Addis Ababa University
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Assessment of Leaching of Selected Toxic Metals in Some Pottery Processed Foods

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This is to certify that the thesis presented by Mahlet Tadesse entitled "Assessment of Leaching of Selected Toxic Metals in Some Pottery Processed Foods" submitted in partial fulfillment of the requirements for the degree of Master of Science (Food Science and Nutrition) complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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Declaration

I, the undersigned, hereby declare that this thesis is my original work and all sources of materials used for the thesis have been correctly acknowledged.

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The thesis has been submitted with my approval as supervisor:

Tetemke Mehari (PhD)	Dawd Gashu (PhD)
Signature ________________	Signature _______________
Date ___________________	Date __________________

DEDICATION

This work is dedicated to my parents who I love most in this world more than anything

To: Taddese Berhe (My father)

Mulu Tatere (My mother) and

Abiselom Getachew (My husband).

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May the Lord bless you!!

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List of Abbreviations

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
CAC	Codex Alimentarius Commission
CSEM	Case Studies in Environmental Medicine
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FASS	Flame Atomic Absorption Spectroscopy
GMP	Good Manufacturing Practice
JECFA	Joint Expert Committee for Food Additives
IOM	Institute of Medicine
NAS	National Academy of Science
NCC	National Codex Committee
NRC	National Research Council
PTWI	Provisional Tolerable Weekly Intake
SCF	Scientific Committee for Food
WHO	World Health Organization

Abstract

Raw materials from uninspected sources used for making of cooking utensils could be a potential source for high toxic metal in food prepared in these utensils. Therefore, in the present work, the level of selected metals (Pb, Cd, and Al) in clay pots was determined. The clay pots were obtained from two sites *kechene* and *legeberi the* areas are *selected* because of the raw materials used to make them were brought from suspected places for metal contamination. In addition, leaching of those metals in some foods (bean stew, tomato sauce and coffee) prepared using these materials were investigated. Furthermore, factors that can influence metal leaching like frequent usage of the pots were also studied. Clay pots were crushed while the food and coffee samples were lyophilized and digested in a closed microwave digestion system. The levels of metals were determined using flame atomic absorption spectrophotometer (FAAS). The results showed that level of metals in clay pot from *legeberi* were Pb (26.7±0.0 mg/kg), Cd (5.6±0.1 mg/kg) and Al (38.74±0.7 mg/kg) and clay pot from *kechene* had Pb content (0.6±0.3 mg/kg), Cd (0.3±0.0 mg/kg) and Al (8.92±0.3 mg/kg). In addition, the level of metals leaching in food samples prepared using clay pots from *legeberi* showed tomato sauce Pb in range (from 9.6±0.0 mg/kg to 13±0.0 mg/kg), Cd (from 0.5±0.0 mg/kg to 1.2±0.0 mg/kg) and Al (from 11.53±0.4 mg/kg to 15.4 ±0.3). The bean stew prepared using clay pots from *legeberi* had Pb in the range (from1.2±0.0 mg/kg to 3.3±0.0 mg/kg), Cd (from 0.14 ±0.0 mg/kg to 0.38 ±0.0 mg/kg) and Al (from10.8±0.2 mg/kg to 13.5 ±0.3 mg/kg), whereas the coffee samples brewed in coffee pot from l*egeberi* showed Pb in range (from1.6±0.0 mg/kg to 3.9±0.0 mg/kg), Cd (from 0.18 ±0.0 mg/kg to 0.34 ±0.0 mg/kg) and Al (from1.9±0.0 mg/kg to 3.8 ±0.1mg/kg). However, Pb and Cd were not detected in any of the food prepared using clay pot from *kechene*. The level of Al in food samples prepared in clay pot from *kechene* was tomato sauce in range (from10.5±0.5 mg/kg to 11.3±0.6 mg/kg), bean stew (from10.1±0.3 mg/kg to 10.4±0.4 mg/kg) and coffee (from1.1±0.0 mg/kg to 2.1 ±0.0 mg/kg). Results of the present investigation indicates that metal (Pb, Cd and Al) content of clay pot from *legeberi* was significantly higher ($p<0.05$) than clay pots from *kechene*. In this manner, food samples prepared using clay pot from *legeberi* had high level of metals (Pb, Cd and Al) beyond the permissible limit allowed by WHO, for Pb (0.01 mg/kg), Cd (0.003 mg/kg), and Al (1mg/kg) in food. Similar trend was observed for Al content of food prepared using clay pot from *kechene*.

Therefore, metals (Pb, Cd, and Al) leaching due to cooking in *legeberi* clay pot and Al in clay pot from *kechene* could be a public health concern.

Keywords: leaching; toxic metals; clay pot; cooking frequency

1. INTRODUCTION

1.1 Back ground

Contamination of foods by toxic metals has become an unavoidable challenge these days. Air, soil, and water pollution are contributing to the presence of harmful elements, such as cadmium, lead, and mercury in foodstuff (Arora *et al*., 2008), and this is a principal concern to public health, as food is the primary source of essential nutrients for man (Pennington, 2000; Jigam *et al*, 2011).

Humans are exposed to these metals through different exposure pathways but, the most common are inhalation of contaminated air and ingestion of products such as water, medicinal herbs, and food (Abou *et al*., 2000; Bocio *et al*.,2005; Yun *et al*.,2007; Zukowska *et al*., 2008). Regardless of the source of the metals found in the food, many of them play an important role in all life forms. For example, cobalt, iron, chromium, zinc, and manganese are essential to plants and animals. However, elements such as lead, cadmium, arsenic, beryllium, mercury, and barium have not shown any beneficial function in human beings (Trichet & Defarge, 1995). Although the toxicity of a metal depends on the amount ingested, chronic exposure to certain metals, such as arsenic, lead and cadmium can cause severe toxic effects, even in low amounts.

In recent years, advancement in technology has led to high levels of industrialization leading to the discharge of effluents and emissions containing heavy metals into the environment. Sources of heavy metals in soils in urbanized areas mainly include natural occurrence derived from parent materials and human activities which are associated with activities such as atmospheric deposition, industrial discharges, waste disposal, waste incineration, urban effluent, long-term application of sewage sludge, fertilizer application in soil, and vehicle exhausts (Bilos *et al*., 2001; Koch & Rotard, 2001).

Food can be contaminated with toxic metals in the different stages of agricultural production, particularly in the soil where toxic metals may be naturally present (Zhuang *et al.,* 2009).The preparation of these agricultural products into palatable food is also another source of food contamination by heavy metals. Indeed, the preparation process requires the use of utensils and ingredients which are also responsible for the presence of heavy metals in food. Located at the end of the chain of food preparation, packaging and cooking utensils contaminate food based on materials used in their design (Cabrera *et al*., 2003). Clay pots, apart from other sources of dietary toxic metals, are considered to be a potential source of metal to human beings. Since they are made of clay, contain major potential contaminants include all metals from the earth crust (Dabonne *et al*, 2010). Human being since early times, especially after the discovery of "making fire" had always milled and cooked using different cooking techniques and (cook wares) utensils. Pottery is one of the most ancient hand craft and first synthetic cook ware to be discovered by man (Anquanda, 2008).

Traditional potters do not perform any chemical analysis on the raw materials before using for commercial production of pots. All they look out for in terms of the suitability of the clay for making pots is its plasticity. Unfortunately, if the geochemistry of the clay has high levels of heavy metals above the recommended levels, then the quality of these products becomes questionable as these elements have a higher tendency to enter the human body through food (Hight*,* 2001). Furthermore, unlike modern utensils, traditional ones do not have protective layer of inert material to prevent contamination of food. The problem with the presence of heavy metals in clay pots lies in the fact that these contaminants can be transferred to food by a leaching process, which is directly related with the physical and chemical conditions of the food, such as temperature and pH (Alper *et al.,* 2008).

1.2. Statement of the problem

Recent study by different scholar in different country have shown traditional cooking pots can contaminate the food with metal based on the material they are designed. For example, studies conducted by Dabonne *et al.,* (2010) in Cote d'Ivoire shows that traditional utensils made out of clay and traditional Al pots are the potential sources of metals like Al and iron. Another study by Omolaoye *et al.,* (2010) in Nigeria also shows that food items and beverages prepared in ceramic products imported from China had show high levels of lead. Case this point, in our country research conducted by Adish et *al.,* (1991) consumption of foods prepared in iron pots increased iron status of the population because of leaching process. However, data on leaching of metals from clay pots commonly used in Ethiopia is scanty. Despite the fact that food processing in traditionally made clay pot is common all over the country the safeness of the food in concern to contamination with heavy metal wasn't studied. Heavy metals are potential environmental contaminants with the capability of causing human health problems if present in excess in the food we eat. They are given special attention throughout the world due to their toxic effects even at very low concentration since, several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Jarup, 2003).Various research groups report heavy metal leaching from clay pot experimental results with food, beverages, and water is affected by different factors pH and temperature of the food. A low pH is found to enhance leaching of toxic metal from the utensils (Alper *et al.,* 2008).

1.3. Significance of the study

Monitoring the presence of toxic metals in foods intended both for human and animal is of interest because of their toxic effects, as heavy metals bioaccumulate and pose health risks. Heavy metals have mostly been studied in soil, water, paints, and food. Clay pots have not really been investigated as one of the sources of lead, cadmium and other heavy metals. Moreover, recent study in different countries have shown that they are one of the most public health hazard constituent but to the best of my knowledge no work has been done in Ethiopia to investigate the problem.

In Ethiopia, as in most developing countries, much of the urban population and almost all the rural population still use traditional clay pots on daily basis especially to prepare local food and beverage such as *injera* and coffee. Considering the above-mentioned problem, efforts should be focused on the evaluation of dietary intakes of heavy metals by consumers in order to assess risk. Therefore, it is very important to study the level of toxic metals found in these utensils to make sure that they are safe and do not pose a health risk if used as a food contact material on our daily basis.

1.4. Objectives

1.4.1. General objective

- To determine the level of leaching of selected toxic metals (Pb, Cd and Al) from locally made cooking clay pot into the food matrix.

1.4.2. Specific Objectives

- To determine and compare the concentration of selected toxic metal (Pb ,Cd and Al) in clay pots from *Kechene* and *Legeberi*.
- To determine the amount of toxic metals leaching from clay pots to commonly consumed Ethiopian foods during processing.
- To find out the influence of cooking frequency or repeated use of the utensil on toxic metal (Pb ,Cd and Al) leaching.

2. LITERATURE REVIEW

2.1. Metals

Metals are naturally-occurring components of the earth’s crust that are, as a rule, neither created nor destroyed, but are simply redistributed (Lenntech *et al.*, 1998). Distribution of toxic metals is not uniform, such that some soils may contain higher amounts of any of these chemicals, either due to natural processes or to pollution factors wherein toxic metals have been disbursed in to the environment through human activities or anthropogenic means, such as mining, power generation, manufacturing, the former use of leaded gasoline, urban runoff, sewage effluents, pest or disease control agents applied to plants, and fertilizers (Khlifi & Hamza , 2010; Ming-Ho, 2005).

The most appropriate manner to classify metals of interest by their impact on health effects are: nutritionally essential, nonessential but with a possible beneficial effect, or nonessential with no beneficial effects (Gloub *et al.*, 2004).

Nutritionally essential metals: Metals that are generally regarded as nutritionally essential for humans are cobalt (Co), chromium III (Cr III), copper (Cu), iron (Fe), molybdenum (Mo), selenium (Se), and zinc (Zn) (Goyer & Clarkson, 2001). For instance iron being required for the haemoglobin; manganese and copper are linked to superoxide dismutase (SOD): chromium stabilizes blood sugar and zinc is important in the healing of wounds but they can also have harmful effects when their intake exceeds the recommended limits (Llobet *et al*, 2003; Jimoh *et al.*, 2012).

Metals with No Known Essential or Beneficial Effects: The most potentially toxic metals in the environment are arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) as well as their inorganic compounds (Lar *et al.,* 2014). They have no known nutritional or beneficial effects on human health but they are present in air, water, and soil, so that some level of exposure is not readily preventable. Other metals of concern to Environmental Protection Agency (EPA) include aluminum (Al), antimony (Sb), barium (Ba), beryllium (Be), silver (Ag), strontium (Sr), and thallium (Tl).

Some other metals are not known to be essential to human health at larger dose but may have some beneficial effects at low levels of exposure. These include silicon, nickel, boron, and vanadium. Some have said arsenic has beneficial effects (NAS/NRC, 1999), but a recent critical review does not support this view for human exposure (NAS/IOM, 2003).

Even though, the possible beneficial metabolic functions of As for humans have not been established, carcinogenicity effect can result at extremely low levels of exposure, some organic arsenic compounds have been used as growth factors in different animals; (NAS/NRC, 1999). Boron (B), nickel (Ni), silicon (Si), and vanadium (V) have been shown to have biological functions in plants and some animals but essentiality for humans has not been demonstrated (NAS/IOM, 2003).

Metals are present in the solid phase and in solution, as free ions, or adsorbed to soil colloidal particles. The heavy metal concentration in topsoil is a result of soil-forming processes, as well as agricultural or human activities (Hem, 1992). Toxic metals are currently of much environmental concern. These metals are dangerous because they tend to bioaccumulate in the food chain and they are harmful to humans and animals. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. The threat that toxic metals pose to human and animal health is aggravated by their long-term persistence in the environment (Alloway, 1994).

2.2. Toxic metals in the environment

Environmental pollution is a major cause of the presence of toxic metals into the food chain. Food can be contaminated during the different stages of agricultural production, particularly in the soil where heavy metals may be naturally present (Zhuang *et al*., 2009). The occurrence of toxic metals-enriched ecosystem components, firstly, arises from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings. These agents have led to metal dispersion in the environment and, consequently, impaired health of the population by the ingestion of victuals contaminated by harmful elements. Thus environmental pollution is a major cause of the presence of heavy metals into the food chain (Nnorom *et al*., 2007).

Recently the relation between adverse health effects and heavy metals in the environment has gained considerable attention in various professional journals as well as in the broader public media. Heavy metals or potential pollutants have been termed ‘geogenic contaminants’ and include such elements as As, Pb, Cd and Hg. Elevated levels of these and other potential pollutants have been recorded in many areas of the world including Canada, USA, India, China and Bangladesh to name a few examples. The recognition that an intimate relationship exists between geology, as measured by geochemistry, and human/animal health, has led to the development of a new field of science called Medical Geology (Bowman *et al.,* 2003).

Many adverse effects have been observed on human health by the environmental pollution of metals. Heavy metals condition is problematic due to their persistence and non-degradability in the environments (Yuan *et al.,* 2004). Theoretically, every 1000 kg of “normal soil” contains 200 g chromium, 80 g nickel, 16 g lead, 0.5 g mercury and 0.2 g cadmium. The development of technology, large-scale and indiscriminate consumption of fuel, and industrial chemical waste have caused the presence of considerable metal concentrations in the environment, which can exert several different effects on the ecosystem (Picado *et al.,* 2010; Youns *et al.,* 2010).

Sources of toxic metals in soils in urbanized areas mainly include natural occurrence derived from parent materials and human activities which are associated with activities such as atmospheric deposition, industrial discharges, waste disposal, waste incineration, urban effluent, long-term application of sewage sludge, fertilizer application in soil, and vehicle exhausts (Bilos *et al*., 2001).

Soil has long been recognized as a repository for pollutants due to the adsorption processes which bind inorganic and organic pollutants to it. It has been reported that topsoil and roadside soil near heavy traffic in urban areas are indicators of heavy metal contamination from atmospheric deposition (Amusan *et al*., 2003). Metals such as Cd, Cu, Pd and Zn are good indicators of contamination in soils because they appear in gasoline, car component, oil lubricants and industrial incinerator emissions. Generally, metals are introduced into the environment either by natural means or human activities (Anthropogenic).

Natural sources: excessive levels of trace metals may occur naturally by geological phenomena like volcanic eruptions, weathering of rocks (acid rock drainage) and leaching into rivers, lakes and oceans due to action of winds and precipitation erosion and find their way into plants and animals as a result of direct input (Bilos *et al*., 2001).

These toxic metals may adversely affect soil ecology, agricultural production or product quality, and ground water quality, and will ultimately be harmful to mankind by food chain. Chemicals like heavy metals once introduced to the environment by one particular method may spread to various environmental components, which may be caused by the nature of interactions occurring in this natural system. Heavy metals may chemically or physically interact with the natural compound, which changes their forms of existence in the environment. In general they may react with particular species, change oxidation states and precipitate (Dube *et al.,* 2000).

Exposure to heavy metals such as mercury, cadmium and lead is a critical problem throughout the world. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products (Jarup, 2003).

Vehicular pollution in most developing countries of the world has not been checked properly by environmental regulating authorities leading to increase levels of pollution. This situation is alarming and is predicated on the poor economic disposition of developing countries, poor vehicle maintenance culture and importation of old vehicles which culminate to an automobile fleet dominated by a class of vehicles with high emission of harmful pollutants .Therefore, to reduce metal concentration of food chain and ecosystem from vehicular emissions, an appropriate land use policy which prohibits the use of road side lands, especially within 30 m either side of the road, for farming, sun-drying of food stuff, grazing or as a source of pasture to feed livestock is required (Amusan *et al*., 2003).

2.3. Metals contamination of food

Contamination of food by heavy metals is of paramount concern to public health, as food is the primary source of essential nutrients for man .Monitoring the presence of heavy metals in foods intended both for human and domestic animals is of interest because of their toxic effects, as heavy metals bioaccumulate and pose health risks (Cabrera *et al*., 2003). The most frequently reported heavy metals with regard to potential hazard and occurrence in contaminated food samples are copper (Cu), lead (Pb), zinc (Zn), nickel (Ni), cobalt (Co), chromium (Cr), iron (Fe), and cadmium (cd) (Jarup, 2003).

Food can be contaminated during different stages of agricultural production, particularly in the soil where heavy metals may be naturally present (Zhuang *et al.,* 2009) and from contaminated soils and surface water may be due to use of (fertilizers & pesticides) and direct deposition of contaminants from the atmosphere on to plant surfaces can lead to plant contamination by toxic metals. Many people could be at risk of adverse health effects from consuming vegetables cultivated in contaminated soil. Many researchers have shown that some vegetables are capable of accumulating high levels of metals from the soil (Cobb *et al.,* 2000). The problems of heavy metal contamination in the vegetable farms of Addis Ababa and their accumulation on the vegetables have been well recorded by (Fisseha, 2002). These agents have lead to impaired health of the population by the ingestion of foodstuff contaminated by harmful elements (Zukowska *et al*., 2008).

Other sources of food contamination by heavy metals include techniques and materials used in food processing and transformation. The transformation process involves the use of cook wares that contaminate food with heavy metals based on the materials of their design (Cabrera *et al*., 2003). Hence, metal poisoning could result, because of safety of food contact materials including from drinking contaminated water resulting from lead pipes or clay pots (Clay pots are used in storing water) the contamination chain of toxic metals almost always follows a cyclic order: industry, atmosphere, soil, water, foods and human (Cabrera *et al*., 2003). Generally, potential contaminants from the use of locally made clay pots include heavy metals such as As, Cd, Co, Cr, Cu, Fe, Pb, Se and Zn among others (Valadez *et al*. 2011).

2.4. Selected toxic metals (heavy metals) for the study

The term heavy metal refers to chemical elements with a specific gravity of at least 5 times the specific gravity of water (Amin *et al*., 2014). However, being a heavy metal has little to do with density but concerns chemical properties. Commonly, the term heavy metal is used to refer elements that are associated with pollution and toxicity problems. Some well known toxic metallic elements with a specific gravity of 5 or more times than of water are arsenic, 5.7; cadmium, 8.65; lead, 11.34; and mercury, 13.546 (Amin *et al*., 2014) . Heavy metals are dangerous because they tend to bioaccumulate (Mason *et al*., 2000). The toxicity of heavy metals for humans is mainly caused by their persistence in the environment and hinged strongly upon the chemical form in which they are ingested (Mclaughlin *et al*., 1999).

Although they can be found in high concentrations in the body, a number of heavy metals such as aluminum, beryllium, cadmium, lead and mercury have no known biological function. Others, such as arsenic, copper, iron and nickel are considered essential at low concentrations but are toxic at high levels (Jomova & Valko, 2010). Ingested lead accumulates and affects the nervous systems (Fang *et* al., 2003; Gasparik *et* al., 2004). Lead and cadmium are considered potential carcinogens and are associated with etiology of a number of diseases, especially cardiovascular, kidney, blood, nervous, and bone diseases (Jarup, 2003; Mclaughlin *et al*., 1999). As a result of these elements have no beneficial effects in humans, and there is no known homeostasis mechanism for them (Vieira *et al.,* 2011).
The most important toxic metals focused in this study are: Lead (Pb), Cadmium (Cd)and Aluminum(Al).

2.4.1. Lead (Pb)

Lead is a metal element that belongs to the group IV and period 6 of the periodic table, with atomic number is 82, atomic mass 207.2, density 11.4 g/cm3, melting point 327.4 °C and boiling point 1725°C. It is a naturally occurring; bluish-gray metal usually found as a mineral contaminated with other elements (such as sulphur or oxygen) (Wuana & Okieimen, 2011). It has four naturally occurring isotopes with ratios depending on the various mineral sources. The usual oxidation state of Pb in inorganic compounds is +2. Most inorganic Pb (II) salts are poorly soluble in water. Lead (Pb) is toxic to the blood, nervous, urinary, gastric and genital systems (Zraly *et al.*, 2007). Furthermore, it is also implicated in causing cancer, mutation and teratogenesis in experimental animals (Enb *et al*., 2009).

It is considered to be among the most dangerous metals for human health because it affects the central nervous system, causing anemia and gastrointestinal damage, and is associated with alterations in genetic expression (Lepper *et al*., 2010).

Lead is used intensively in industries such as storage-battery manufacture, printing, pigment manufacturing, petrochemicals, phosphate fertilizer, pesticides, fuel combustion and photographic materials. The current annual worldwide production of Pb (II) is approximately 5.4 million tons and continues to manufacturing of batteries (automobile batteries, in particular), while the remainder is used in the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products (Zraly *et al.*, 2007).

This metal is a highly toxic and has the ability to accumulate in living organisms through long time exposure. Assimilation in the human body of relatively small amounts of Pb over a long period of time can lead to malfunctioning of certain organs and chronic toxicity. It can damage practically all tissues, particularly the kidneys and the immune system. Intense exposure to high Pb levels causes encephalopathy with the following symptoms: vertigo, insomnia, migraine, irritability, and even convulsions, seizures, and coma. Lead as a toxicologically relevant element has been brought into the environment by man in extreme amounts, despite its low geochemical mobility and has been distributed worldwide (Oehlenschlager, 2002).

Lead still has a number of important uses in the present day; from sheets for roofing to screens for X - rays and radioactive emissions. Like many other contaminants, lead is ubiquitous and can be found occurring as metallic lead, inorganic ions and salts (Harrison, 2001).

Food is one of the major sources of lead exposure; the others are air (mainly lead dust originating from petrol) and drinking water. Plant food may be contaminated with lead through its uptake from ambient air and soil; animals may then ingest the lead contaminated vegetation. In humans, lead ingestion may arise from eating lead contaminated vegetables or animal meats. Another source is ingestion of food and water contaminated through the use of lead-containing vessels or lead-based pottery glazes (Ming-Ho, 2005).

Recent reductions in the use of lead in petrol (gasoline), paint, plumbing and solder have resulted in substantial reductions in lead levels in the blood. However, significant sources of exposure to lead still remain, particularly in developing countries. (Fewtrell *et al*., 2003). Gasoline, lead-acid batteries, old paint, pesticide and industrial applications are sources of lead pollution. Demolition waste processed into wood products can be a source of lead found in fertilizers.

Lead is neither essential nor beneficial to living organisms (Tsuji *et al.*, 1997). It is a neurotoxin metallic element that can be absorbed by the body, primarily through the lungs and stomach. It can replace calcium in bones (Wayne & Ming-Ho, 2004). Lead can pass the placental barrier and may reach the foetus, resulting in miscarriages, abortions and stillbirths (Opeolu *et al*., 2010).The effect of lead differs with age, gender and nutritional status. Those with malnutrition, anemia and colic are prone to Pb poisoning with some symptoms like low intelligent quotient (IQ) (Munoz *et al*., 1993), impaired speech and hearing, hyperactivities. Higher doses generate fatigue, lethargy, abdominal discomfort, irritation, headache, vomiting and constipation (Bellinger *et al.,* 1994).

Excessive amounts of lead in the body can cause disorders in the metabolism of other micro elements, *e.g.,* iron, which may be manifested as anemia, copper or zinc, which has a negative effect on the function of heart and kidneys. Children may be particularly vulnerable to lead exposure because they spend a significant part of their time on the streets, put everything into their mouths and often lack proper nutrition that may also increase their susceptibility to lead poisoning (Jankiewicz *et al*., 2001). According to 'code of practice for the reduction and prevention of Pb contamination in food': Chronic exposure to lead at relatively low levels can result in damage to the kidneys and liver, and to the reproductive, cardiovascular, immune, nervous and gastrointestinal system (Schwartz, 1994). Short term exposure to high amount of Pb can cause gastrointestinal distress, anemia, encephalopathy, and death. Young children are particularly vulnerable to Pb poisoning because of their tendency to put chips of peeling paints into their mouth or sucking of fingers contaminated with lead-laced dust. Studies revealed that children absorb lead into their body at a higher rate than adult just as children naturally absorb more nutrients from their diet. They absorb more toxicant than a mature body could flush away. The most critical effect of low level lead exposure is reduced cognitive and intellectual development in children (Lanphear *et al.,* 2005).

Lead has been recognized as a poison for millennia and has recently been the focus of public health regulations in most of the developed world (Zaki *et al*., 2010). Although lead naturally occurs in soils in relatively low concentrations, its concentration in the environment has been increasing for several decades due to human activities particularly the use of lead additives to gasoline (Sharma & Prasad, 2010). Reduction of lead additives in gasoline drastically lowered the metal contamination in the roadside environment in Hong Kong, China (Ho, 1990).

Due to the low safety factor, all use of lead in food contact materials should be abandoned or avoided**.** JECFA (1993) has established a PTWI at 0.025 mg/kg bodyweight. SCF at its 91st meeting held on December 9-10, 1993 agreed with the JECFA conclusion. Estimated daily dietary intake for adults range from 0.015-0.1mg, depending on the composition of the diet and where the consumer lives (Codex, 1995). According to the guidelines on metals and alloys used as food contact materials , parts made wholly or partly of lead and lead solder for repair should not be used in materials and articles intended to come into contact with foodstuffs including the use of lead in soldered cans and utensils.

2.4.2. Cadmium (Cd)

Cadmium is naturally present in the environment: in air, soils, sediments and even in unpolluted seawater. Cadmium is emitted to air by mines, metal smelters and industries using cadmium compounds for alloys, batteries, pigments and in plastics, although many countries have stringent controls in place on such emissions (Harrison, 2001). Tobacco smoke is one of the largest single sources of cadmium exposure in humans. Cadmium exposure may cause kidney damage. The first sign of the renal lesion is usually a tubular dysfunction, evidenced by an increased excretion of low molecular weight proteins or enzymes (WHO, 1992). It has been suggested that the tubular damage is reversible, but there is overwhelming evidence that the cadmium induced tubular damage is indeed irreversible (Jarup *et al*, 1998). Cadmium is highly toxic and is even more dangerous, being 10 times more toxic than lead. It is responsible for several cases of poisoning through food. Small quantities of Cd cause adverse changes in the arteries of human kidney. It replaces Zn biochemically and causes high blood pressures, kidney damage, and so forth (Divrikli *et al*., 2006).

It is used in nickel cadmium batteries, PVC plastic and paint pigments. It can be found in soils because insecticides, fungicides sludge, and commercial fertilizers that use cadmium are used in agriculture. Cadmium may be found in reservoirs containing shell fish. Inhalation accounts for 15-20% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Cadmium sulphide and selenide are commonly used as pigments in plastics (Eaton, 2005).

Cadmium occurs in the surface of the Earth in all soils and rocks, including coal, in very low concentrations (<0.1 mg/kg) (Lind, 1997). Also, zinc ores contain cadmium, which is emitted during the melting of zinc. Cadmium is a relatively rare element and current analytical procedures indicate much lower concentrations of the metal in environmental media than did previous measurements due to improved sampling and analytical techniques (WHO, 1992). Phosphate fertilizers and sewage sludge used on agricultural land may be significant sources of cadmium. Cadmium is found in most foodstuffs in the range of 0.005-0.1 mg/kg certain foodstuffs, e.g. mushrooms, kidney and oysters, may contain much higher concentrations (Friberg *et al.,* 1986). The lowest levels of cadmium are found in fruits and beverages. Vegetables, cereals and cereal products make the greatest contribution to cadmium intakes. Cigarette smoking has been shown to contribute significant amounts of cadmium to the intake (Friberg *et al.*, 1986).

JECFA (1993) has established a PTWI at 0.007 mg/kg of body weight stating that "the PTWI does not include a safety factor" and that "there is only a relatively small safety margin between exposure in the normal diet and exposure that produces deleterious effects". Cadmium can also be used as pigments in certain enamels in food contact materials. Leachable cadmium in enamel pottery and glazes may be a source of contamination. Cadmium plated utensils in food processing and preparation is forbidden according to Directive 91 /338/EEC.

2.4.3. Aluminum (Al)

Although aluminum is not a heavy metal (specific gravity of 2.55 -2.80), it makes up about 8% of the surface of the earth and is the third most abundant element. It is readily available for human ingestion through the use of food additives, antacids, buffered aspirin, astringents, nasal sprays, antiperspirants and from drinking water. Studies suggested that aluminum might have a possible connection with developing Alzheimer's and Parkinson's disease when researchers found what they considered to be significant amounts of aluminum in the brain tissue of Alzheimer's patients (Bakare& Odunola, 2005).

Food is one of the sources of aluminum; its concentration in unprocessed food can be 0.1-20 mg/kg. About 0.1 mg/kg of Al is found in unprocessed aliments like egg, apple, raw cabbage, corn, and cucumber (Agency for Toxic Substances and Disease Registry (ATSDR), 1997) and 4.5 mg/kg in tea powder (Pennington and Jones, 1989). Much higher values are found in some industrially processed foods with aluminum salts added as food additives. Significant amount of Al can be transferred into the food through food contact materials contact materials such as saucepans, Al lined cooking utensils, coffee pots, and packaging products such as food trays, and cans (Codex,1995). Aluminum compounds used in pigments of utensils also the other sources of Al contamination in the food.

Al is not known to be of any nutritional value to human beings and it was previously regarded as non-toxic (Metwally & Mazhar, 2007). However, aluminum as a chelator has the ability to capture and prevent the uptake of essential elements such as calcium, zinc and copper, and disrupt the proper use of many of them (Dabonne *et al*, 2010). However, over the past 20 years more evidence has come to light to link Al with certain neurodegenerative diseases (Gray, 2002).

According to JECFA (1989) established the Provisional Tolerable Weekly Intake (PTWI) is 7 mg/kg bodyweight for the total intake, including food additive uses of aluminum. Even if there is no health based guideline a certain value is recommended by WHO (1992) : Its directive 98/83/EC intended to set a standard value of 0.2 mg/l ion in drinking water for human consumption considering the practical use of aluminum for treatment and discoloration of distributed water can be seen as a guideline. According to the Agency for Toxic Substance and Disease Registry (1997), exposure to aluminum is usually not harmful. Al is excreted by the kidneys, and only a small amount of Al is absorbed (JECFA, 1989).

2.5. Metal leaching from utensils

One of the sources of food contamination by metal includes techniques and materials used in food processing (Dabonne *et al.,* 2010). The domestic preparation of food as a potential source of metal contamination has been afforded little importance. However, there are reports that indicate that certain kitchen utensils used for food preparation can represent a significant risk because they are manufactured with materials that can be hazardous or contaminated by toxic metals (Hight*,* 2001). Leaching of toxic elements from cooking utensils is a long recognized problem, and covers a wide range of elements, the traditional examples include materials such as Pb (mainly from earthenware) and Cu (typically from coffee pots with defective tin-plating) (Baba & Kaya*,* 2008).

Metals can accumulate in foods stored or cooked in uncoated utensils. The amount and types of metal that accumulate in foods during preparation depend on the pH of the foods, the length of cooking periods and the types of utensils. Acidic foods had a great ability to transfer metals into the food according to a research conducted in Mexico by Valadez-Vega, 2011, examining containers obtained from four regions' in the Mexican state of Hidalgo. Metals and alloys can come in contact with food materials mainly in processing equipment, containers, household utensil and also in foils for wrapping foodstuffs.

They are often covered by a surface coating, which reduces the migration in foodstuffs. When they are not properly coated these metals can give rise to migration into the foodstuffs and therefore could either endanger human health or bring about an unacceptable change in the composition of the foodstuffs (Marsh & Bogus, 2007).The use of metallic utensils for cooking and storage of foods is known to contribute metals into the food, and leaching of these metals from cooking pots is influenced by various factors, such as pH, temperature, frequency of usage, duration of contact or heating and the presence of various food ingredients (Semwal *et al*., 2006). In principle, the maximum quantity of metals which can be absorbed by individuals from all sources should not exceed daily or amount of weekly the TDI's and the PTWI's (multiplied by a bodyweight of 60 kg) established by JECFA or the Scientific Committee for Food of the EU (SCF) or any other international bodies.

The other influential factors for the migration of metals into foodstuff are temperature and storage. In a migration study with 3% acetic acid (Gramiccioni *et al*., 1996), the migration was approximately 10 fold higher at 40°C compared to 5°C after 24 hours. The other one that greatly also contributes to the migration of metals from utensils into the food is cooking time. (Greger ,1985) reported that, tomatoes heated in aluminum pans for a few minutes accumulated only 0.02- 0.03 mg Al/100 g serving, while tomato sauces cooked for 3 hours in aluminum pans accumulated 5.7 mg Al/100g serving. Greger (1985) estimates that the amount of Al added to the daily diet through the use of aluminum pans, tray, and foil during food and beverage preparation to be about 3.5 mg/day.

Clay or ceramics may be considered to be materials made from naturally occurring clay or earth. Scientifically, these utensils are compounds of metallic and non-metallic elements. Such utensils are produced either industrially or handcrafted as a cottage industry. Due to the fact that, metals are natural components of the earth crust and used as decorative and protective enamel, these utensils have a great chance to be contaminated. Food items and beverages prepared in such utensils are likely to show high levels of Pb. Some ceramic food wares have been found to leach significant quantities of Pb from the potential food contact surfaces (Sheets, 1997).

2.6. Mechanism of metal toxicity

Although the toxicity of a metal depends on the amount ingested, chronic exposure to certain metals, such as arsenic, lead and cadmium can cause severe toxic effects, even in low amounts. In general, heavy metals disrupt basic metabolic functions in two ways. On one side, they disrupt the functioning of vital organs and gland such as the heart, brain, kidney, bone or liver and secondly they alter nutrients that are essential minerals preventing them from fulfilling their biological function (Valadez-Vega, 2011).

Metallic toxicant in food through ingestion may find their way in to the body and act through one or more of these possible mechanisms (Iyengar and Nair, 2000).

Inhibition of enzymatic activities: this is so because some metal such as Pb, Hg, and Cd have affinity for sulphur and therefore attack sulphur bonds in enzyme, thus immobilizing them. Other sites of attack include the free amino and carboxyl groups in protein (Alka, 2000).

Attacks on cell membrane and receptor: the heavy metals bind to cell membrane and receptor, thereby altering their structures. This affect transport and other inter or intra cellular activity in the body. Cd inhibits oxidative phosphorylation in the body (Alka, 2000).

Interference with metabolic cations: Heavy metals interfere with the metabolism of essential cations such as absorption, transportation, decomposition and storage. Cd follows the pathway of Zn and Cu metabolism. Pb replaces Ca in the bones. In our human body, aluminum ion can inhibit different metabolic processes by competition reactions with other ions such as iron, magnesium, calcium, phosphorus, fluoride, and others. It is also reported that aluminum is associated with anemia, osteomulacia, and a neurologic syndrome. This is most crucial aspect that leads to manifestation of toxic effect (Alka, 2000). Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Jarup, 2003). Consuming food contaminated with Pb and Cd can seriously deplete body stores of Fe, vitamin C and other essential nutrients, leading to decreased immunological defenses, intra-uterine growth retardation, impaired psycho-social faculties and disabilities associated with malnutrition.

Generally, heavy metals disrupt basic metabolic functions in two ways: on one hand, they disrupt the functioning of vital organs and glands such as the heart, brain, kidney, bone or liver, on the other hand, they move nutrients that are essential minerals and prevent them from fulfilling their biological functions.

Table 1.The source, interacting element, chronic effect and PTWI of the most toxic metals can be summarized in the table below; (Source : EPA,2004).

Toxic Metal	**Source**	**Interacting element**	**Chronic effect**	**PTWI mg/kg body weight**
As	Wood preserving, Pesticide production and application, Coal/oil production and processing, Volcanic action, Mining, Burning fossil fuel, Smelting non ferrous metal.	Cd, Se, Zn	Peripheral and vascular disease, skin cancer	0.015
Cd	Consumption of tobacco products (a cigarette box contains 2 to 4 μ g Cd), phosphate fertilizer sewage sludge, industrial emissions	Zn, Cu, Ag, Hg, Se, Fe, As, Mg	Lungcancer, bone fracture, kidney dysfunction hyper tension	0.007
Hg	Power plants, Manufacturing industries crematories, Various biocidal coatings, Coal-fired power station, Fossil fuel burning.	Se, Ag	Renal, pulmonary, reproductive, and cardiovascular toxicity Insomnia, Memory loss, paresthesias	0.005
Pb	The production and disposal of storage batteries, some phosphate fertilizers and pesticides, exhaust fumes from cars and industrial emissions	Fe, Zn, Cu, Se, Ca	Anemia, central nervous system disorder	0.025

There are a number of different factors that can influence a metals' toxicity but generally the poisonous effect of metals is a function of a concentration which is attained in a target organ in the human body. Thus, similar effects will occur following long- and short-term exposure to low and high cadmium levels, respectively. Consequently, both acute and chronic systemic toxic effects may be induced. Among possible target organs of heavy metals, soft tissues such as the kidney and liver and the central nervous system appear to be especially sensitive (Apostoli, 2002).

3. MATERIALS AND METHODS

3.1. Study design

In a laboratory based work or experiment, metal content of traditional clay pots and the leaching of, Pb, Cd and Al from the repeated use of these pot (clay pot and coffee pot) were analyzed using FAAS (Flame Atomic Absorption Spectrophotometer) by preparing two kinds of stew (tomato sauce and bean stew) and coffee beverage which are commonly consumed Ethiopian foods. The same types of food in a similar manner were also cooked in a beaker to control the effect of metal leaching from the cookware. Sample preparation for instrumental analysis and moisture quantification values were done at research laboratory of the Center for Food Science and Nutrition, and Department of Chemistry of Addis Ababa University.

3.2. Sample collection

Traditional clay cooking pots (n=24) were collected randomly from two sites located in Addis Ababa particularly "*kechene*" and in "*legeberi*" which is far from Addis Ababa to the east around 25 km . Besides, the ingredients used in cooking stew and coffee beverages were purchased from local market. All clay pots were washed with detergent simulating everyday use, rinsed with deionized water, and air dried for 24 hr (following the laboratory conditions established by De Mejía and Craigmill (1996).

3.3. Sample preparation

- **Preparation of clay pot sample**

The clay pot samples to be analyzed were rinsed with deionized water to remove dirt because of their newness and air- dried in the laboratory then, ground in porcelain mortar using a pestle and sieved through a 5 mm stainless sieve (mesh), and then the resulting homogenized, well-mixed samples were stored at room temperature in polyethylene bags for further analysis.

- **Preparation of sauces**

Types of commonly consumed sauces bean stew and tomato sauce were prepared in each clay pots come from the two sites (*kechene* & *legeberi*) in accordance to house hold preparation procedures. Tomato sauce, this was standardized and prepared by mixing 128 g of onion (*Allium cepa*), 55 ml of oil and 27 g of Berbere (*Piper nigrum mixed* with different spices), 290g of tomato (*Lycopersicon esculentum*) and 140 ml of deionized water and cooked for 45 min. pH value of the stew was recorded at the end of cooking. Bean stew*:* prepared based on the proportion stated on Federal Democratic Republic of Ethiopia Ministry of Health; Nutrition Extension Package, were prepared. Briefly, the sauce was standardized and prepared by cooking a mix containing 60gm of onion (*Allium cepa*), 25 ml of oil and 125g of *Shiro* powder and 800 ml of deionized water. pH value of the sauce was recorded at the end of cooking.

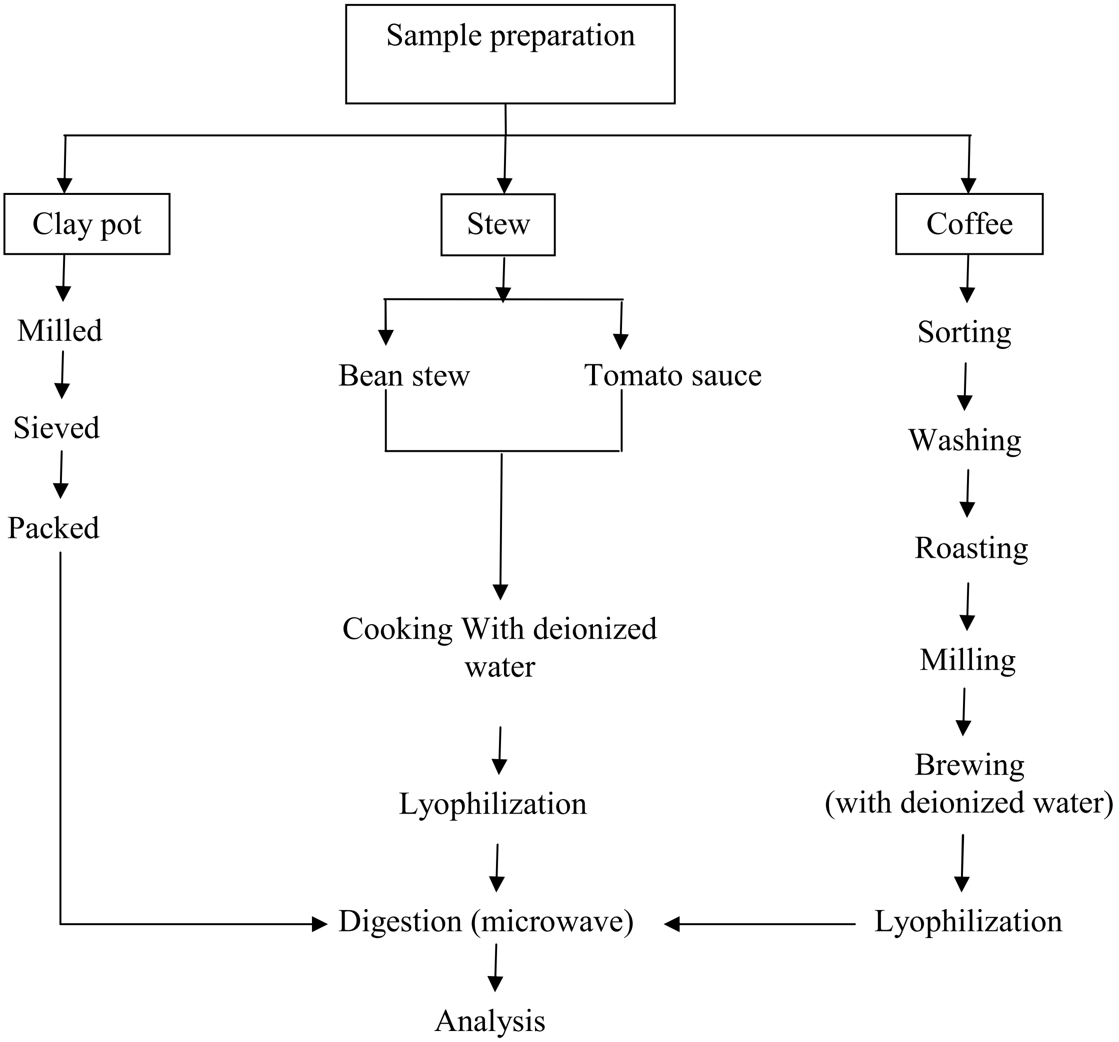
At the same time, to compare and decide whether the metals were from the pots or ingredients the method described by Odularu *et al*., (2013) was used. Briefly, both the bean stew and tomato sauces were also prepared in a beaker using the same procedure as used for traditional pot as a control with the assumption of no leaching. In addition, similar foods were also cooked repeatedly for 3 times in the same cookware to determine the leaching of metals during the repeated use of the utensil.

- **Preparation of coffee sample**

Coffee was also prepared in each coffee pot in accordance to household preparation procedures by mixing 80 g of roasted and powdered coffee with 300 ml of deionized water and boiled. To see the effect of brewing frequency on metal leaching from the cooking pot, samples were regularly drawn every 35 minutes. Mimicking the traditional coffee brewing, the first round is locally named "*Abol"*, the second round "*Tona"*, and the third round is known as "*Bereka"* the whole processes were repeated in the second brewing time. The coffee was also brewed in a beaker using the same procedure used for traditional coffee making as a control with the assumption of no leaching. The process was carried out in triplicate for each sample. Ingredients;

samples of the ingredients used to cook the foods and coffee were taken for digestion and analyzed for the presence of metals,

Generally sample preparation procedures can be summarized in the following flow chart;



3.4. Moisture content determination

Moisture content of sauce was determined following the AOAC, (2006) method. Briefly, empty drying dishes made of porcelain were dried using a drying oven for 1 hour at 105 ^{0}C. The dishes were cooled for 30 minutes in desiccators with granular silica gel and weighed using a digital analytical balance to the nearest milligram (W_1). Duplicates of about 5.0 g of fresh samples were weighed (W_2) in dried and pre-weighed drying dishes. The dishes and their contents were then placed in drying oven and dried for 5 hrs at 105^{0}C. The dishes and their contents were cooled in desiccators to room temperature and weighed (W_3). The procedure was repeated until a constant weight was recorded (APPENDIX 2).

Moisture content is expressed as:

$$\boxed{\text{\% Moisture Content} = (W_2-W_3) / (W_2-W_1) * 100}$$

Where:

W_1=weight of the dish

W_2= weight of fresh sample and the dish

W_3= weight of dried sample and the dish

3.5. Sample digestion

The food samples were digested according to the method described by the Association of Official Analytical Chemists (AOAC, 2006). About 0.5 g of each well homogenized sample was digested with 1 ml of H_2O_2 and 7 ml of concentrated HNO_3 in Teflon with a Milestone microware digestion system. For the digestion of soil we used 1ml of HCl^+ rather than H_2O_2. Then the resulting solution was cooled and filtered by what man filter paper 41 to remove the insoluble particles. The filtrate was collected in volumetric flask and brought to a final volume of 50ml with deionized water. Blank sample was also prepared similarly. Afterwards, the samples were stored at 4 °C until analysis. The process was carried out in triplicate for each sample.

3.6. Metal determination

The measurements of Pb, Cd and Al, was carried out with Atomic Absorption Spectrophotometer (Vario 6 spectrometer, Analytic Jena, Germany) at Ethiopian Public Health and Nutrition Research Institute. Air acetylene gas mixture was used as source of flame. Standards of these metals from stock solution were prepared at the concentration of 0, 1.25, 2.5, 5 and 10 mg/l and set for the calibration of the AAS reading and blank were run in each procedure for quality assurance. Maximum absorbance was obtained by adjusting the Cathode lamps at specific slit and wave lengths as indicated in Table 1. To quantify the level of metal in a sample in Atomic Absorption Spectrometry it is important to keep the linearity of concentration against absorbance curve to ensure the accuracy and reliability of the instrument.

The different concentrations of metals obtained during analysis were converted in to mg/kg dry matter using the formula below. $\frac{\mathbf{Mg}}{\mathbf{kg}} = \frac{(\boldsymbol{a}-\boldsymbol{b})*\mathbf{Vs}}{\boldsymbol{Wt}} * \frac{\mathbf{100}}{\mathbf{100}-\boldsymbol{c}}$

Where, b - Concentration of a sample
a - concentration of blank
Vs - Volume of solution
Wt - weight of sample (fresh weight)
c – Moisture content

Table 2**.** Instrumental Conditions

Parameter	Slit width (nm)	Wavelength (nm)	Detection limit (µg/ml)
Pb	1	217.0	0.05
Cd	0.5	228.0	0.01
Al	0.5	309.2	0.03

3.7. Statistical analysis

Data analysis was computed using SPSS software version 20. Descriptive statistics like range, and arithmetic mean ± SD were used to express the level of each metal. Independent sample t - test was used to compare the metal content of clay pots come from the two sites. Means of the metals (Pb, Cd and Al) in coffee prepared first and second as well as in sauces (bean and tomato) prepared first, second and third were computed using one way ANOVA(Analysis of variance) .The statistical significance of the differences was assessed by applying the Tukey test. A level of $p<0.05$ was considered as significant. Calibration graphs and tables were drawn using Microsoft Office Excel 2010.

4. RESULTS AND DISCUSSION

❖ Results

The calibration curves for Pb, Cd, and Al were fairly linear approximately ($r^2=0.999$).

4.1. Metallic content of various food ingredients

The level of Al in different food ingredients used to make both foods (bean stew and tomato sauce) and coffee is summarized in Table 3. The Pb and Cd level were below the detection limit of the instrument.

Table 3. The level of Al in various food Ingredients

Ingredient	Pb ,	Cd	Aluminum (mg/kg)
Coffee	ND	ND	*0.89±0.0
Beriberi	ND	ND	5.2±0.8
Shiro	ND	ND	8.43±0.1
Tomato	ND	ND	2.3±0.0
Onion	ND	ND	0.9±0.0

* All values are means of Triplicate ± SD.

ND= Not Detectable

4.2. Metal content of clay pots

Results of Pb, Cd, and Al content of clay pot samples manufactured in two sites ("*Kechene* and *Legeberi"*) are presented in Figure 1.The Pb (26.72±0.0 mg/kg), Cd(5.6±0.1 mg/kg) and Al(38.74±0.7 mg/kg) level of clay pots from *legeberi* had significantly higher ($p<0.05$) than the Pb (0.6±0.3 mg/kg), Cd(0.3±0.0 mg/kg) and Al (8.92±0.3 mg/kg) level in clay pots from *Kechene* .

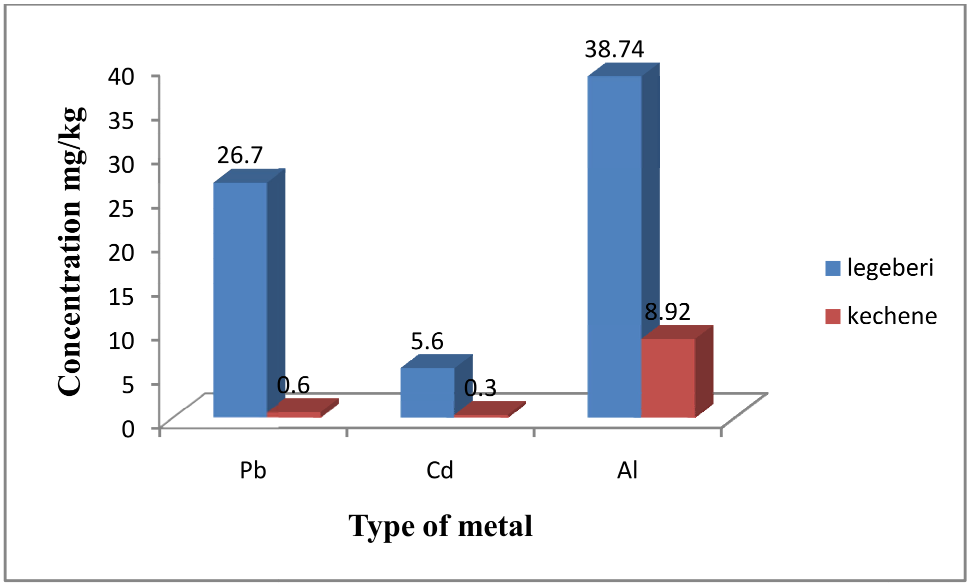


Figure 1. Pb, Cd, and Al concentration (mg/kg) in *Kechene* and *Legeberi*

4.3. Leaching of metals from the traditional clay pot into the food

4.3.1. Lead

Leaching of Pb during the preparation of stews in a traditional clay pot and their repeated usage are illustrated in table 4. As it is shown in table 4 no leaching was observed in both food samples prepared in clay pot from *kechene* and in the beaker with their repetative usage. Pb leaching in clay pots from *legeberi* used for tomato sauce cooking for the first (9.6 ±0.0 mg/kg), second(12±0.0 mg/kg), and third (13±0.0 mg/kg) time was increased significantly ($p<0.05$). In addition, the Pb level of bean stew was also significantly ($p<0.05$) increase from first (1.2±0.0 mg/kg) to second (2.8±0.0mg/kg) and third cooking time (3.3±0.0mg/kg).

Table 4. Pb concentration (mg/kg) in tomato sauce and bean stew

	Stew in *legeberi* Clay pot Pb (mg/Kg)		Stew in *kechene* Clay pot Pb (mg/Kg)		Stew in beaker (control) Pb (mg/Kg)	
Frequency	Tomato sauce	bean stew	Tomato sauce	bean stew	Tomato sauce	bean stew
First	9.6 ±0.0^a	1.2±0.0^a	ND	ND	ND	ND
Second	12±0.0^b	2.8±0.0^b	ND	ND	ND	ND
Third	13±0.0^c	3.3±0.0^c	ND	ND	ND	ND

ND= Not Detectable. All values are means of Triplicate ± SD.
Means designated by different letter in the same column shows significance difference ($p<0.05$).

4.3.2. Cadmium

Leaching of Cd from clay pots due to cooking of stews and repetative usage of these pots is summerized in table 5. No leaching was observed in tomato sauce and bean stew prepared in clay pots from *kechene* and in beaker used as a control.

The Cd content of tomato sauce prepared in clay pots from *legeberi* showed no significance difference ($p>0.05$) between first (0.5±0.0mg/kg) and second (0.8±0.0 mg/kg) cooking time Similar trend was observed in the second(0.8±0.0 mg/kg) and third(1.2±0.0 mg/kg) cooking time. However, third cooking time (1.2±0.0 mg/kg) showed significanctly ($p<0.05$) higher Cd level than first cooking time (0.5±0.0mg/kg).

The Cd level of bean stew in the first (0.14±0.0 mg/kg) and second cooking time (0.15±0.0 mg/kg) was not different significantly ($p>0.05$). But bean stew sample of the third cooking time (0.38±0.0 mg/kg) was significanctly ($p<0.05$) higher than first (0.14±0.0 mg/kg) and second (0.15±0.0 mg/kg) cooking time .

Table 5. Cd concentration (mg/kg) in tomato sauce and bean stew

Frequency	Stew in *legeberi* clay pot Cd (mg/Kg)		Stew in *kechene* clay pot Cd (mg/Kg)		Stew in beaker (control) Cd (mg/Kg)	
	Tomato sauce	Bean stew	Tomato sauce	Bean stew	Tomato sauce	Bean stew
First	0. 5±0.0^{a}	0.14±0.0^{a}	ND	ND	ND	ND
Second	0. 8±0.0^{ab}	0.15±0.0^{a}	ND	ND	ND	ND
Third	1.2±0.0^{b}	0.38±0.0^{b}	ND	ND	ND	ND

ND= Not Detectable All values are means of Triplicate ± SD.
Means designated by different letter in the same column shows significance difference ($p<0.05$).

4.3.3. Aluminum

Result of Al leaching during the preparation of two types of stews in a traditional pot as well as and their repeated usage are presented in the table 6. The cooking pot from *legeberi* used for first and consecutive time of cooking of tomato sauce resulted significantly ($p<0.05$) higher Al content (11.53±0.3 mg/kg) for the first, (13.1±0.2 mg/kg) second and (15.4±0.3 mg/kg) third time. In addition, each reading was significantly ($p<0.05$) higher than the control sample prepared in beaker (8.45±0.5 mg/kg).

Aluminum content of the bean stew was significantly ($p<0.05$) increased from first (10.8±0.21 mg/kg), to second (12.2±0.1 mg/kg) and third (13.5±0.3 mg/kg) cooking time. The reading of control sample (9.34±0.6 mg/kg) was also significantly lower ($p<0.05$) than each reading of Al content in bean stew sample prepared using clay pot.

The control tomato sauce sample (prepared in a beaker) had (8.45±0.5 mg/kg) Al content and bean stew prepared in a beaker had (9.34±0.6 mg/kg) Al content which was significantly ($p<0.05$) lower from the mean Al content of tomato sauce samples and bean stew prepared in clay pot from *kechene.* However, no significance ($p>0.05$) difference was observed between the

Al content in the tomato sauces as a result of using clay pots from *kechene* for the first (10.5±0.5 mg/kg), second (10.9±0.7 mg/kg) and third (11.2±0.6 mg/kg) cooking time .

In addition, no significance ($p>0.05$) difference was observed in Al content of bean stews prepared for first (10.1±0.3 mg/kg), second (10.3±0.5 mg/kg) and third (10.4±0.3 mg/kg) cooking time using clay pots from *kechene.*

Table 6. Al concentration (mg/kg) in bean stew and tomato sauce

Frequency	Stew in *legeberi* clay pot Al (mg/Kg)		Stew in *kechene* clay pot Al (mg/Kg)		Stew in beaker (control) Al (mg/Kg)	
	Tomato sauce	bean stew	Tomato sauce	bean stew	Tomato sauce	bean stew
First	11.53±0.35^{a}	10.8±0.21^{a}	10.5±0.51^{a}	10.1±0.30^{a}	8.45±0.53	9.34±0.67
Second	13.1±0.21^{b}	12.2±0.10^{b}	10.9±0.72^{a}	10.3±0.51^{a}	8.45±0.53	9.34±0.67
Third	15.4±0.30^{c}	13.5±0.30^{c}	11.2±0.61^{a}	10.4±0.37^{a}	8.45±0.53	9.34±0.67

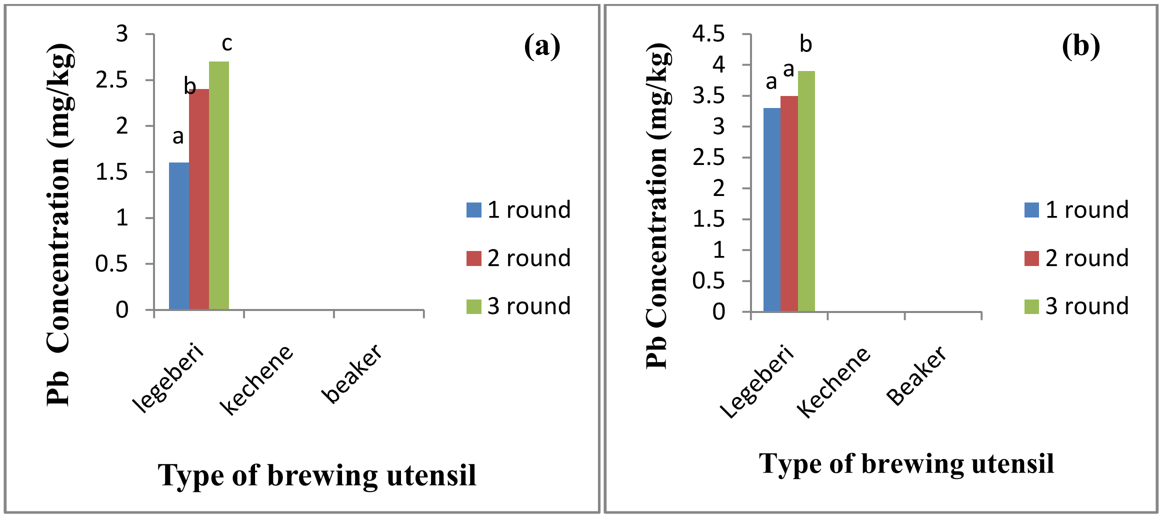
Means designated by different letter in the same column shows significance difference ($p<0.05$).
All values are means of Triplicate ± SD.

4.4. Leaching of metals from the clay pot into the coffee

4.4.1. Lead

The mean levels of Pb of the coffee samples processed in coffee pot from *legeberi* and *kechene* as well as prepared in a beaker is indicated in Figure 2. As clearly presented, in the figure no leaching of Pb was found in the coffee samples processed in coffee pots from *kechene* and in a control (prepared in a beaker) as well as in their repeated usage.

However, there was in coffee samples prepared in coffee pot from *legeberi* .In this manner in the first brewing time of the first round coffee had a mean Pb content was significantly ($p<0.05$) increased from first round (1.6±0.0 mg/kg) to second round (2.4±0.0 mg/kg) and third round (2.7±0.01mg/kg). In the second brewing time no significance difference ($p>0.05$) was observed between the Pb level of first round (3.3±0.0mg/kg) and second round (3.5±0.0mg/kg) of the coffee samples. However, Pb content of the third round (3.9±0.0mg/kg) coffee sample was significantly ($p<0.05$) higher than first round (3.3±0.0mg/kg) and second round (3.5±0.0mg/kg).



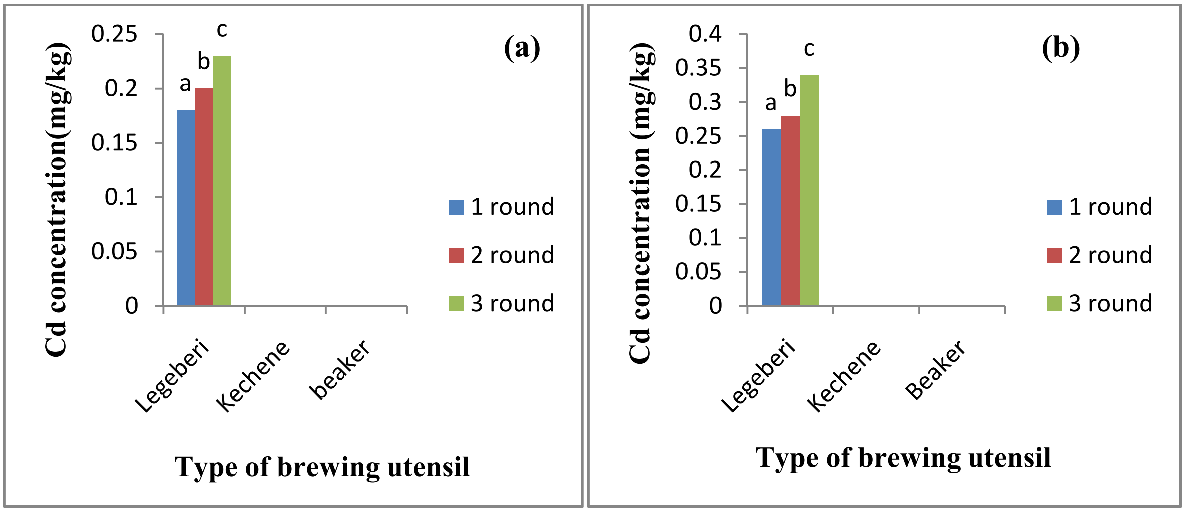
Means designated by different letter in the same bars shows significance difference ($p<0.05$).

Figure 2. Lead concentration (mg/kg) coffee brewed in coffee pot from Legeberi, Kechene and in beaker (a) for the first brewing time (b) for the second brewing time.

4.4.2. Cadmium

The Cd content of coffee samples brewed in coffee pot from *legeberi* and *kechene* as well as coffee prepared using beaker is presented in Figure 3. As shown below in the figure, no leaching of Cd was detected in the coffee samples prepared in coffee pot from *kechene* and in the control (beaker).

Cadmium was detected in all coffee samples prepared using coffee pots from *legeberi,* thereby, in the first brewing time the Cd level of the coffee samples significantly ($p<0.05$) increased from first round (0.18±0.0 mg/kg) , second round (0.20±0.0 mg/kg) and third round (0.23 ±0.0 mg/kg).Similar trends was observed in the second brewing time of the coffee samples was significantly ($p<0.05$) increased from the first round (0.26 ±0.00 mg/kg) to second round (0.28±0.0 mg/kg) and third round(0.34±0.0 mg/kg).



Means designated by different letter in the same bars shows significance difference ($p<0.05$).

Figure 3. Cadmium concentration (mg/kg) coffee brewed in coffee pot from *Legeberi* , *Kechene* and in beaker (a) for the first brewing time (b) for the second brewing time.

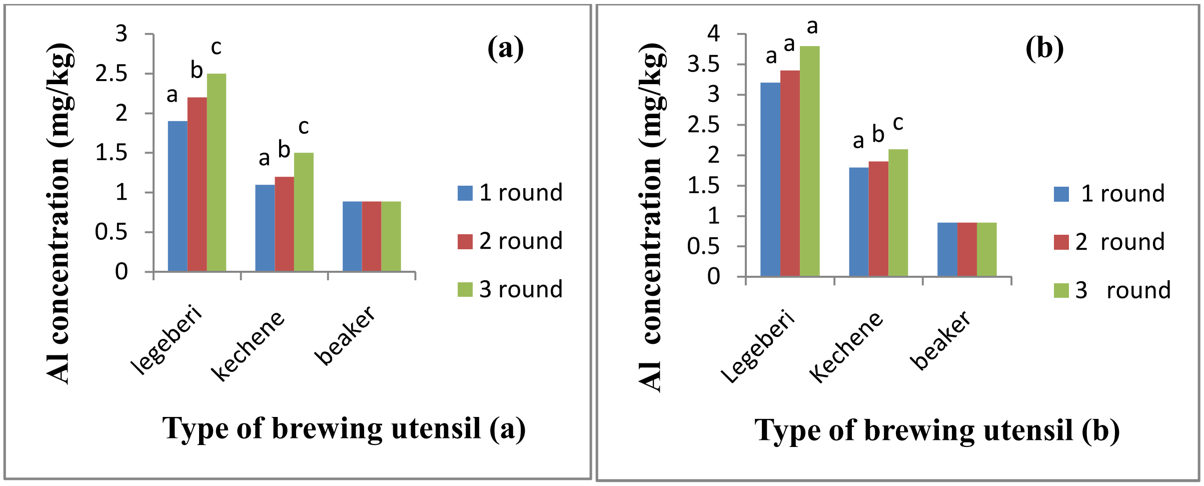
4.4.3. Aluminum

Leaching of Al during the preparation coffee in traditional coffee pots from (*Kechene and Legeberi*) as well as prepared in a beaker (control) and their repeated usage are presented in

Figure 4. The control coffee sample (prepared in a beaker) had a mean Al content of (0.89±0.0 mg/kg) which was significantly ($p<0.05$) lower from the Al content of coffee prepared in both types of coffee pots, in first and second brewing time.

In the first brewing time the coffee samples brewed in coffee pot from *kechene* significantly ($p<0.05$) increased from the first round (1.1±0.0mg/kg), to second round (1.2±0.0mg/kg) and third round (1.5±0.0 mg/kg), similar trend was observed in the second brewing time, in this case, Al content was significantly ($p<0.05$) increased from the first round (1.8±0.0 mg/kg) to the second round (1.9±0.0 mg/kg) and third round (2.1±0.0 mg/kg) .

In case of coffee samples prepared in coffee pot from *legeberi* at the first brewing time the mean Al level significantly ($p<0.05$) increased from the first round (1.9±0.0 mg/kg), to the second round (2.2±0.0 mg/kg) *and* third round (2.5±0.0 mg/kg). However, no significance ($p>0.05$) difference was observed among the Al content of coffee samples in the first round (3.2±0.0 mg/kg), the second (3.4±0.0 mg/kg), and third round (3.8±0.1mg/kg) at the second brewing time.



Means designated by different letter in the same bars shows significance difference ($p<0.05$).

Figure 4. Aluminium concentration (mg/kg) of coffee brewed in coffee pots from *Legeberi, Kechene* and in beaker (a) for the first brewing time (b) for the second brewing time.

❖ Discussion

In general, the increase in concentration of the metal (Al) in the experimental sample over the control (prepared in the beaker) sample suggests the contribution of the metal added by the traditional cookware.

In addition to the natural metallic content of the foodstuffs, additional contamination can occur through utensil that come into contact with food that contain metals (Semwal *et al*., 2006). Metals and alloys can come in contact with food materials mainly in processing equipment, containers, household utensil and also in foils for wrapping foodstuffs. They are often covered by a surface coating, which reduces the migration in foodstuffs. When they are not properly coated these metals can give rise to migration into the foodstuffs and therefore could either endanger human health or bring about an unacceptable change in the composition of the foodstuffs (Marsh & Bogus, 2007).

According to the study conducted by Valadez-Vega, (2011) traditional clay pot can constitute a public health hazard if they are used as a food contact material. This is because clay pots can be manufactured from materials such as clay, and water which are contaminated by pollutants especially toxic metals such as Pb and Cd. Since the geochemistry of the clay is different from regions to region depending on the natural background of the soil and pollution factors the utensil made from such raw materials also shows variation in the concentration. The present study also confirmed that traditional clay pot manufactured in *legeberi* could contaminate the food with the toxic metals such as Pb , Cd and Al.

Clay pots which were produced in *kechene* are relatively safe as compared to clay pots manufactured in *legeberi*. This may be due to the source of raw materials that potters used to make a pot. The clay pot from *legeberi* showed high level of metals which may be because the source of clay soil as a raw material is found close to the road side and agricultural practices are being practiced there. Furthermore, the water used in making cooking pots is also came from the river known as *legeberi* because of flooding and poor waste management the water can also be contaminated. However, clay pots from *kechene* are made with tap water and the soil from "*intoto*" is less likely polluted. The high concentration of lead and cadmium contained in soils and vegetables from towns of were attributed to vehicular emissions (Itanna, 2002; Gzyl, 1990).

Although, lead naturally occurs in soils in relatively low concentrations, its concentration in the environment has been increasing for several decades due to human activities particularly in the roadside by the use of lead additives to gasoline (Sharma & Prasa, 2010). Along, in agricultural places due to the application of phosphate fertilizers as well as pesticides can be contaminated. In Ethiopia, the motor gasoline imported and distributed was not lead free (with 0.6 g/L of lead) until July 2003, when the government promulgated new specifications for unleaded gasoline (ULG) and decided that all the gasoline imported into Ethiopia shall be unleaded gasoline (WB, 2003). Even if at present the lead content of the gasoline imported and distributed by the Ethiopian Petroleum Enterprise is low (0.013 g/L), the lead from previous use could persist in the soils and may have long term effects through food chain.

According to Directive, (1989), incompliance with Article 2 of Directive 89/109/EEC food contact materials under normal and foreseeable conditions, should meet the following conditions: They should be manufactured in accordance with good manufacturing practice and they should not transfer their constituents to foodstuffs in quantities, which could: endanger human health and/or bring about unacceptable change in the composition of the foodstuffs or deterioration in the organoleptic characteristics. Traditional cook wares may not meet this criteria can posse's public health because they are made under no quality control.

Generally depending on the quality of the raw materials traditional hand crafted clay pots can constitute a public health hazard for people in poor countries who use them daily. For instance, studies conducted by Dabonne *et al.,* (2010) in Cote d'Ivoire shows that traditional utensils made out of clay and traditional Al pots are the potential sources of metals like Al and iron. Another study by Omolaoye *et al.,* (2010) in Nigeria also shows that food items and beverages prepared in ceramic products imported from China had show high levels of lead. Similarly this study revealed that traditional clay pot made from contaminated clay soil in *legeberi* can *potentially* increase in take of toxic metals level from food. Similar to the results of the present work, other researcher Belgaied, (2003**)** have observed that the cadmium levels in clay pot can be very high due to the contamination of the raw materials. Both clays and enamels demonstrate high which and leached from the container to the food in greater concentrations during processing the more the container is used, yielding levels above those permitted by the WHO, (2006).

The use of some utensils for cooking and storage of foods is known to contribute metals into the food, and leaching of these metals from cooking pots is influenced by various factors, such as pH, temperature, frequency of usage, duration of contact or heating and the presence of various food ingredients (Semwal *et al*., 2006). The present study also confirmed that pH and frequency of usage can affect the migration of metals into the food.

The pH of food is an important factor during the study of leaching of metals from the food into the pot as migration of metals into the food is higher in acidic foods followed by alkaline (Semwal *et al*., 2006). The study by, Semwal *et al*., (2006) reported that leaching of metals under the preparation of lower pH (4.25) food in an Al cooking pot increase the concentration of Al metal by 20.3 mg/kg. Another study by Valadez-Vega, (2011) showed that boiling of acetic acid for 45 hr lead to the migration of 127.52 ppm for Cd, 70.38 ppm for Co, and 188.08 ppm for Pb. Another study by Hermogene *et al*., (2012) on assessment of leaching of heavy metals from traditional clay pots "*inkoko*" and "*ibibind*" shows that; those traditional utensils were found to be high in concentration of heavy metals leached during preparation of tomato sauce and 4% acetic acid. From this point of view acidic foods and temperature have a great influence on leachability of metals from cook ware.

Acidic foods such as tomatoes, cabbage, coffee, and many soft fruits are among the most that frequently take up more metals from cook ware (Lenntech *et al.,* 2009). Similarly, the finding of the present work showed Preparation of tomato sauce with a pH value of about 4.3 had a greater leaching than preparations of bean stew with a pH value of about 6.2. Due to the fact that metals are attacked by most dilute mineral acids and solubility increases markedly at pH below 4.5 and above 8.5. However, at neutral pH solubility becomes negligible (Elinderand , 1986).

Frequency of usage is the other factor in the migration of metals into the food. Study by Odularu *et al*., (2013) showed that old metallic pots had the lowest concentration (Al- 289 mg/kg) of leaching while new steel pots had the highest leaching of Al (295 mg/kg). Similar observations have also been reported earlier (Semwal *et al.*, 2006). However, another study by Valadez-Vega, (2011) has revealed that repeated usage of cook wares enhanced metal migration in to the food. Correspondingly, the present study documented frequent usage of the cook wares enhanced the toxic metal leaching in such case, leaching in both food types was found to be increased ($p<0.05$) from first time preparation (new cooking pot) to the second time, and third subsequent preparations. Therefore, is a toxicological risk when the containers are constantly reused because increasing their use degrades the utensil due to acidity and to the friction between the food and the vessel walls, which consequently causes a greater concentration of metals to leach.

As was observed in this work, the Pb, Cd, and Al content in the analyzed food samples prepared using clay pot from *legeberi* was above the maximum levels permitted in food by WHO, (2006) for Pb (0.01 mg/kg), Cd (0.003 mg/kg) and Al (1mg/kg). The results indicated that the use of these pots contributes significantly to the daily intake of metals through food. In addition, Al content of food sample prepared using clay pot from *kechene* was also beyond the maximum permissible limit. However, the presence of Al in the soil because of it is the most abundant element on the earth and the natural phenomenon of metal accumulation in some plants (Zhuang *et al*., 2009) could also contribute to the recorded value which may not originate from the cook ware.

The experimental food samples of the food that have been used in this study were most frequently consumed by most people as a daily food. Therefore, Pb, and Cd levels of food stuffs processed in *legeberi* clay pot are alarming and calls for concern as they are the most toxic metals for man. However, the same thing is required for Al Level of foods processed in both types of clay pots from *legeberi* and *kechene* even if Al is not as much as toxic as Pb and Cd. Because prolonged consumption of contaminated foodstuffs may lead to chronic diseases. Accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (FAO,2001). The effect of Pb differs with age, gender and nutritional status. Those with malnutrition, anemia and colic etc are prone to Pb poisoning with symptoms like low intelligent quotient (IQ) (Munoz *et al.*, 1993), impaired speech and hearing, hyperactivities, decreased verbal activities, decrease learning and memory activities.The most critical effect of low level Pb exposure is reduced cognitive and intellectual development in children (Lanphear *et al*., 2005).

5. CONCLUSIONS AND RECOMMENDATIONS

From the present study one can conclude that there is a possibility of contaminant in traditional cook wares depending on the materials they are designed. In this manner, toxic metal accumulation in soils and water is an important concern in pottery production as they are major raw materials.

Unfortunately Ethiopia does not exert toxicological control over the clay pots used to make cooking pots; thus, the final products of the cooking pot can show various levels of toxic metals. The natural diversity of each clay lots means that its composition varies considerably, and the cook wares made from these clays therefore also vary in composition.

The results clearly indicate that the use of traditional clay pots as cooking material produced in *legeber*i contributes high amount of lead, cadmium and aluminum significantly to the total daily intakes than in *kechene* .This can be due to difference in the source of raw materials that potters used to make a pot.

Leaching of Pb, Cd and Al from cook ware manufactured in *legeberi* in to all food was above the maximum permissible limit allowed by WHO and call for concern. However, no leachate of Pb and Cd were observed in all experimental food samples prepared in *kechene* clay pots, where as Al leachate of food samples were found to be above the maximum permissible in food allowed by WHO. The results showed that clay pot manufactured in *kechene* were safe in respect to contamination of food by the most toxic metals Pb and Cd as compared to *legeberi* clay pot but in case Al they need concern.

Frequent usage of these cook wares also enhance leaching of these metal in to the food thereby reaching the body through food chain, the concentration of metal leached in the first cooking time was lower as compared to subsequent cooking time. The study also suggested that acidic foods like tomato sauces and coffee increase amount of metal leachate from the cook ware.
Based on the findings I need to recommend the following things;

- There is a need to create awareness in the community on the danger evoked by high levels of metals.

- Consumption of foods processed especially in *legeberi* clay pot should be avoided in order to prevent toxic metal poisoning due to accumulation over time. Not only that other toxic metal like arsenic, which are not studied in this work, should be investigated.

- As clay pots are made from clay soil and water their quality depends on the back ground of the raw materials, because metal content of soil and water is different from region to region by nature or due to pollution factors. So therefore, further investigation is required to assess the safety of these traditional cook wares especially for the one which is manufactured from the raw materials suspected for pollution.

- The high metal content observed indicates the absence of quality control and makes the need evident for the government to exercise preventive measures in this situation by enforcing the quality control of raw materials such as clay.

- It was therefore imperative to monitor comprehensively and periodically heavy metals raw materials in this area in order to advise and safeguard the health of the populace.

- Considering the above mentioned problems, efforts should be focused on the evaluation of dietary intakes of toxic metals by consumers in order to assess risk.

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APPENDIX-1

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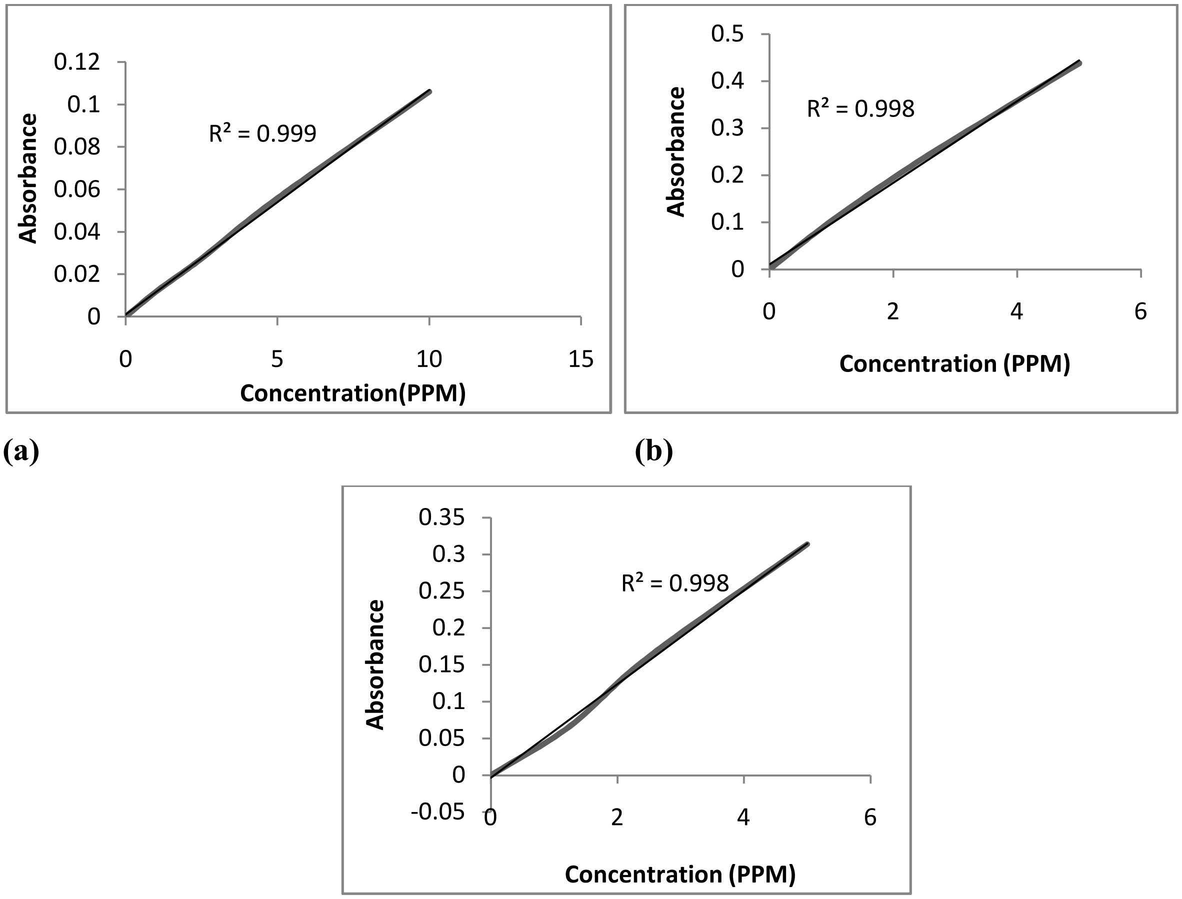
Figure 1. *Jebena* (claypot)display in the place where it is produced “*legeberi”*

Figure 2. *Jebena* (claypot) display in the market (*shola*)

(a) **(b)**

Figure 3. *shiro wet* cooked in *shekla dist* (a) and in beaker (b) as a control.



(a) **(b)**

(c)

Figure 4. Calibration curves: Pb (a), Cd (b), and Al (c)

APPENDIX-2

Table 1.The moisture content of clay pot, ingredients, food cooked in a beaker and clay pot pot.

Sample type	Wt. of Crucible	Wt. of fresh Sample	Wt. Cru. + Sample	Wt. Cru. + Dry Sample	wt. Dry	% of moisture	Mean of mois.	dry matter=100-%moisture
Clay soil k	13.90	5.01	18.91	18.588	0.318	6.35	6.4	93.6
Claysoil L	13.42	5.03	18.45	18.039	0.323	6.75	6.75	93.25
Coffee1	13.06	5.04	18.10	16.57	5.054	93.33	93.33	6.67
Coffee 2	13.45	5.012	18.462	15.995	4.907	92.45	92.45	7.55
Oni 1	13.73	5.07	18.80	14.62	4.39	87.10	87.18	12.82
Oni 2	13.94	5.05	19.00	14.59	4.40	87.25		
Shiro Wot 1	13.56	5.00	18.56	14.31	4.39	85.94	86.33	13.67
Shiro Wot 2	13.558	5.01	18.591	14.245	4.42	86.72		
Shiro Sa (Beaker) 1	13.24	5.09	18.42	14.19	4.22	84.40	85.77	14.24
Shiro Sa (Beaker) 2	13.64	5.04	18.68	14.299	4.392	87.13		
Tom Sau. 1	13.22	5.00	18.22	14.71	3.63	72.63	79.89	20.11
Tom Sau. 2	13.81	5.111	18.929	14.475	4.45	87.14		
Tom. Sa (Beaker) 1	13.53	5.004	18.621	14.49	4.12	82.54	82.70	17.48

Tom. Sa 2	13.33	5.011	18.599	14.445	4.15	82.99		
Berber powder1	13.05	5.002	18.052	18.003	0.49	9.24	8.91	91.09
Berber powder2	13.08	5.01	18.09	18.43	0.40	8.58		
Shiro powder 1	13.74	5.05	18.79	18.66	0.43	7.35	7.19	92.81
Shiro powder 2	13.44	5.14	18.58	18.77	0.42	7.02		

Table 2. pH value of the bean stew and tomato Sauce

No. of cooking	**bean stew**	Tomato Sauce
First cooking	6.2 ± 0.01	4.2 ± 0.03
Second cooking	6.3 ±. 0.03	4.4 ± 0.01
Third cooking	6.1 ± 0.04	4.3 ± 0.2

Appendix-3

Results and statistical analysis

Table 1. Independent sample T test for significance Pb, Cd, and Al content of clay pot

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Pb	Equal variances assumed	3.101	.153	87.782	4	.000
	Equal variances not assumed			87.782	2.064	.000
Cd	Equal variances assumed	4.000	.116	138.242	4	.000
	Equal variances not assumed			138.242	2.000	.000
AL	Equal variances assumed	1.082	.357	69.506	4	.000
	Equal variances not assumed			69.506	2.750	.000

Table 2. Tukey test for significance Pb, Cd, and Al content of tomato sauce cooked in l*egeberi* clay pot

Pb concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05		
		1	2	3
First	3	9.6000		
Second	3		12.0000	
Third	3			12.9667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 3.000.

Cd concentration

SD

sample code	N	Subset for alpha = 0.05	
		1	2
first	3	.5000	
second	3	.5000	
third	3		1.3000
Sig.		1.000	1.000

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 3.000.

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	8.4500			
First	3		11.5333		
Second	3			13.1000	
Third	3				15.4000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 3. Tukey test for significance Pb, Cd, and Al content of *shiro stew* cooked in l*egeberi* clay pot

Pb concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05		
		1	2	3
first	3	1.2000		
second	3		2.8000	
third	3			3.3000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 3.000.

Cd concentration

SD

sample code	N	Subset for alpha = 0.05	
		1	2
first	3	.1400	
second	3	.1500	
third	3		.3800
Sig.		.165	1.000

/leans for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	9.3400			
First	3		10.8000		
Second	3			12.2000	
Third	3				13.5000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 4. Tukey test for significance Pb, Cd, and Al content of first coffee brewed in l*egeberi* clay pot

Pb concentration

SD

sample code	N	Subset for alpha = 0.05		
		1	2	3
a abol	3	1.6000		
Atona	3		2.4000	
Abereka	3			2.7000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Cd concentration

SD

sample code	N	Subset for alpha = 0.05		
		1	2	3
a abol	3	.1800		
Atona	3		.2000	
Abereka	3			.2300
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Aluminum concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05			
		1	2	3	4
Acontrol	3	.8900			
Aabol	3		1.9000		
Atona	3			2.2000	
Aberaka	3				2.5000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 5. Tukey test for significance Pb, Cd, and Al content of second coffee brewed in l*egeberi* clay pot

Pb concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05	
		1	2
babol	3	3.3000	
btona	3	3.5000	
bereka	3		3.9333
Sig.		.128	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Cd concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05		
		1	2	3
Babol	3	.2600		
Btona	3		.2800	
Bereka	3			.3400
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05	
		1	2
Bcontrol	3	.8900	
Babol	3		3.2000
Btona	3		3.4000
Bbereka	3		3.8000
Sig.		1.000	.104

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 6. Tukey test for significance Al content of tomato sauce cooked in *kechene* clay pot

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05	
		1	2
Control	3	8.4500	
First	3		10.5000
Second	3		10.9000
Third	3		11.2000
Sig.		1.000	.584

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 7. Tukey test for significance Al content of *shirostew* cooked in *kechene* clay pot

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05
		1
Control	3	9.3400
First	3	10.1000
Third	3	10.3033
Second	3	10.4067
Sig.		.103

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 8. Tukey test for significance Al content of first brewed coffee in *kechene* clay pot

Aluminium concentration

Tukey HSD

Samplecode	N	Subset for alpha = 0.05			
		1	2	3	4
Acontrol	3	.8900			
a abol	3		1.1000		
Atona	3			1.2000	
Abereka	3				1.5000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 9. Tukey test for significance Al content of second brewed coffee in *kechene* clay pot

Aluminium concentration

Tukey HSD

sample code	N	Subset for alpha = 0.05			
		1	2	3	4
Bcontrol	3	.8900			
Babol	3		1.8000		
Btona	3			1.9000	
Bberaka	3				2.1000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.