DILLA UNIVERSITY

SCHOOL OF POST GRADUATE STUDIES

CHEMISTRY DEPARTMENT

DETERMINATION AND COMPARISON OF Si, Na, Cu, Ni, AND Co CONTENTS OF GARDEN COFFEE AND MODERN FARM COFFEE OF ABAYA DISTRICT, WEST GUJI ZONE, OROMIA REGION OF ETHIOPIA

MSc THESIS

BY

KITE DUBE

JUNE, 2023

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KITE DUBE

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

JUNE, 2023

DILLA

AUTHOR'S DECLARATION

I, the undersigned, declare that this thesis, entitled "DETERMINATION AND COMPARISON OF Si, Na, Cu, Ni, AND Co CONTENTS OF GARDEN COFFEE AND MODERN FARM COFFEE OF ABAYA DISTRICT, WEST GUJI ZONE, OROMIA REGION OF ETHIOPIA" is my original work, that it has not been presented for any other institution for the award of any academic degree, diploma or certificate, that I followed all ethical and technical principles of scholarship in the data analysis and preparation of the report, and that all sources of material used in this thesis are duly acknowledged .

Name **Kite Dube**

Signature___________

Date ___________

ADVISOR’S APPROVAL SHEET

I the undersigned certify that I have read and evaluated this thesis entitled DETERMINATION AND COMPARISON OF Si, Na, Cu, Ni, AND Co CONTENTS OF GARDEN COFFEE, AND MODERN FARM COFFEE OF ABAYA DISTRICT, WEST GUJI ZONE, OROMIA REGION OF ETHIOPIA prepared under my guidance by KITE DUBE. I recommend that it be submitted for defense as fulfilling the thesis requirements.

_____________________	_____________	_____________
Advisor’s name	Signature	Date

EXAMINERS' APPROVAL SHEET

DILLA UNIVERSITY

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We the undersigned certify that the thesis prepared by KITE DUBE entitled DETERMINATION AND COMPARISON OF Si, Na, Cu, Ni, AND Co CONTENTS OF GARDEN COFFEE AND MODERN FARM COFFEE OF ABAYA DISTRICT, WEST GUJI ZONE, OROMIA REGION OF ETHIOPIA and submitted in partial fulfillment of the requirements for the degree of master of science complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Internal examiner 1	Signature	Date
Internal examiner 2	Signature	Date
External examiner	Signature	Date
Advisor	Signature	Date

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LIST OF ACRONYMS AND ABBREVIATIONS

AI	Adequate Intake
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ICP-OES	Inductive coupled plasma-optical spectrometry
EDA	Established Recommended Daily Amount
RNA	Ribonucleic Acid
SOD	Superoxide Dismutase
MDL	Method of Detection Limit
Na	Sodium
Cu	Copper
Ni	Nickel
Co	Cobalt
B_{12}	Vitamin B_{12}
Si	Silicon
δ	standard deviation

ABSTRACT

The concentrations of five metals (Na, Cu, Ni, Co and Si) were determined in coffee bean that cultivated in Garden and Modern farm method that obtained from Abaya District by using Inductive coupled plasma-optical spectrometry, Optima 8000 (ICP-OES). The digestion procedure was carried out using 5 mL of HNO3 and 5 mL of HClO4 with 3 hours total time at temperature about 120 oC for digestion of 0.5 g of powder sample of both coffee farms. The validity of the optimized procedure was evaluated by the analysis of spiked samples whose recovery of analytes was in the range of 85−113 %. The mean concentration of each metal in the two farms of coffee bean samples was Na (192.23, 193.20mg/l), Cu (4.86, 8.33mg/l), Co (0.63, 0.99mg/l) and Ni (0.44, 1.76mg/l) Garden coffee and Modern coffee farms respectively but, Si is under the detection limit. The metal concentrations of the modern coffee farm were higher than those of garden coffee farm, when expressed in the same units. The extraction was highest for Na and intermediate for Cu lowest for Co and Ni. The concentrations of metals in the garden coffee farms were compared to the values modern coffee farms that the concentration of Modern coffee is relatively higher than the Garden coffee farms.

KEY WORDS: *Modern Coffee, Garden coffee, metals, ICP-OES*

CHAPTER 1

1. INTRODUCTION

1.1 BACK GROUND OF THE STUDY

Coffee is the fruit and dried seed of the coffee plant regardless of whether it has been roasted or ground of the plant of the genus Coffee, generally of the cultivated species, and the products of these in their different stages of the process and use intended for human consumption (Perez-Sarinana et al., 2017). Kalid or Khalid was a legendary Ethiopia Sufi goatherd in Ethiopia according to popular legend, Kalid, noticing that when his goats were nibbling on the bright red berries of a certain bush, they become more energetic (jumping goats), chewed on the fruit himself. His exhilaration prompted him to bring the berries to an Islamic monk in a nearby Sufi monastery, but Sufi monk disapproved of their use and threw them in to a fire, from which an enticing aroma billowed. The roasted beans were quickly raked from the embers, ground up, and dissolved in hot water, yielding the world's first cup of coffee (Ukers, 1935).

Ethiopia is the center of origin and diversity of coffee. Coffee plays a pivotal role in the socio-economy of the country. It employs more than 20% of the economically active population and contributes more than 25% of the country's foreign exchange earnings. Currently, Ethiopia is the leading Arabica coffee producer in Africa, the fifth largest worldwide and the tenth in coffee exports worldwide. The average annual production amounts to about 350,000 tons. The average yield is about 0.71ton/ha. Ethiopian coffee is intrinsically organic and renowned for its superior quality. Small holder farmers' account for more than 95% of the total coffee produced in Ethiopia, but still traditional farming systems. In Ethiopia, Coffee is produced under four broad production systems, i.e. Forest coffee 8-10%, semi forest coffee 30-35, cottage or garden coffee 50-57% and modern coffee plantation 5% (Alemayehu et al., 2017).

There are different coffee species exploited in the world including coffea Arabica, coffeaconephora (Robusta), coffeaLiberica, coffeaabeakutyae, coffeadewevrel, coffeacongensis, etc. The two main coffee species exploited in the world today are coffea Arabica (~75%) of the world production, coffeaconephora named as robusta (~25%), coffeaLiberica (<1%) and the others are less cultivated type (Martin et al., 1998).

Coffee is one of the most important commodities in the international agricultural trade, representing a significant source of income to several countries of Africa, Asia and Latin America. Ethiopia is a leading Arabica coffee producer in Africa, the third largest coffee producer in the world after Brazil and Vietnam, beating out Colombia (dos Santos et al., 2009).

Coffee grows in Ethiopia, almost in all administrative regions. Even though, the whole coffees cultivated in Ethiopia are coffea Arabica wide variability are noted among coffee cultivates in the country (Gure et al., 2017). Currently it contributes about 30 present of the country's foreign currency earnings. More than 15 million people directly or indirectly depend on coffee value chain for their income and employment. About 35% of the total production is consumed within the producing areas (Chauhan et al., 2015), and in general, over 50% of the coffee produced is consumed within Ethiopia. The total area coverage of coffee in Ethiopia is estimated to be around 800,000 Hectares, of which about 95% is produced by 4 million small scale farmers (Alemayehu et al., 2017).

Some metals are biologically crucial in low concentrations for living organisms, including copper (Cu), chromium (Cr), cobalt (Co), manganese (Mn), nickel (Ni), zinc (Zn); The coffee plants may absorb these metals and store them in the roots or transport them into the shoots and grains. The metals vary in concentration in the different plant tissues, and normally, the grains contain lesser concentrations than the vegetative plant parts (Bettiol and Campbell, 2006).

Coffee is the complex mixture of potential "neutriceuticals." The chemical composition of coffee varies based on species (Arabica or robusta), country of origin, system of cultivation (organic or conventional) and its physical form as raw or roasted (Gutu, 1998,). The most important constituents are minerals, lipids, caffeine, proteins, fats, carbohydrates, and water. Green coffee contains about 250 different volatile molecular species (Tagliaferro et al., 2003).

According to the report on coffee varieties in different country like: Ethiopia, southeast Brazilian and others there is a difference in metals content among the samples of coffee varieties. These variations in metal compositions among samples of coffee can indicate the differences in the cultivation of the coffee plants; such as the type of soil, the use of fertilizers with different chemical compositions and the environmental conditions (Martinet al., 1998, Gure et al., 2015).

The amount of elements in plants, generally, depend on many factors; species, age, root distribution of the plant, physical and chemical nature of the soil, proportions and distributions of elements and the general climatic conditions. Due to the health benefits and economical relevance of coffee extensive research has been published on the elemental composition of roasted and green coffee beans for geographical origin and species discrimination (Rajaganapathy et al., 2011).

Some recent studies have reported the mineral contents in roasted coffee. Dos Santos et al. have evaluated some metals in Brazilian coffees cultivated during the process of conversion from conventional to organic agriculture (dos Santos et al., 2009). Pohl et al. have described different techniques for the determination of the elemental composition of coffee (Pohl et al., 2013), Gure et al also reported the mineral contents of green and roasted coffee variety in Ethiopia. The results indicated that higher concentrations of these metals in roasted coffee than in the corresponding raw varieties (Gure et al., 2015).

1.2 Statements of the Problem

Coffee is a widely consumed stimulant beverage prepared from roasted seeds, commonly called coffee beans, of the coffee plant. The popularity of coffee as a beverage is ever increasing, despite the fact that there are reports attesting that it is not necessarily good for health. The part of the coffee, which is important for household consumption and commercial purposes, is the bean, which naturally contains proteins, carbohydrates, vitamins, and mineral substances. However, the chemical content of the coffee varies with coffee types and environment in which they are cultivated and in raw and roasted coffee beans of the same coffee type collected from the same environment.

In Ethiopia specially Oromia region west Guji zone Abaya District coffee used for consumption of human with unknown concentration of Na, Si, Cu, Ni and Co in almost the whole District. However, this metal is used for human being at low concentration of this metal. Therefore, this study can be identify the level of this five metal and the levels of the metals in Garden farm coffee and Modern farm coffee beans can be compared with each concentration of five metals.

1.3 Objective of the Study

1.3.1 General Objective of the Study

The general objective of this study was to determine and compare the concentration of metals (Si, Na, Cu, Ni and Co) in Garden coffee and Modern farm coffee beans collected from Abaya District.

1.3.2 Specific Objectives of the Study

The specific objectives of this study were:

- To determine the level of concentration of Si, Na, Cu, Ni and Co in Garden coffee and Modern farm coffee samples of the Abaya District.
- To compare the concentration level of, metals in coffee beans of Garden Coffee and Modern Farm Coffee of Abaya District.

1.4 Significance of the Study

It is believed that the results of this study may have the following importance for its users. First this study will be intended to give clear information on the concept of the concentration level and characterization of the important features and application of the Na, Si, Cu, Ni and Co in coffee beans of West Guji Zone Abaya District. Secondly, the Significance of this study were motivates other researchers to work further on this area, to investigate the possibility of conception of coffee.

1.5 Scope of the Study

In this study, much of the works described were concerned with the concentration level of Na, Si, Cu, Ni and Co metals in coffee beans of Abaya District. After collection of the sample from specified area, the concentration levels of the five metals were identified, and the concentration of the metal in different cultivation method were compared.

1.6 Organization of the Thesis

The thesis was reported with five chapters following one with another. In this sequences of chapters, background of the study with the relevant history of Ethiopian coffee bean were organized in chapter one. The statement of the problem, General objective and specific objectives, significance and scope of the study were explained also in this chapter. In chapter two the related literature were reviewed from different references. In chapter three Materials and chemicals used for this thesis with appropriate procedure and chemical gradient mentioned. The results of the thesis were expressed in many tables and figures with detail discussion and explanation. Lastly conclusion of the results and recommendations are written in the chapter five.

CHAPTER 2

2. RELATED LITERATURE REVIEW

2.1 The Origin and Structure of Coffee

Coffee is one of the most consumed beverages in the world. This is due to its distinctive aroma and flavor which readily attract although some are intrigue for its caffeine content (Higdon and Frei, 2006).The familiar coffee bean which consumer is used to is actually only a small part of the plant. The time between flowering and fruit picking varies between 6 to 8 months for Arabica according to site and variety. Hence, good plantation management encompasses appropriate timing of all cultivation steps at the different development stages, from growth, flowering, fruiting to full ripening. Ecology and growing conditions, the natural habitat of all Coffea species is the African tropical forest. Coffee is a well-adapted plantation crop to different eco-physiological conditions of tropical highlands Arabica. The original fruit of coffee has the same look and size of cherry with an outer fleshy portion of the pericarp. Beneath the flesh is tissue like parchment known scientifically as endocarp. Underneath endocarp is spermoderm which referred to silver skin that coat the entire endosperm (coffee bean) (Esquivel and Jimenez, 2012).

Coffee quality depends on many factors, which range from species choice and crop variety to the method of preparation of coffee for consumption. Among these factors, processing, drying, storage, milling, and transportation are fundamental for obtaining and maintaining a differentiated final product. Of all the post-harvest stages, drying is the most relevant from energy consumption and a processing cost formation perspective, as well as from a quality preservation point of view. If the drying is not conducted correctly, it will impair the quality due to undesirable physical, chemical, and sensorial alterations, causing degradation of chemical components that may come in contact with hydrolytic and oxidative enzymes, thereby affecting the characteristic color, flavor, and aroma of the beverage (Borem, 2008)

Both altitude and latitude influence the optimal or near-optimal edaphic-climatic conditions for cultivation and must be considered along with temperature, rainfall and water supply, soil, slope and aspect when determining where to plant coffee. Arabica coffee requires an elevation between 1,200 and 2,200 m above sea level at the equator, but the optimal altitude can be less at higher latitudes. Arabica coffee is cultivated between 25°N and 24°S, ranging from more inclined subtropical areas to tropical regions with greater elevations as far as the temperature requirements (15 to 30° C) are fulfilled. Robusta coffee, consequent to its central African origin, is best adapted to less elevated hot and humid forests of tropical regions. The crop is preferably grown between 15°N and 12°S in flat areas and elevations between 300 and 800 m.Arabica coffee is well adapted to cooler temperatures, needs more than seven months of rainy season and high temperatures for an abundant differentiation of flower buds; it tolerates drought when grown in deep soils (Anthony et al.,2001).

2.2 Chemical Profile and Composition of Coffee Beans

Coffee beans are harvested when the fruits are bright red and glossy and dried to 10- 14% moisture levels prior to dehusking. The seeds, commonly called green beans, the ripe coffee cherries are usually picked by hand. The main exception is Brazil, where the relatively flat land scope and immense size of the coffee fields allow for machinery use, are then marketed for roasting. The chemical components of green beans largely depend on the species and variety of the coffee used whereas the brew properties largely depend on the procedure adopted for roasting. Since the coffee aroma is dependant largely on the roasting process, the amount and type of volatiles follow temperature-time relation during the roasting process (Gogoasa et al., 2013). The coffee bean is composed of many components, including carbohydrate, proteins, lipids, tannin, polyphenols and caffeine. It also contains an important number of vitamins very few calories and minerals. Minerals include potassium, magnesium, calcium, sodium, iron, manganese, sulphate, zinc, copper, strontium, chromium, barium, nickel, cobalt, lead, cadmium, cesium, lanthanum, rubidium, scandium, and phosphorus (Tsegaye et al., 2014).

2.2.1 Sodium (Na)

Sodium is an important mineral and electrolyte necessary for many functions in the body. It has an important role in maintaining water balance within cells, and is involved in proper functioning of both nerve impulses and muscles within the body. Along with potassium, sodium also plays a crucial role in blood pressure regulation. Sodium is only needed in small quantities and it is also found in many other ingredients used in food processing. Many commercially prepared condiments and seasonings are high in sodium. The Adequate Intake (AI) of sodium is 2,300 milligrams daily. Specific health claims can be made about sodium for food products that meet certain requirements. Deficiency usually only occurs with prolonged bouts of fluid loss due to diarrhea, vomiting, or perspiration. Those who have kidney problems may also be more likely to develop a sodium deficiency. Symptoms of deficiency include nausea, dizziness, and muscle cramps (Taylor and Francis, 2009).

2.2.2 Silicon (Si)

High quality of this element is found in hard water as compared to soft water. The important sources of silicon are; fruit: Apples, Cherries, and oranges, Vegetables: raw cabbages, carrots, onions, cucumbers, and pumpkins, nuts, peanuts etc. Si is known as a beautifying mineral and there are many health benefits associated with it. It not only helps strengthen connective tissues and bones but is also useful in skin, nail and hair cares. This element also plays a vital role in the prevention of atherosclerosis, insomnia, and tuberculosis, and aluminum toxicity. It is present in the human body in the form of a derivative of either silicate or silicic acid. It is also necessary for the diet as it increase the overall benefits of vitamin D, glucosamine, and calcium (Harry et al., 2008).

Earlier in human history, it was not regarded as a physiologically important element due to its substantial presence in the animal and plant tissues. However, with ongoing research, the health benefits of this element have been clearly demonstrated and used for strengthen of bones, treats alopecia, skin care, prevents brittle nails, prevent atherosclerosis, restores mucosa, promotes healing, prevent aluminium toxicity. The deficiency symptoms are quite apparent as they are closely associated with the connective tissues. A few of the visible symptoms are thinning of the hair, brittleness of nails, wrinkle formation (Robberecht et al., 2008).

2.2.3 Copper (Cu)

Copper is an essential trace element in plants and animals. The human body only contains about 150 mg of this vital mineral. The established RDA for Cu in normal healthy adults is 2 mg/day. Cu is absorbed in the gut and then transported to the liver bound to albumin. After processing in the liver, Cu is distributed to other tissues in a second phase. Cu transport in liver involves the protein ceruloplasmin, which carries the majority of Cu in blood. Ceruloplasmin also carries Cu that is excreted in milk and is particularly well absorbed as a Cu source. 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 (Osredkar and Sustar, 2011).

Cu is an essential constituent of several enzymes such as cytochrome oxidase, monoamine oxidase, catalase, peroxidase, ascorbic acid oxidase, lactase, tyrosinase, and superoxide dismutase (SOD). 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, helps in the incorporation of Fe in hemoglobin, assists in the absorption of Fe from the gastrointestinal tract, and in the transfer of Fe from tissues to the plasma (Linder et al., 1998).

Cu deficiency is rare among healthy people, but it may occur among infants. The most common symptoms of Cu deficiency include fatigue, anemia, and a decreased number of white blood cells. Sometimes, osteoporosis develops or nerves are damaged. Nerve damage can cause tingling and loss of sensation in the feet and hands. Muscles may feel weak. Some people become confused, irritable, and mildly depressed. It has been found that the most common cause of Cu deficiency is the remote gastrointestinal surgery, such as gastric bypass surgery, due to malabsorption of Copper (Tan et al., 2006).

2.2.4 Nickel (Ni)

It is well accepted that nickel is as essential ultra-trace nutrient in plants, animals, and humans. It has been reported that the nickel is essential for the active synthesis of urease in plant cells. In several species of higher plants such as jack beans, soybeans, rice, and tobacco, it is required for effective urea metabolism and urease synthesis. 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. More research is needed to reveal the properties of this interesting mineral in the human body. It has been reported that the estimated daily intake of nickel from food and water worldwide is 80-130 µg/day. Ni is contained in many foods such as, beans, chocolate, soybeans, lentils, split, green peas, oats, buckwheat, barley, and corn. Many vegetables and some fruits, such as bananas and pears, have moderate amounts of nickel (Anke et al., 1995).

Ni deficiency has not been shown to be a concern in humans, despite this it may cause biochemical changes, such as reduced Fe re-sorption that leads to anemia. It can disturb the incorporation of calcium into skeleton and lead to parakeratosis-like damage, which finds expression in disturbed Zn metabolism. It has found that nickel deficiency particularly affects carbohydrate metabolism. More researches are required to see the benefits of, and what effects nickel deficiency can cause on the human body (Anke et al., 1995).

2.2.5 Cobalt (Co)

Co is an essential trace element for the human body, where it is a key constituent of cobalamin (the scientific name of vitamin B_{12}). It also has a substantial role in the formation of amino acids and neurotransmitters. Human body can get Co ions through several pathways: With food, by the respiratory system, by the skin, and as a component of biomaterials. The largest source of exposure to cobalt for the general population is the food supply. The estimated intake from food is 5-40 μg/day, most of which is inorganic cobalt. Green vegetables and fresh cereals are the richest sources of cobalt, whereas dairy products, refined cereals, and sugar contain the least cobalt. Inorganic forms of cobalt are toxic to the human body, and the longer they stay in the body, the more the detrimental effects they cause in cells (Uniceet al., 2012).

It has been found that the cobalt deficiency is associated with disturbances in vitamin B_{12} synthesis. It might cause anemia and hypothyroidism, as well as increase the risk of developmental abnormalities and failure in infants. The excess level of this metal in the human body might cause hypothyroidism and overproduction of erythrocytes, fibrosis in lungs and asthma (Battagliaet al., 2009).

2.3 Properties of Coffee

The food and therapeutic properties of coffee is given by its main chemical, biochemical, plastic, and energetic properties which meet the physiological needs of the human body, among which minerals. Therefore, the beneficial effects of coffee are determined by their content of macro- and micro-elements that are deeply involved in the human body functioning: hence, the interest in knowing the distribution of these minerals in the different coffee assortments. Literature contains numerous data on the distribution of minerals in the different assortments of cultivated coffee in different geographical areas, as well as a series of mineral analysis techniques. Trace elements as essential bio-elements, even in very low concentrations are particularly important roles micro-elements serve as catalysts in the metabolism of plants and animals (Chu, 2012).

Coffee has taken on several physical transformations, initially serving as an energy source when nomadic tribes combined coffee berries with animal fat as early form of an energy bar. Later it was consumed as a tea, then wine, and finally to the beverage we've come to identify today. So far that’s great news for people with an unbalanced diet but there is another therapeutic benefit to coffee that is even more surprising. Recently Italian scientists discovered that drinking coffee may lower our incidence of dental caries. According to researchers trigonelline works by preventing the adhesion of mucus-like acid by products onto teeth which would otherwise lead to dental caries (Archana, 2016).

2.4 Health Benefit of Raw Coffee

The most coffee we consumed have been roasted, a process that changes the colour, flavour, and odour of green coffee bean to become roasted coffee that will be ready to brew. However, the roasting process introduced causes 8-10% chlorogenic acid degradation and transformation per each 1% loss of dry matter and 11 to 45% polyphenol degradation. In term of weight loss, oral administration of green coffee bean extract to the rats was found to reduce visceral fat and body weight. This anti weight gain property is due to the activation of fat metabolism in the liver and by inhibition of fat absorption which is governed by green coffee bean extract constituents. Green coffee extract daily intake effectively decrease blood pressure in hypertensive subjects in rats and humans and may help to prevent stroke. This property was claimed due to the presence of chlorogenic acid in green coffee bean extract (Shimoda et al., 2006).

Green coffee also exhibits anti-cancer property due to antioxidant property of chlorogenic acid content and also plausibly due to direct modulation of chlorogenic acid and polyphenol that increase the detoxification activity of phase II enzyme while inhibiting the activity of the enzyme that activates carcinogen (Kozuma et al., 2005). Though the position of coffee in functional food groups is debatable, there are many reports of beneficial effects of drinking moderate amounts of coffee. Coffee consumption, in general, improves mood and performance of the brain. Coffee consumption is proven to have potent hepatoprotective effects on the consumer by lowering the incidence of liver cirhossis and reversing the ill effects of alcohol on hepatic tissue. Coffee drinking is also associated with lower risks of Parkinson’s and Alzheimer’s diseases (Ebtihal and Khojah. 2016).

Overweight and obesity have become a serious health concern. Different weight management strategies are presently utilized and a variety of weight loss supplements sold as “slimming aids” are readily available. However, the efficacy of some of these food supplements remains uncertain. One of the common traditional forms of coffee is green coffee extract that prepared from green or raw (unroasted) coffee been. It is also present in roasted coffee, but much of the green coffee extraction is destroyed during the roasting process .Green coffee extraction has been introduced as the richest sources of cholorogenic acid and most of weight losing effects of green coffee extraction has proposed to be related to its cholorogenic acid content (Higdon and Frei, 2006).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Description of Sample Collection Site

Oromia region is one of the areas where the coffee can be cultivated. The Oromia region West Guji Zone is located in the south part of Ethiopia. Oromia region West Guji Zone has 11 Districts. The possible efforts would be made to collect the representative samples of coffee from Abaya District of the West Guji zone.

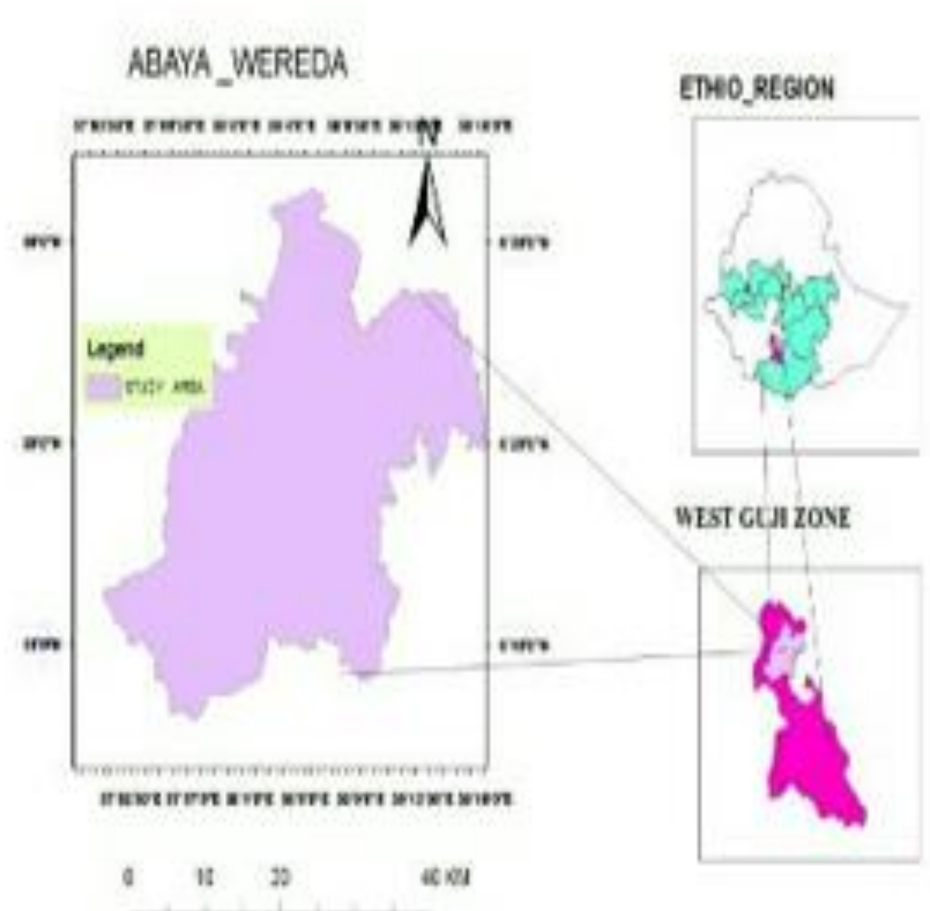

Figure 1: Location map of the Abaya wereda

Two different coffee samples were collect from six (Bochessa, Shara, Foge, Woxemba, Bukisa and Dabaka) Keble of Oromia region West Guji Zone Abaya District, Ethiopia. As indicated in figure 2 and figure 3

Figure 2 Garden coffee farm samples

Figure 3 Modern coffee farm samples

3.2 Chemical and Reagent

The Chemicals and reagents used for digestion nitric acid (HNO_3) (70%), perchloric acid, ($HClO_4$) (70%), raw grounded coffee, distilled water. All chemicals are analytical grade and were used without further purification (Suseelaet al.,2003).

3.3 Instruments and Apparatus

Mortar and Pestle, electrical blending device, hot plat digester, rounded bottle flask (100ml), volumetric flask (50ml), funnels, measuring cylinders, whatman filter paper, ICP-OES (Inductive coupled plasma-optical spectrometry, Optima 8000) for determination of metals concentration in Garden and Modern farm coffee were used. All beakers, volumetric flasks, measuring cylinder, funnel used were washed with tap water using detergent. The apparatus were rinsed with distilled water and dried before use. The apparatus were also washed with distilled water before the next analysis.

3.4 Procedure

3.4.1 Sample Preparation

The coffee bean sample were collected from both farms, are dried in the room to control the decomposition of metals in the sun light. The coffee bean were separated from the ripe coffee cherries by mortar and pestle and about 100g of each coffee sample were ground in to fine powder using electrical blending device and kept in plastic ready for digestion.

3.4.2 Digestion of Coffee Sample

Exactly 0.5 gm powders of each raw coffee sample were placed in 100 ml round bottom flask and 5ml of concentrated HNO_3 (70%) and 5ml of concentrated $HClO_4$ (70%) was added (Suseela*et al.*, 2003). The sample were heated for 30 min at 80 oC and then the temperature was increased up to 120 oC after the sample were boiled for at least 2:30 hrs until a clear solution was obtained. Concentrated HNO_3 and $HClO_4$ were added to the sample (10 ml would add at least three times) and the digestion occurred until the volume reduced. After cooling, the digested solution were filtered with Whatman filter paper in to 50 ml volumetric flask and diluted with distilled water up to the mark.

3.4.3 Digestion of the Blank Sample

Estimation of the metal concentration of the blank is important for the determination of the detection limit of the analytical method used during the study (Fong et al., 2006). Thus, one reagent blanks were digested for each sample following the same procedure used for the coffee powder.

3.4.4 Digestion of Coffee Samples Spiked With Standard Metal Solution

A spiking experiment was due to evaluate the efficiency of the procedure used. Known amounts of standard metal solutions were added to the coffee samples taking care of the dilution of the final solution. Aliquots of 0.05 mL (0.2 ppm) Na and Si were taken from 100 ppm of standard solution and spiked at once into a 0.5 gm of coffee powder samples in one digestion flask and 1.75 mL (0.7 ppm) Cu, Ni and Co also taken from 10 ppm of standard solution in same digestion flask. The digested spiked samples were analyzed for their respective metals contents using ICP OES.

3.4.5 Method detection limit (MDL)

Method detection limit (MDL) is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, but it may not necessarily be quantified as an exact value. The method detection limits was calculate by multiplying the standard deviation of the reagent blank by three (Butcher and Senddon, 1998).

CHAPTER 4

4. RESULTS AND DISCUSSION

4. 1. Analysis of Coffee Samples for Metal Level

The samples were analyzed using ICP-OES (Optima 8000) for determination of the Si, Na, Cu, Ni and Co at their corresponding intensity and the concentrations of these metals in the bean coffee samples were obtained in mg/l after calibration was carried out using standard metal solutions. The standards were prepared from the intermediate metal solution (mg/L) prepared from the stock solution (1000 mg/L) of each metal. The correlation coefficient (R^2) of each element was determined by plotting calibration curves intensity versus concentration of prepared standards. The linear correlation coefficients obtained were within the range of 0.990 - 0.993.

The calibration plots of metals analysed are shown in Figure 4.

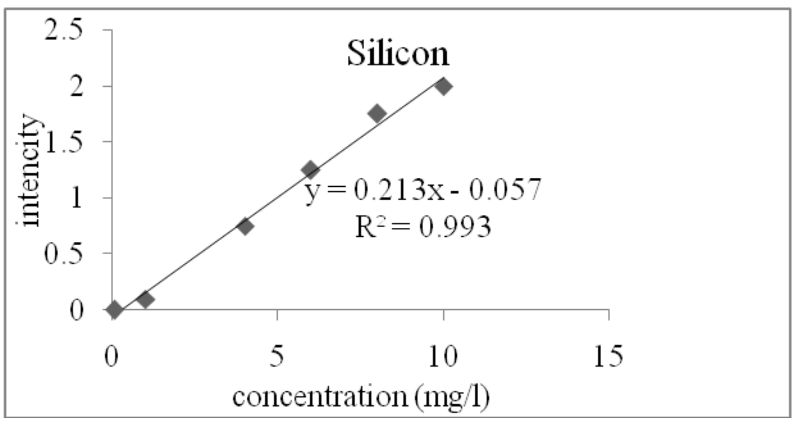

Figure 4(a): Silicon calibration curve

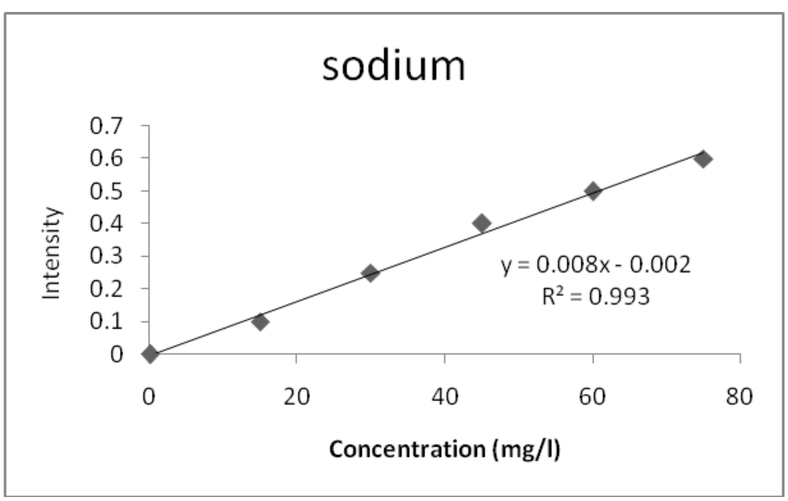

Figure 4(b): Sodium calibration curve

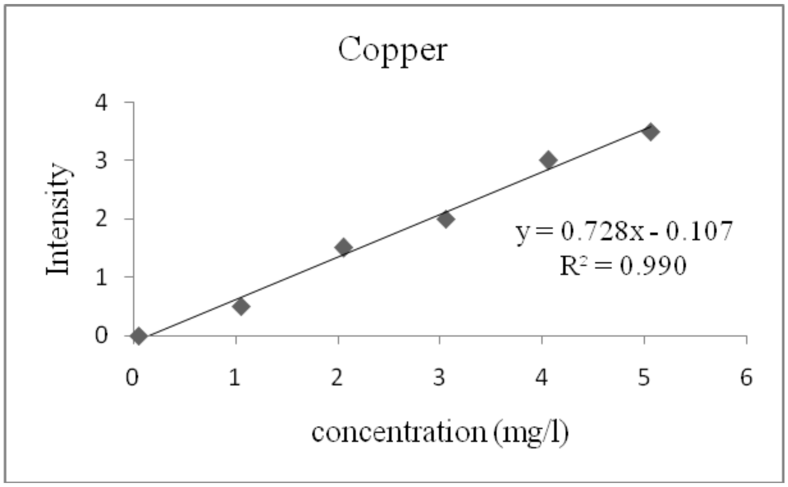

Figure 4(c): copper calibration curve

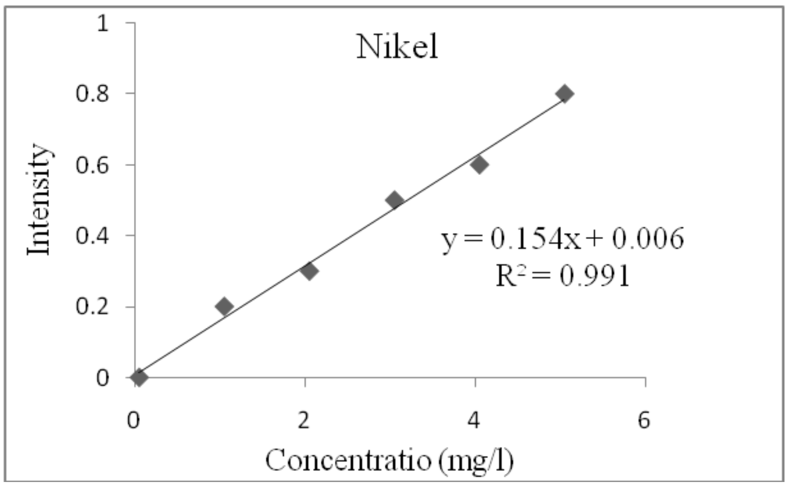

Figure 4(d): Nickel calibration curve

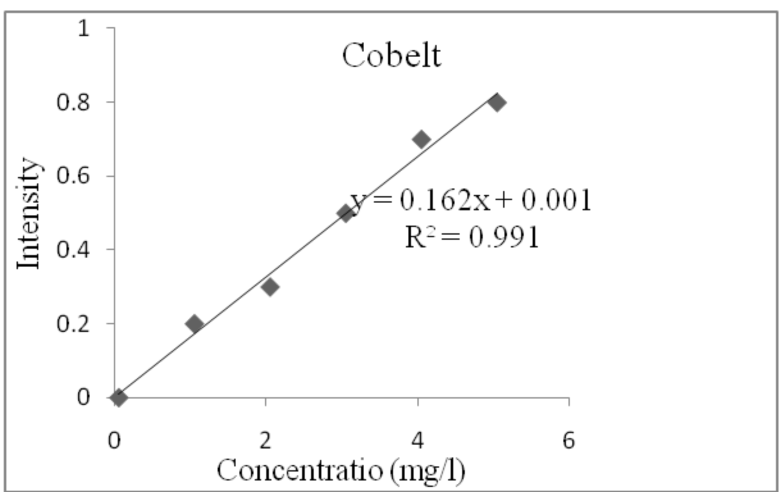

Figure 4(e): Cobalt calibration curve

Figure 4: Calibration curves (Plots of intensity versus metal concentration)

Y = Mean intensity ICP-OES C = Concentration in mg/L

Table 1: Concentration of working sample (mg/L), correlation coefficients of the calibration curves and Equation of the calibration curve.

Metals	Concentration of working sample (mg/L)	Correlation coefficient of calibration curves	Equation for correlation of calibration curve
Si	0.05, 1, 4, 6, 8, 10	0.993	Y=0.213X-0.057
Na	0.1, 15, 30, 45, 60, 75	0.993	Y=0.008X-0.002
Cu	0.05,1.05,2.05,3.05,4.05,5.05	0.990	Y=0.728X-0.107
Ni	0.05,1.05,2.05,3.05,4.05,5.05	0.991	Y=0.154X+0.006
Co	0.05,1.05,2.05,3.05,4.05,5.05	0.991	Y=0.162X+0.001

The result showed in Table 1 that the values of correlation coefficient ranged between 0.990 for Cu and 0.993 for both Si and Na. The correlation coefficients of all calibration curves were >0.990 and these correlation coefficients showed that there was a good correlation between concentration and intensity.

4.2. Method Detection Limit

Method detection limit (MDL) is defined as the amount of analyte that gives a signal equal to three times the standard deviation of the blank. The standard deviation for each element was calculated from the blank measurements to determine method detection limit.

Table 2: Method of detection limit of elements ($nx\delta_{blank}$, n=3 δ,=standard deviation) determined using the blank solution.

Metals	Si	Na	Cu	Ni	Co
MDL (mg/l)	0.04	0.44	0.001	0.001	0.001

From the above table it is possible to conclude that the MDL of elemental value ranged between 0.001 mg/L for (Si) and 0.44 mg/L for Na. The low MDL values clearly demonstrate that there is the high sensitivity and linear range of ICP-OES method for elemental analysis of sample (Mitra et.al, 2003)

4.3 Recovery Test

Method validation is the process of providing that analytical method is acceptable for its intended purpose. The percentage recovery was calculated by using the following formula given below and the value is shown in Table 3.

% Recovery = C (Spiked) – C (non-Spiked)/C (added) × 100

Where: C (Spiked) is metal content of the spiked sample concentration, C (non-Spiked) metal content of non-spiked sample concentration and C (added) is metal content of metal added concentration.

Table 3: Recovery test results of metals for the analysis of coffee samples.

Metals	Concentration in the sample (mg/l)	Amount added (mg/l)	Concentration in spiked sample (mg/l)	Recovery (%)
Si	ND	0.2	ND	--
Na	12.226	0.2	12.41	92
Cu	0.181	0.7	0.774	85
Ni	0.238	0.7	1.004	109
Co	0.008	0.7	0.800	113

From Table 3 the results of these recovery tests were between 85% and 113%. These results revealed that all the percent recovery values were within the acceptable range of metal analysis (85 –120%) (Chauhan, A., Mittu, B. and Chauhan, et.al.2015) and the method employed for metal analysis was valid.

4.4 Concentration of Metals in Coffee Samples

The concentration of five metals in the Abaya District coffee sample were determined by using ICP-OES. The result showed that the metal content of Garden coffee and Modern farm coffee of the five elements were determined having variation concentration in two cultivation techniques. Therefore, the concentrations of Modern coffee farm metals are higher than that of the Garden farm coffees. But Si was not detected in the two selected method of coffee farms as it was found below the detection limit i.e. <0.0003 of inductive couple plasma of emission spectrometry (ICP-OES). The concentrations of the metals in the two coffee samples are summarized in Table 4.

Table 4: concentration of metals in Garden and Modern coffee farms samples

Trial	Concentration of metals (mg/L) in Garden coffee farms					Concentration of metals (mg/L) in Modern coffee farms				
	Si	Na	Cu	Ni	Co	Si	Na	Cu	Ni	Co
Trial 1	ND	189.66	4.72	0.44	0.59	ND	188.29	7.64	1.88	1.00
Trial 2	ND	194.00	4.93	0.40	0.62	ND	195.96	8.67	1.69	0.96
Trial 3	ND	193.04	4.95	0.47	0.67	ND	195.34	8.68	1.72	1.02
Average	ND	192.23	4.86	0.44	0.63	ND	193.20	8.33	1.76	0.99
Standard deviation	--	2.28	0.13	0.03	0.04	--	4.26	0.60	0.10	0.03

From the above table it was concluded that the average concentration of metals in Garden coffee farms was in the range of 0.44mg/L concentration of Ni which is the lowest value to 192.23mg/L concentration of sodium which is the highest value among the specified metals. In the same ways the average concentration of metals in Modern farms coffee was 0.99mg/L for Co, which the lowest value, 1.76 mg/L for Ni and 8.33mg/L for Cu which is the medium value and 193.20mg/L for Na which is the highest value among the specified metals in this study.

4.4.1 Concentration of Metals in Garden Farm Coffee Samples

The coffee sample collected from Garden coffee farms have contains Na in the highest amount with concentration (192.23, 2.28 mg/l) followed by Cu (4.86, 0.13 mg/l), Co (0.63, 0.04 mg/l) and Ni (0.44, 0.03 mg/l) average and standard deviation respectively. Whereas Si was found to be below detection limits their relation shown in the Figure 5 below.

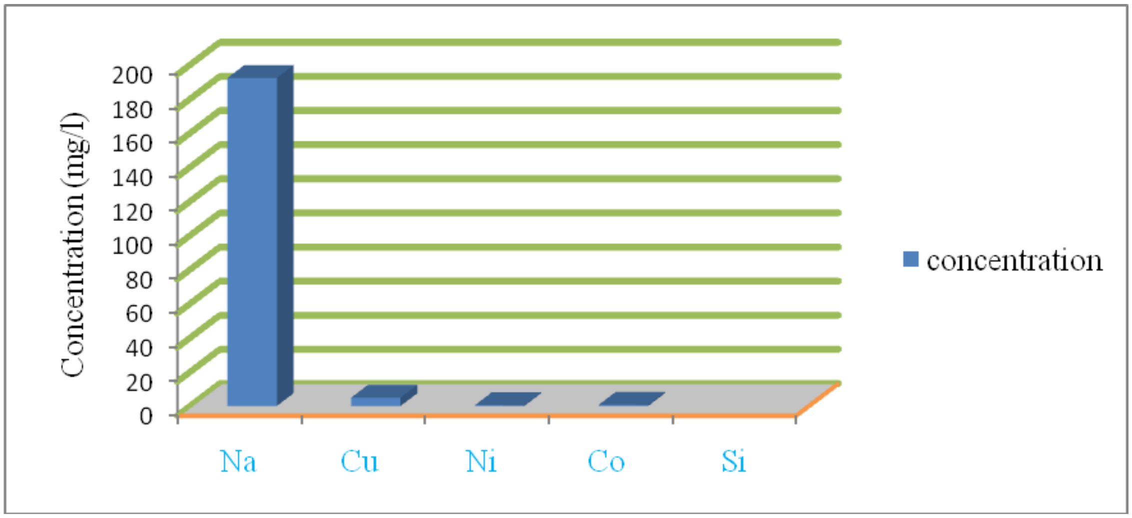

Figure 5: Garden coffee farm concentrations

From figure 4 it was conclude that the concentration of metals are high for sodium and very low for silicon which is under detection limit.

4.4.2 Concentration of Metals in Modern Farm Coffee Samples

Modern coffee also rich in its contents of Na (193.20, 4.26 mg/l) followed by Cu (8.33, 0.60 mg/l), Ni (1.76, 0.10 mg/l), and Co (0.99±0.03 mg/kg). Si was not detected as it was found below the detection limit,

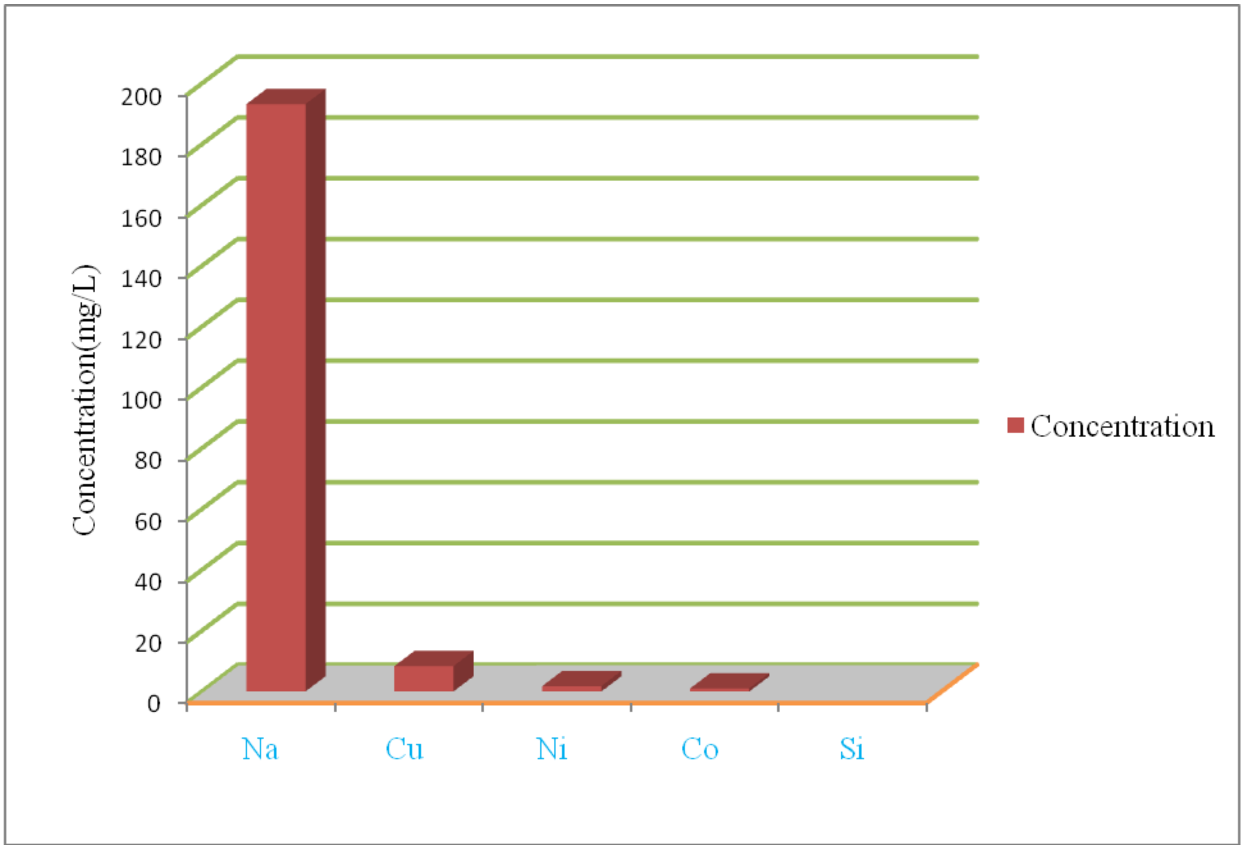

Figure 6: Modern coffee Farm concentrations

From figure 6 it indicates that the concentration level of metals are vary because of their solubility of metals in the soil and absorbed by routs of the coffee plant then transported to the coffee bean to found in high concentration level.

4.5 Comparison of Concentration of Metals in Two Different Farm Coffee Samples

The average concentrations of metals in the two coffee farms were summarized in next Figure.

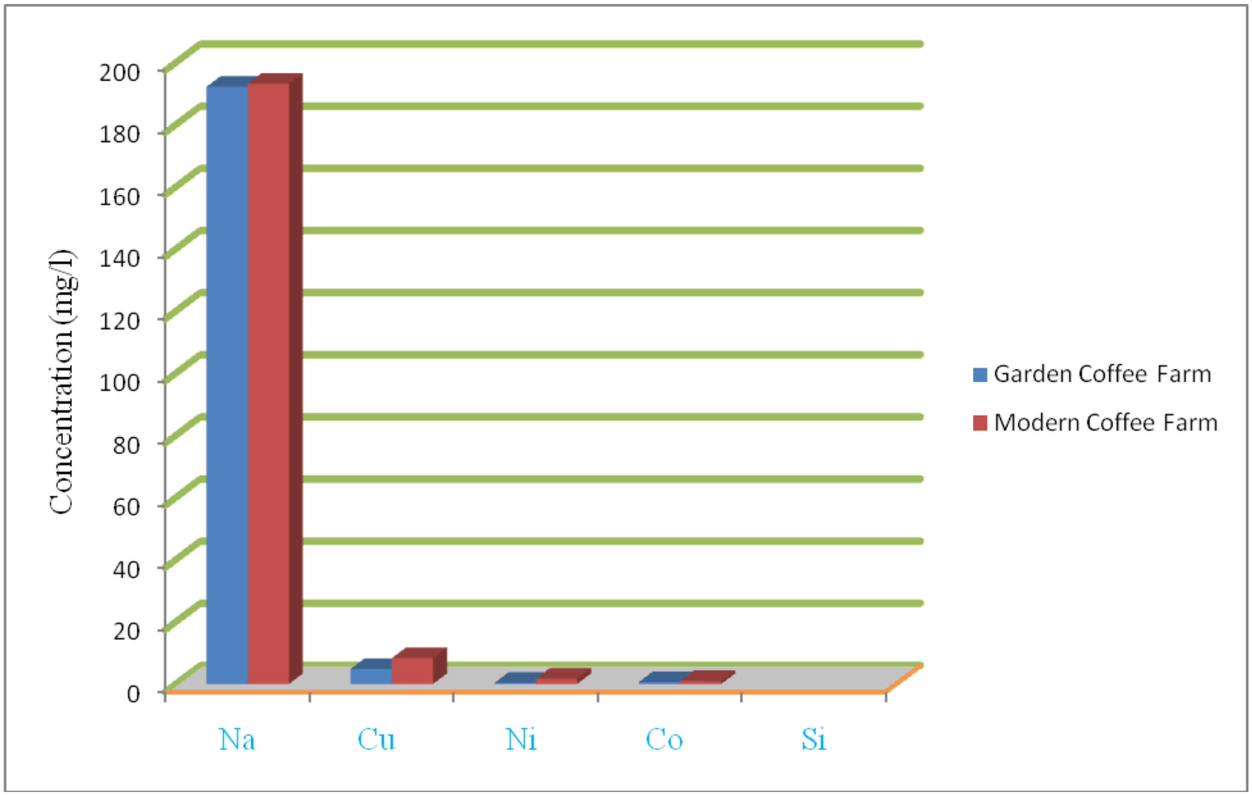

Figure 7: comparison of Modern and Garden farm coffee sample concentration.

From figure 7 it indicates that the concentration of each metal is varies with the agricultural techniques as follows:

Na: Modern farm coffee >Garden farm coffee

Cu: Modern farm coffee >Garden farm coffee

Ni: Modern farm coffee >Garden farm coffee

Co: Modern farm coffee >Garden farm coffee

Si: undetermined

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Coffee plays a significant role in human nutrition even though Effects of coffee on human health are still under strong debate. In this study the level of Si, Na, Cu, Ni and Co in coffee samples collected from Oromia region West Guji Zone Abaya District were determined by ICP-OES. The digestion method used for metals analysis in coffee sample was found to be efficient and it was validated through recovery experiment and good percentage recovery was achieved in the range of (85-113%) except Si. The levels of metal in coffee samples determined in this study were found in the following order: Na (192.23 and 193.20 mg/l), Cu (4.86 and 8.33 mg/l), Ni (0.44 and 1.76 mg/l) and Co (0.63 and 0.99 mg/l) from Garden coffee and Modern coffee farm respectively. Si found to be below the detection limits. Generally coffee bean sample contain higher amount of Na followed by Cu, Ni and Co in selected Abaya District. From the present investigation shows that variation of elemental concentration of coffee subspecies which is collected from the same soil composition and from the same environment but different agricultural practices. There have been suggestions that certain minerals predispose to higher quality and others to lower quality due to ability to uptake mineral from the soil through their roots and transport them to coffee bean. Finally the investigation of the present study concluded that from the analysis in various coffee bean subspecies constituents it showed that coffee bean is a good source of minerals. For further studies it could be suggested that coffee beans under investigation could be a source of dietary minerals and complementing food composition.

5.2 RECOMMENDATION

The concentration level of Silicon is under the detection limit of ICP-OES which is about less than 0.003. This is due to less solubility of silicon in the soil to absorbed by routs of the coffee trees and transported to the bean coffee fruits. The recovery of silicon was an expectedly high and hence, we recommend applying high sensitive equipment like GFAAS to detect Si in these coffee samples.

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