et al*., 1993).

However, some heavy metals have been reported to play important biological roles if exposed to in trace quantities. For example; Iron facilitates the transportation of oxygen in the red blood cells, Zinc enhances the removal of carbon dioxide from the body. Metals such as zinc, copper, iron, manganese, and chromium are essential nutrients; and are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic (WHO, 1998).

In general, a number of health problems were linked to excessive uptake of dietary heavy metals. These include a decrease in immunological defences, cardiac dysfunction, foetal malformation, impaired psychosocial and neurological behaviour, gastrointestinal cancer, and many others (Steenkamp *et al*., 2005).

2.6 Heavy Metals in Herbal Medicines

Plants used in the making of herbal medicines absorb heavy metals when grown in contaminated soils, traffic emissions and industrial effluents. Also toxic metals have a possibility of occurring in these medicinal preparations when used as active ingredients. For example lead in some Chinese, Mexican and Indian herbal medicine and when plants are grown in polluted areas, such as roadsides and use of contaminated fertilizers (Khan & Allgood, 2001).

2.6.1Lead

This metal is toxic to many tissues, bones, heart, reproductive and interferes with the metabolism and lethal in children. Lead is among the most widespread toxic metals due to the fact that it has been used as an anti-knock in gasoline over centuries. These herbal medicines are also used and obtained from plants grown in countries that do not use unleaded fuel an example Uganda, there is a risk that the herbal medicines used are contaminated with lead (Wong *et al.,* 1993).

Prolonged exposure to lead reduces the functioning of the nervous system and lowers renal clearance. Lead poisoning is considered one of the significant environmental health threats for children even at low levels of exposure (Annan *et al*., 2010).

It is associated with impairment of childhood cognitive function. High lead levels during pregnancy may also result into complications such as spontaneous abortion, low birth weight and impaired neuro development of the unborn child. Lead poisoning occur when the level ranges between 100-140 µg/*l* (Annan *et al*., 2010). Many clinical cases had been reported regarding lead poisoning due the consumption of different types of traditional medicine (Elgorashi, 2004). Cases of lead poisoning had been reported in Bangalore for a 45-years-old man, he was admitted twice to the hospital due to vomiting and abdominal pain. The patient's history revealed that he had been consuming 12 different Ayurvedic medicines for stress relief for the past 4 years. The patient's blood lead level (BLL) was 1224 µg/*l*, which confirmed lead poisoning. Analysis of the 12 Ayurvedic products revealed that 75% of the products contained high levels of lead, arsenic and mercury in concentration higher than the daily permissible limits.

Many studies were preformed regarding the detection of lead in herbal medicines in different parts of the world. In Karachi Pakistan, herbal medicine samples were collected from three different parts of the city (southern, eastern and western) for the determination of eight heavy metals including lead.

In Malaysia, different batches of four types of natural herbal medicines manufactured by local pharmaceutical factories, were analysed for lead content. The results indicated that all samples contained lead in concentration range of 0.125-4.79 µg/g. Lead has been reported as a prevalent heavy metal constituent of most Indian and Asian herbal medicines. It has been reported to cause a number of health effects to patients taking such remedies (Garvey, *et al.*2002).

Lead poisoning is associated with abdominal pains and the effect of toxic metals depends on exposure time and concentration. In India, herbal medicines are reported to induce several cardiac effects such as hypertension, and arrhythmias (Parry & Eaton, 1995). In China and India, cases of brain damage, kidney, nervous and red blood cells have been reported to be attributed to lead poisoning in herbal medicines. Also in infants, large amounts of lead are reported to cause delays in physical and mental development (Garvey *et al.*, 2002).

Lead is commonly used in the building industry for roofing, flashing and for soundproofing. It's used in pipes, and when combined with tin, it forms solder which is used in electronics (Annan *et al*., 2010). Lead is used in batteries and sinkers in fishing, in paints. For example; Lead chromate is a yellow pigment used in paints and usually applied to school buses. Lead salts are used as colouring agents in various foods. At high levels, inorganic lead is a general metabolic poisons.

Lead poisoning affects the neurological and reproductive systems (Annan *et al*., 2010). Lead breaks the blood-brain barrier and interferes with the normal development of the brain in infants. Lead is observed to lower IQ levels in children and lead is transferred post-natally from the mother in her breast milk.

2.6.2 Cadmium

This metal is very toxic and inhalation of cadmium leads to respiratory problems as well as tremendous damages to the liver and kidneys. Researchers in other countries such as Argentina, Pakistan, Poland, India where herbal medicines are used reported heavy metal contents in plants. These plants have a tendency to absorb metals when grown under natural conditions and their extracts are used for preparations of herbal medicines (Ray *et al*., 2009).

Cadmium metal originates from different activities such as uncontrolled discharge of wastes, sewage, burning of fossil fuels, agricultural chemicals containing cadmium and phosphate fertilizers. These contaminate plants from which these herbal medicines are obtained (Annan *et al*., 2010). Coal burning is the main source of environmental cadmium. The metal is also combined in pigments and in paints (yellow colour), photovoltaic devices and television screens, Cigarette smoke, fertilizers and pesticides**.**

The greatest proportion of our exposure to cadmium comes from our food supply-seafood, organ meats, particularly kidneys, and also from potatoes, rice, and other grains.

Severe pain in joints, Bone diseases, Kidney problems, and its lifetime in the body is several years. In very high levels it poses serious health problems related to bones, liver and kidneys and can eventually cause death (Annan *et al*., 2010).

2.6.3 Manganese

Manganese is essential for normal development and body function across the lifespan of all mammals. Manganese binds to and/or regulates several enzymes throughout the body. It is a required co-factor for arginase, which is responsible for urea production in the liver, superoxide dismutase, which is critical to prevent cellular oxidative stress (Street *et al.,* 2000). The brain is the major target organ for manganese toxicity as it retains manganese much longer than other tissues. Long-term exposure to manganese can cause a permanent neurodegenerative disorder, with few options for treatment.

2.6.4 Iron

Iron intake enhances the incidence of carcinogen-induced mammary tumours in rats. In humans, increased body stores of iron have been shown to increase the risk of several oestrogen-induced cancers. Iron acts as a catalytic centre for a broad spectrum of metabolic functions. Its also a component of various tissue enzymes such as the cytochrome that are important for energy production. However, Iron has an effect on copper metabolism. Iron deficiency includes symptoms such as reduced resistance to infection, work productivity, physical fitness, weakness, fatigue, impaired cognitive function, and reduced learning ability (Elgorashi, 2004).

2.6.5 Mercury

Mercury is an environmental toxin that produces a wide range of adverse health effects in humans. The most common natural forms of mercury found in the environment are metallic mercury, mercuric sulphide and mercuric chloride.

Metallic and inorganic mercury enters the air from mining deposits of ores that contain mercury, from the emissions of coal-fired power plants. Also burning municipal and medical waste, from the production of cement, and from uncontrolled releases in factories that use mercury (Elgorashi, 2004). Mercury is a liquid at room temperature, but some of the metal will evaporate into the air and can be carried long distances. In air, the mercury vapour can be changed into other forms of mercury, and can be further transported to water or soil (Adepoju *et al*., 2012).

2.6.6 Nickel

Nickel is an essential trace element in animals basing on reports of nickel deficiency in several animal species and manifested primarily in the liver (Das, 2009). Effects include abnormal cellular morphology, oxidative metabolism, and fluctuations in lipid levels. Decrease in growth, haemoglobin concentration and impaired glucose metabolism are a result of Nickel accumulation in the tissues. Nickel compounds are known carcinogens in both human and animal models (Harman, 1988; Grannum *et al* 1986).

2.7 Persistent Organic Pollutants (POPs)

POPs include organic chemicals, such as the synthetic aromatic chlorinated hydrocarbons. These are partially soluble in water and are persistent or stable in the

presence of sunlight, moisture, air and heat. In the past, they were extensively used in agriculture as pesticides (Robert *et al*., 2008).

The use of persistent pesticides, such as DDT and benzene hexachloride (BHC) in agriculture has been banned for many years in many countries. However, they are still used in some areas and often contaminate plants with residues that promote fungal growth to plants growing nearby. Many of these substances are still being used for public health purposes and example the control of disease vectors such as malaria-carrying mosquitoes (Robert *et al*., 2008). Therefore, care should be exercised with checking the quality of the medicinal plants grown in areas where these persistent pesticides are still being used.

2.8 Radioactive Contamination

According to WHO (2007), a certain amount of exposure to ionizing radiation is unavoidable. This is because various sources including radio nuclides occur naturally in the ground and the atmosphere. Dangerous contamination may be the consequence of a nuclear accident. It may also arise from the emissions that pollute the environment with toxic metals. WHO (1998) in close collaboration with several other international organizations, has developed guidelines for use in the event of widespread contamination by radionuclide's resulting from major nuclear sources (Robert *et al*., 2008).

The guidelines for minimum daily permissible limits of heavy metal uptakes in herbal medicines by WHO (2010) consider the health risks posed by herbal medicines accidentally contaminated by radionuclide. They depend not only on the specific

radionuclide and the level of contamination but also on the dose and duration of use of the product consumed.

2.9 Solvents occurring as Contaminants

Solvents used in industries other than the manufacturing of herbal medicines, are often detected as contaminants in water used in irrigation, for drinking and for industrial purposes. These find their way into medicinal plants at various stages of growth which are the major raw materials in the making and processing of herbal medicines. In most countries, herbal medicines are continuously used to treat wounds and various bacterial infections (Namukobe, 2011).

Herbal medicinal use is prominent in Uganda and most patients use both traditional and western medicine (Abba *et al*.2007; Okonlola *et al*. 2009). Most herbs used for treatment are obtained from plants and other components such as animal products. The environment from which these components are obtained is highly polluted with industrial fumes, microbes such as fungi and bacteria, sewage, dust, smoke and other contaminants (Rao *et al*. 2009).

2.9.1 Agrochemical Residues

The main agrochemical residues in herbal medicines are derived from pesticides and fumigants (Okunlola *et al*., 2007). Pesticides can be grouped on the basis of their intended use. Forexample insecticides, fungicides and nematicides, herbicides, as well as other pesticides e.g. acaricides, molluscides and rodenticides.

Examples of fumigants include ethylene oxide, ethylene chlorohydrin, methyl bromide and sulphur dioxide (Okunlola *et al*., 2007). A variety of agrochemical agents

and some organic solvents also constitute of common residues in herbal medicines (Anyanwu, 2010).

Chemical and microbiological contaminants result from the use of human excreta, animal manures and sewage as fertilizers (Okunlola *et al*., 2007). According to the WHO guidelines on the recommendations of medicinal plants, human wastes must not be used as a fertilizer, and animal manures should be thoroughly composted. Toxic elements and other chemical contaminants, including solvents originating from households as well as industrial chemicals, can be concentrated in composted sewage (Esimone *et al*., 2002).

Phillips, (2007) contends that contamination of medicinal herbs and herbal products with bacterial strains resistant to known antibiotics poses a prominent health risk. Brown and Jiang studied the prevalence of antibiotic-resistant bacteria in twenty-nine herbal supplements purchased from local stores in the USA. They isolated the following resistant species: *Bacillus spp., Erwinia spp., Ewingella americana, Staphylococcus aureus Enterobacter aerogenes and Stenotrophomonas maltophilia.*

The prevalence of antibiotic resistance was high to ampicillin, nalidixic acid, trimethoprim, ceftriaxone, and streptomycin (Vidhyasekaran *et al*., 2002). Opportunistic microbial species such as; bacteria and moulds in teas can cause infection and pose a threat to immune-suppressed patients, especially those with AIDS, (Kineman *et al*.2012).

Herbal products obtained from a national grocery store chain, and a local grocery cooperative, including purple cone-fleta (*Echinacea purpurea* L), Moench, pepper

(*Piper methysticum* G. Forst.), St. John's wort (*Hypericumperforatum* L.), and milk thistle (*Silybummarianum*), (Wong *et al*., 1993) were found with micro-organisms. These microbial contaminants originate from the following sources. The surrounding into which these plants and other components used in herbal medicines are located. Also the facilities of storing these finished products and raw materials. Finally the condition during transportation of these therapies and the process of preparing these remedies. It's through such ways that these microbial contaminants find their way into these herbal medicines subjecting risks to the users (Kineman *et al*., 2012).

CHAPTER THREE

RESEARCH METHODS

3.1Study areas

Figure 1: A Google map of Kampala showing KCCA markets where samples were collected.

KEY

★ Sampling area

3.2 Collection of Samples

Twenty samples of herbal medicines used for the treatment of cough, ulcer, diabetes and malaria were bought from three local markets in KCCA in USAFI, Nateete and Mengo located in Rubaga division. These include Kombucha cough syrup, Kisakyamuzadde among others. The samples in liquid form (18) were packed in tightly covered plastics and glass bottles while solid samples (2) were in form of clay bars locally called Mumbwa. For each disease, five different samples were bought for analysis.

The collected samples were kept in their bottles and stored in a cupboard within the university laboratory. The samples collected were given codes that correspond to the respective diseases for microbial analysis as shown in the table 1.

Table 1: Collection of Samples

Disease	Sample code	Point of collection
Cough	C1 C2	Katwe Market
	C3 C4	Nateete market
	C5	Mengo
Malaria	M1 M2	Mengo
	M3 M4	Katwe
	M5	Nateete
Ulcer	U1 U2	Nateete
	U3 U4	Katwe
	U5	Mengo
Diabetes	D1 D2	Katwe
	D3 D4	Mengo
	D5	Nateete

3.3 MICROBIAL CONTAMINATION.

3.3.1 Sample Preparation.

Liquid samples of each herbal medicine (1 ml) was dissolved in buffered peptone water (10 ml) followed by tenfold serial dilutions (0.1m) under sterile conditions.

3.3.2 Sterilization of Materials

All glassware and media used were sterilized by autoclaving at 121^{o}C for 15 minutes. The nutrient agar and potato dextrose agar were prepared in glass bottles, covered and wrapped using aluminium foil.

3.3.3 Preparation of media for microbial growth.

Nutrient agar was dissolved in sterile water and autoclaved in glass bottles. It was then transferred to petri plates and allowed to solidify for growth of bacteria. For growth of fungus and moulds, potato dextrose agar was dissolved in sterile water, autoclaved and poured petri plates for solidification.

3.4 Sample Analysis for Biological Contaminants

The analysis of biological contaminants was carried out at Kyambogo University Biotechnology laboratory and during the study, the following reagents were used;

Nutrient agar for the general culture of micro-organisms, Sterile water, Sabouraud Dextrose Agar (SDA), MacConkey Agar, Eosin-Methylene Blue (EMB) Agar, Potato Dextrose Agar (PDA) for isolation of fungi and moulds.

3.4.1 Culturing of micro-organisms

Micro-organisms were grown in culture media enriched with nutrient agar for bacterial growth and potato dextrose agar for growth of fungus and moulds. Liquid samples of herbal medicines (1ml) was added to sterile distilled water (9mls) to

obtain ten folds dilution of original sample. 1ml from each dilution was poured into petri- plate which contains molten nutrient agar and was smeared to distribute the sample uniformly in the media.

The mixture on the petri-plates was allowed to solidify and then the plates incubated at 37^0c for 24 hrs for growth of bacterial colonies (Okunlola *et al.*2007).

3.4.2 Identification of bacteria

Bacterial colonies were identified basing on colony pigmentation, growth pattern, mycelia structure, morphological and biochemical tests. Bacterial colonies obtained on nutrient agar plates were further sub- cultured on separate nutrient plates so as to obtain pure cultures.

3.4.2.1 Bacterial Colony characteristics

These were identified by microscopy for morphological study. Other biochemical tests such as carbohydrate fermentation, starch hydrolysis, Catalase test, and growth in nutrient broth with 6% NaCl, Triple Sugar-Iron Agar, and growth in SPDA agar were carried out for further characterization of bacterial colonies (Holt *et al.,* 1994).

3.4.2.2 Microbiological and Biochemical Analysis

For the identification of bacterial species, the microbiological techniques which were used included: Inoculation, Gram, staining, colony and morphological characterization for analysis of structural features of organisms. Biochemical tests carried out included; catalase, oxidase citrate, tri-iron sugar and fermentation tests.

Fungal identification was carried out using Potato Dextrose Agar (PDA) and was based on colony pigmentation, mycelia texture and growth pattern and microscopy.

3.4.3. Characterization and Identification of Organisms

The pure cultures of micro-organisms were obtained by growing mixed microbial culture isolates on fresh nutrient agar. The stock cultures were prepared for particular micro-organism using nutrient agar and were incubated at30°C for 24 hours, and then stored in the refrigerator.

Pure cultures of isolates from nutrient agar were subjected to catalase test, gram stain test and oxidative test to specifically determine the identity of bacteria.

3.4.4 Microscopy

A smear of bacteria culture was applied on a clean slide and heat fixed by passing it over an ethanol flame and Gram-stained. A drop of cedar wood immersion oil was applied to the dry smear and observed under high power objective of a light microscope. Rod shaped bacterial cells, clustered purple colonies, chain shaped cells, spherical-shaped cells were observed for the different slides indicating microbial contamination in the samples.

3.4.5 Test for specific Microbes using selective Media

Selective media were used to test for specific micro-organisms as follows:-

3.4.6 Staphylococcus aureus

A sample from different colonies in Nutrient Agar was sub-cultured on a plate of bloody agar base which was freshly prepared was incubated at 33 °C for 72 hours.

The growth of golden colonies indicated the presence of *Staphylococcus aureus.* Further biochemical tests were carried out for its presence as shown in Table2

Table.2: Biochemical tests for identification of *Staphylococcus aureus*

Biochemical test	**Reaction**
Catalase	+
Gram staining	+
Oxidase	-
Methyl red	+
Citrate	+

3.4.7 Escherichia coli

A pure culture from nutrient agar was inoculated into MacConkey agar which was freshly prepared and where incubated at 33 °C for 72 hours. Colonies with dark centres and golden green colonies indicated the presence of *Escherichia coli* .Further biochemical tests were also carried out as shown in Table 3 below.

Table.3: Biochemical tests for identification of *Escherichia coli*

Biochemical test	**Reaction**
Catalase	+
Lactose fermentation	+
Gram staining	+
Tri-iron sugar test	+
Nitrate reduction	+
Indole production	+

3.4.8 Enterobacteraerogenes

A pure culture from Nutrient Agar was inoculated into MacConkey agar and incubated at 33°C for 72h. The growth of pink large mucus-like colonies confirmed the presence of *Enterobacter aerogenes*. Catalase tests were carried out to investigate the presence of Catalase enzyme in each of the bacterial isolates that catalyzes the hydrolysis of hydrogen peroxide to oxygen and water that can be toxic to the body tissues and nucleic acids.

3.4.9 Identification of Fungal Isolates

Identification of fungal isolates was based on colony morphology such as growth pattern, pigmentation, hyphae structure, spore colour and shape.

3.5 Heavy Metal analysis

3.5.1 Sample Preparation

During preparation, the following were used:

Beakers made of borosilicate glass, Hydrochloric acid(37%), Nitric acid (65%), Hydrogen peroxide (30%), Measuring cylinder (10, 50 and 500 ml), Funnels (6cm in diameter), conical and Volumetric flasks of 250ml. The glass wares used were rinsed with 1M nitric acid.

Powdered sample (2g) of the air dried and ground sample was transferred to a beaker. HCl/ HNO_3 3:1(50 ml) was added, mixed and filtered. The filtrate was heated to 100^0C and maintained for 1 hour so as to concentrate the solution to 5ml. Then 30% H_2O_2(3ml) were added and allowed to settle for 10minutes so that ions are set free in solution. Water (50 ml) and HCl (25ml) were added, mixed and heated till boiling.

The filtrate was Cooled and transferred to a volumetric flask (100ml) and filled up to the mark, mixed and left to stand for 15hours.

For liquid samples of herbal medicines, digestion with aqua regia (HNO_3 67% HCl 37% in a ratio of 3:1) was carried out. The volume of the digested sample (50mls) was transferred to a volumetric flask (100mls) and the solution made to the mark with 1% nitric acid.

3.5.2 Sample Analysis

The analysis of heavy metals was carried out at the Uganda government analytical laboratories Wandegeya. The absorbance of the clear supernatant was measured using AAS model AA-6300 Shimadzu cooperation. The amount of ions were calculated using the formulae below.

$\textbf{Amount of ions} = \frac{\mathbf{C\ X\ D}}{\mathbf{M}}$**,** Where: C is the Concentration of sample solution, M is the Mass of sample in grams, D is the Dilution and for liquids.

$\textbf{Amount of ions} = \frac{\mathbf{C\ X\ V_1}}{\mathbf{V_2}}$, Where: C is the Concentration of sample solution (mg/l), V_1 is the Final volume (250ml), V_2 is the Volume of sample.

Standard reference solutions of lead, cadmium, Nickel and Chromium (Merck) were used for calibration and quality assurance.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Biological Contaminants

The results of study showed that all herbal medicine samples analyzed were contaminated with micro-organisms as shown in Table 4. The samples of cough were highly contaminated, followed by ulcer samples, diabetes and malaria samples were least contaminated. Fungus and *Escherichia coli* were the most predominant micro-organisms in the samples analyzed followed by *staphylococcus aureus*, moulds, *Enterobacter aerogenes* and least was penicillium.

Different samples of herbal medicines showed a variety of microbial contaminants and the number of samples for each disease contaminated is summarised in Table 4.

Table 4: Number of micro-organisms in various herbal medicines for different diseases

Micro-organisms	Number of samples contaminated			
	Cough	**Malaria**	**Ulcer**	**Diabetes**
Escherichia coli	04	03	02	04
Enterobacter aerogenes	02	-	03	02
Fungus	04	03	04	03
Penicillium	-	03	-	-
Moulds	02	02	04	02
Staphylococcus aureus	03	01	02	03

The results above indicate that the percentage contamination of the total analysed samples with fungus was high to about 70%, *Escherichia coli* with 65%, moulds with

50%, *staphylococcus aureus* with 45%, *Enterobacter aerogenes* 35% and least contamination observed in penicillium with about 15% for all the samples analysed.

Samples of malaria were the least contaminated with micro-organisms, followed by diabetes and a high rate of contamination was observed in cough and ulcer samples with a percentage of about 75%.

4.1.1 Morphological characteristics of bacterial isolates

Several bacterial isolates were identified and characterized from different culture media as shown in Table 5.

Table 5: Morphological characteristics of bacterial isolates

Organisms identified	shape	colour
Escherichia coli	spherical	Pink
Staphylococus aureus	Rod shaped	White
Enterobacter aerogenes	Spherical	Purple
Fungus	Filamentous	white
Moulds	Filamentous	Dark

From all the isolated microbial species, *enterobacter aerogenes* was least abundant while *staphylococcus* and *Escherichia coli* were noted most predominant. This shows that herbal medicines may increase infections and as a result cause other diseases since the risks of aerial contamination are high as they are prepared and sold in open places.

Plate counting was carried out for microbial and fungal isolates for the samples analyzed. The numbers were obtained in terms of average values of colony forming units (cfu g-1) of micro-organisms. Fungal species in samples of different herbal medicines of cough, malaria, diabetes and ulcer were also obtained as shown in the Table 6 below.

Samples of cough and ulcer showed most contamination with micro-organisms while samples of diabetes had moderate and malaria samples showed least contamination.

Table 6: Microbial and Fungal Counts for Samples Obtained from Usafi, Nateete and Mengo Markets

Diseases	Sample Code	Microbial Count (cfu/ml)	Fungal Count (cfu/ml)
Cough	C_1	5.00×10^6	4.20×10^6
	C_2	2.61×10^3	2.04×10^7
	C_3	2.21×10^5	2.00×10^7
	C_4	2.50×10^7	2.84×10^6
	C_5	3.80×10^7	2.23×10^7
Malaria	M_1	1.56×10^7	1.07×10^7
	M_2	2.03×10^8	7.1×10^8
	M_3	2.01×10^4	2.05×10^7
	M_4	1.56×10^7	2.48×10^7
	M_5	3.14×10^7	1.14×10^7
Ulcer	U_1	6.00×10^5	5.30×10^4
	U_2	4.50×10^5	4.40×10^5
	U_3	2.50×10^7	6.80×10^7
	U_4	1.50×10^8	7.40×10^6
	U_5	2.50×10^5	4.10×10^6
Diabetes	D_1	1.94×10^7	2.63×10^7
	D_2	1.80×10^7	1.56×10^9
	D_3	2.6×10^3	4.00×10^5
	D_4	2.73×10^7	3.07×10^7
	D_5	4.66×10^5	2.86×10^7

Escherichia coli and *Staphylococcus aureus* were isolated in the higher number of counts compared to other species with samples of ulcer and cough showing higher

levels of contamination while *Enterobacter aerogenes*, were isolated in low numbers and herbal medicines of diabetes and malaria showed the least contamination. Although least bacterial contamination was noted in the samples of diabetes and malaria, these herbs showed a high contamination of fungus and moulds.

In comparison to the other herbal medicines, samples of diabetes and cough showed the lowest fungal and moulds count but with the highest bacterial counts. The microbial levels of herbal medicines used in this study as depicted in Table 6 showed that all the samples had microbial contaminants. Microbial counts by plate method ranged between 1.5×10^8 ($cfug^{-1}$) and 6.0×10^5(cfu g-l).

Among all isolated species, *Enterobacter aerogenes* was recorded as least abundant whereas *Staphylococcus aureas* and *Escherichia coli* were found most abundant in herbal medicines of ulcer and cough analysed together with high levels of moulds and fungal species.

Table 7: Microorganisms identified in different samples

Diseases	Sample Code	Micro Organism Identified
Cough	C_1	*Escherichia coli*, *Enterobactera erogenes*, fungus,
	C_2	*Escherichia coli*, *Enterobacter aerogenes staphylococcus aureus*, moulds.
	C_3	*Staphylococcus aureus* , *Escherichia coli*, fungus.
	C_4	*Staphylococcus*, Moulds, Fungus
	C_5	*Escherichia coli*, *Enterobacter aerogenes*, fungus.
Malaria	M_1	*Penicillium*, fungus
	M_2	*Escherichia coli*, moulds.
	M_3	*Escherichia coli*, *Staphylococcus aureus*, moulds.
	M_4	*Penicillium*, fungus
	M_5	*Penicillium*, *Escherichia coli*, fungus.
Ulcer	U_1	*Enterobacter aerogenes*, Moulds, Fungus
	U_2	*Staphylococcus aureus Escherichia coli*, Moulds, Fungus
	U_3	*Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, moulds*
	U_4	*Enterobacter aerogenes, Fungus*
	U_5	Moulds, Fungus
Diabetes	D_1	*Escherichia coli*, Fungus, *Enterobacter aerogenes*
	D_2	*Staphylococcus aureus, Escherichia coli*, fungus.
	D_3	*Staphylococcus aureus, Escherichia coli*
	D_4	Moulds, *Enterobacter aerogenes*
	D_5	*Staphylococcus aureus, Escherichia coli*, Moulds, Fungus

Microbial isolates showed different bacterial types and fungal types as shown in Table7.

Staphylococcus aureus was the most frequently isolated bacterial contaminant from most of the analysed samples while *Enterobacter aerogenes* and *Escherichia coli* followed in the rate of contamination respectively. Fungus and moulds were isolated in the samples of a cough, ulcer while samples of diabetes and malaria displayed least fungal growth.

This study showed that there are high levels of microbial contamination in herbal medicines and may be as a result of poor methods of preparation. Micro-organisms might be introduced to the products during processing, handling of the raw materials and packaging of products. Also the environment and conditions that medicinal plants are grown and collected subject them to contamination. Reports have suggested that unhygienic equipment and materials could also be a source of microbial contaminants (Abba *et al*., 2009).

All herbal medicines of the cough, ulcer, diabetes analysed were contaminated with micro-organisms. *Escherichia coli* and *staphylococcus aureus* were the most abundant in the samples. These are commonly transmitted through body contact, unhygienic equipment, use of polluted water, which are rampant in our communities and the areas where these herbal medicine products are processed. Contamination with fungus and moulds may be a result of a high rate of pollution in the environment and this also includes market areas.

As a result, the finished products and the raw materials may be subjected to contamination. Therefore, there is a need for good Manufacturing Practices of harvesting, drying, storage, handling and preparation of the herbal medicinal products.

These organisms are common causative agents of childhood diarrhoea of bacterial origin (Bonkoungou *et al*., 2013). When *Staphylococcus aureus* enters into the body it causes diseases and is a threat to people with depressed immune systems, chronic diseases, such as HIV, and in those undergoing chemotherapy, which suppresses the activity of the immune system. When it enters the blood stream, it can result into complications such as pneumonia, meningitis, osteomyelitis, endocarditis and some strains produce toxins which can cause food poisoning.

Enterobacter aerogenes and *Staphylococcus aureas* are known micro-organisms for causing a number of complications most especially to immune-compromised individuals. These organisms also have the ability to produce proteins that disable the immune system and damage the tissues releasing exotoxins which cause gastrointestinal disorders (Abba *et al*., 2009). This research showed that *Staphylococcus aureas* and *Escherichia coli* were the predominant bacteria in the herbal medicines and these results are comparable with those obtained by Oyetayo (2008). Oyetayo (2008) carried out an analysis of Nigerian herbal medicines and reported that almost all herbal medicines tested were contaminated with Bacillus species which are commonly found in soil, air and dust.

Many aerobic species of Bacillus produce endospore that makes them adaptive to environmental stress for their sustenance and long-term survival under adverse conditions. About 20% of the herbal medicines analysed showed antibacterial property and this is attributed to the presence of chemical compounds known as phytochemicals such as Tannins and Alkaloids which are produced by plants. Phytochemicals have been known to be secondary metabolites that are usually produced by plants and accumulated in different parts of the plant (Kaingu *et al*., 2013). These phytochemicals have been thought to be responsible for the antimicrobial activity shown by extracts from various herbal plants. This is shown in *Aloe secundiflora* that has been used in treating ailments including; chest problems, polio, malaria and stomach ache by herbalists in the Lake Victoria region .

Aloe secundiflora leaf components have been credited for antibacterial, antifungal and antiviral and antihelmintic medicinal properties (Kaingu, *et al.*, 2013). Extracts of Aloes especially from its leaves have shown antibacterial activity by inhibiting the growth of both Gram-negative bacteria and Gram-positive bacteria.

Specific compounds isolated from Aloevera such as anthraquinones and dihydroxyanthraquinones as well as saponins have been proposed to have antibacterial activity (Reynolds &Dweck, 1999).

The plants in the genus of *Asteraceae* usually have a bitter taste and in english, they are called bitter leaf.

Some of the common African names of plants in this genus are Olusia (Luo), Mululuza (Luganda), and Chusar-doki (Hausa) (Kokwaro, 2009), are also examples that produce phytochemicals. Vernonialasiopus decoctions from the stems and leaves have been traditionally been used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems (Kareru *et al*., 2008). Reports have shown that some of the phytochemicals found in extracts of Mululuza have antimicrobial capability (Koul, *et al*., 2003). Its extracts have also been used in treating some of the sexually transmitted diseases while in Uganda, its extracts are used in the treatment of malaria.

This is in line with the findings of this study as observed from malaria herbal medicines analysed which showed low counts of microbial loads .This is because some plants whose extracts are commonly used in preparation of malaria herbal medicines show antimicrobial properties as reported in various studies (Alzoreky & Nakahara,2003).

Reports have shown that some edible plants extracts also have antimicrobial activity against *Staphylococcus aureus* (Alzoreky & Nakahara, 2003). Further studies have shown a great synergistic activity of plant extracts and spices when used against pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and other bacteria organisms, both Gram positive and Gram negative.

Medicinal plants are known to produce a variety of phytochemicals with antibacterial activity belonging to chemical structural classes such as, phenolic, terpenoids, alkaloids, lectins, polypeptides, and polyacetylenes.

However, the most bioactive compounds are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Microbial contamination usually occurs because of poor methods of drying and storage of the raw materials which results in the degradation of these materials. Thus, microbial contamination can make plant material toxic, either by transforming the chemicals in the plant material or through the production of toxic compounds by the micro-organisms (Ramakrishnan *et al*.,2006).

4.2 Heavy metal Contaminants

The concentration of lead in the analyzed samples ranged from 0.0120 to 2.0808 µg/g, nickel ranged from 0.0120 to 0.9548 µg/g. Cadmium and chromium were not detected in this study for all the samples analyzed.

Samples of malaria showed a high metal concentration with lead predominating than nickel followed by ulcer samples which showed a high nickel concentration compared to lead. Samples of diabetes showed low metal concentration compared to other samples analysed

Quality control

All the calibration plots were linear with regression values ranging from 0.9953 to 1.0000 for all metal standards of lead, chromium, nickel and cadmium (refer to appendix for calibration curves). The concentrations obtained for each herbal

medicine sample was measured in µg/g as shown in Table 8 and it shows the summary of the analysis..

Table 8: Mean metal concentrations (mg/l) in herbs for n=20

Disease	Sample code	Pb	Cd	Ni	Cr
		Mean concentration mg/l ± sd)	**Mean concentration mg/l ± sd)**	**Mean concentration mg/l ± sd)**	**Mean concentration mg/l ± sd)**
Malaria	F	0.7292 ± 0.340	< 0.0001	0.2260 ± 0.100	< 0.0001
	H	0.0001 ± 0.000	< 0.0001	0.5308 ± 0.250	< 0.0001
	J (mumbwa)	2.0808 ± 0.980	< 0.0001	0.9548 ± 0.480	< 0.0001
Cough	A	0.1568 ± 0.005	< 0.0001	0.3200 ± 0.150	< 0.0001
	E	1.0448 ± 0.492	< 0.0001	0.2980 ± 0.140	< 0.0001
	E_1	0.8010 ± 0.377	< 0.0001	0.7940 ± 0.273	< 0.0001
Ulcer	D	0.0964 ± 0.045	< 0.0001	0.3396 ± 0.160	< 0.0001
	G	0.6716 ± 0.045	< 0.0001	0.7292 ± 0.340	< 0.0001
	G_1	0.1270 ± 0.060	< 0.0001	0.0228 ± 0.021	< 0.0001
	I	0.0120 ± 0.004	< 0.0001	0.0001 ± 0.000	< 0.0001
	I_1	0.1808 ± 0.084	< 0.0001	0.8010 ± 0.377	< 0.0001
Diabetes	B	0.0001 ± 0.000	< 0.0001	0.0120 ± 0.004	< 0.0001
	C	0.4132 ± 0.194	< 0.0001	0.3396 ± 0.159	< 0.0001
	C_1	0.5320 ± 0.245	< 0.0001	0.4630 ± 0.218	< 0.0001

NB: <0.0001≡ND

Table 8 shows relative concentration of metals in herbal medicines.

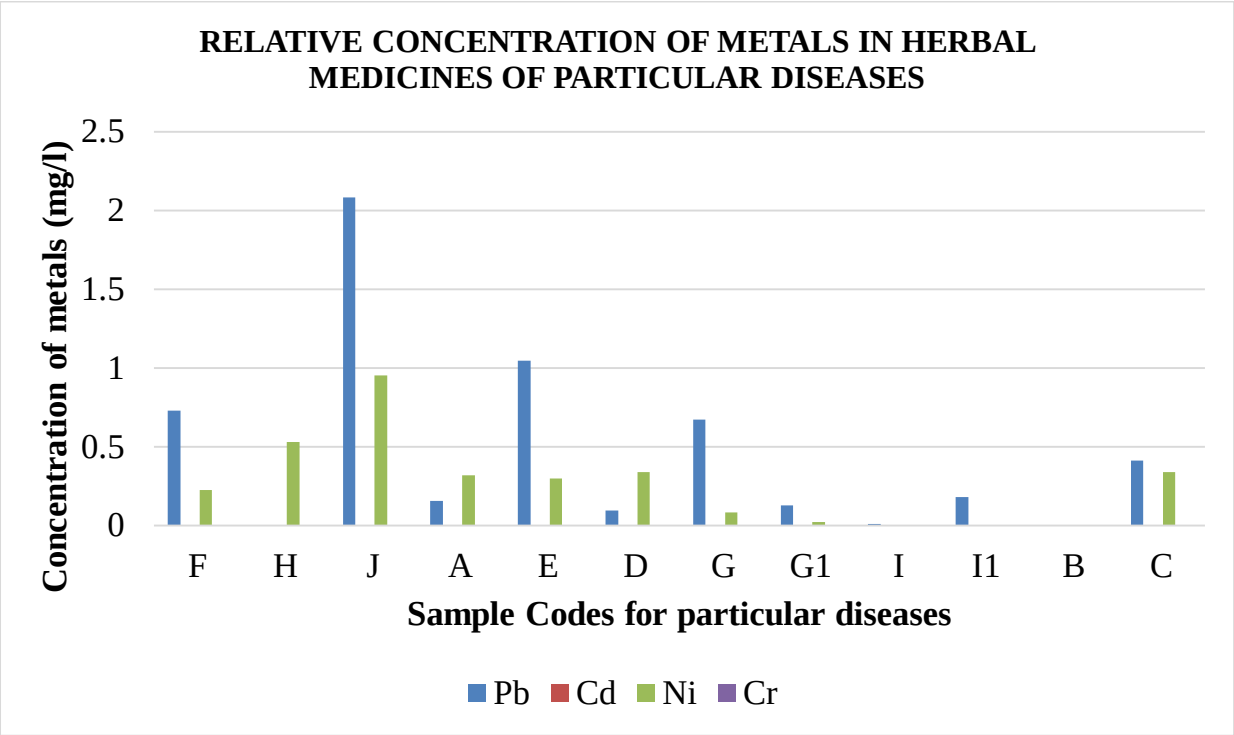
From the study, most herbal medicines analysed showed that the most prevalent metals were lead and nickel ranging from 0.012 ± 0.004 to 2.0808 ± 0.980.Mumbwa showed a high concentration of lead of 2.0808 ± 0.980 and nickel of 0.9548 ± 0.480 which is beyond minimum limits of 0.001mg/litre and for nickel of 0.003mg/litre. This is of great concern since lead, nickel and their compounds are known human carcinogens.

The methods of prescribing dosage for herbal medicines are not standard as every herbalist prescribes in any way. This subjects patients to intake of large doses of these therapies exposing them to much intake of toxins such as heavy metals. As a result, health risks are likely to increase. Also the labelling and packaging of these herbal medicines may provide deceptive information as it gives patients a false sense of the product safety. The information on contraindications, manufactured and expire date is not shown on most herbal medicines sold on market increasing risks to patients.

Malaria herbal medicines analysed showed high metal concentration with lead being most prevalent. This was followed by ulcer nickel concentration. Diabetes with relatively high amount of lead while cough samples analysed showed low metal concentrations but with much lead than nickel.

Generally the concentrations of lead and nickel varied for each type of herbal medicine analysed and this is likely to be attributed to the differences in components used in the preparations and market areas. The above findings are further illustrated in figure 4. 2 below.

Figure 4.2: Bar graph showing a relative concentration of metals in herbal medicines of particular diseases.



4. 3 Heavy metal contamination in herbal medicine products

Heavy metals are introduced in herbal medicines during preparation stages such as harvesting, collecting, cleaning and drying of the medicinal plants. Accidental contamination during the manufacturing process such as grinding, mixing and exposure to heavy metals from metal-releasing equipment used in different stages of processing these herbal medicines also exposes them to contamination (Okatch *et al*., 2012). For the safety of the human populations, a number of organizations such as USEPA, WHO, have set up parameters to regulate the presence of heavy metals in herbal medicines. Parameters such as permissible daily exposure (PDE), the rationale for reference doses (RFD's) as well as oral component limit (OCL) are measures set to minimise contaminations in herbal medicines (WHO, 2000).

4.4 Toxicity of heavy metals

Cadmium (Cd), lead (Pb), nickel (Ni) and Chromium are reported in a number of countries to be found in herbal medicines (Elgorashi, 2004). Long exposure to heavy metals may cause adverse health effects and toxicity. These metals have the ability to bio-accumulate, hence interfering with the functioning of important organs in the body such as brain, kidneys and liver.

4.4.1 Cadmium (Cd)

Cadmium is a non-essential element, soluble in the biological systems and known for its high toxicity and similar to other heavy metals since it has a tendency to bio accumulate and disrupts the functioning of body organs (Street *et al*., 2000). Both acute and chronic exposure to cadmium has a negative impact for human health.

Cadmium may cause high blood pressure and destruction of red blood cells. Cadmium metal ion in the body's metallo-enzyme can easily replace another metal ion due to the chemical similarities and competition for binding stage. Therefore, Cd^{2+} can replace Zn^{2+} which in some dehydrogenating enzymes, causes cadmium toxicity.

Cadmium has been detected in herbal medicines in various studies conducted in different parts of the world (Street *et al*., 2000). In Nigeria, a study was conducted on cadmium content in herbal medicines and was reported that cadmium concentration were found in the range of 16.438 - 29.796 mg/g. However, in this study, cadmium was not detected in the samples analysed using atomic absorption spectrophometer (AAS).

4.4.2 Lead

In this study, the concentrations of lead in the samples analysed were in the range of 0.0120-2.0808 mg|l and a high concentration was found in MUMBWA. This high concentration was observed in clay bars since they are made of soil most especially clay. Reports show that most of these heavy metals are drained in swamps hence having high levels in such soils and are the major components of clay bars.

These lead concentrations are above the permissible daily intakes of lead according to WHO (2005) of 0.001mg|l hence toxic to human health.

This high lead concentration in herbal medicines is most likely to result from use of leaded petrol in developing countries, untreated waste disposal which find their way in natural ecosystems. Also plant uptake from contaminated soils and a high rate of pollution in our environment are possible sources of lead contamination (Wong *et al.,* 1993).

The concentrations of lead varied considerably among the samples analysed other than mumbwa, with herbal medicines of malaria having highest concentrations compared to other medicines. This variation is likely to result from the different sources from which these raw materials for the different herbal medicines were obtained. In other herbal medicines analyzed, the lead concentrations were in the range of 0.0120 ± 0.004 to 0.8010 ± 0.377 which is beyond the permissible limits hence risky for human consumption as the metal is reported to be carcinogenic (Garvey *et al*., 2002).

Lead is a heavy metal that has been reported to cause undesirable effects on different body organs. Prolonged exposure to lead reduces the functioning of the nervous system and lowers renal clearance. Lead poisoning is considered one of the significant environmental health threats for children even at low levels of exposure (Annan *et al.*,2010). Many clinical cases had been reported regarding lead poisoning due the consumption of different types of traditional medicine (Elgorashi, 2004). In China different batches of four types of natural herbal medicines manufactured by local

pharmaceutical factories, were analyzed for lead content, the results indicated that all samples contain lead in concentration range of 0.125-4.79 µg/g (Annan *et al.*, 2010).

4.4.3 Nickel (Ni)

In this study, the concentrations of Nickel in the samples analysed were in the range of 0.0120-0.9548mg|l and a high concentration was found in "MUMBWA". These Nickel concentrations are above the permissible daily intakes of Nickel according to WHO (2007) of 0.0003mg-l hence toxic to human health.

Numerous medicinal plants have the potential to accumulate heavy metals when grown under their natural conditions. An example is Asteraceae species a common plant used for preparation of malaria herbs is one of the nickel hyper accumulating plant (Przybylowics, 1995). Exposure to Ni may result in a variety of pathological effects. Oral exposure to large doses of nickel mainly targets the cardiovascular system.

The common adverse health effect of nickel in humans is allergic skin reaction in those who are sensitive to nickel. Most of the toxicity of nickel might be attributed to its interference with the physiological processes of zinc and calcium (Przybylowics,1995). Generally, the results of analysis indicated that lead and Nickel were present in varied concentrations ranging between 0.012±0.004 to 2.0808±0.980 for lead and 0.012±0.004 to 0.9548±0.480 for nickel in most of the herbal medicines analysed. The concentrations of lead and nickel in cough, ulcer, mumbwa and diabetes exceeded the internationally accepted permissible levels.

These variations in metal concentrations in the analysed herbal medicines are attributed to the level of pollution, differences in the plant metal uptake and translocation abilities.

Uptake of heavy metals by plants depends on a number of factors such as plant species and their stage of growth, the soil type, and the type of metals absorbed. Reports have indicated variations in concentrations of different metals in a variety of plant species (Street *et al.*, 2000). For example, reports on plants have shown high transfer values of nickel which are obtained from soils irrigated with wastewater, indicating a stronger accumulation of this metal by the food crops. The presence of metals in herbal medicines may be attributed to several factors which include soil pH, metal levels in the soil, redox potential of the soil, and other chemical and physical factors of soil.

Another study that monitored the metallic micronutrients and heavy metals in herbal medicines from Austria found out that species such as St. John's wort, poppy, showed higher tendency to accumulate Nickel. Similarly, results of this study have shown higher Nickel concentrations in herbal medicines (Garvey *et al.*, 2002).

4.4.4 Chromium

Reports have been made in various parts of the world on the presence of chromium in herbal medicines for example; Nigeria (Street, *et al.*, 2000). However, in this study chromium was not detected in all the samples analysed.

The results of this study show that the sources of heavy metal contamination in herbal medicines are linked to water used during preparation, polluted soils, fertilizers and pesticides, industrial emissions, transportation, harvesting and storage processes. The health risk due to metal contamination depends on the average daily dietary intake of these herbal medicines.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

This study has shown that herbal medicines sold on Ugandan markets are not entirely safe and reveals a potential health risk associated with consuming of these medicines. This is because the study revealed a high rate of contamination with micro-organisms of *Escherichia coli*, *Staphylococus aureus*, fungus and moulds and heavy metals of lead and nickel whose concentrations were above the maximum permissible limits.

These herbal medicines analysed during the study may be taken as representative samples of others sold in the different markets throughout the country.

5.2 Recommendations

In the light of findings of the study and discussion the researcher has the following recommendations.

1. It is recommended that there is a need to extend government regulation to monitor herbal medicinal products to ensure that their processing, preparation methods, manufacturing processes comply with Good Manufacturing Practices, and thus reduce on the risks to consumers.
2. There is need for more work to be done on the contamination of herbal medicines in other parts of the country to ensure safety of the consumers of these remedies since these herbal medicines are used without considerations of bio safety in terms of contaminants such as microbial and heavy metals.
3. There is need for more work to be done on the possible sources of these contaminants in herbal medicines

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APPENDIX A1.

Definition of sample codes for analysis of metals.

A – Cough

B – Diabetes

C _ Diabetes

D – ulcer

E – Cough

F – Malaria

G – Ulcer

H – Malaria

I – ulcer

J – mumbwa

APPENDIX A11.

Recomended limits for heavy metals according to WHO.

Recommended limits for heavy metals in herbal medicines.

	Raw products	Finished Products
Lead	10ppm	0.02mg/l
Cadmium	3ppm	0.006mg/l
Nickel	3ppm	0.006mg/l
Chromium	2ppm	0.01mg/

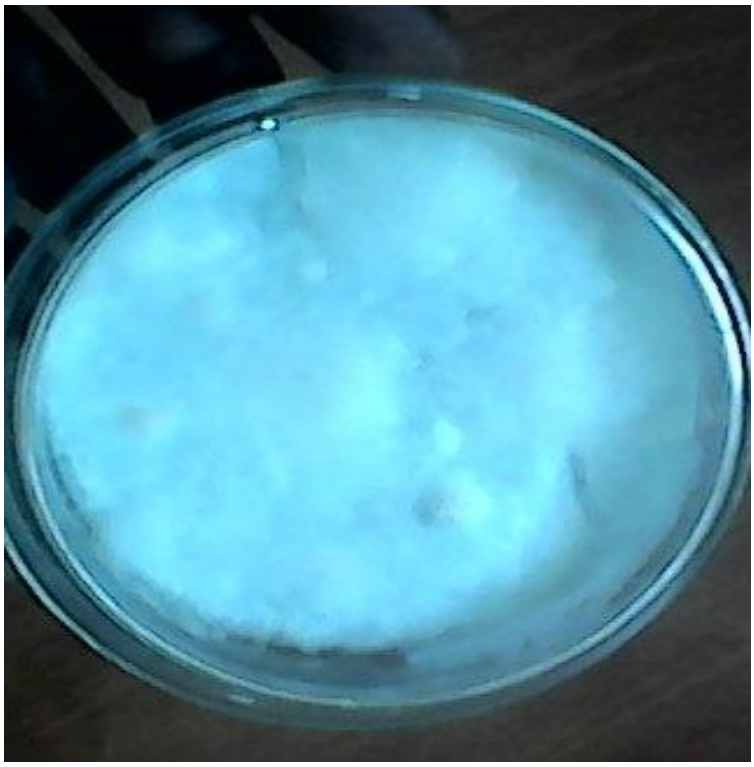
APPENDIX B.

PLATES SHOWING MICROBIAL GROWTH

PLATES SHOWING FUNGAL GROWTH IN COUGH SAMPLES

Fig.b2:SAMPLES OF MALARIA HERBS SHOWING ANTI-MICROBIAL CHARACTER

Fig.b3:SAMPLES OF DIABETES HERBS SHOWING FUNGAL AND MICROBIAL GROWTH



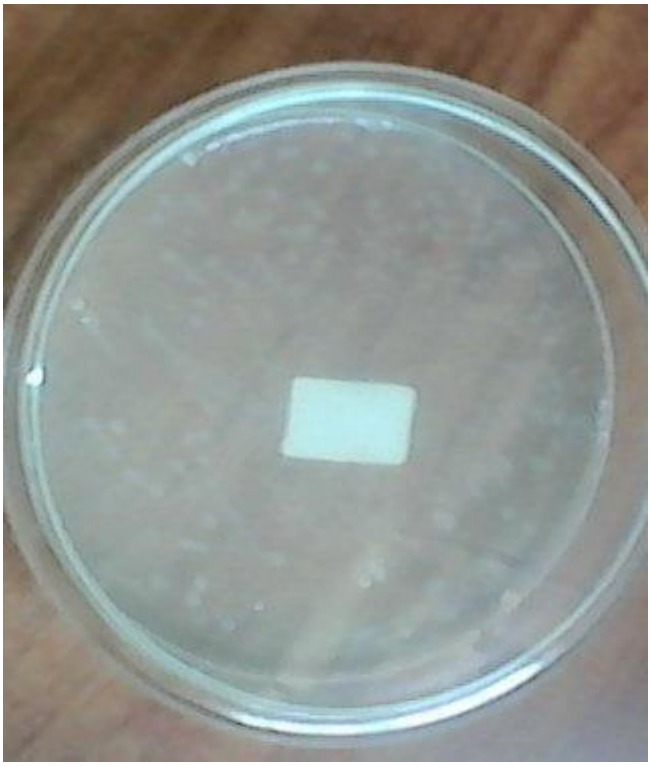
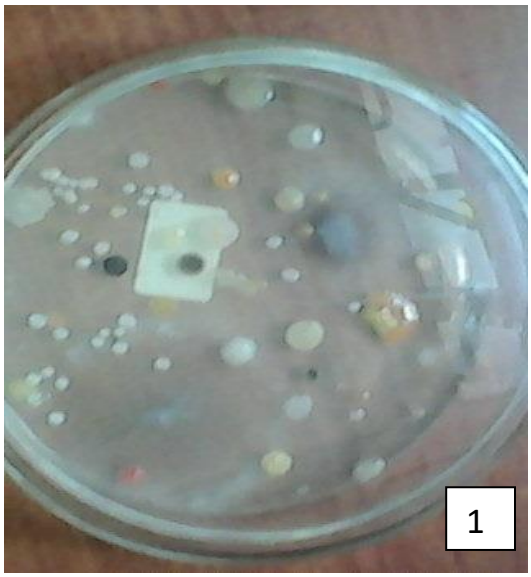


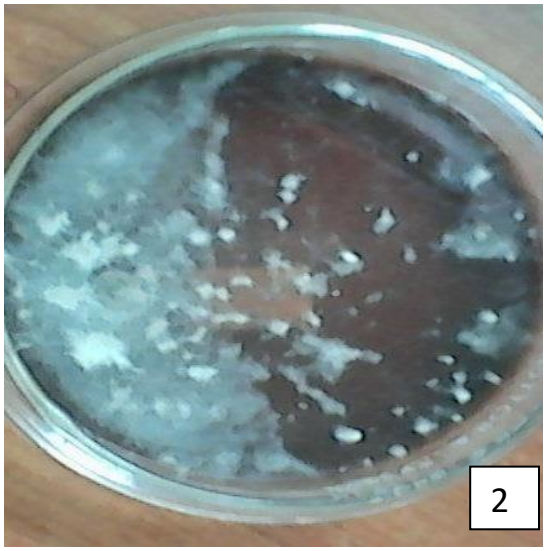
fig.b4:MALARIA SAMPLES SHOWING LESS CONTAMINATION

White mould cells spread in the nutrient agar an indication of less contamination

fig.b5:PLATES SHOWING CONTAMINANTS IN THE DIFFERENT SAMPLES UNDER NUTRIENT AGAR
SAMPLES OF COUGH



Bacteria colonies grown under nutrient agar and incubated at 32^0C for 24hours
White spherical colonies of bacterial cells spread in the media with yellow zones showing contamination with micro-organisms.



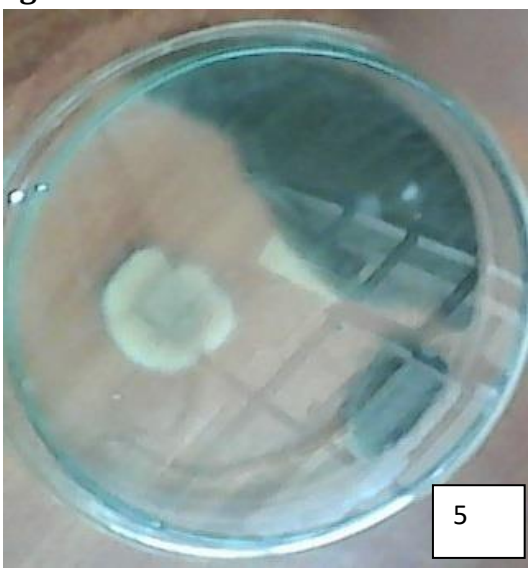
Growth of fungus moulds under potato dextrose Agar media showing contamination.

fig.b6:SAMPLES OF DIABETES

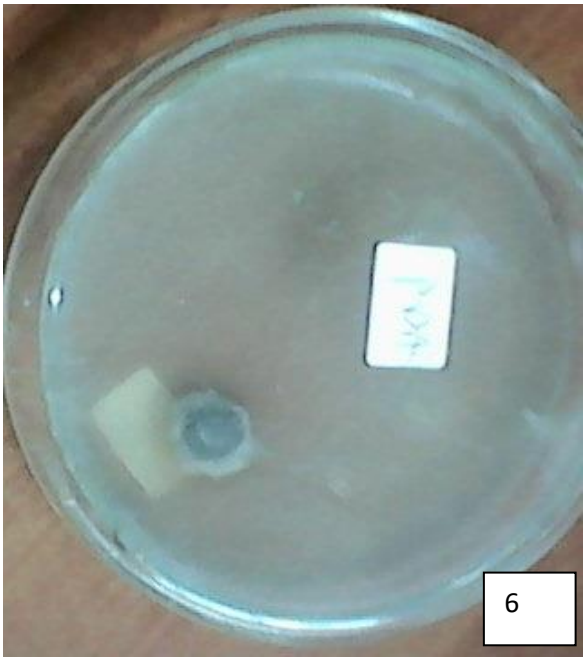
White filaments cells grown under potato dextrose Agar on incubation for 72 hours showing moulds and fungus.

On incubation for 24hours under nutrient agar, white spherical colonies, yellow colonies and pink colonies grow in the media showing different microbial species grown

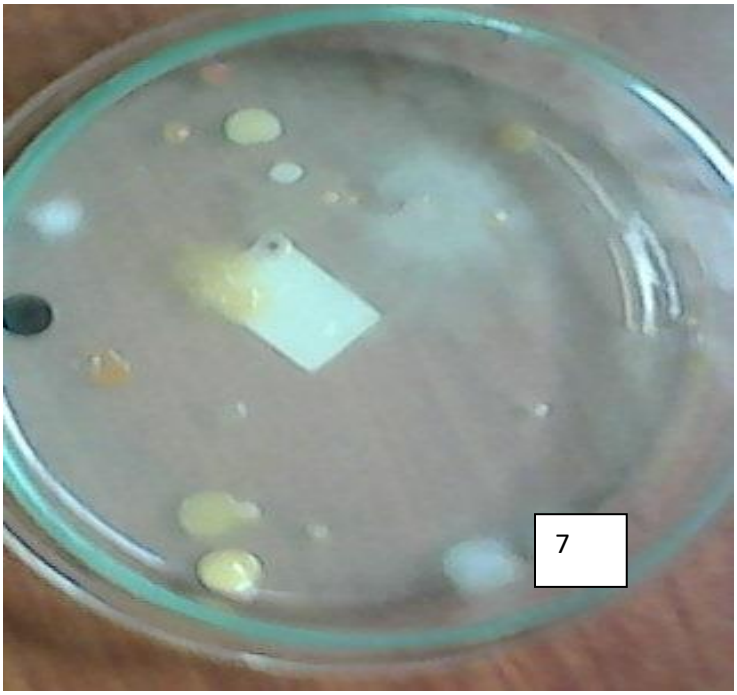
fig.b7:SAMPLES OF MALARIA



A sample grown under potato-dextrose agar showed growth of white clustered colonies with dark centres at the end of the plate showing mould growth



Anti-microbial activity is observed by a patch of cells clustered in the centre of the media

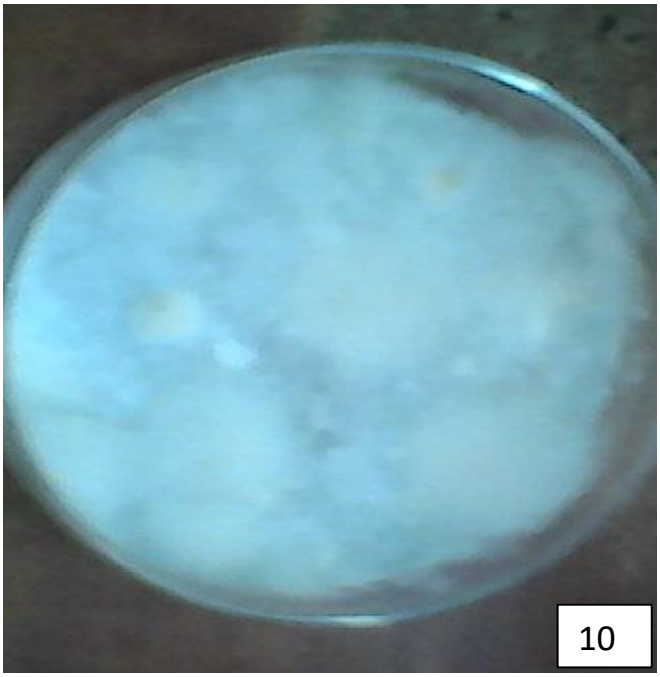


Growth of different bacterial colonies when cultured in nutrient agar

White mucoid cells spread in the nutrient agar an indication of less contamination.

fig.b8:SAMPLES OF ULCER

White clustered bacterial colonies but spread in the media on incubation under nutrient agar at 32^0 for 24hours showing bacterial contamination.



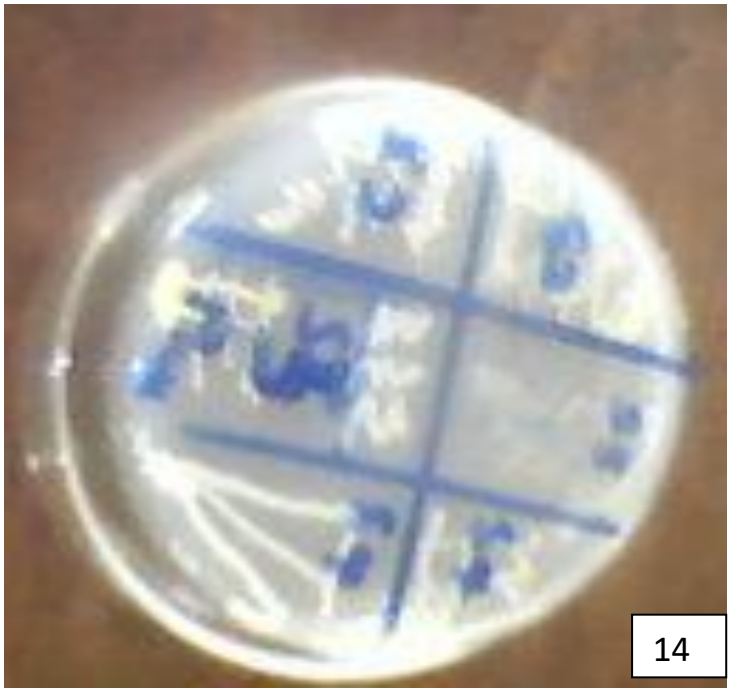
Samples show growth of white clustered filamentous colonies and some colonies are oval-shaped on incubation under potato dextrose agar for 72hours

fig.b9: SPECIFIC ISOLATION OF BACTERIAL COLONIES

Bacterial colonies isolated from nutrient agar and grown under macconkey agar for specific media for specific identification of *E.col.i*

Colonies grown under mannitol agar for specific identification of staphylococcus

Colonies under Eosin methylene blue agar for specific identification of *enterobacteraerogenes*.

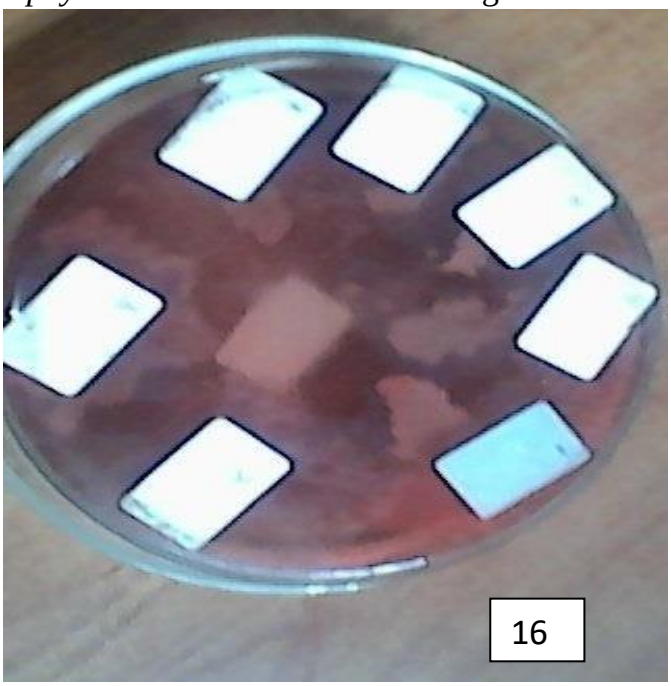


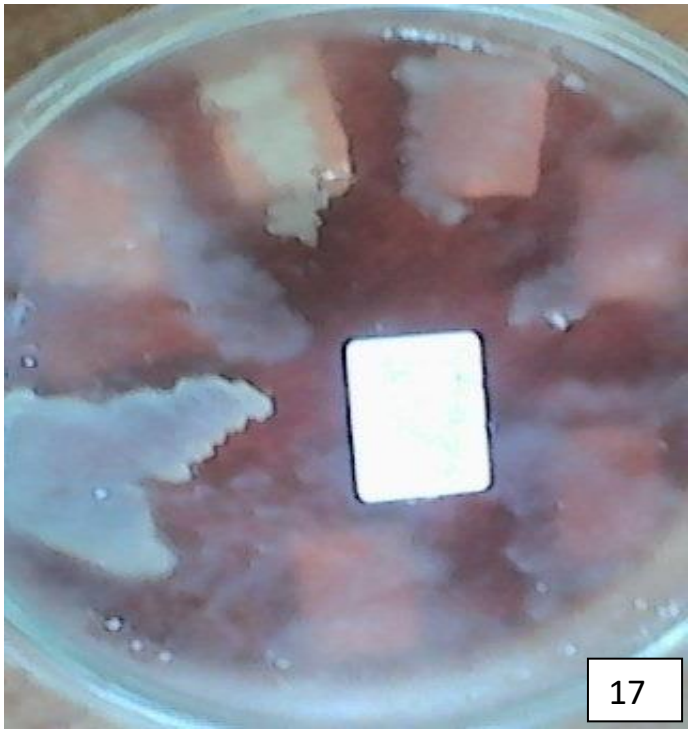
Colonies under tetralthionate broth base for specific identification of *salmonella*.

Isolated colonies under sahoroud potato dextrose agar for identification and growth offungus and moulds.

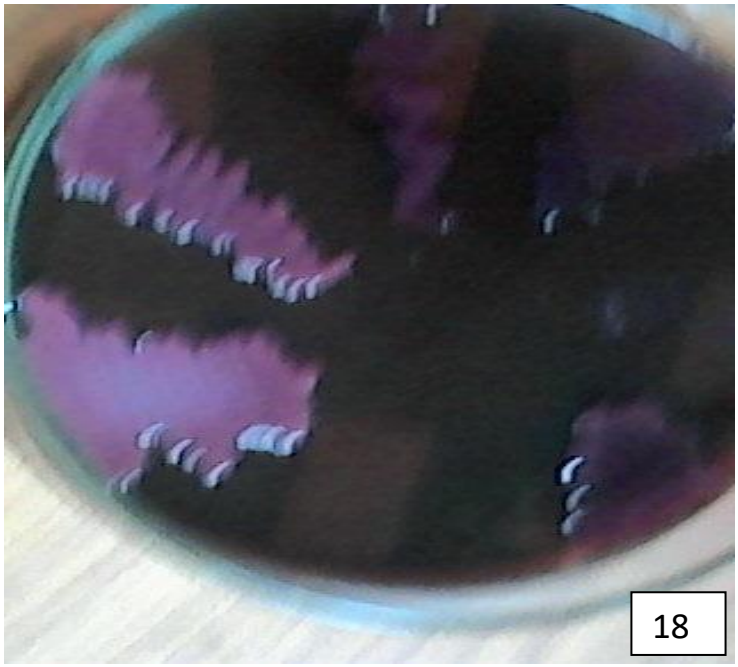
Fig.b10: APPEARANCE OF BACTERIAL COLONIES UNDER SPECIFIC IDENTIFICATION CULTURE MEDIAS

Staphylococcus aureus observed as golden mucoid colonies

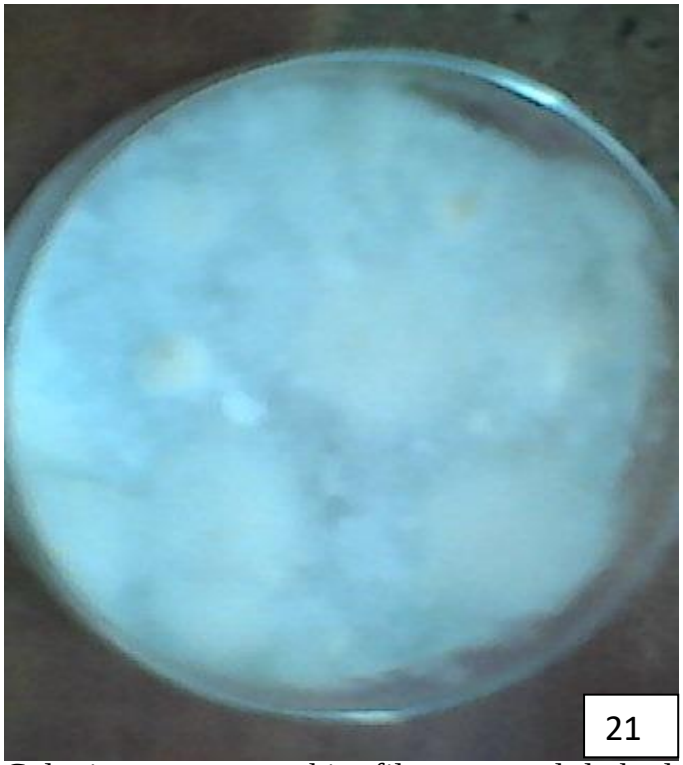




Colonies in bloody agar base appear as golden colonies that shows *staphylococcus aureus* grown under the media.



Purple colonies grown under macconkey agar showing *Escherichia coli* and after incubated for 72hours at 33^0C.



Colonies appear as white filaments and dark clustered filaments in sabouroud potato dextrose agar showing growth of moulds and fungus after incubation at 27^0C for 72hours.

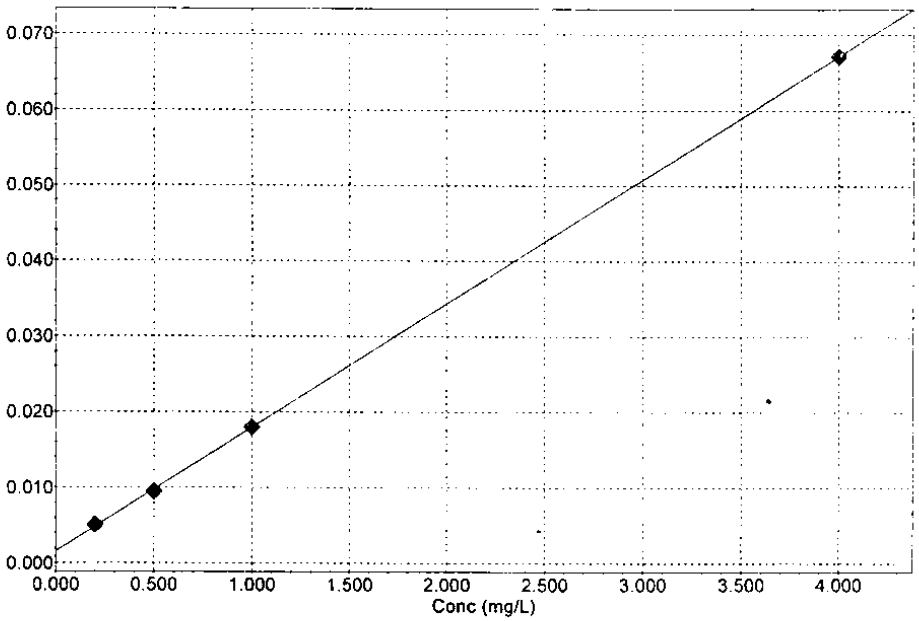
Moulds grow and appear as dark clustered filamentous cells in saboroud potato dextrose agar (SPDA) after incubation for 72hours.

APPENDIX C: AAS CALIBRATION CURVE FOR METALS

CALIBRATION CURVE FOR LEAD ANALYSIS

Wednesday, April 25, 2018

Calibration Curve(Element:Pb:Flame C#:01)



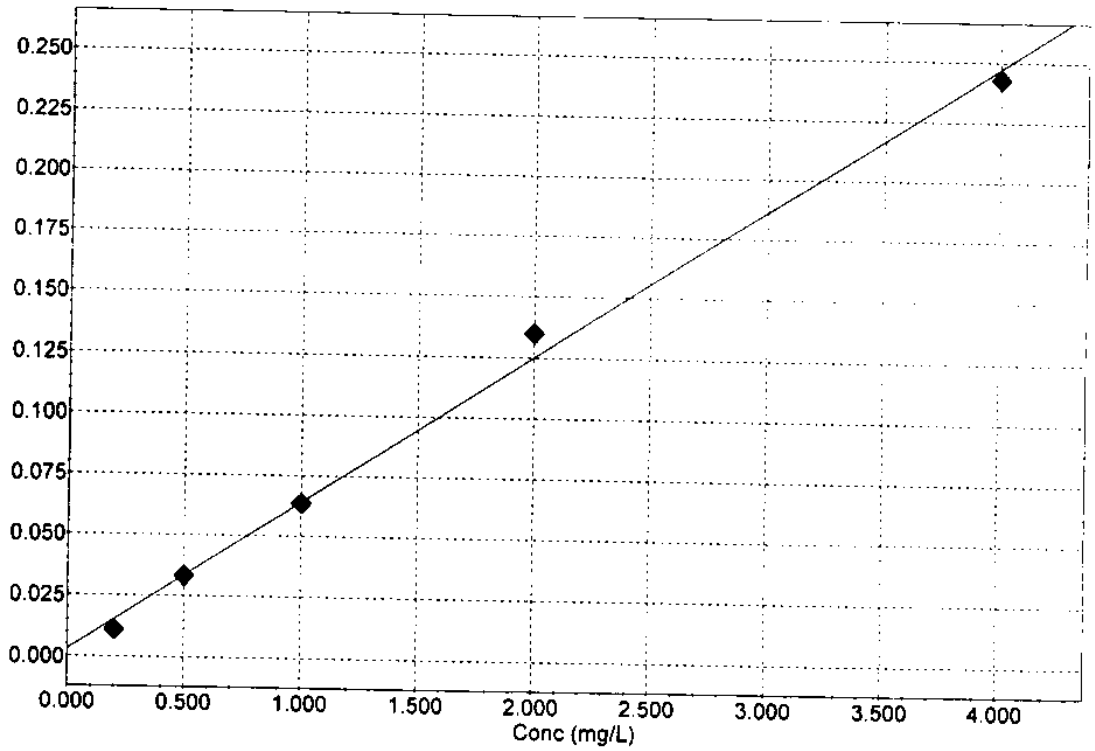
Abs=0.016401Conc+0.0015788 r=1.0000

CONC	ABS
0.2000	0.0051
0.5000	0.0095
1.0000	0.0180
4.0000	0.0672

CALIBRATION CURVE FOR NICKEL ANALYSIS

Wednesday, May 16,

ıtion Curve(Element:Ni:Flame C#:01)



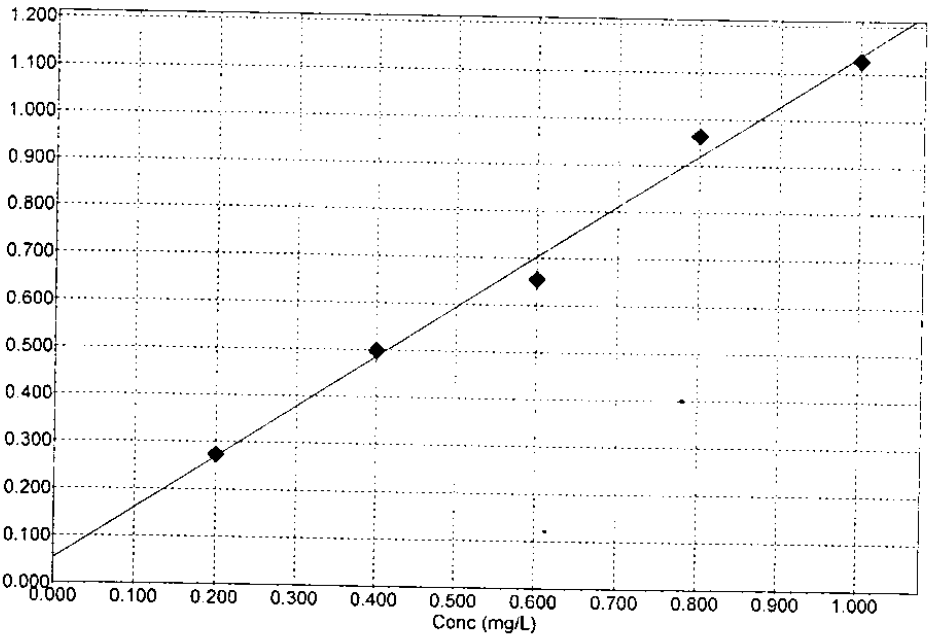
Abs=0.061056Conc+0.0030531 r=0.9982

CONC	ABS
0.2000	0.0110
0.5000	0.0332
1.0000	0.0636
2.0000	0.1347
4.0000	0.2429

CALIBRATION CURVE FOR CADMIUM ANALYSIS

Wednesday, April 29, [illegible]

Calibration Curve(Element:Cd:Flame C#:01)



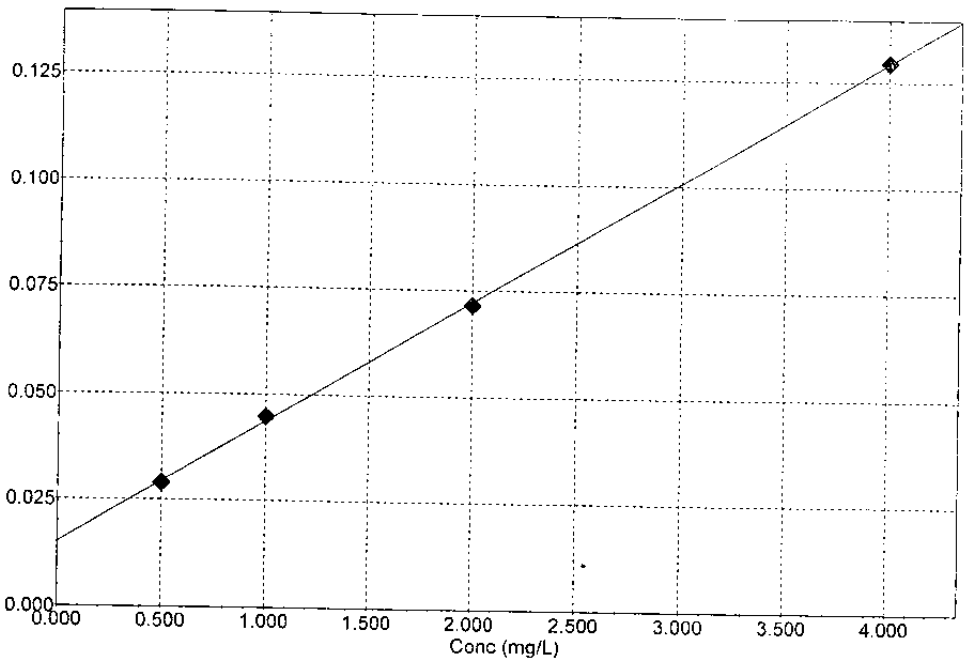
Abs=1.0856Conc+0.051990 r=0.9953

CONC	ABS
0.2000	0.2731
0.4000	0.4990
0.6000	0.6548
0.8000	0.9635
1.0000	1.1265

CALIBRATION CURVE FOR CHROMIUM ANALYSIS

Wednesday, April 25, 2018

ibration Curve(Element:Cr:Flame C#:01)



Abs=0.028560Conc+0.015100 r=0.9998

CONC	ABS
0.5000	0.0289
1.0000	0.0447
2.0000	0.0715
4.0000	0.1295