ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Determination of some Selected Metals in Peanut (Arachis hypogaea L.) Seed samples collected from three different areas of Ethiopia using Flame Atomic Absorption Spectrophotometry (FAAS)

By: Mekdes Abebe

Advisor: Dr. Merid Tessema

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DECLARATION

I, declare that this thesis work entitled as "Determination of selected metals in peanut (*Arachis hypogaea L.*) Seed samples collected from three different areas in Ethiopia using Flame Atomic Absorption Spectrophotometry (FAAS)" is original report of my research, prepared under the guidance of Dr. Merid Tessema and the thesis has been written by me and has not been submitted in part or fully for any previous degree. All sources of materials used for the thesis were recognized accordingly.

Name: Mekdes Abebe

Signature:----------

This research work has been submitted for the examination with my approval as university advisor.

Advisor: Dr. Merid Tessema

Signature----------

DEDICATION

This thesis is dedicated to my beloved mother Ejigayehu Abebe.

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APPROVAL OF THE THESIS

Approved by the examining committee

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LIST OF ABBREVIATIONS

AAS----------Atomic Absorption Spectrometry

ANOVA----------Analysis of variance

AOAC----------Association of Official Analytical Chemists

DNA----------Deoxyribonucleic acid

FAAS----------Flame Atomic Absorption Spectrophotometry

FAO----------Food and Agriculture Organization

LOD----------Limit of detection

LOQ----------Limit of Quantification

MoA----------Ministry of agriculture

MP ----------Microwave plasma

RNA----------Ribonucleic acid

RSD----------Relative standard deviation

SD----------Standard deviation

USAD----------United states agriculture department

WHO----------World Health Organization

ABSTRACT

Peanut (Arachis hypogaea L.) is an important legume oilseed. Peanut is mainly used to make oils and can be consumed directly (roasted and salted) or processed into oil or cake. Peanut is nutrient rich source of protein, fat, vitamin and minerals. The main objective of this study was to determine some selected metals in peanut seeds cultivated in three different areas in Ethiopia. The peanut seed samples were collected from Babile (Oromia region), Dibate (Benishangul Gumuz region) and Zala Mela (SNNP) of Ethiopia. After the pretreatment, the samples were digested for optimized volume of reagent, temperature and time. FAAS was used to determine the metals. The accuracy of the optimized procedure was evaluated by analyzing the digestion of spiked samples with standard solution and percentage recoveries varied from 90.5 to 98. The concentration of metals in peanut seed samples were in the following range. Ca (48.4-83.4), Fe (9.3-11.3), Mn (3.15-7.3), Na (784.7-1146), Pb (1.59-1.96) and Zn (12.2-19.3) mg/kg, respectively. The concentration of Cd, Cu and Ni is below detection limit. ANOVA indicated that there is no significant difference between the mean concentrations of Fe among the peanut seed samples at 95% confidence. There is good correlation between Na-Ca, Mn-Na, Fe-Ca, Mn-Ca and Mn-Fe. Poor correlations were observed between Fe-Zn, Mn-Zn, Pb-Fe and Zn-Ca.

Keywords: Peanut (*Arachis hypogaea L.),* Ethiopia, FAAS

Chapter One: Introduction

1.1 Background

Peanuts *(Arachis hypogaea L.)* are important monoecious annual legume used for oilseed, food and animal feed all over the world. It is the main source of food in various forms and used as a component of crop rotation in many countries. Peanuts are grown on 26.4 million ha worldwide with a total production of 38.2 million metric tons. Peanut is a high value crop that can be marketed with little processing; however, it is extremely versatile and can be used in a wide range of products. Peanut is used to make oils and it is second largest source of vegetable oils next to soybeans. Peanuts are also a significant source of cash income in developing countries that contribute significantly to food security and alleviate poverty. As a legume, peanuts improve soil fertility by fixing nitrogen and thereby increasing productivity of the semiarid cereal cropping systems [1].

Peanut is also known as groundnut, earthnut, monkey nut and goobers [2]. Peanut is the sixth most important oilseed crop in the world and the 13^{th} most important food crop [3] cultivated in nearly 100 countries, over 90% of which are developing countries. The peanut is a food staple and valuable cash crop for millions of households. They can be consumed directly (roasted and salted), processed into oil or cake/meal, or further processed into confectionary products or snack food [4]. The productions of peanuts with developing countries are in Asia (66 %) and Africa (25 %) as the major producers [5]. Peanut plants grow best in well-drained sandy soils and sunny warm temperatures with moderate rainfall [6].

Peanut cultivation began in South America dating back to 7500 years ago. In the 1^{st} century, the plant reached Mexico where it further spread to North America, China, and Africa. The peanut is currently a common crop planted around the world. China grows more peanuts than anywhere else in the world. China managed to produce 16,685,915 metric tons of groundnuts according to FAO. The country accounts for 8% of the world peanut export. India is the second largest peanut producer in the world. India produced 6,857,000 metric tons of peanuts in 2016. Nigeria is the

largest peanut producer in Africa accounting for 30% of the total Africa's nut production. Nigeria produced 3,028,571 metric tons of peanuts according to the FAO report [7].

The lowland areas of Ethiopia have considerable potential for increased oil crop production including peanut [1]. Peanut requires a long and warm growing season. The best soil for peanut production is well-drained, light colored sand, loamy sand, or sandy loam. Peanut produces good yields in soils with pH of 6.0- 6.5. Although peanut is considered to be tolerant to acid soils, some cultivars grow well in slightly alkaline soils with a pH up to 8.0 which helps in nitrogen fixation [8].

As a whole food and as an ingredient, peanuts are nutritionally dense. Peanuts are nutrient-rich sources of protein, fat, vitamins and minerals. 100 grams of raw peanut seed contain 567 Calories, 7%Water, 25.8 g Protein, 16.1 g Carbs, 4.7 g Sugar, 8.5 g Fiber, 49.2 g Fat, and 6.28 g Saturated 24.43 Monounsaturated, 15.56 Polyunsaturated, 0 g Omega-3 and 15.56 g Omega-6. Peanuts are a good option for people with diabetes for these reasons [9]. Peanuts contain many essential macro, trace and ultra-trace elements. Like calcium, magnesium, sodium, potassium, iron, zinc, manganese, nickel, chromium and copper [10]. The objective of this study is to determine selected metals such as Na, Ca, Mn, Fe, Ni, Cu, Zn, Cd and Pb using Flame Atomic Absorption Spectrophotometry (FAAS).

1.2 Objective

1.2.1 General objective

The main objective of this study is to determine some selected metals concentration in Peanut *(Arachis hypogaea L.)* seed samples cultivated in three different areas of Ethiopia using Flame Atomic Absorption Spectrophotometry (FAAS).

1.2.2 Specific objectives

1. To develop an optimum digestion procedure for the determination of selected metals in peanut *(Arachis hypogaea L.)* seed samples using Flame Atomic Absorption Spectrophotometry (FAAS).

2. To determine the concentration of selected metal in peanut *(Arachis hypogaea L.)* seed samples using Flame Atomic Absorption Spectrophotometry (FAAS).
3. To compare the levels of identified metals in peanut *(Arachis hypogaea L.)* seed samples in the three different areas in Ethiopia.
4. To compare the concentration of each metal in peanut *(Arachis hypogaea L.)* seed samples in the three different areas in Ethiopia with that reported in the literature.
5. To compare the concentration of metals in peanut *(Arachis hypogaea L.)* seed samples with other oil seeds (Pulses).

1.3 Statement of the problem

Peanuts are very important oil seeds and they are the main source of edible oil. Peanuts have been consumed in different varieties like roasted peanuts, peanuts butter, peanuts oil, peanuts paste, peanuts sauce, peanuts flour, peanuts milk, peanuts snack and peanut cheese analog. Raw peanuts are consumed all over the world. The oil of peanuts is mainly used as cooking oil and for the production of soap and cosmetics [11]. But in Ethiopia people do not know about the different uses of peanut. The production of peanuts is very small and limited to some lowlands areas of the country. Most people use peanuts while chewing chat and the people also do not commonly plant it like other oil seeds. Most of the Ethiopian people do not have enough information about the nutritional contents of peanuts. So this study gives information and creates awareness about the peanuts in the country.

Chapter two: Literature review

2.1 General overview of Peanuts

2.1.1 Origin and Geographical distribution

Peanut (sometimes referred to as groundnut) and the genus *Arachis,* originated in central Brazil in South America. Early archaeological evidence suggests that peanut was domesticated in northern Argentina and eastern Bolivia, and subsequently grown in Mexico, the Caribbean Basin and throughout Brazil, and the coastal regions of Peru. It is believed that the crop did not reach other parts of the world until after Columbus arrived in America, after which time it was taken from Brazil to Africa and the Far East by the Portuguese. The Spaniards are believed to have taken the crop to the western Pacific, Indonesia, and China early in the sixteenth century [12].

Figure 1: Picture of peanut *(Arachis hypogaea L)* seeds

2.1.2 Botanical information of peanuts

The peanut belongs to the family *Leguminosae,* subfamily *Papilionoidae*, tribe *Aeschnomeneae,* sub-tribe *Stylosanthinae*, genus *Arachis* and species *hypogaea*. The genus name *Arachis* stems from a-rachis (Greek, meaning without spine) in reference to the absence of erect branches. The species name *hypogaea* stems from hupo-gè (Greek, meaning below earth) and relates to the gynophore (flower stalk or peg) that grows downward into the earth so that the pod develops underground [8].

Based on its growth habit, branching pattern, presence or absence of reproductive nodes on the main stem, position of vegetative and reproductive axes on the primary branches, pod and seed characteristics, and seed dormancy the species *hypogaea* is classified in to two sub species. These are Subspecies *hypogaea* and Subspecies *fastigiata.* Subspecies *hypogaea* has a central axis that never bears inflorescence and has laterals where vegetative branches alternate regularly with reproductive branches. In Subspecies its main stem holds flowers and lateral branches on which the reproductive and vegetative branches present them in no specific order [8, 13].

2.2 Uses of Peanuts

About 41% of the world peanut production is used for oil production, whereas 45% is used directly as human food [14]. Peanut kernels, usually cooked or roasted, are appreciated worldwide as a flavourful snack food. Peanuts are also the primary ingredient of many finished products such as peanut butter, confections and nutritional bars, and are used in numerous dishes. Peanuts are usually too valuable to be used as animal feed [15].

Peanut is used to make oils and it is second largest source of vegetable oils next to soybeans. The oil can be used for cooking, and to make peanut butter. Processed peanut is used in diversified ways including peanut butter which is used as spread for bread or biscuits, in cookies, Sandwiches and candies. Peanut is also used to prepare children's food ("fafa") [1].

As a legume, peanut fixes atmospheric nitrogen in soils and thus improves soil fertility [2, 4] and thereby increasing the productivity of other crops when used in rotation or in intercropping [16]. In Eastern Ethiopia peanut seeds are used to make local cake known as Halawa [17].

2.3 Peanuts in Ethiopia

Peanut is relatively new to Ethiopia. It was introduced from Eritrea to Hararghe in the early 1920s by Italian explorers [5, 18]. In Ethiopia, peanut is grown in the lowland areas and is the second important lowland oilseed of warm climate [2]. In Ethiopia, peanut is one of the five widely cultivated oilseed crops [19]. The estimated production area and yield of peanut in Ethiopia in 2015/2016 cropping season were 75,255.73 hectares and a total production of well over 115,180 tones [20]. The major peanut producer region in Ethiopia is Oromia region (41,089 ha), followed by Benshangul-Gumuz (14,759 ha) and Amhara (3,161 ha) regional states [16]. Peanut is planted both during the "Belg" season (March) and also during the main season (June), in some parts of western Ethiopia [21].

2.4 Nutritional composition of peanuts

All foods are composed of chemical compounds, which can be defined as macro- or micro-nutrients such as proteins, carbohydrates, fats, or vitamins, minerals and phytonutrients [22]. Peanuts are very rich in proteins, oils, fiber, vitamins, carbohydrate and are consumed all over the world due to its availability and affordability compared to other types of nuts [23]. According to the United States Department of Agriculture (USDA), 100 grams of raw peanuts contain 567 calories energy, macronutrients (protein 25.8 g, carbohydrate 16.13 g, fiber 8.5 g, sugars 4.72 g), fats 49.1 g minerals (Calcium 44 mg, Magnesium 178 mg, phosphorous 363 mg, copper 0.428 mg, iron 1.58 mg, manganese 1.79 mg and zinc 2.77 mg) and vitamins (Thiamin 0.152 mg, Riboflavin0.197 mg, Niacin14.355 mg, Vitamin B60.466 mg, Folates97 μg and Pantothenate 1.01 mg)[24].

Fats

The peanut kernel generally contains 44–54% fat. The fatty acid in peanut includes saturated fatty acids (palmitic acid 6–18%, stearic acid 1.3–6.5%, arachidic acid 1.0–3.0%, behenic acid, wood tar, and myristic) and unsaturated fatty acids (oleic acid 35–72%, linoleic acid 20–45%, and arachidonic acid), and the total amount of unsaturated fatty acid reaches over 85%, which is a healthful type of fat [25].

Proteins

Plant proteins are inherently more efficient to produce than animal protein, and increased future emphasis is expected to be placed on producing more plant protein more efficiently [24]. The peanut seed contains 32 different proteins [22]. Peanuts contain all the essential amino acids necessary for normal body growth and metabolism such as tryptophan, phenylalanine, methionine, tyrosine [26]. Epidemiological evidence consistently reveals that individuals with the highest mixed nut intake have a roughly 35% reduced risk of coronary heart disease as a result of improved blood lipid profiles and vascular reactivity [27].

Carbohydrates

Carbohydrates are biomolecules, which are differentiated based on their properties as sugars and non-sugars, and the sugars are poly hydroxyl aldehyde/ketones, soluble in water, crystalline in appearance, and can be easily digested. The major carbohydrate present in peanuts is starch which is a homopolysaccharide made up of α-D glucose residues joined together by glycosidic bonds [26].

Vitamins

Peanuts contain vitamin such as vitamin E also known as alpha-tocopherol. Vitamin E is a fat soluble vitamin and is an anti-oxidative vitamin. Peanuts are a good source of Thiamine (B_1), Vitamin B_2 Riboflavin, vitamin B_3 Niacin, Vitamin B_6 Pyridoxine, Vitamin B_5 Pantothenic acid and Vitamin B_9, more commonly known as folate or folic acid. These Vitamins are water-soluble vitamins [22, 26].

Minerals

Minerals are elements that originate in the soil and cannot be created by living things, such as plants and animals. Yet plants, animals and humans need minerals in order to be healthy. Plants absorb minerals from the soil, and animals get their minerals from the plants or other animals they eat. Most of the minerals in the human diet come directly from plants, such as fruits and vegetables, or indirectly from animal sources. Minerals from plant sources may also vary from place to place, because the mineral content of the soil varies according to the location in which the plant is grown [28]. Minerals can be divided in to macro (major) minerals, micro (trace) minerals and ultra-trace minerals.

Peanuts are a good dietary source of the macro minerals which are the minerals needed daily in a quantity greater than 100 mg/day. These Macro minerals are Potassium, Magnesium, Sodium, Calcium and Phosphorus. Peanuts also provide a source of trace minerals, which are minerals needed daily in a quantity less than 100 mg/day. These Trace minerals are Zinc, Iron, Manganese, Copper and Selenium [22].

2.5 Health benefit of peanut consumption

The limited available data indicate peanuts consumed as a snack do not have any differential impact on appetitive ratings and energy intake when compared to peanuts ingested as part of a meal. However, they tend to promote more precise dietary compensation when consumed as a snack. That is, individuals eat somewhat less energy over the day when peanuts are consumed as a snack compared to when they are included as part of a meal [27].

Nut consumption has been reported to be significantly associated with reduced risk of cancer, cardiovascular, respiratory, infectious, renal and liver disease mortality but not with diabetes or Alzheimer’s disease mortality [23].

Peanut consumption may help to moderate blood sugar concentrations and reduce the risk of developing type 2 diabetes. Daily consumption of peanuts may also lead to a decrease in blood pressure. The benefits of nut consumption on the cardiovascular system have been well studied

in both epidemiological and interventional studies. Epidemiological evidence consistently reveals that individuals with the highest mixed nut intake have a roughly 35% reduced risk of coronary heart disease as a result of improved blood lipid profiles and vascular reactivity [27].

2.6 Essential and Non essential Elements

2.6.1 Essential elements

A number of definitions of the concept of essentiality have been proposed by different investigators. One of the simplest is that an essential element is a ‘metabolic or functional nutrient. A somewhat more sophisticated definition is that an essential element is one that is required for the maintenance of life, and its deficiency causes an impairment of a function from optimal to suboptimal. The impairment can lead to disease, metabolic anomalies or perturbations in development [29].

Essential minerals are sometimes divided up into major minerals (macrominerals) and trace minerals (microminerals). These two groups of minerals are equally important, but trace minerals are needed in smaller amounts than major minerals [30].

Macro elements are the natural elements of which the body needs in more amounts and are more important than any other minerals. Macrominerals includes sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) which are cations. The trace metals are dietary mineral that is needed for the proper growth, development, and physiology of the organism, which are needed by the human body in very small quantities (generally less than100 mg/day). Trace elements include the transition metals manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), chromium (Cr), and molybdenum (Mo) and the nonmetals selenium (Se) [31]. The physiological importance of essential metals is discussed below.

Calcium (Ca)

Calcium is the most abundant mineral element in our body. Calcium is important for healthy bones, teeth and a normal heart rhythm, helps muscles relax and contract; important in nerve functioning, blood clotting, blood pressure regulation, hormone function immune system health.

The major source of calcium in the diet is milk and milk products, providing over 40% of calcium intake in adults, followed by cereals and cereal products providing 30% [32, 33].

Magnesium (Mg)

Mg is the fourth most abundant mineral in the human body. Mg is the most copious macro-nutrient which is vital for the continuation of proper health. It is needed for the activity of more than 300 enzymes, which serve several essential physiological functions in the human body. The Mg containing enzymes are concerned in the glucose homeostasis, nerve transmission, DNA and RNA production. The Mg deficiency might lead to a decrease in insulin mediated glucose uptake. Inadequate Mg intake frequently causes muscle spasms and has been associated with cardiovascular disease, diabetes, high blood pressure, anxiety disorders, migraines, osteoporosis, and cerebral infarction [34, 35].

Sodium (Na)

Sodium is an important mineral and electrolyte necessary for many functions in the body. Sodium is the principal cation in extracellular fluid in the body, and is an essential nutrient necessary for maintenance of plasma volume, acid–base balance, transmission of nerve impulses and normal cell function. Sodium is only needed in small quantities, and the kidneys are responsible for excreting extra sodium from the body. Increased sodium consumption is associated with increased blood pressure, whereas lower sodium consumption appears to decrease blood pressure. Increased sodium has also been associated with cardiovascular diseases. WHO recommends a reduction in sodium intake to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. WHO recommends a reduction to <2 g/day sodium (5 g/day salt) in adults [36, 37].

Potassium (K)

Potassium is an essential nutrient. It is the most abundant cation in intracellular fluid, where it plays a key role in maintaining cell function, particularly in excitable cells such as muscles and

nerves acid and electrolyte balance. Potassium concentration is higher in fruits and vegetables than in cereals and meat. Reduced potassium consumption has been associated with hypertension and cardiovascular diseases, and appropriate consumption levels could be protective against these conditions [38, 39].

Copper (Cu)

Cu is an essential trace element in plants and animals. The human body only contains about 150 mg of this vital mineral. The best dietary sources of Cu to human body include wheat, barley, sunflower seeds, almonds, pecans, walnuts, peanuts, cashews, prunes, raisins apricots, various dried beans, mushrooms, chicken, and most fish. Cu is an essential micronutrient necessary for the hematologic and neurologic systems. It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems. Cu deficiency includes fatigue, anemia, and a decreased number of white blood cells [34].

Iron (Fe)

The majority of Fe in the body is contained within hemoglobin, and is an essential component of myoglobin. Iron is found in most any seafood, meat, poultry or organ food like liver, and also in many vegetables such as beans and peas, nuts, seeds, and green vegetables. People with deficiencies suffer from anemia and weak blood function. Symptoms of low iron can include heartburn, dizziness, headaches, sore tongue, hair loss, digestive problems, nausea and sensitivity to cold, irritability, and loss of appetite [34, 40].

Manganese (Mn)

Manganese (Mn) is an essential element in the human body that is mainly obtained from food and water. Manganese acts as an activator of many enzymes and as a component of metalloenzymes. It is involved in the glucose and lipids' metabolism, acceleration of protein synthesis, vitamin C, and vitamin B, catalysis of hematopoiesis, bone and tissue formation, skeletal growth, reproduction, and immune function improvement [41].

Cobalt (Co)

Cobalt is an essential trace element for the human body. It forms an integral part of vitamin B12 and has a substantial role in the formation of amino acids and neurotransmitters [42]. Deficiency of cobalt is strongly related to disturbances in vitamin B12 synthesis, so it might cause anaemia and hypofunction of thyroid and increase the risk of develop-mental abnormalities and failure in infants. Excess of this metal might increase the action of thyroid and bone marrow [43].

Chromium (Cr)

The activity of Cr depends on its valence state and chemical complexes it forms. Trivalent form of Cr has high biological activity which is necessary for the optimal glucose uptake by cells. Cr regulates insulin and blood glucose levels by stimulating insulin signaling pathway and metabolism by up regulating glucose transporter translocation in muscle cells. Cr deficiency results in the elevation of blood glucose levels and if it is continued for longer period, it may lead to the progress of diabetes. The Cr supplements decrease the blood sugar level in diabetes. Dietary sources are whole grains, broccoli, meat, green beans and spices [35].

Nickel (Ni)

Nickel is essential for the active synthesis of urease in plant cells. The biological function of nickel is still somewhat unclear in human body, however, nickel is found in the body in highest concentrations in the nucleic acids, particularly RNA, and is thought to be somehow involved in protein structure or function. Small quantities of nickel are essential for the body. High content of nickel may cause health problems such as liver, kidney, spleen, brain and tissue damage, vesicular eczema, lung, and nasal cancer [34].

Zinc (Zn)

Zinc is the second most prevalent vestigial element in the human body, and it is essential for individuals to remain healthy. Since the human body does not store excess zinc, it must be consumed regularly as part of the diet. Common dietary sources of Zn include red meat, poultry

and fish. Zinc performs a wide variety of functions in the human body, such as maintenance of physiological processes, metabolism, signalling, transduction, cell growth and differentiation. [30] Zinc is involved in numerous aspects of cellular metabolism. Zinc is required for the catalytic activity of more than 200 enzymes, and it plays a role in immune function, wound healing protein synthesis, DNA synthesis and cell division. Zinc is required for proper sense of taste and smell and supports normal growth [44]. Deficiency of zinc can lead to stunted growth, diarrhea, impotence, hair loss, eye and skin lesions, impaired appetite, and depressed immunity [45].

2.6.2 Non essential elements

Non essential elements are considered to be toxic and their presence in the body can cause profound biochemical and neurological changes in the body [46].

Cadmium (Cd)

Sources of cadmium in the food supply include shellfish, leafy vegetables, potatoes and grains, peanuts, soybeans and sunflower seeds as well as tobacco and organ meats. Cd bioaccumulates and this represents the primary source of cadmium exposure aside from occupational exposure and tobacco smoke. High and chronic inhalational exposure results in kidney disease and death. Symptoms following oral exposure are irritation of the stomach, vomiting, diarrhea, Lung cancer has been documented in workers exposed to Cd in the air and Cd is listed as a probable human carcinogen [47, 48, 49].

Lead (Pb)

Lead is a bright silvery metal, slightly bluish in a dry atmosphere. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources. Lead is an extremely toxic heavy metal that disturbs various plant physiological processes and unlike other metals it does not play any biological functions. The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system [50].

2.7 Flame Atomic Absorption Spectrophotometry (FAAS)

Atomic Absorption Spectrometry (AAS) dated back to nineteenth century, the modern form of this technique was largely developed during the 1950s by Alan Walsh [51]. AAS is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample [52].

Atomic absorption spectrometry has become one of the principal tools of analytical chemistry because of the high sensitivity and ease with which many samples can be analyzed. Can detect different metals (70–80 elements) in concentrations as low as and frequently lower than 1ppm. Mainly, the sample is diluted to reduce concentrations to the ppm level to analyze the major constituents of an unknown. The technique requires a different light source (hollow-cathode lamp) and wavelength for each element being analyzed [51].

It is normal in atomic spectroscopy for the sample to be found in one of two forms solid or liquid. The liquid case would seems to be the easiest form in which to handle the sample, with maybe a requirement for filtration being all that is required [53].

Flame Atomic Absorption is a very common technique for detecting metals present in samples. The technique is based on the principle that ground state metals absorb light at a specific wavelength. Ions in a solution are converted to atomic state by means of a flame. When light of the correct wavelength is supplied, the amount of light absorbed is measured and a reading for concentration can be obtained.

Chapter Three: Experimental

3.1 Chemicals, Reagents and Standard solution

HNO_3 69-72% and $HClO_4$ 70% (Research lab fine chemical industry, India) were used for digestion of peanut seed samples. Stock standard solutions (BDH) containing 1000 mg $L^{-1}$of the metals Na, Ca, Mn, Fe, Ni, Cu, Zn, Cd and Pb were used for preparation of calibration standards and in the spiking experiments, for Fe, Zn, Na, Ca, standard solution were prepared from the compound $FeCl_3$, NaCl, $ZnCl_2$ and $CaCl_2$ respectively. Deionized water was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware.

3.2 Apparatus and instrumentation

Digital drying oven for drying and removal of the moisture, Ceramic Mortar and pestles for grinding and homogenizing the samples and an analytical balance with a precision of ± 0.0001 g were used for the weighing of the peanut seed samples. Round bottomed flasks fitted with reflux condensers set on Kjeldahl heating apparatus (Gallenhamp, England) were used to digest the samples. Pipettes, micro-pipettes, measuring cylinder and volumetric flasks were used to measure, keep and dilute sample solution and prepare standard solution. Muffle furnace and crucible were used in the determining of ash contents of the sample. Analytikjena (Model ZEEnit 700P, Made in Germany) Flame Atomic absorption spectrophotometry (FAAS) was used for the analysis of metals.

3.3 Working Procedures

3.3.1 Study Area

The peanut seed samples were collected randomly from three different areas in Ethiopia: South Nations, Nationalities and People, Gofa Zone, Zala woreda (Mela), Oromia Region East Harargaha, Babile and Benshangul Gumuz Region Metekel zone, Dibate woreda. The selected areas were based on levels of production. Babile and Dibate are high production areas where as Zala (Mela) is a low production area in Ethiopia.

Table 1: Geographical description of sample collection areas

No.	Sample site	Region	Approximate geographical location			
			Latitude	Longitude	Altitude above sea level (m)	Distance from Addis Ababa (km)
1	Zala (Mela)	SNNPR	6^0 33' 39"N	$37^0$6'13"E	1315	510
2	Babile	Oromiya	9^0 12'0" N	$41^0$0'00"E	1648	544
3	Dibate	Benshangul-Gumuz	10^0 39' N	$36^0$13'E	1438	542

3.3.2 Short descriptions about sampling areas

Zala woreda (Mela)

Zala is one of the woredas in the Southern Nations, Nationalities, and Peoples' Region of Ethiopia. Part of the Gofa Zone, Zala is bordered on the southwest by Uba Debretsehay, on the northwest by Demba Gofa, on the northeast by Kucha, on the east by Deramalo, and on the southeast by Kemba. Zala was part of former Zala Ubamale woreda. The district has 36 kebeles (Mela is one kebele) from which 96% is a low land and 4% mid land [54].

Babile

Babile is a town in eastern Ethiopia, located 30 kilometers east of Harar. The town has an elevation of 1648 meters above sea level. It shares boundary with woredas of the Somali region and Fadis Woreda of Oromiya regional state. The total size of the Woreda is about 1,325 km^2. It has 17 kebeles and 42 sub-kebeles. The average temperature is 26.5℃ with uneven rainfall distribution. Babile is known for its hot springs, mineral water and elephant sanctuary. Maize, sorghum and sweet potato are the major staple foods. Other food crops that include barley, wheat, teff and pulses are also used.

Dibate

Dibate is one of the 20 woredas in the Benishangul-Gumuz Region of Ethiopia. Part of the Metekel Zone, it is bordered by Mandura on the north, by the Dura River on the east which separates it from the Amhara Region, by the Abay River on the south which separates it from the Kamashi Zone, and by Bulen on the west. People in Dibate district base their livelihood on agriculture and rely largely on rain-fed agriculture and livestock rearing [55].

3.3.3 Collection of samples

Peanut seed samples were collected from three different areas in Ethiopia based on high production (Babile and Dibate) and low production area (Zala Mela).The sample were collected from farmer store using random sampling technic. From each sampling area 1kg of peanut seed samples were collected. The samples collected from the sampling areas were stored in clean plastic bags. The samples of peanut seeds were labeled as Go, Ba and Me for Zala Mela, Babile and Dibate sample areas respectively. The sample were transported to the laboratory and stored there prior to sample preparation, digestion and analysis.

3.3.4 Apparatus cleaning

To prevent contamination all apparatus used for the analytical methods such as volumetric flasks, measuring cylinder, funnels, spatula, digestion flasks and beakers were washed with detergent and tap water, rinsed with deionized water, soaked in dilute nitric acid for 24 hrs, rinsed with deionized water, dried at room temperature and kept in clean and dust free place until needed.

3.4 Preparation of peanut seed samples

Peanut seeds are covered with two shells. The outer shell was carefully removed and allowed to dry in the sun. Since peanut seeds are oil seeds they require much time to dry. After drying in the sun the brown color shell of peanuts were removed. The peanut seeds samples were then placed in clean crucibles labeled according to the sample site and allowed in an oven to dry for 24 hrs at 105℃. After the moisture was removed from the seed, it was ground to powder with mortar and pestle and sieved using 0.5 mm sieve to prepare fine powder of peanuts for digestion.

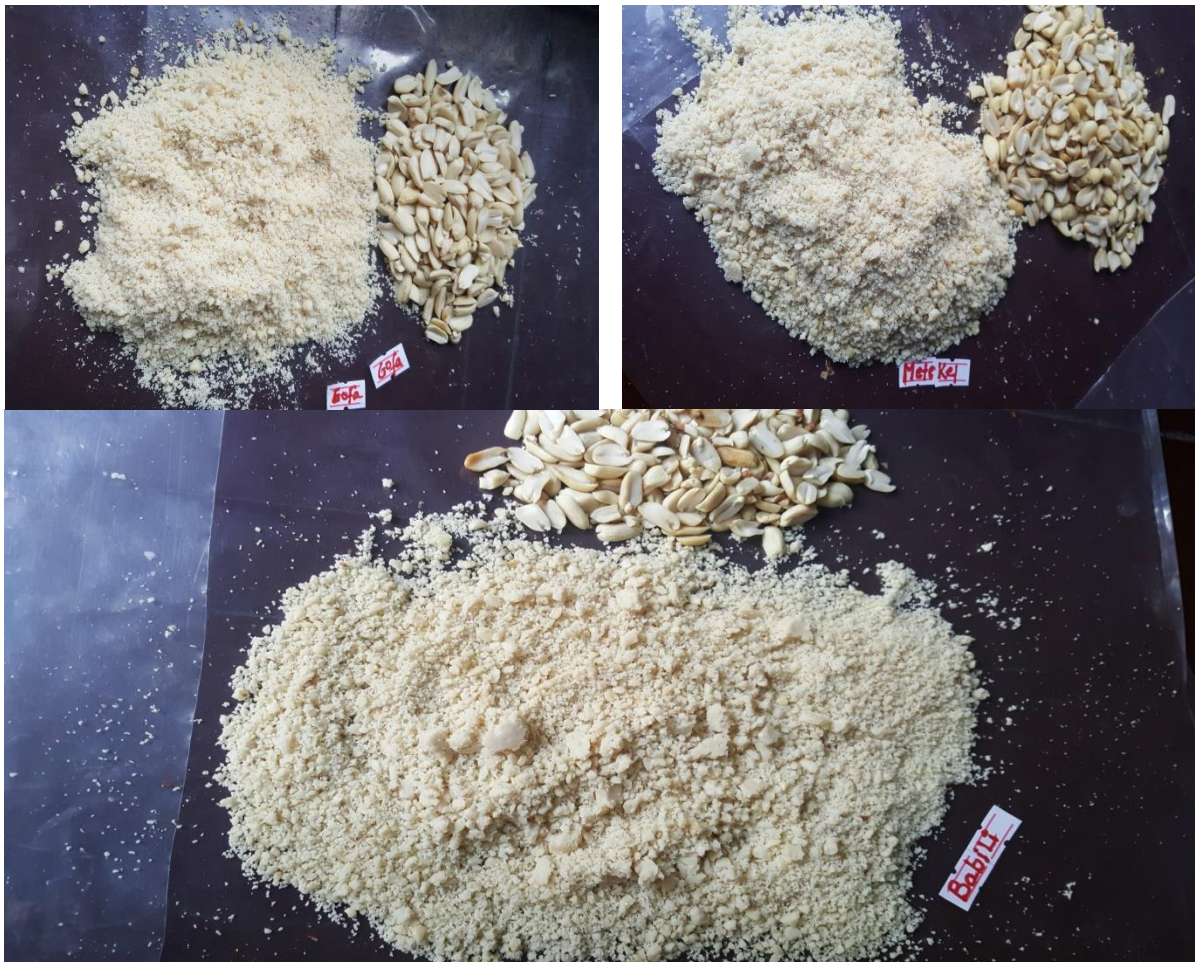

Figure 2: picture of powdered peanut seed samples

3.4.1 Optimization of digestion procedure

In the wet acid digestions carried out using Kjeldahl apparatus organic components are assumed to decompose and metallic elements are left in the solution except easily volatile metals [56]. To select an optimum procedure for digestion, parameters such as, digestion temperature, volume ratio of reagent, digestion time and mass were optimized by varying one parameter and keeping the others constant. Parameters that produced clear solutions with no residue and suspended matter was selected for the routine digestion of the peanut seed samples. Parameters that give clear solution at lower temperature with minimum reagent volume and digestion time were selected as the optimum procedure for the digestion of peanut seeds. Based on these criteria 3 hrs digestion time of 0.5 g peanut seed samples with a mixture of 3 mL of 69-72% HNO_3 and 1.0

mL 70% $HClO_4$ and 300 ℃ of temperature was chosen as an optimum digestion procedure. The results are given in the following table.

Table 2: Optimization of reagent volume for the digestion of 0.5 g peanut seed samples at constant temperature and time.

Trial	Mass (g)	Time (hrs)	Temperature (^{0}C)	Volume (mL) HNO_3 : $HClO_4$	Observation
1	0.5	3	300	1:1	Slightly yellow with residue
2	0.5	3	300	2:1	Slightly yellow
3	**0.5**	**3**	**300**	**3:1**	**Clear and colorless***
4	0.5	3	300	4:1	Clear and colorless
5	0.5	3	300	5:1	Clear and colorless
6	0.5	3	300	2:1.5	Slightly yellow with residue
7	0.5	3	300	3:1.5	Colorless with residue
8	0.5	3	300	4:1.5	Colorless with suspension
9	0.5	3	300	5:1.5	Colorless with suspension
10	0.5	3	300	2:2	Slightly yellow
11	0.5	3	300	3:2	Colorless and turbid
12	0.5	3	300	4:2	Slightly yellow
13	0.5	3	300	5:2	Colorless and colorless

*indicates optimum digestion volume ratio of reagent

Table 3: Optimization of time for the digestion of 0.5 g peanut seed samples at constant volume and temperature.

Trial	Mass (g)	Time (hrs)	Temperature (^{0}C)	Volume (mL) HNO_3: $HClO_4$	Observation
1	**0.5**	**3**	**300**	**3:1**	**Clear and colorless***
2	0.5	2:30	300	3:1	Colorless with turbid
3	0.5	2	300	3:1	Slightly yellow
4	0.5	1:30	300	3:1	Slightly yellow
5	0.5	1	300	3:1	Slightly yellow with residue
6	0.5	0:30	300	3:1	Yellow with residue

*indicates optimum digestion time

Table 4: Optimization of temperature for the digestion of 0.5 g of peanut seed samples at constant volume and time

Trial	Mass (g)	Time (hrs)	Temperature (^{0}C)	Volume (mL) HNO_3: $HClO_4$	Observation
1	0.5	3	30	3:1	Yellow with oily residue
2	0.5	3	60	3:1	Yellow with oily residue
3	0.5	3	90	3:1	Yellow with residue
4	0.5	3	120	3:1	Slightly yellow, cloudy with residue
5	0.5	3	150	3:1	Cloudy with residue
6	0.5	3	180	3:1	Cloudy with residue
7	0.5	3	210	3:1	Slightly yellow
8	0.5	3	240	3:1	Colorless and turbid
9	0.5	3	270	3:1	Colorless with suspension
10	**0.5**	**3**	**300**	**3:1**	**Clear and colorless***

*indicates optimum digestion temperature

3.4.2 Digestion of peanut seed samples

0.5 g peanut seed samples was weighed with analytical digital balance and transferred into 250 mL round bottomed flask. Then 3 mL of HNO_3 (69-72%) and 1mL $HClO_4$ (70%) were added to the sample and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature at 300°C for 3 hrs. Then, the digested solution was allowed to cool at room temperature for 15 min without dismantling the condenser from the flask and 10 min after removing the condenser. The solution was diluted with 10 mL of distilled water and filtered with whatman filter paper (110 mm diameter) in to 50 mL volumetric flask. The round bottom flask was further rinsed with distilled water and the washing was added to the filtrate. The solution was filled with distilled water up to the mark. Each sample was digested in triplicate following the same procedure. The digestion was carried out in triplicate for each blank sample. Digestion of reagent blank was also performed keeping all digestion parameters the same. The digested samples were kept clean until the level of all the metals in the sample solutions were determined by FAAS.

3.5 Calibration of the instrument and Analysis of peanut seeds samples using FAAS

3.5.1 Analysis of peanut seed samples using FAAS

Standard solution of metal were prepared from FAAS standard solution containing 1000 mg/L. these intermediate standard were diluted with deionized water to obtain working standard solution of metals. The metals: Ca, Na, Pb, Zn, Fe and Mn were analyzed with FAAS. The correlation coefficients for each metal indicate that absorbance with concentration was good. Concentration of working standards, Correlation coefficient of calibration curves and Correlation coefficient of calibration curves were summarized in table 5.

Table 5: Working standards, Correlation coefficients and Calibration curve Equation of the Calibration curves for the determinations of metals using FAAS

Metals	Concentration of working standards (mg/L)	Correlation coefficient of calibration curves	Calibration curve Equation
Ca	0.25, 0.5, 1, 2	$R^2 = 0.9982$	y = 0.0109x - 0.0019
Cd	0.25,0.5, 0.75, 1	$R^2 = 0.9994$	y = 0.0284x - 0.003
Cu	0.25, 0.5, 1, 2	$R^2 = 0.9984$	y = 0.0132x - 0.0006
Fe	0.25, 0.5, 1, 2	$R^2 = 0.9977$	y = 0.0119x - 0.0019
Mn	0.25, 0.5, 1, 2	$R^2 = 0.9996$	y = 0.0217x - 0.001
Na	1,2,4,8	$R^2 = 0.9987$	y = 0.0891x + 0.0276
Ni	1, 2, 3, 4	$R^2 = 0.9963$	y = 0.0045x + 2E-05
Pb	1, 2, 3, 4	$R^2 = 0.9988$	y = 0.0027x + 0.001
Zn	0.25, 0.5, 0.75, 1	$R^2 = 0.9963$	y = 0.051x + 0.0008

3.5.2 Calibration curves of working standard concentrations

The calibration curves for the metals are shown as follow.

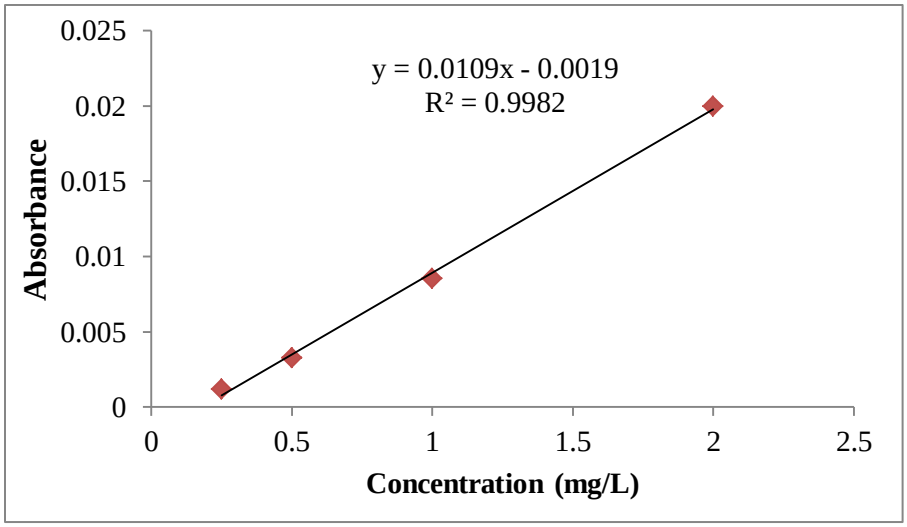

Figure 3: Calibration curve of standard solution of calcium

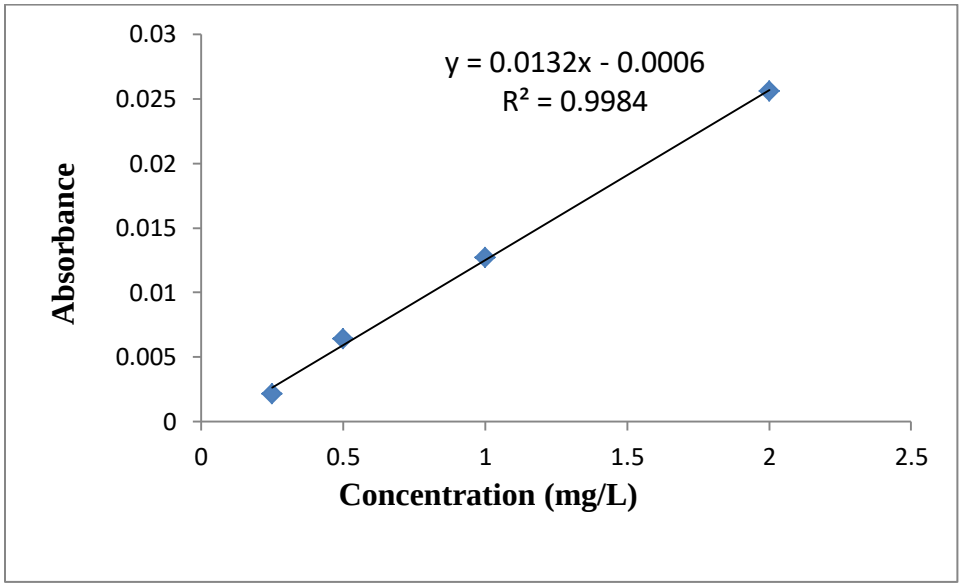

Figure 4: Calibration curve of standard solution of Copper

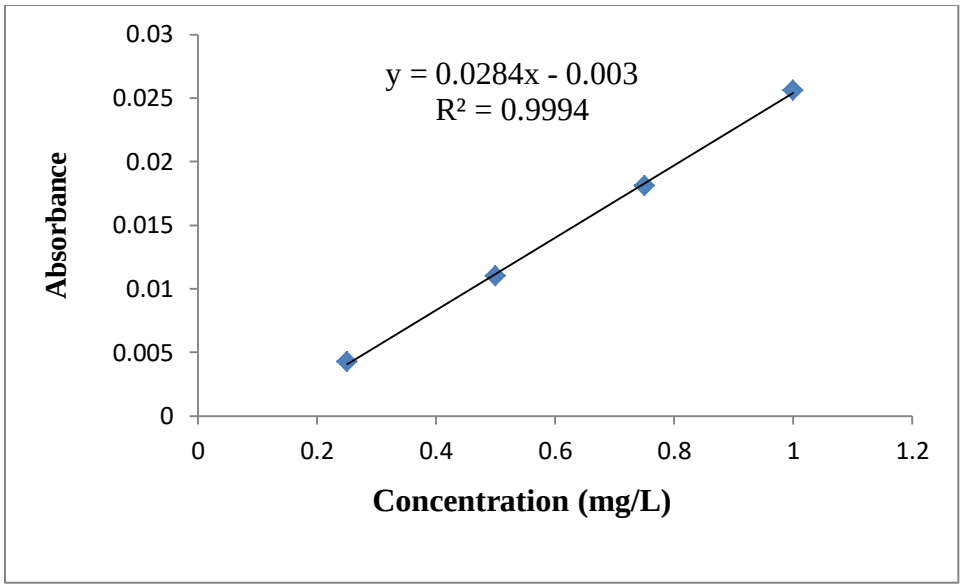

Figure 5: Calibration curve of standard solution of Cadmium

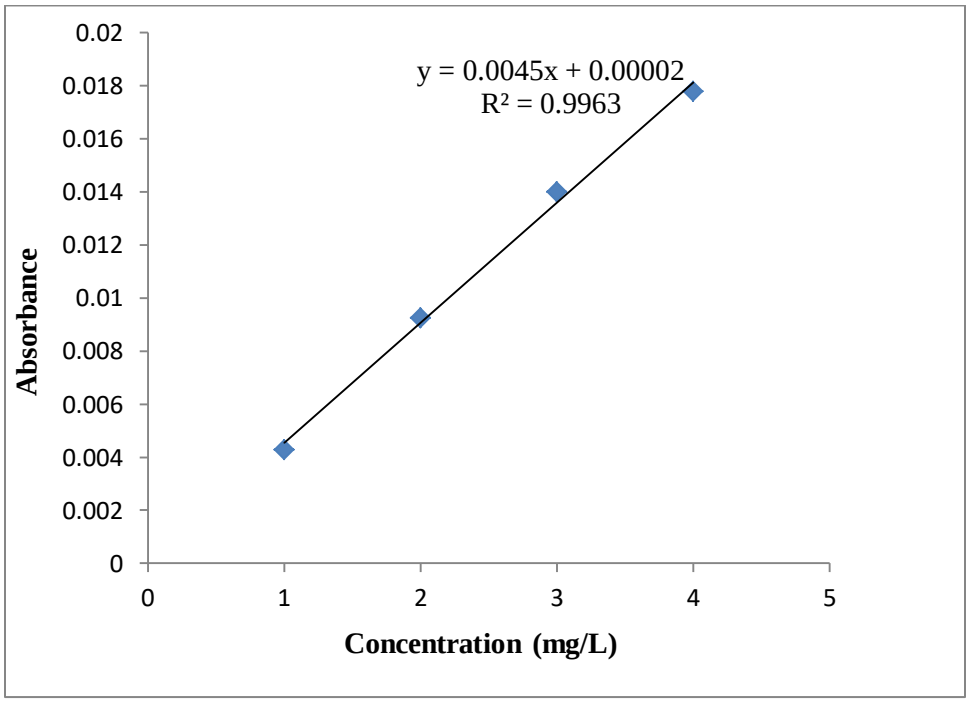

Figure 6: Calibration curve of standard solution of Nickel

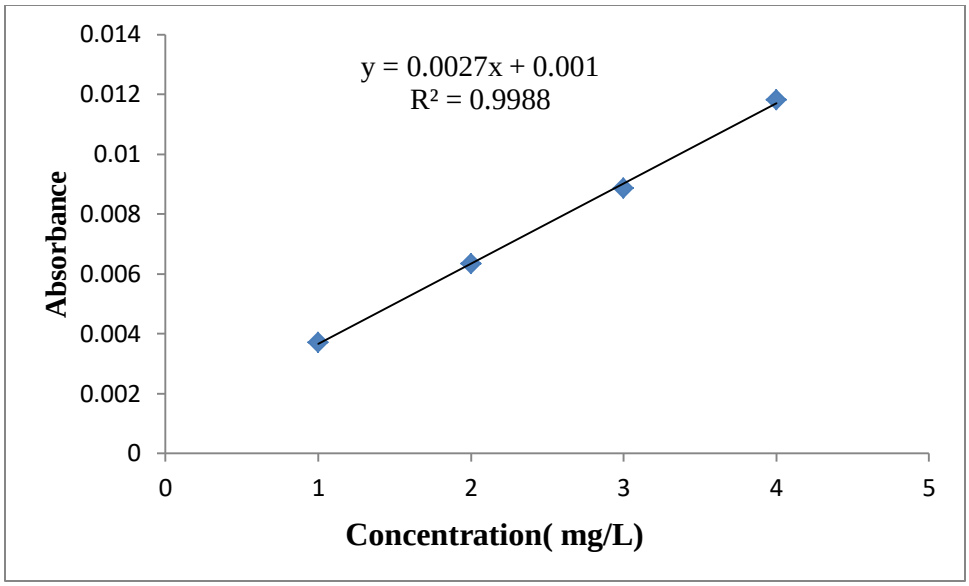

Figure 7: Calibration curve of standard solution of Lead

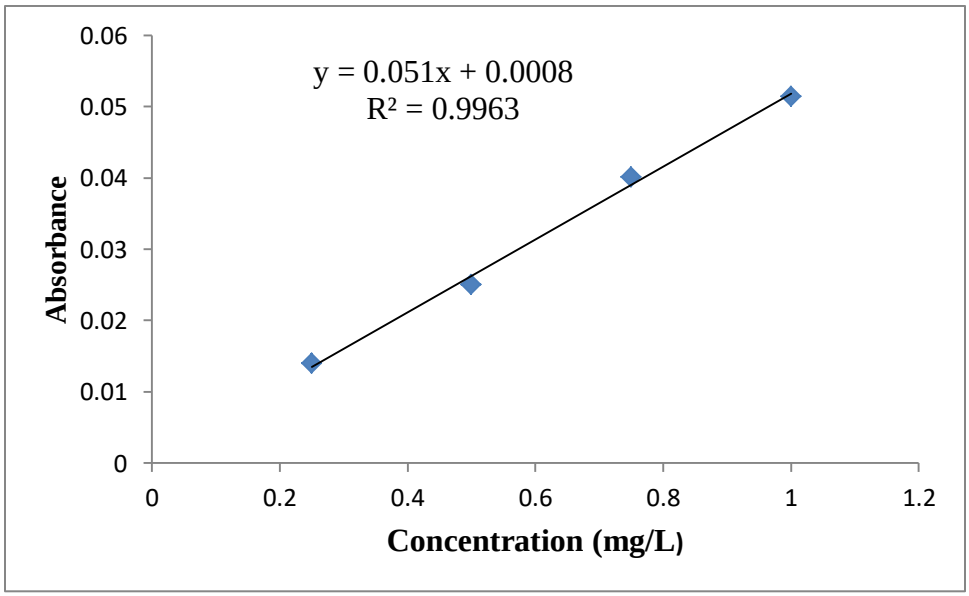

Figure 8: Calibration curve of standard solution of Zinc

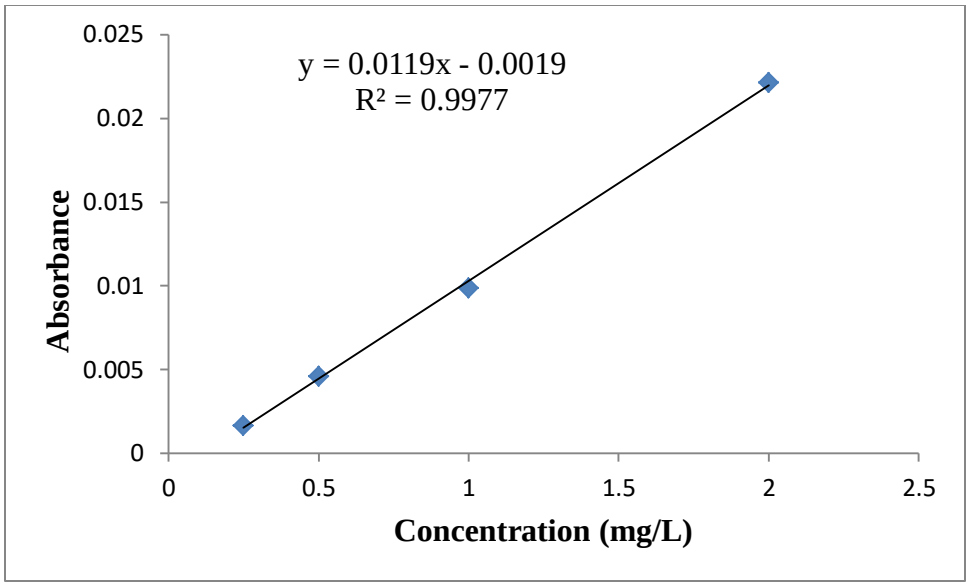

Figure 9: Calibration curve of standard solution of Iron

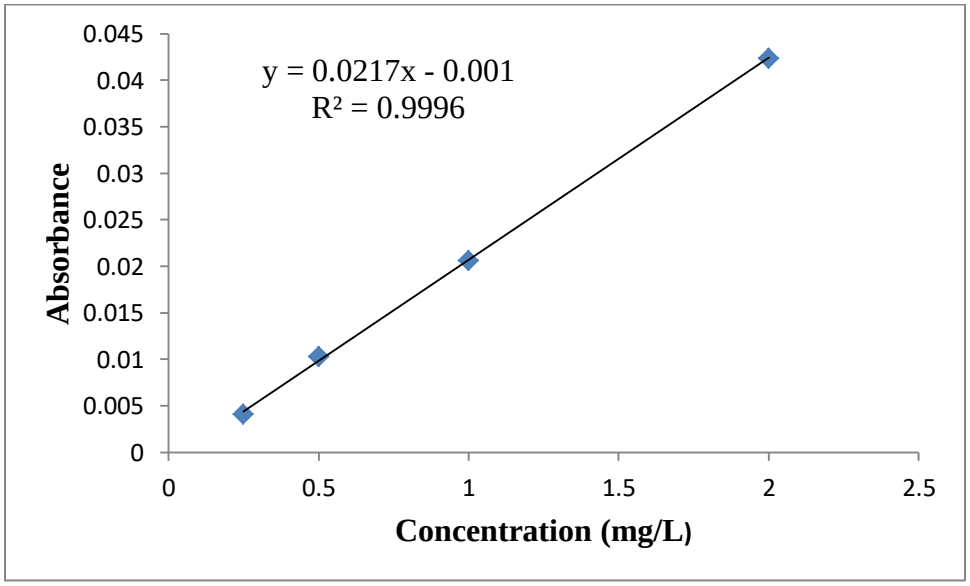

Figure 10: Calibration curve of standard solution of Manganese

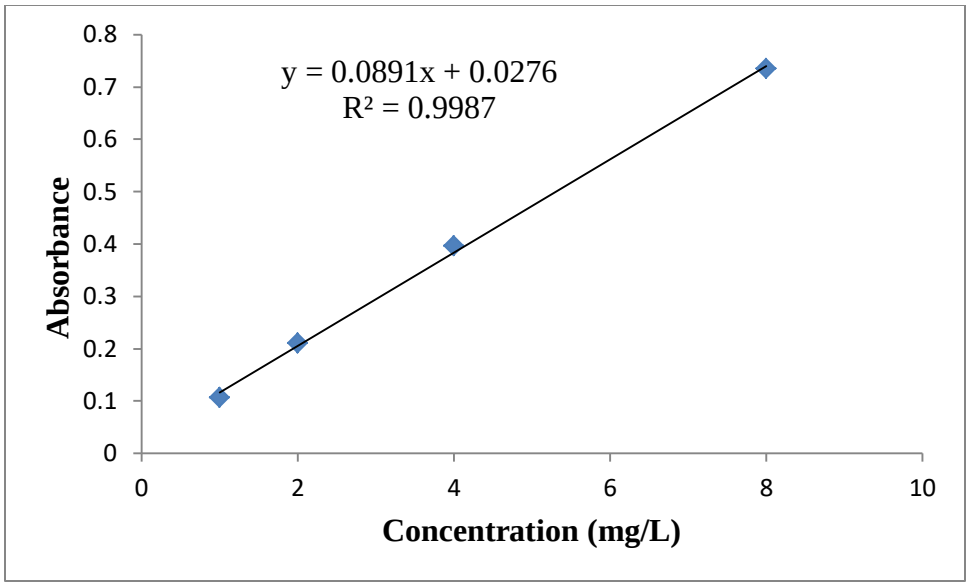

Figure 11: Calibration curve of standard solution of Sodium

3.6 Physico-chemical properties of peanuts

3.6.1 Moisture Content of peanut seed samples

Moisture content was determined by the method of the Association of Official Analytical Chemists by drying the sample in an electric controlled oven to constant weight. 5.0 g of the sample was accurately weighed into a previously cleaned, dried and weighed glass crucible. The crucible with its content was put in an oven at 105°C for 24 hrs. The sample was then cooled and weighed. The loss in weight expressed as a percentage of the initial weight of the sample gives the moisture content of the sample [11, 57]. The following formula was used to calculate the moisture content of peanut seed samples.

$$\%MC = \frac{W2-W3}{W2-W1} \times 100$$

Where: W_1= Weight of empty crucible

W_2= Weight of crucible + Fresh sample and

W_3= Weight of crucible + Sample after drying

3.6.2 Ash Content of peanut seed samples

The ash content was determined by the method of Association of Official Analytical Chemists. A 3.0 g sample was weighed into a previously dried and weighed porcelain crucible. The crucible with its content was placed in a furnace preheated to 600°C for 3 hrs. The sample was allowed to cool in the muffle furnace to 250°C. The crucible and the ash were then transferred into an oven at 100°C for 30 min cooling. The crucible with its content was weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample [11, 57]. The following formula was used to calculate the ash content of peanut seed samples.

$$\%AC = \frac{W3-W1}{W2-W1} \times 100$$

Where: W_1= Weight of empty crucible

W_2=Weight of crucible + sample and

W_3=Weight of crucible + ash

3.7 Accuracy and Precision

Accuracy is defined as closeness or agreement between a quantity obtained by measurement and the true value of the measurement. The precision of an analytical procedure expresses the closeness or agreement between replicate measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (repeatability or reproducibility). The common term used to measure variability is the relative standard deviation (RSD), which may also be expressed as a percentage and it is the parameter of choice for expressing precision in analytical sciences. In this study, the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of triplicate sample measurements for a given blank sample (i.e. three samples (n = 3) and triplicate readings for each sample) [58, 59].

3.8 Validation of optimization procedure

Method validation is the process used to confirm the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can also be used to judge the quality, reliability and consistency of analytical results; and it is considered as an integral part of any good analytical practice [60]. The parameters accuracy, precision and limit of detection were used for method validation.

Optimization procedure was validated using spiking or recovery experiment. In this study standard solution of 1000 mg/L for each metal were used. For recovery experiments the sample collected from Zala Mela is preferred. From 1000 mg/L stock solution 13.9 µL of Ca, 98.1 of µL Na, 2.44 µL of Zn and 2.16 µL of Fe were spiked and added to round bottomed flask containing 0.5 g of peanut seed sample and 4 mL of mixture of acids (3 mL of HNO_3 and 1 mL of $HClO_4$). The spiked and un-spiked samples were digested using the same procedure for the sample analysis. The percent recoveries were calculated using the following formula.

$$\%\text{Recovery} = \frac{\text{spiked sample} - \text{unspiked sample}}{\text{amount added}} \times 100$$

The results of recovery analysis are shown in table 6. The percentage recovery varies in the range of 90.5 to 98 and it is in acceptable range for the analyzed metals.

Table 6: Recovery study for the optimized procedure of peanut seed sample

Metal	Concentration in sample (mg/kg)	% Spiked	Amount added (mg/kg)	Concentration of Spiked sample (mg/kg)	Recovery (%)
Ca	79.4	35	27.79	105.7	95
Fe	9.6	45	3.8	13	90.5
Na	784.7	25	196.5	970.3	94.5
Zn	12.21	40	4.88	17	98

3.9 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is a measure of how sensitive the analytical method is and is the lowest concentration or weight of analyte that can be measured at a specific confidence level. For the determination of limit of detection of the analytical method (LOD), triplicate blanks were prepared and analyzed for their metal contents. The standard deviation (SD) of the three blanks was calculated and multiplied by three (LOD = 3SD) to determine the method detection limit [61, 62]. According to Association of Official Analytical Chemists (AOAC) limit of quantitation is the lowest amount of analyte in a sample, which can be quantitatively determined with precision and accuracy appropriate to analyte matrix considered. The LOQ is mathematically defined as 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection. LOQ =10 SD Where SD= standard deviation of the blank [62, 63]. In this study the limit of detections for all of the elements considered in the peanut seed samples are given in Table 7.

Table 7: Limit of detection and limit of quantitation for metals determined in the peanut seed samples.

Metals	Wavelength (nm)	LOD (mg/L)	LOQ (mg/L)
Ca	422	0.3	1
Cd	228	ND	ND
Cu	324	ND	ND
Fe	248	0.6	2
Mn	279	0.15	0.5
Na	589	0.06	0.2
Ni	232	ND	ND
Pb	283	0.15	0.5
Zn	213	0.09	0.3

*ND indicates not detected

Chapter Four: Results and Discussion

4.1 Concentration of metals in peanut seed samples

The concentration of nine metals (Ca, Cd, Cu, Fe, Mn, Na, Ni, Pb and Zn) in peanut seed samples collected from the three areas (Babile, Dibate and Zala Mela) in Ethiopia were determined by using FAAS. The mean values were determined from triplicate analysis of each sample and triplicate sample were used for each sample site. The results were expressed in terms of mean ± SD. Precision of the results was checked by calculating the relative standard deviation. Results obtained from FAAS were converted in to mg/kg. Mean, standard deviation, relative standard deviation and rang of metals concentration are shown in table 8.

Table 8: Metals concentration (mg/kg, mean ± SD, n=3) and %RSD in the different peanut seed samples

Metal	Sample site concentration (mean ± SD in mg/kg)						Range of metals concentration (mg/kg)
	Babile	%RSD	Dibate	%RSD	Zala Mela	%RSD	
Ca	83.4 ± 1.5	1.8	48.4± 0.8	0.2	79.36± 4.8	6.05	48.3-86.11
Cd	BDL	_	BDL	_	BDL	_	_
Cu	BDL	_	BDL	_	BDL	_	_
Fe	9.3±0.7	7.8	11.3±0.6	5.04	9.55±0.8	8.9	8.56-12.08
Mn	3.31±0.3	9.1	7.3±0.3	4.1	3.15±0.2	6.4	2.89-7.6
Na	983.9±2.9	0.3	1146±30.6	2.7	784.7±1,5	0.2	782.8-1176
Ni	BDL	_	BDL	_	BDL	_	_
Pb	1.59± 0.1	6.3	1.63± 0.03	1.8	1.96± 0.07	3.6	1.49-2.04
Zn	19.3± 0.9	4.7	14.5± 0.4	2.8	12.2± 0.6	4.9	11.35-20.09

BDL: below detection limit.

As shown in table 8 metals (Ca, Fe, Mn, Na, Zn, and Pb) are found in peanut seed samples and Cd, Cu and Ni are below detection limits. The mean concentration of Na in the range of 784.7 to 1146 mg/kg is the highest in each sample area followed by Ca. This indicates peanut seed is good source of sodium. The mean concentration of the non essential element Pb is relatively low as compared to the other metals concentration in peanut seed samples of each site. The maximum mean concentration levels in peanut seed samples for elements Na, Ca, Zn, Fe, Mn and Pb are 1146, 83.4, 19.3, 11.3, 7.3 and 1.96 mg/kg respectively. The results show that the essential elements are found in higher amount and non essentials are found in small amounts.

4.2 Comparison of the concentration of metals in the three different peanut seed Samples

As indicated in figure 12, the concentration of Na collected from Dibate sample site is highest (1146 mg/kg) followed by Babile (983.9 mg/kg) and relatively lowest amount in Zala Mela sample area (784.7 mg/kg). The order of levels of Na in peanut seed samples based on sample areas are Dibate > Babile> Zala Mela.

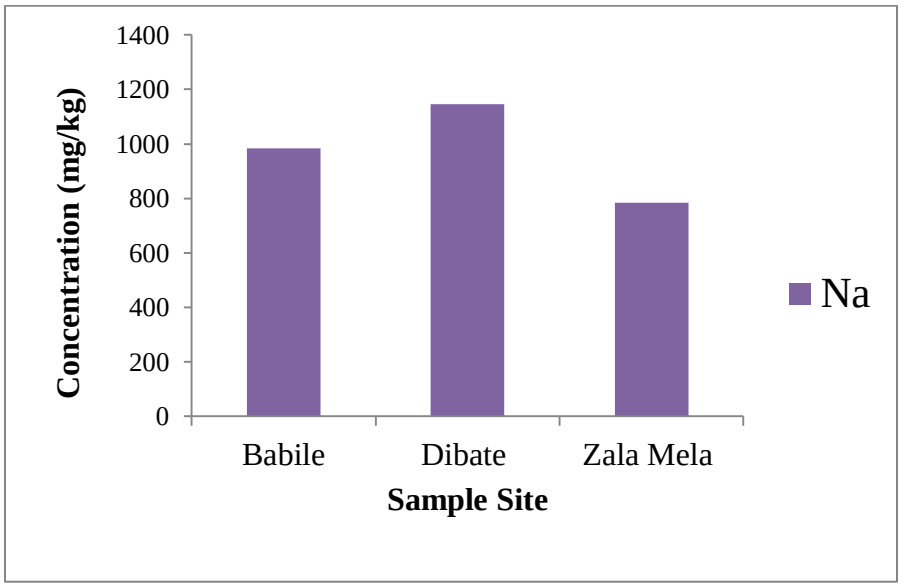

Figure 12: Bar graph of mean concentration of Na in the three sample areas for peanut seed samples

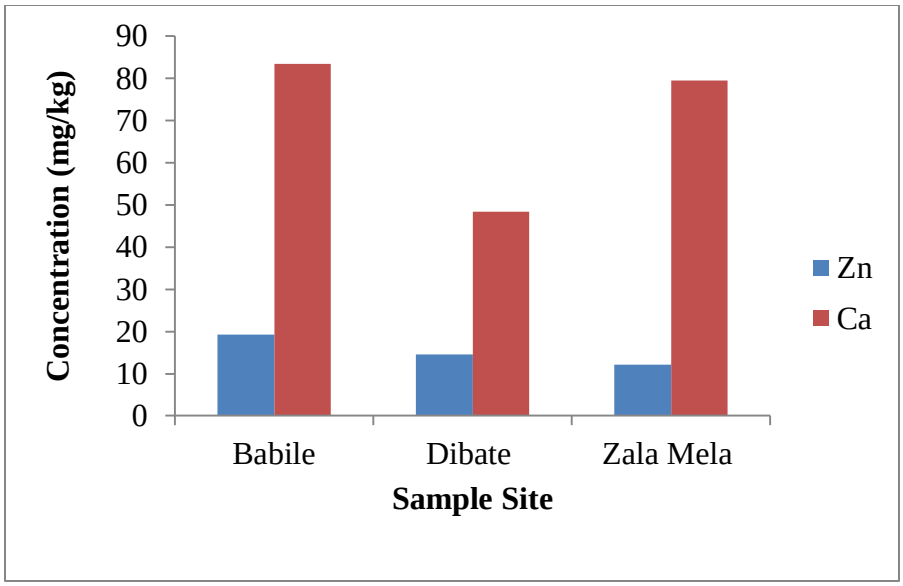

Figure 13: Bar graph of mean concentration of Ca and Zn in the three sample areas for peanut seed samples

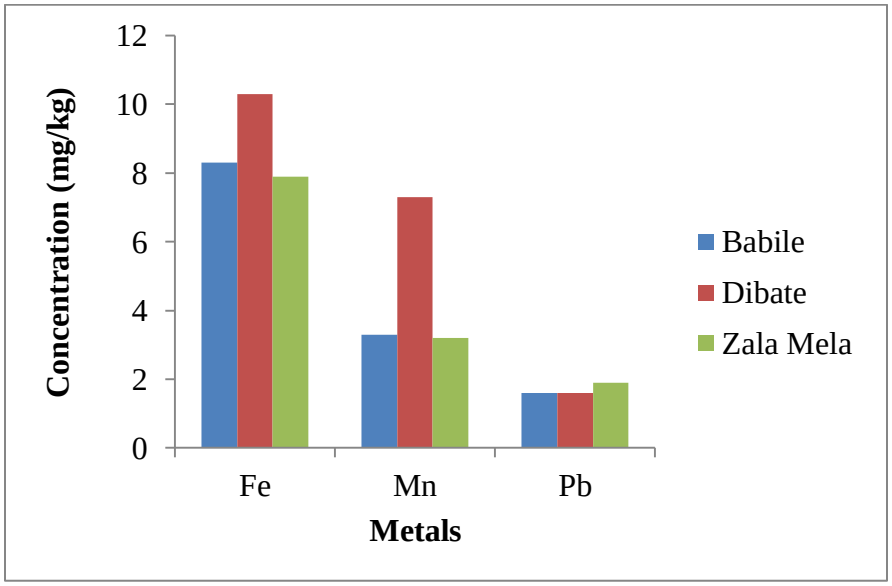

Figure 14: Bar graph of mean concentration of Fe, Mn, and Pb in the three sample areas for peanut seed samples

As indicated in table 8 and figure (12-14) in Babile sample area the order of concentration of metals: Na (983.9 mg/kg) > Ca (83.4 mg/kg) > Zn (19.3 mg/kg) >Fe (9.34 mg/kg) >Mn (3.3 mg/kg) >Pb (1.6 mg/kg). In Dibate sample area the order of concentration of metals: Na (1146 mg/kg) > Ca (48.4 mg/kg) > Zn (14.5 mg/kg) > Fe (11.3 mg/kg) > Mn (7.3 mg/kg) > Pb (1.6 mg/kg). In Zala Mela sample area the order of concentration of metals: Na (784.7 mg/kg) > Ca

(79.4 mg/kg) >Zn (12.2 mg/kg) > Fe (9.55 mg/kg) > Mn (3.2 mg/kg) >Pb (1.9 mg/kg). Generally the order of metals in the three sample areas are the same Na> Ca> Zn> Fe > Mn> Pb. The concentration of calcium in Babile area is higher followed by Zala Mela and Dibate sample area has a low concentration compared to the others. The concentration of manganese in Babile and Zala Mela sample areas are approximately equal and also the concentration of lead in Zala Mela is higher but in Babile and Dibate sample areas are equal.

4.3 Comparison of the concentration metals in peanut seed samples with literature values

Table 9: Comparison of metals concentration in mg/kg of peanut seed sample with reported values.

Range metals concentration(mg/kg)						Method	Reference
Ca	Fe	Mn	Na	Zn	Pb		
511	17.4	14.1	13.6	26.5	NR	ICP-OES	[64]
NR	NR	NR	NR	39	13.1	FAAS	[65]
602	59	18	74	53	NR	FAAS	[66]
1148	42.3	55.	628	45.2	NR	FAAS	[67]
NR	0.03	NR	NR	0.04	0.1	FAAS	[68]
48.4-83.4	**9.3-11.3**	**3.15-7.3**	**784.7-1146**	**12.2-19.3**	**1.59-1.96**	**FAAS**	**This study**

NR: not reported

As shown in Table 9, the concentration of metals in this study indicated the maximum and minimum values found in the peanut seed sample collected from the three sampling areas. The concentration of Na in peanut seed samples in this study is higher compared to other. But the concentration of metals determined in this study is lower compared to the literature values. The concentration of Fe and Zn in this study is higher than indicated in [68]. The concentration of lead in this study is lower compared with what is reported [65]. The variation of concentration of metals may be due to mineral contents of soil, the amount of fertilizer that is used and weather conditions.

4.4 Comparison of the concentration metals in peanut seed samples with other pulses

Table 10: Comparison of metals concentration (mg/kg) of peanut seed samples with other oilseed (pulses) reported in the literature values

Plant seed	Country	Method	Metals						References
			Ca	Na	Fe	Mn	Zn	Pb	
Linseed	Ethiopia	FAAS	635	242	198	23	33	32	[56]
Sesame	Ethiopia	FAAS	NR	NR	38.2	NR	60.4	0.14	[60]
Castor	Nigeria	-	NR	NR	16.667	3.750	2.700	13.05	[69]
Peanut	Ethiopia	FAAS	48.4-83.4	784.7-1146	9.3-11.3	12.2-19.3	12.2-19.3	1.59-1.96	This study

NR: not reported

As indicated in table 10, the concentration of Na determined in peanut seed samples is higher than in other pulses. The concentration of Fe is relatively small compared to the other pulses. The concentration of Mn and Zn in peanut seed sample is higher than the concentration of Mn and Zn in castor seed. The concentration of Pb in Linseed and Castor seed sample is higher compared to peanut seed sample.

4.5 Moisture and Ash contents of peanut seed samples

4.5.1 Moisture contents of peanut seed samples

Table 11: Moisture content of peanut seed samples from the three areas

Samples	W1(g)	W2(g)	W3(g)	MC%
Babile	189.85	194.85	194.51	6.8
Zala Mela	139.31	144.31	144.01	6
Dibate	207.63	212.63	212.27	7.2

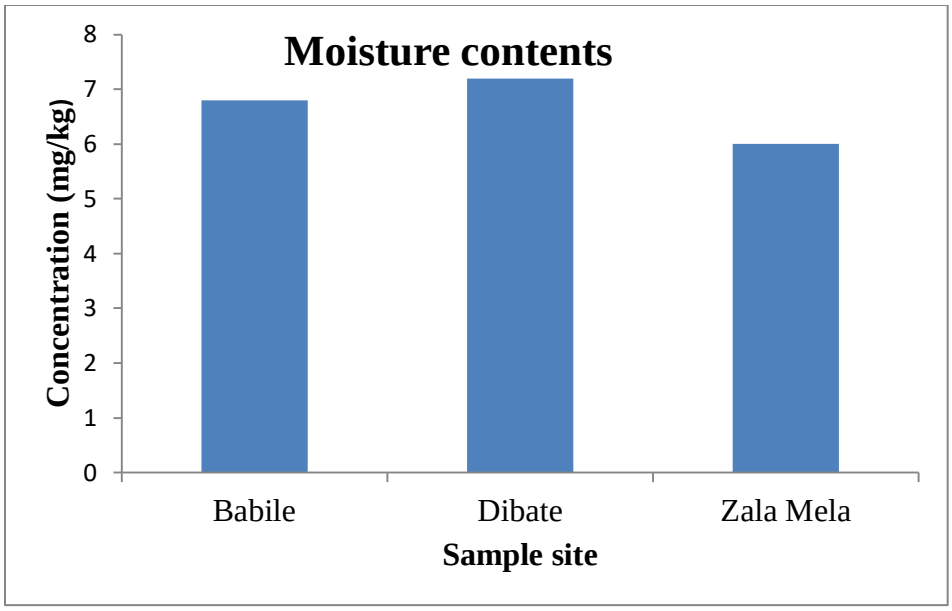

Figure 15**:** Moisture contents of peanut seed samples from the three areas

Table 11 and Figure 15 indicate the moisture contents of peanut seed samples from three areas. The moisture contents of peanut seed samples are in the range of 6% to 7.2%. From this data the samples collected from the Dibate area have high moisture content followed by Babile. This indicates that the shelf lives of peanut seed of the two areas are short compared to Zala Mela sample of peanut seeds. Low moisture contents make the shelf life long and contribute to the stability of seeds preventing rancidity [67]. According to International Nut and Dried Fruit Council, the moisture contents of peanuts in-pod is ≤ 10% and in peanut kernels is ≤ 9%. Based on this the result of moisture contents of the three samples agree with the reports. [70]

4.5.2 Ash contents of peanut seed samples

Table 12: Ash content of peanut seed samples from the three areas

Sample	W1 (g)	W2 (g)	W3 (g)	%AC
Babile	25.89	28.89	25.94	1.67
Dibate	25.80	28.80	25.87	2.33
Zala Mela	26.69	29.69	26.75	2

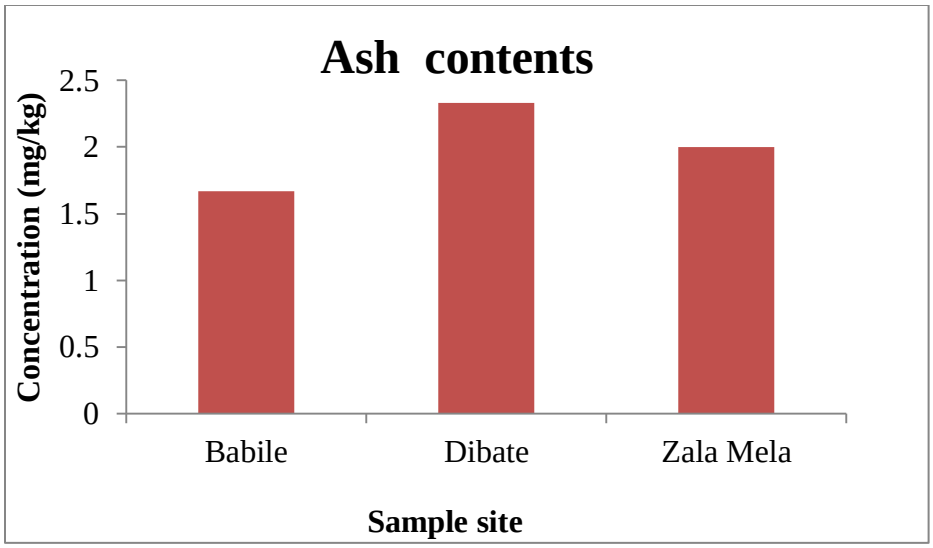

Figure 16: Ash contents of peanut seed samples from the three areas

Table 12 and figure 16 indicate the ash contents of peanut seed samples of the three areas. The ash content is a measure of the total amount of minerals present within a seed or the inorganic residue remaining after the water and organic matter have been removed. Higher ash contents indicate it is rich in different minerals [71]. The value of ash content of Babile, Dibate and Zala Mela are 1.67%, 2.33% and 2%, respectively. Peanut seed sample collected from Dibate has higher mineral followed by Zala Mela. The ash content of Babile is small compared to the other samples.

4.6 Statistical Analysis

4.6.1 Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a statistical tool concerned with comparing means of several samples. It can be thought of as an extension of the F-test for two independent samples to more than two groups. The purpose is to test for significant differences between class means, and this is done by analysis the variances. The ANOVA test of the hypothesis is based on a comparison of two independent estimates of the population variance. A one-way analysis of variance is used when the data are divided into groups according to only one factor [72].

The F-distribution arises in tests of hypotheses concerning whether or not two population variances are equal and concerning whether or not three or more population means are equal.
In this study one way ANOVA (Single factor) was used with Microsoft Excel 2010 for the data analysis. As indicated in table 13: the statistical analysis of ANOVA shows there is no significant difference among the sample mean concentration of Fe at 95% confidence intervals and there is significant difference among the sample mean concentration of Ca, Mn, Pb, Zn, and Na at 95% confidence intervals. This significant difference may be caused due to the soil mineral composition, soil pH, rainfall, harvesting and storing system and the fertilizer that is used.

Table 13: Analysis of variance (ANOVA) between and within peanut seed samples at 95% confidence level.

Metal	Comparison	SS	Df	*p*-value	Fcal	Fcrit	Remarks
Ca	Between Groups	2198	2	3.6×10^{-5}	87.6	5.14	Significant difference between sample mean
	Within Groups	75.3	6				
Fe	Between Groups	6.99	2	0.067	4.4	5.14	No Significant difference between sample mean
	Within Groups	4.73	6				
Mn	Between Groups	32.6	2	1.4×10^{-5}	121.8	5.14	Significant difference between sample mean
	Within Groups	0.8	6				
Na	Between Groups	196529	2	2.9×10^{-6}	207.6	5.14	Significant difference between sample mean
	Within Groups	2840.3	6				
Pb	Between Groups	0.34	2	0.016	9.02	5.14	Significant difference between sample mean
	Within Groups	0.08	6				
Zn	Between Groups	77.3	2	1.4×10^{-4}	54.7	5.14	Significant difference between sample mean
	Within Groups	4.2	6				

4.6.2 Pearson correlation of metals within peanut seed samples

The correlation coefficient *r* is the specific measure that quantifies the strength of the linear relationship between two variables in a correlation analysis and its value between -1 and 1. If the two variables are in perfect linear relationship, the correlation coefficient will be either 1 or –1. The sign depends on whether the variables are positively or negatively related. The correlation coefficient is 0 if there is no linear relationship between the variables. A positive correlation coefficient indicates that an increase in the first variable would correspond to an increase in the second variable, thus implying a direct relationship between the variables. A negative correlation indicates an inverse relationship where in one variable increases the second variable decreases [73, 74].

Correlation coefficient is classified as exactly –1 a perfect downhill, –0.70 a strong downhill –0.50 a moderate downhill, –0.30 a weak downhill, +0.30 a weak uphill linear relationship +0.50 a moderate uphill +0.70 a strong uphill and exactly +1 a perfect uphill linear relationship [75]. As shown in table 14 a strong uphill linear relationship was observed between Mn-Na, Mn-Fe, and Pb-Zn. A strong downhill linear relationship was observed between Na-Ca, Mn-Ca and Pb-Na. A moderate uphill was observed between Fe-Na. A weak uphill linear relationship was also observed between Zn-Na, Pb-Ca, Pb-Mn, Zn-Ca, Fe-Zn, Mn-Zn and a weak downhill linear relationship between Pb-Fe.

Table 14: Pearson correlation coefficients of the metals

	Ca	Na	Zn	Fe	Mn	Pb
Ca	1					
Na	-0.7538	1				
Zn	0.2639	0.3667	1			
Fe	-0.8003	0.6316	0.1477	1		
Mn	-0.9758	0.8482	0.1534	0.7547	1	
Pb	0.3052	-0.7178	0.7718	-0.2680	0.3818	1

Chapter Five: Conclusion

In this study, the concentration of metals in peanut seed samples collected from three different areas in Ethiopia were analyzed (Ca, Cu, Cd, Fe, Mn, Na, Ni, Pb and Zn) using flame atomic absorption spectrophotometry. The mean concentration of metals in the peanut seed samples collected from three different areas indicates that concentration of Na was the highest among the metals and Pb was found in a relative small amount in the three sampling areas. Cu, Cd and Ni were also analyzed but they are below detection limit.

The optimized method for the analysis of peanut seed sample was efficient and it was evaluated by recovery experiment. The percentage recoveries for the detected metals varied from 90.5 to 98. The mean concentration of metals determined in peanut seed samples were found to be in the following order: Na (784.7-1146 mg/kg) >Ca (48.4-83.4 mg/kg) > Zn (12.2-19.3 mg/kg) >Fe (9.3-11.3 mg/kg) >Mn (3.15-7.3 mg/kg) >Pb (1.59-1.96 mg/kg).

At 95% confidence level, one way ANOVA indicates that there is no significant difference between the mean concentrations of Fe but there is significant difference between the mean concentrations of Na, Mn, Pb, Zn and Ca. This significant difference may be caused due to soil mineral composition, soil pH, rainfall, harvesting and storing system and the fertilizer that is used.

Based on this, the results indicate the peanut seed sample contain high concentration of essential macro elements, Na and Ca. The levels of metals investigated in peanut seed samples collected from three sampling areas were comparable.

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