LEVELS OF SELECTED ESSENTIAL AND NONESSENTIAL METALS IN ROASTED HARARGHE COFFEE BEAN VARIETIES

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I hereby certify that I have read and evaluated this Thesis entitled **Levels of Selected Essential and Nonessential Metals in Roasted Hararghe Coffee Bean Varieties** Prepared under my guidance by Dugassa Ayana. I recommend that it be submitted as fulfilling the thesis requirement.

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As a member of the Board of Examiners of the MSc Thesis Open Defense Examination, I certify that I have read and evaluated the Thesis prepared by Dugassa Ayana and examined the candidate. I recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Chemistry.

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DEDICATION

This thesis manuscript is dedicated to my dear wife Diribe Debelo and my children Milkessa, Naol and Ebisse Dugassa whose understanding and support have truly made this Thesis possible.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and completion of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

The author, Mr. Dugassa Ayana, was born from his father Ayana Nikus and his mother Shashina Tufa in East Welega Zone, Oromiya Regional State, in 1976. He attended his primary school (grade 1-6) at Kekero Primary School. He attended his junior secondary school (grade 7-8) at Hinde Primary and Junior Secondary School. Then senior secondary school was (grade 9-12) at Gida Ayana Senior Secondary School.

After completing the Ethiopian Schools Leaving Certificate Examination (ESLCE) in 1991, he attended teachers training institute (TTI) for primary school at Assela in 1994/95, following his graduation he was employed by the Ministry of Education as primary school teacher in West Hararghe Zone Chiro district in 1995. After serving for four years as primary school teacher, he attended diploma in Chemistry at Adama Teachers training College (TTC) from 2000-2004 under the summer in service program. Then he attended his BEd degree at Haramaya University from 2005 - 2008 under the summer in service program. After serving for five years at Chiro Number 1 School as chemistry teacher, he joined Haramaya University for his Postgraduate Studies in July 2013.

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ACRONYMS AND ABBREVIATIONS

ECX	Ethiopian Commodity Exchange
FAAS	Flame Atomic Absorption Spectrometry
FRAP	Ferric Reducing Antioxidant Power
HCB	Hararghe Coffee Bean
HR-CS FAAS	High resolution Continuum Source Flame Atomic Absorption Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
INAA	Instrumental Neutron Activation Analysis
MDL	Method Detection Limit
MQL	Method Quantification Limit
RDI	Recommended Daily Intake
SD	Standard Deviation

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LEVELS OF SELECTED ESSENTIAL AND NONESSENTIAL METALS IN ROASTED HARARGHE COFFEE BEAN VARIETIES

ABSTRACT

The present study was conducted to determine the levels of essential and non-essential metals present in Hararghe coffee bean varieties. Three samples of Hararghe coffee bean (HCB) (Harar A, Harar B and Harar C) were obtained from the Ethiopian commodity exchange (ECX) Dire Dawa branch. The determination of the levels of selected metals in roasted coffee bean (K, Mg, Ca, Mn, Zn, Cd, and Pb) was conducted using Flame Atomic Absorption Spectrophotometer (FAAS). The digestion procedure was optimized and it was validated using spiking experiments. Recoveries of the spiked samples varied from 94% to 112% for the roasted coffee samples. Then the digestion process was carried out using an open vessel system with 3 mL HNO_3 (70 %), 1 mL $HClO_4$ (70%), 1 mL H_2SO_4 (98%) and 1 mL H_2O_2 (30 %) reagents. Generally, the levels of metals in all roasted coffee bean varieties were: K > Mg > Ca > Zn > Cd, but the metals Mn and Pb were found to be below the method detection limit. The concentrations of metals in Hararghe coffee bean varieties were comparable to the values reported in the literature. The mean concentration of each metal in the three coffee samples of Hararghe coffee bean varieties were: K (13.6669 – 15.7859 mg/kg), Mg (7.1473 – 8.3676 mg/kg), Ca (3.6691 – 5.569 mg/kg) and Zn (0.3897 – 2.3367 mg/kg). The toxic metal Cd was not detected in Harar A and B coffee varieties while a concentration of (0.2282 ± 0.02 mg/kg) was found in Harar C coffee variety. However, Mn and Pb were not detected in all coffee samples. The concentrations of the metals were in the recommended maximum permissible limits indicating no risk of health by drinking the coffee made from Hararghe coffee bean with respect to the metals considered in the present study.

Keywords: Coffee, Essential metals, Non-essential metals, Risk of health, Roasted coffee bean, Toxic metal.

1. INTRODUCTION

1.1. Background of the Study

Coffee is one of the most consumed beverages worldwide and ranked as the second most traded global commodities after petroleum and is the second most popular drink after water in the world (Pohl *et al*., 2013; Romano *et al*., 2014). The term ‘‘green coffee” refers to raw or unroasted beans of *Coffea* fruits; the coffee that we know is produced by processing the green coffee beans in several stages (Valentin and Watling, 2013; Farah and Santos, 2015). Coffee culture greatly influences world trade. Coffee is consumed mostly for its sensory characteristics, besides various other social factors (Carvalho *et al*., 2016). The annual world coffee consumption exceeds 5 billion kilograms, which corresponds to 500 billion cups. In the year 2015, Brazil ranks first in the world as a coffee producer and exporter while Ethiopia is the 5^{th} world coffee producer (ICO, 2015).

The genus *Coffea* contains more than 100 species, only two of which, *Coffea Arabica* (known as Arabica coffee) and *Coffea canephora* (known as Robusta coffee) are commercially cultivated. Coffee Arabica produces high quality coffee compared to Robusta, and contributes about 75% of the total world coffee production, being sold 2-3 times higher prices than the Robusta. Coffee Arabica is originated from Ethiopia, where it grows in very large stands on the highland plateaus (1300 m – 1900 m). It is an evergreen plant, having about 8 to 10 m tall. The size and the shape of beans of this type coffee differ depending upon the varieties, environmental conditions and cropping practices (Abera, 2006). Biniyam *et al* (2013) Classified Hararghe coffee bean (HCB) in to four predefined categories (Harar A, Harar B, Harar C and Harar D) depending on three major features i.e. color, texture, shape, and a combination of these features. Coffee is an economically important crop, which is contributing the highest of all export revenues in Ethiopia and has become a brand item in the country. For quite a long time, it was the dominant foreign currency earner and still its share is big enough to generate 60 % of the total earnings (Abera *et al*., 2017).

It is also the major cash crop of the country and farmers of the coffee producing regions. Ethiopia is endowed with immense diversity of coffee quality types and contrasting ecological

conditions to produce high quality coffees for sale at an exceptionally higher price. Ethiopia has diverse and favourable environments in five major coffee growing regions for the production of Arabica coffee and coffee types with unique flavour and taste, variable specialty distinguished as Sidamo, Yirgachefe, Hararghe, Gimbi and Limu types (Abera, 2006). These specialty coffees have good opportunities in international market to fetch premium prices as far as their distinct, unique aroma and flavour are maintained and improved. Hararghe coffee has a unique quality that fetches premium prices in the world market. The first step to improve the crop is to understand the exiting variability in the genotype /accession. Coffee is the single most important cash crop of Ethiopia, whose entire branches of industries and services is linked with transportation, trade, processing and exportation of this commodity. In the year 2010, an estimated 1.2 million Ethiopians depend directly and approximately 15 million indirectly on coffee for their livelihoods, which stand for about 20 percent of the country's total population (EEA, 2010).

For the purpose of fair local and international trade, in addition to the geographic and botanical origin of coffee it is very crucial to identify the concentration of essential and nonessential or toxic metals. Therefore, identifying, the concentration of essential and nonessential metals in addition to the geographic and /or botanic origin of coffee supports the claims to prevent any deliberate or accidental substitution of a product of reputed origin by a cheaper coffee originating from another country or region. However, to exploit this good opportunity the distinct unique shape, color, size and metal contents of the Hararghe coffee need to be identified and improved.

Green coffee has total elemental content of approximately 5% (m/m), including essential and non-essential or toxic ones. These contents depend mainly on growing region of the coffee which is the factor primarily associated with soil type, coffee variety, field practices, climate and processing (Pohl *et al*., 2013; Debastiani *et al*., 2014). Minerals are more stable than organic compounds and reflect the soil type and growing conditions (Oliveira *et al*., 2015).

A lot of work has been done and reported in the literature on levels of selected essential and nonessential metals determination in roasted coffee beans of different parts of the world. These include determination of essentials and toxic metals in raw and roasted coffee in Bule

Hora Woreda, Borena Zone, Ethiopia (Tewodros and Nigus, 2014); levels of selected essential and nonessential metals in roasted coffee beans of Yirgacheffe and Sidama, Ethiopia (Tesfay et al., 2015); concentration levels of metals in commercially available Ethiopian roasted coffee powders and their infusions (Abyssinia, Alem and Pride) (Ashu and Chandravanshi, 2011), and determination of heavy metals in the roasted and ground coffee beans and brew, Cerrado Mineiro region (Alto Paranaíba – MG, Brazil) (Sabrina *et al*., 2017). However, there is no reported work in the literature on the levels of selected essential and nonessential metals in roasted Hararghe coffee bean varieties.

The concentration of various elements in green and roasted Coffee bean can be determined using different analytical techniques such as flame atomic absorption spectrometry (FAAS), high resolution continuum source (HR-CS FAAS), and solid sampling electro-thermal (SS-ET AAS) (Amorim *et al*., 2007; Stelmach *et al*., 2015), particle induced X-ray emission (PIXE) (Debastiani *et al*., 2014), neutron activation analysis (INAA), inductively coupled plasma-optical emission spectrometry (ICP-OES) (Martín *et al.*, 1998; Oleszczuk *et al*., 2007; Valentin and Watling, 2013) and inductively coupled plasma mass spectrometry (ICP-MS) (Rodrigues *et al*., 2011; Santato *et al*., 2012; Valentin and Watling, 2013). Therefore, in the present study the levels of selected essential and nonessential metals (K, Mg, Ca, Mn, Zn, Cd and Pb) in roasted Hararghe coffee bean varieties were determined by FAAS. As reported in the literature, FAAS is found to be efficient for the determination of selected metals in roasted and ground coffee samples.

1.2. Objectives of the Study

1.2.1. General Objective

The main objective of this research is to determine the level of selected essential and nonessential metals (K, Mg, Ca, Mn, Zn, Cd and Pb) in roasted Hararghe coffee bean varieties, Hararghe, Ethiopia, using FAAS.

1.2.2. Specific Objectives

- Optimization of the analytical procedure for the digestion of the coffee samples.
- To validate the analytical procedure for the digestion of the coffee samples.
- To determine the level of selected metals: five essential (K, Mg, Ca, Mn, and Zn) and two nonessential (toxic) metals (Cd and Pb) using FAAS in samples of different coffee bean varieties of Hararghe, collected from the Ethiopian Commodity Exchange Center (ECX), Dire Dawa branch, Dire Dawa, Ethiopia.

2. LITERATURE REVIEW

2.1. Coffee

There are over 120 species of coffee (genus Coffea). However, the only two species of coffee have economic importance: Arabica coffee (Coffea arabica) and Robusta coffee (Coffea canephora). Ethiopia is the center of origin and diversity of Arabica coffee. Arabica coffee is the most widely consumed, dominating over 70 % in volume of production and over 90% of traded value globally. More than 80 developing countries mainly earn their foreign currency from coffee. Coffee is an economically important crop, which is contributing the highest of all export revenues in Ethiopia.

As the country of origin for the crop, Ethiopia produces premium quality coffee. Ethiopia is the leading producer in Africa, and the 5th in the world, following Brazil, Vietnam, Colombia and Indonesia. If we consider Arabica alone, Ethiopia is the 3rd largest producer after Brazil and Colombia (ICO, 2013). Ethiopia also has large highland area suitable for Arabica coffee production, and hence has the potential to be a leading producer in both quality and quantity.

Coffee is a key crop in generating rural income, providing significant employment and earning foreign exchange revenues. Uniquely, Ethiopia and coffee are synonym as coffee provides a livelihood for 15 millions of Ethiopians (EEA, 2010). Currently, about 800,000 hectares of land is covered with an annual production capacity of 500,000 tons. Coffee farms account for 25 percent of the workforce in the country. It is a nation where coffee has been indigenous cultural traditions for centuries and 50 percent of its production consumed locally.

The brew from this well sought for cash crop is readily consumed due to its good sensory qualities owing to the presence of many micronutrients. Some of these chemical compounds possess biological activities, including anti-proliferative, antioxidant, and antimicrobial effects. Four representative groups of these micronutrients, namely, caffeine, chlorogenic acid, diterpenes, and trigonelline, play key roles in these bioactive effects of coffee. In order to guarantee the quality of coffee products and to protect consumer interest and safeguard their wellbeing, it is extremely important to employ sensitive and accurate analytical methods in the characterization and quantitative determination of these bioactive constituents.

2.2. Coffee Processing

The term ‘‘green Coffee” refers to raw or unroasted beans of Coffea fruits; the Coffee that we know is produced by processing the green coffee beans in several stages. In this production process, the green coffee beans are firstly cleaned and dried, then seeds are roasted, grounded, and brewed, and then just getting ready for drinking (Valentin and Watling, 2013; Farah and Santos, 2015). The aroma of green coffee seeds is quite different from what we imagine when we hear the word coffee. It is only through roasting that the seeds gain the characteristic aroma and flavor of coffee. Although roasting appears to be simple in terms of processing conditions, the chemistry underlying this flavor development is highly complex and not completely understood. The high roasting temperatures cause a series of physical and chemical changes in the seeds. The specific roasting conditions strongly influence these changes and consequently affect the bioactivity and flavor of the beverage. Chemical changes in this initial phase are relatively small compared to those that occur at the end of the roasting process. At temperatures higher than 160 ℃, a series of exothermic and endothermic reactions take place; the seeds become light brown, their volume increases considerably, and aroma formation begins. The chemical reactions responsible for the aroma and flavor of roasted coffee are triggered at approximately 190 ℃ .

The caffeine content in the coffee drink also vary according to the used brewing procedure, being observed a considerable increase on caffeine yields when higher amounts of coffee grounds and volumes of coffee prepared are used. Depending on the length of coffee boiling time, similar or higher caffeine contents can be found when comparing with filtered coffee. The water quality plays also a crucial role in coffee brewing, being considered as the second most important ingredient for coffee brewing. Water with an altered composition, such as some mineral spring waters, excessively hard water, and chlorinated water, might reduce the quality of the coffee brews (Belitz *et al*., 2009). Besides water, the pH of brewed coffee is another factor with great influence on the flavor characteristics of the coffee beverage.

In addition, roasting parameters such as the amount of coffee in the roaster, temperature, roasting time, and speed of hot air circulation (in the case of fluid and spouted bed roasters) used to reach a single roasting degree can vary considerably. The speed at which the seed

reaches the desired color affects a number of physical and chemical parameters and therefore the flavor and bioactivity of the beverage. The most common brewing methods worldwide are simple percolation, boiled coffee, electric coffee maker, espresso machine, Italian coffee maker, and French press (Toci *et al*., 2009). Arabica coffee provides superior cup quality and aroma compared with Robusta, which commonly possesses a more aggressive flavor and, in light roast coffee, has a flat popcorn-like aroma. For most consumers, some Arabica seeds are needed for a blend to seem like coffee. As a result, the value of Robusta seeds is approximately half that of Arabica. Low to moderate caffeine intake is generally associated with increased alertness, learning capacity, exercise performance, and perhaps better mood, but high doses can produce negative effects in some sensitive individuals (e.g., anxiety, tachycardia, and insomnia) during its halflife, which is 2–6 hours after coffee intake (Ogita *et al*., 2003; Toci *et al*., 2006; Farah *et al*., 2006).

Figure 1. Caffeine and other organic contents of coffee brew

2.3. Mineral Composition of Raw and Roasted Coffee

Heavy metals are widely dispersed in the environment. They enter the food chain and occur in varying concentrations in human food (Roychowdhury *et al.,* 2003). The contamination of food is a serious problem as heavy metals are taken up from the digestive tract and exhibit harmful influence on many tissues. Some metals exhibit toxic properties in relatively low doses and, moreover, they gradually accumulate in tissues (Beckett *et al.,* 2007). Metals disturb the ionic balance and mineral regulation, induce oxidative damage to cell structures, produce injury to DNA and induce cancer transformations (Wallkers, 2003).

Samples of laboratory roasted and ground coffee beans, natural powdered, or soluble coffees (0.1–5.0 g) are commonly wet digested in pressurized closed vessel microwave assisted

systems (Santos *et al*., 2008; Castro *et al.*, 2009; Santos *et al*., 2010), a Berghof apparatus and open vessel systems with hot plates or heating blocks (Ribeiro *et al.*, 2003 and Filho *et al*., 2007). In case of hot plates and heating blocks applied for open vessel systems, samples are heated at much higher temperatures (≈180 ℃) for 1–4 h (Anderson and Smith, 2002; Filho *et al*., 2007). In open vessel system the predigestion can be used to avoid a rapid decomposition of samples (Ribeiro *et al.,* 2003; Oleszczuk *et al*., 2007). Residues after the digestion are diluted with water to 50 mL (Santos *et al*., 2009; Ashu and Chandravanshi, 2011).

The chemical composition of coffee is very complex and depends on the place of origin and species /cultivar of the coffee plant. The technology used in the preparation and industrial processing of green beans, as well as the methods consumers use to prepare their coffee modify the concentrations of the substances in the final product. Additionally, potential contamination may derive from package and storage. The chemical composition of coffee is so complex that it is not possible at present to provide a complete list of all constituents. Heavy metals are the most evaluated elements in food or any other product due to their ability to accumulate in the food chain.

The maximum levels to which they are present therefore becomes the standard of quality across the world. As these elements are stable, they remain in the environment, accumulating in the soil due to the weathering process of rocks and soil formation, environmental conditions, technological practices and/or chemical usage (Ashu and Chandravanshi, 2011). Some metals are biologically crucial in low concentrations for living organisms, including copper (Cu), chromium (Cr), cobalt (Co), manganese (Mn), nickel (Ni) and zinc (Zn); however, because elements such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), titanium (Ti) and uranium (U) are not essential and exert harmful effects on different parts of the biosphere, they are termed toxic metals.

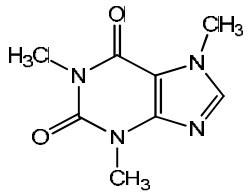
The Coffee plants can absorb these metals and store them in the roots or transport them into the shoots and grains. The heavy metals vary in concentration in the different plant tissues, and normally, the grains contain lesser concentrations than the vegetative plant parts (Bettiol and Camargo, 2006). On reaching the coffee beans, these metals form the vehicles of contamination for humans inducing adverse health effects like severely decreased neurological

and hepatic functions, as well as mutagenesis and carcinogenesis (Matés *et al.,* 2010). The levels of Mn, Co, Ni, Cr, and Ag were shown to be too low to influence health. Lead was estimated to account for 0.9% of the total global disease burden, due to lead-induced mental retardation and on consequences of increased blood pressure. Coffee would therefore, be expected to add to this disease burden. However, coffee drinking would not contribute to levels of Pb which are considered to cause lead poisoning.

Table 1. Main mineral component in raw and roasted coffee (Abera, 2006)

g %/100 g on dry mass	Coffee Arabica		Coffee Robusta	
	Green (Raw)	Roasted	Green (Raw)	Roasted
Caffeine	0.9 – 1.2	1.0	1.5 – 2.4	2.0
Minerals	3.0 – 4.2	3.5 – 4.5	4.0 – 4.5	4.6 – 5.0
Proteins	11.0 – 13	13.0 – 15.0	11.0 – 13.0	13.0 – 15.0
Fat	12.0 – 18.0	14.5 – 20.0	9.0 – 13.0	11.0 – 16.0
Oligosaccharides	6.0 – 8.0	0.0 – 3.5	5.0 –7.0	0.0 – 3.5
Water	10.0 – 13.0	1.0 – 5.0	10.0 – 13.0	1.0 – 5.0

The chemical composition of coffee varies according to species (Arabica or robusta), country origin (Ethiopia, Brazil, Kenya, etc), system of cultivation (organic or conventional) and the way it exist (raw or roasted). Main coffee components are given in Table 1 (Abera, 2006). Caffeine ($C_8H_{10}N_4O_2$), an alkaloid that is chemically known as 1, 3, 7-trimethylxanthine (or 1, 3, 7-trimethyl- 1H-purine-2, 6(3H, 7H)-Dionne), is among the most commonly consumed stimulants worldwide and Contains two fused rings (Figure 2) that are related to pureness (Amaresh *et al*., 2011). Caffeine is found in many natural and processed products (Dor´e *et al*., 2011; Heckman *et al*., 2010).



1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
(Caffeine)

Figure 2. Chemical structure of Caffeine

The coffee infusion was prepared as a beverage using roasted and ground coffee in boiling hot water (95 to 100 °C) and filtering, in the ratio of 12 g of powder to 100 mL of water (Tesfay *et*

al., 2015). Over two-thirds of all the research literature on geographic origin commodities involves the analysis of vitamins or other organic molecules (amino acids, triglycerides, volatile aromatic compounds, etc).

2.4. Recommended Daily Intake (RDI) of Metals

Coffee seems to have distinct acute and long-term effects on health. Interestingly, its consumption has been suggested to be beneficial in dementia, Alzheimer's disease, Parkinson's disease and diabetes mellitus type 2 (Gongora, 2010; Hjellvik *et al.,* 2011). However, other researchers have associated coffee drinking with an increased risk of developing coronary heart disease estimated that consumption of more than 5 cups per day increased the risk of coronary heart disease from 40 to 60 % (Higdon and Frei, 2006; and Montagnana *et al.,* 2012). The consumption of large quantities of this beverage is not recommended for pregnant women because it increases the risk of miscarriage (Higdon and Frei, 2006). In order to observe the health risk of any pollutant, it is very important to estimate the level of exposure, by detecting the routes of exposure to the target organisms. There are several possible path-ways of exposure to humans but amongst them the food chain is the most important pathway (Aroraa *et al*., 2008). The daily intake of metals depends on both the concentration and the amount of food consumed.

The provisional tolerable weekly intake suggested by FAO/WHO is 214 μg per day for a 60 kg person or 91 mg per year for a 70 kg person. The leaching of each element present in the roasted and ground coffee samples and their infusions can differ, which makes it crucial to also assess the values of these elements in the beverage (Stelmach *et al.,* 2013). Therefore, it is important to assess the dietary exposure for risk evaluation. The common objective of the above cited article was to analyze the elements in coffee samples in order to examine the origin and the authenticity of the green coffee. Besides, inorganic elements play an important role in nutrition and influence human health in different ways (Oliveira *et al.,* 2012).

The daily dietary intake of coffee for an average Finland population is 12 kg, in Brazil it is 5.8 kg, in Norway 9.9 kg per capital per year. However, in Ethiopia the daily consumption of coffee is very low. It is considered to be 3.56 g/day which is calculated from 1.3 Kg per

capital per year. Assuming a value of 3.56 g of coffee consumption per day, the daily intake of the detected metals from roasted coffee are depicted in Table 2. The last column shows the RDI as set by different international organization (Tewodros and Nigus, 2014). As expressed in table 2 the average daily dietary intake of the detected metals from roasted coffee in Ethiopia is below RDI, this shows coffee drinking serves as supplementary source of metals in food chain.

Table 2. Recommended daily intake (RDI) of metal in coffee sample

Element in Coffee	Concentration in roasted coffee (mg /g)	Daily intake (mg /day) for roasted	RDI (mg/day)	References
Calcium	1.505	5.36	100.00	Tewodros and Nigus, 2014
Magnesium	2.017	7.18	35.00	Tewodros and Nigus, 2014
Potassium	13.922	49.59	35.00	Tewodros and Nigus, 2014
Copper	0.014	0.05	2.00	Tewodros and Nigus, 2014
Manganese	0.018	0.06	5.00	Ogabiela *et al.,* 2011

2.5. Antioxidant Capacity and Elemental Composition of Green Coffee

Food of plant origin contains many antioxidants and bioactive compounds such as phenolics, vitamins /pro-vitamins as well as major, minor and trace elements. Higher intake of antioxidant-rich products is associated with lower oxidative stress in the human body. There are many evidences that diet rich in food antioxidants especially from plants, herbs, spices and beverages shows protective effect on human health and reduces the risk of various diseases (Benzie and Choi, 2014).

Popular beverages contain multitude of antioxidants and between them coffee brew showed high antioxidant capacity in vitro and in vivo tests (Daglia *et al*., 2000; Shahidi and Chandrasekara, 2010). Antioxidant capacity of espresso coffee measured with the use of FRAP assay is even two fold higher in comparison to other beverages such as red wine or pomegranate juice. Coffee, prepared on filter or boiled has the same capacity as red wine (Carlsen *et al*., 2010). Coffee with unique aroma and flavor is one of the most popular beverages in the world. Coffee contains antioxidants, mainly the chlorogenic acids (caffeoylquinic acids). Green coffee extracts show a hypertensive effect on rats and reduces visceral fat and body weight (Suzuki *et al.,* 2002; Shimoda *et al*., 2006 and Igho *et al.,* 2011).

The other bioactive compounds with antioxidant capacity such as caffeine, theophylline and theo-bromine, tocopherols, cafestol, kahweol and trigonelline were also determined (Kuhnert *et al*., 2011; Jeszka *et al*., 2015).

Latest studies on chlorogenic acid (5-caffeoylquinic acid), the main component of green coffee beans, showed the protective role of this compound in neurons, therefore, it could prevent from neurodegenerative diseases such as ischemic stroke (Mikami and Yamazawa, 2015). Antioxidant capacity of green coffee extracts of Arabica depends on its calcium levels (Stelmach *et al.,* 2015). Other trace elements such as copper, manganese and selenium also play a significant role in oxidative metabolism; they are involved in redox processes that are essential for many metabolic pathways as well as for cellular defense against oxidative stress (Brigelius, 2006).

2.6. Coffee Growing Area in Ethiopia

Ethiopia is one of the eight regions in the world considered to have a strikingly high level of diversity in cultivated crop plants (Gole *et al*., 2011). Coffee production in Ethiopia is a long standing tradition. Ethiopia is where coffee Arabica, the coffee plant, originates (Abera, 2006). The plant is now grown in various parts of the world; Ethiopia itself accounts for around 3% of the global coffee market. Ethiopia, as the botanical home of *Coffea arabica*, with almost fertilizer-free environment, produces a number of distinctive varieties of coffee. Coffee grows in Ethiopia, almost in all administrative regions, but the Oromia and Southern regional states are the two major growers. Even though, the whole coffees cultivated in Ethiopia are Coffea arabica wide variabilities are noted among coffee cultivars in the country (Abera *et al*., 2017).

Coffee consumption varies widely according to geographical location. As report of the International Coffee Organization, in 2008 coffee was consumed at a rate of 2.5 billion cups per day (1 cup = 30 mL). This consumption is a model for addictive behavior. Genetic investigations in twins suggested that the heritability of Coffee intake can be estimated to be in the range of 39 to 56% (Laitala *et al*., 2008; Vink *et al*., 2009). Species of *Coffea* genus *Coffea Arabica* and *Coffea Canephora var. Robusta* belong to *Rubiaceae* family. Arabica usually comes from South America (i.e. Brazil) and from the uplands and mountain areas of East

Africa (i.e. Ethiopia, Kenya and Uganda). The main places of Robusta origin are Vietnam and the lowlands of Central and West Africa as well as South Asia (ICO, 2013).

2.7. Elemental Composition of Coffee

2.7.1. Techniques of Determination of Metals in Coffee

The development of instrumental methods suitable for a reliable elemental analysis of coffee, providing measurements of concentrations of mineral nutrients is essential for the whole coffee sector because competently assures the high quality of the final product (Tagliaferro *et al*., 2006).

Flame atomic absorption spectrometry (FAAS) with a deuterium lamp background corrector or intermittently with a Zeeman effect background corrector (Filho *et al*., 2007) is quite often used for selective determinations of different major (Ca, K, Mg, Na), minor (Cu, Fe, Mn, Zn) and trace (Cd, Co, Cr, Ni, Pb) elements of coffee (Grembecka *et al*., 2007; dos Santos *et al*., 2009, 2010; Ashu and Chandravanshi, 2011). High-resolution continuum source flame atomic absorption spectrometry (HRCS- FAAS) can also be used for this purpose (Ca, Fe, K, Mg, Mn, and Na) (Oliveira *et al*., 2012).

2.7.2. The Working Principles of FAAS

Atomic absorption spectroscopy has become one of the most frequently used tools in analytical chemistry. This is because for the determination of most metals and metalloids the technique offers sufficient sensitivity for many applications and is relatively interference free. Flame atomic absorption spectrometry 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. Metal 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.

FAAS is a very accurate quantitative technique and also a good qualitative technique. This is one of the main reasons it is the most widely used of the atomic absorption methods. The set up for most FAAS are relatively simple in design. The most complicated part of the instrument

is the nebulizer. The nebulizer system is highly important in FAAS. The nebulizer converts the sample solution into a mist or aerosol. The nebulized sample is then carried into the flame. The radiation then enters a monochromator, which isolates the line of interest. The light is then measured by a photomultiplier tube (detector). The signal is then processed and the computer system prints the output on screen. FAAS is a sensitive technique for the quantitative determination of more than sixty metals. The hollow cathode lamp is an excellent, bright, stable line source for most elements in FAAS. Hollow cathode lamps have a finite lifetime. With extended use, the sputtering process removes some of the metal atoms from the cathode and these are deposited elsewhere. Fill gas is absorbed in the sputtered metal, on the glass walls and also absorbed into the glass from bombardment (Evans *et al*., 1978).

3. MATERIALS AND METHODS

3.1. Expermental Site

Coffee moisture measuring, roasting process and grinding were carried out at the ECX, Dire Dawa, Coffee Quality and Flavor Inspection Laboratory Center. The sample digestion procedure and FAAS test were carried out at Haramaya University Chemistry Laboratory.

3.2. Sample Collection Method

Coffee samples were collected from ECX, Dire Dawa branch. The ways of naming coffee bean varieties of the samples under study were the same as the naming given by ECX Center Dire Dawa, Ethiopia (i.e. Harar A, Harar B and Harar C). Names of the three collected coffee samples with their moisture contents are given in figure 5.

Figure 3. **Samples of raw Hararghe coffee bean varieties**

3.3. Equipment and Apparatus

Automated moisture measuring apparatus (available at ECX center Dire Dawa branch), Polyethylene plastic bags were used to store the samples; coffee roasting machine, (PROBAT-WERKER EMMERICHAN RHEIN Germany electrical roaster); a blending grinding machine (Guatemala SB Grounder Machine) was used for grinding and homogenizing the roasted coffee samples; round bottom digestion flasks (25 mL) were used for the digestion of coffee

samples in wet digestion; Borosilicate volumetric flasks (25, 50 and 100 mL) were used during digestion and dilution of samples and preparation of metal standard solutions; Measuring cylinders and pipettes were used for measuring the reagents; deionizer was used to remove ions from distilled water; FAAS (**BUCK SCIENTIFIC MODEL 210VGP**) equipped with deuterium background corrector and hollow cathode lamps of Ca, Mg, Mn, Zn, Cd, Pb with air-acetylene flame were used and for K, flame Photometer (buck Scientific model no PFP 7) with butane gas flame was used.

3.4. Chemicals and Reagents

Chemicals and reagents that were used in the analysis are all analytical grades. Nitric acid (70%), Perchloric acid (70%), Sulfuric acid (98%) and Hydrogen peroxide (30%) were used.

3.5. Coffee Sample Roasting

About 100 g of each coffee sample of export standard green or raw coffee bean samples were roasted by using coffee roasting machine (PROBAT-WERKE, Emmerich an Rhein, Germany, Electrical Roaster) at the ECX, Dire Dawa, Coffee Quality and Flavor Inspection Laboratory Center by an expert. Finally, the roasted coffee samples were ground into fine powder using coffee grinding machine or blending device (Guatemala SB Grounder Machine) at the ECX, Dire Dawa and stored in polyethylene plastic bags.

3.6. Instrument Calibration and Operating Conditions

The range of linearity of concentration vs. absorbance curve is of great importance in determining the elemental concentration of roasted coffee samples and ensures the accuracy of the atomic absorption spectrophotometer and to establish that results of the determination were efficient and reliable (Dean, 2003). Stock standard solutions of the metals (K, Mg, Ca, Zn, Mn, Pb, and Cd) (100 ppm), intermediate standard solution and working calibration standards, were prepared from 1000 ppm calibration standard solutions of the metals (K, Mg, Ca, Zn, Mn, Cd and Pb) and a blank (de-ionized water) and standards were allowed to run in FAAS and five points of calibration curve were established. Each sample solution was aspirated into

the FAAS and absorbances of the metals were recorded. Three replicate determinations were carried out on each sample.

3.7. Optimization of Digestion Procedure

At the beginning of this study, series of procedures involving some changes in reagent volume, reagent composition, and digestion temperature and digestion time were tested. Accordingly, from those series of procedures tested for digestion of roasted coffee samples, the optimized one were selected depending upon: clarity of digests, minimal reflux /digestion time, minimal reagent volume consumption and absence of undigested coffee samples, simplicity and acceptable use of masses of coffee samples. Based upon these criteria, the optimal digestion procedure (3 mL HNO_3 (70%), 1 mL $HClO_4$ (70%), 1 mL H_2SO_4 (98%), 1 mL H_2O_2 (30%); 1:30 minutes and temperature of 150 ℃) were selected.

3.8. Method Validation

3.8.1. Digestion of Spiked Roasted Coffee and the Blank Sample

Spiking experiments were done to evaluate the efficiency and accuracy of the optimized method. Known amounts of standard metal solutions were added to the roasted coffee samples taking care of the dilution of the final solution. Aliquots of 1 mL of 12 mg/L K and 2 mL of 6 mg/L Cd, 1 mL of 6 mg/L Mg, Ca, Mn, and Zn and 3 mL of 6 mg/L Pb were added into a 0.5 g of roasted ground coffee samples in the round bottom digestion flask and were digested in triplicate following the same optimized procedure as the unspiked samples. The digested spiked samples were analyzed for their respective metals contents using FAAS.

3.8.2. Recovery Test

The percentage recoveries of metals in the spiked roasted coffee samples were calculated using the following equation. $R(\%) = \frac{F-I}{A} \times 100$ (Harvey, 2000), Where, F = spiked sample, I = un spiked portions, A = is the concentration added to the spiked portion.

3.8.3. Method Detection Limit (MDL)

Detection limit is the lowest concentration level that was determined to be statistically different from an analyte blank or the minimum concentration that can be detected by the analytical method with a given certainty. A general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal (Dean, 2003 and Abera, 2006). In this study, the detection limit of each metal were calculated as three times the standard deviation of the blank (3 σ blank)

3.8.4. Determination of Limits of Quantification

The limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank. Limit of quantification is the lowest limit for precise quantitative measurements. The quantification limit of each element was calculated as ten times the standard deviation of the blank (10 σ blank, n = 8). The results are given in Table 9.

3.9. Digestion of Coffee Samples

The wet digestion procedure was optimized for digestion of powdered roasted coffee samples with a mixture of nitric acid (70 % HNO_3), perchloric acid (70 % $HClO_4$), hydrogen peroxide (30 % H_2O_2) and sulfuric acid (98 %. H_2SO_4). 1000 ppm calibration standard solutions of the metals (K, Mg, Ca, Zn, Cd and Pb) were used for the preparation of intermediate and working standards for the preparation of calibration curves for the determination of metals in the coffee samples. Wet digestion with acid mixtures was performed in loosely covered open flasks on hot plates. Acids in combination are preferred for certain inorganic matrices and are generally more advantageous for the decomposition of organic compounds (Abentroth *et al.,* 2011, Aydin, 2008). The addition of hydrogen peroxide can increase the solubilizing power of mineral acids. Wet digestion is generally carried out in open flasks, covered loosely to avoid atmospheric contamination. A very efficient acid mixture is nitric, sulfuric, and perchloric acid in a volume ratio of ≈ 3:1:1 (Dean, 2003).

In the present study the digestion were carried out based on the selected optimized procedure. Acordingly, 0.5 g of each of the roasted ground coffee samples were digested on hot plates (150 ℃) by addition of 3 mL HNO_3 (70%), 1 mL $HClO_4$ (70%), 1 mL H_2SO_4 (98%) and 1 mL

H_2O_2 (30%) for 1:30 h and the digested samples were kept in the refrigerator, until the level of selected essential and nonessential metals in the sample solutions were determined by FAAS. This sample preparation step mostly aims at reducing matrix effects originating from organic compounds and releasing elements in the form of their simple ions.

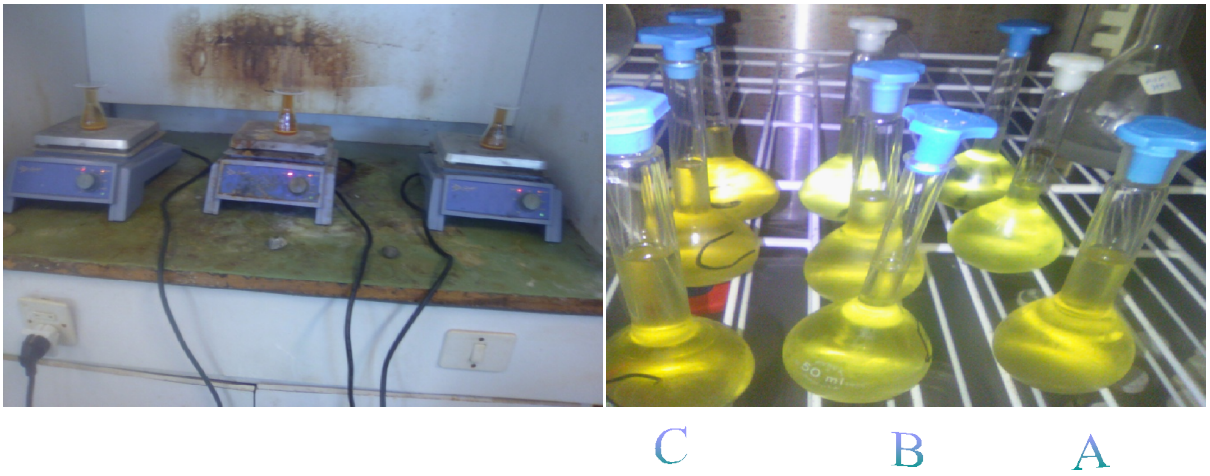


Figure 4. The digestion process and digested coffee samples

3.10. Determination of Metals in Coffee Samples

Optimization of the digestion procedure requires mineralization of 0.5 g roasted coffee samples by addition of mixture of 70 % HNO_3, 70 % $HClO_4$, 98 % H_2SO_4 and 30 % H_2O_2 with appropriate volume ratio that were found from the optimization procedure. Because of this reason, determination of detection limits for the developed procedure was necessary. For this reason, a blank solution consisting of the mixture of digestion reagents was digested following the digestion procedure. The digests were diluted to 50 mL with distilled and deionized water (Ribeiro *et al.*, 2003; Santos *et al.*, 2009; Ashu and Chandravanshi, 2011). Finally, all the seven metallic elements in each digested reagent blank were analyzed with FAAS using calibration curves as described under instrument calibration and operating condition section. Concentrations of the selected metals in the digests and diluted solutions of roasted coffee samples were determined with FAAS employing calibration curves.

3.11. Data Analysis

All the sample analyses in this study were carried out in triplicate and results were reported as mean ± SD. Statistical analyses were carried out for comparing mean concentration of essential and nonessential metals within Hararghe coffee bean varieties. The significance of variation in metals concentration between samples were analysed by oneway ANOVA. The statistical analysis was conducted using Microsoft Office Excel 2007 and oneway ANOVA Fisher's LSD at 0.05.

4. RESULTS AND DISCUSSION

4.1. Moisture Determination of Coffee Samples

Moisture standard for export coffee is less than or equal to 11.5 %, and moisture above this standard is not good for storage. In this study, all the samples collected for the analysis were dry and ready for export. Even though samples were dry, their moisture content may vary depending upon the extent of drying and storage. For these reasons their moisture content was initially determined by using an automated moisture measuring apparatus (*MINI GAC DICKEY JOHN* moisture measuring instrument). The moisture content of the three dry coffee sample varieties varies from 9.2 % to 11.2 %.

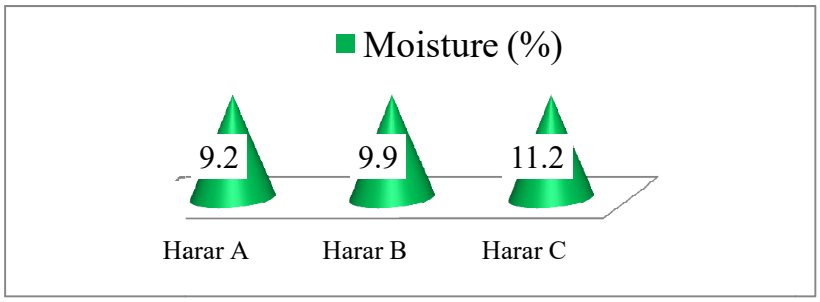


Figure 5**.** Moisture contents of dry coffee samples.

4.2. Roasting of Coffee Beans

All the roasting processes were carried out by an expert on coffee roasting using the proper temperature and time needed for roasting of all coffee samples (Harar A, Harar B and Harar C). Moreover, medium roasting was selected among the degree of roasting coffee beans (light, medium and dark). Finally the roasted coffee samples were ground into fine powder using coffee grinding device. The time and temperature for roasting of all coffee samples were 7-12 minutes and 150 ^{o}C respectively as described in table 3.

Table 3. Temperature and time needed for roasting of coffee samples

Coffee type	Harar A	Harar B	Harar C
Temperature (°**C**)	150	150	150
Time (min)	12	7	9

4.3. Optimization of Experimental Procedures

For the evaluation of the total concentration of elements using FAAS methods, samples of coffee require to be solubilized to reduce matrix effects originating from organic compounds and releasing elements in the form of their simple ions. The optimum digestion procedure for complete digestion of 0.5 g roasted ground coffee was selected depending upon: minimum values for the reagent volume, digestion temperature and digestion time to give a clear digest (Table 4, 5, 6).

Table 4. Procedures for optimization of reagent volume for digestion of coffee samples

No	Amount of coffee sample	Reagent added	Temp. (℃)	Digestion time (h)	Nature of the digest after filtration
1	0.50 g	4 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	Clear but pale yellowish color
2	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	*Clear and Colorless (Optimum)
3	0.50 g	5 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	Clear and Colorless
4	0.50 g	3 mL HNO_3 (70%) 1.5 mL $HClO_4$ (70%) 1.5 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	Turbid
5	0.5 g	4 mL HNO_3 (70%) 1.5 mL $HClO_4$ (70%) 1.5 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	yellowish color

As can be seen in the above table the reagent volume of **(**3 mL HNO_3 (70%), 1 mL $HClO_4$ (70%), 1 mL H_2SO_4 (98%) and 1 mL H_2O_2 (30%)) was selected for the next experiment to optimize the digestion temperature.

Table 5. Procedures for digestion temperature optimization for selected reagent volume

No	Amount of coffee sample	Reagent added	Temp. (℃)	Digestion time (h)	Nature of the digest after filtration
1	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	90	2:00	Turbid
2	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	120	2:00	Clear but pale yellowish color
3	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	150	2:00	*Clear and Colorless (Optimum)

As can be seen in the above table the digestion temperature for the selected reagent volume (150 ℃) was selected for the next experiment to optimize the digestion time.

Table 6. Procedures for digestion time optimization for selected reagent volume

No	Amount of coffee sample	Reagent added	Temp. (℃)	Digestion time (h)	Nature of the digest after filtration
1	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	150	1:00	Turbid
2	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	150	1:30	*Clear and colorless (Optimum)
3	0.50 g	3 mL HNO_3 (70%) 1 mL $HClO_4$ (70%) 1 mL H_2SO_4 (98%) 1 mL H_2O_2 (30%)	150	2:00	Clear and colorless

As can be seen in the above table the digestion time of the selected reagent volume and temperature (1:30 h) was selected for the next experimental procedure.

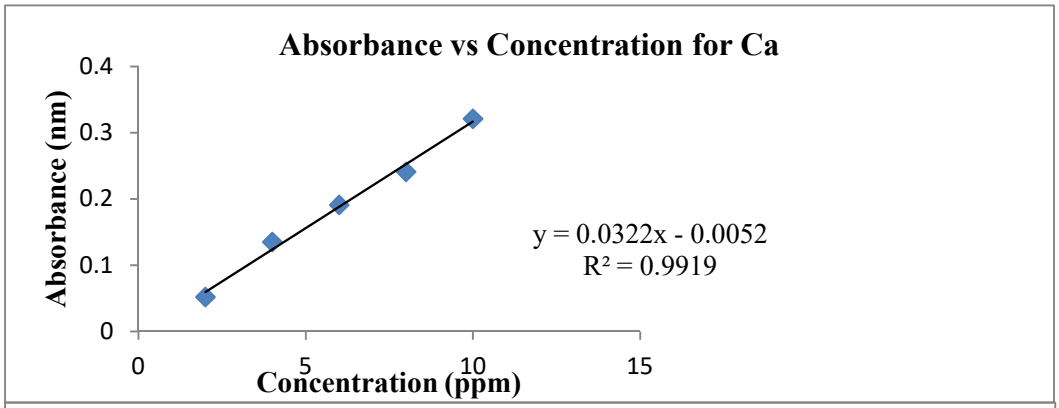
4.4. Instrument Calibration and Operating Conditions

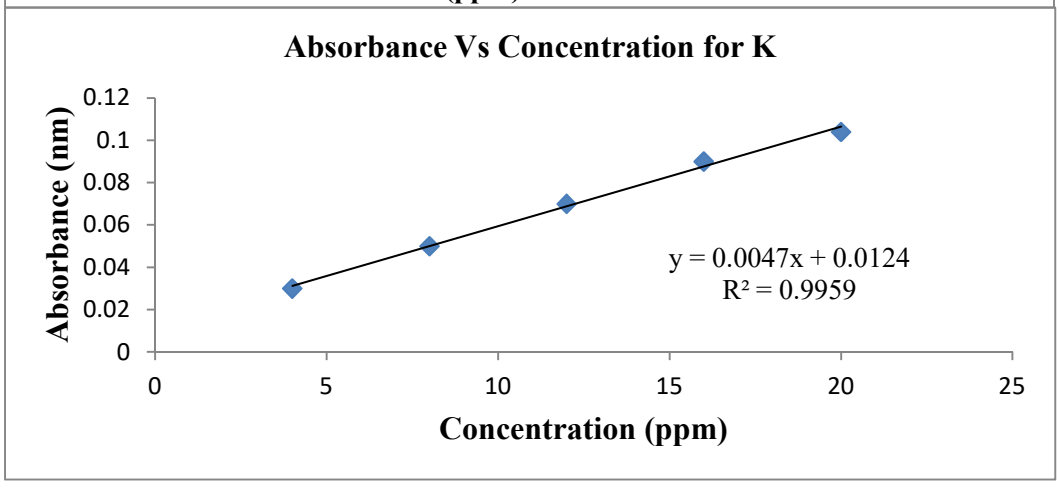
Standard aqueous solutions of selected metals were used to calibrate the FAAS machine. The coefficients of determination (R^2-values) ranges from 0.9911 – 0.9959 from the calibration

curves (Figure 6) and (Table 7). Thus, the obtained calibration curves were fairly linear, which assured that linearity of instrumental response for individual analyte.

Table 7. Instrumental parameter, coefficient of determination of the calibration curves and method detection limits.

Element	Wavelength (nm)	coefficients of determination (R^2)	Instrumental detection limit (µg/g)	Method detection limit for coffee powder samples (µg/g)
K	766.5	0.9959	0.01	0.02
Mg	285.2	0.9945	0.005	0.099
Ca	422.7	0.9919	0.05	0.1
Mn	279.5	0.9919	0.03	0.11
Zn	213.9	0.9915	0.005	0.11
Cd	228.9	0.9911	0.01	0.11
Pb	283.3	0.9918	0.04	0.09





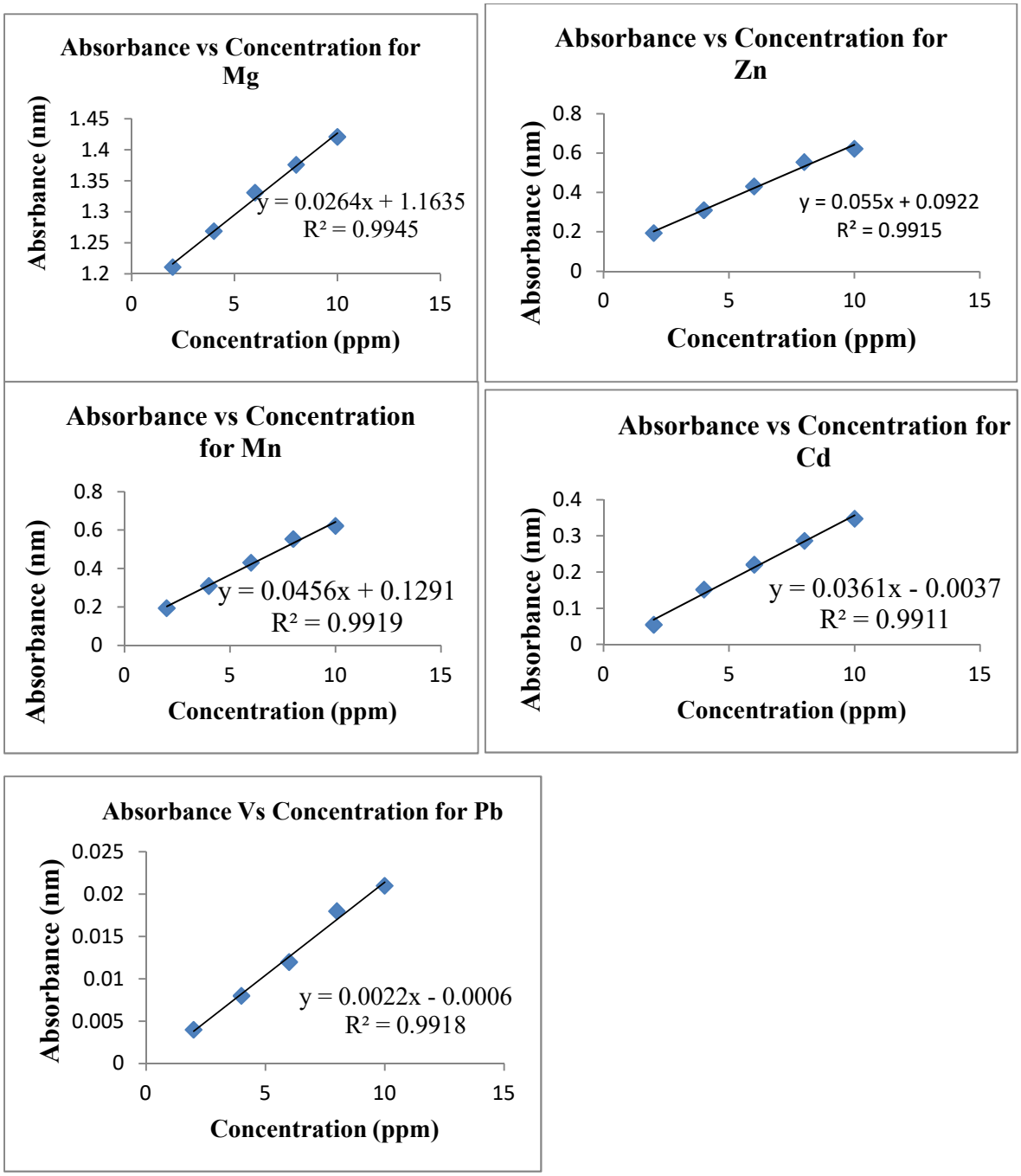


Figure 6. Calibration curves of standard solution for the selected metals

4.5. Validation of the Optimized Procedure

All the seven metallic elements in each digested blank were analyzed with FAAS calibration curves. Then the limits of detection and quantification were calculated as three and ten times the SD of the blank (3 σ blank and 10 σ blank, n = 8), respectively. The values of limits of

detection and quantification for each element are summarized in Table 9. It is likely that the large volume (6 mL) of the mixture of reagents (HNO_3, $HClO_4$, H_2O_2 and H_2SO_4) used in the digestion of coffee samples could have contributed to the high observed method detection and quantification limits. Despite such high blank values, the concentrations of analytes in the samples were above both method detection and quantification limits, except for Mn, Cd and Pb. Similarly, concentrations of these metals in the digests and diluted solutions of roasted coffee samples were determined with FAAS employing calibration curves.

The efficiency of the method used was validated by studying the recovery of the particular metals. The samples and the added standard solutions were subjected to similar analytical procedure as all other samples. The recoveries of the detected metals (Ca, Mg, K and Zn) in the spiked samples for roasted coffee lies within the range of 94 % – 103 % with SD (0.02 – 0.11) (Table 8). Generally, good recoveries were obtained for metals which were detected in the present study. Thus, the optimized procedure has good accuracy and precision.

Table 8. Recovery (%) of metals in the samples

Element	Concentration in the sample (X± SD) (mg/kg)	Amount added (mg/kg)	Concentration in the spiked sample (X± SD) (mg/kg)	Recovery (%) (X± SD)
K	15.7860 ± 1.2	0.24	16.0332 ± 1.22	103 ± 0.05
Mg	7.1473 ± 0.9	0.12	7.2618 ± 0.9	95 ± 0.02
Ca	5.5687 ± 0.24	0.12	5.6817 ± 0.24	94 ± 0.11
Mn	ND	0.12	0.122 ± 0.002	102 ± 1.70
Zn	0.3897 ± 0.03	0.12	0.5099 ± 0.04	100 ± 0.09
Cd	0.2282 ± 0.02	0.24	0.4977 ± 0.04	112 ± 0.08
Pb	ND	0.36	0.3798 ± 0.03	105 ± 0.07

*Note, X = mean concentration, SD = Standard deviation

The percentage recoveries of metals in the spiked samples of the present study (Ca, Mg, K, Zn, Mn, Cd and Pb) for roasted coffee lies within the range of 94 % – 112 % with SD (0.02 – 1.7). Eight reagent blank samples were digested following the same procedure as the coffee samples and each of the samples were analyzed for metal concentrations of K, Mg, Ca, Mn, Zn, Cd and Pb by FAAS (Table 8). The SD for each element was calculated from the eight reagent blank measurements to determine method detection limit of the instrument. The method detection limit is less than the results of real sample for detected metals and greater

than those of not detected metals as can be seen from Table 9, the method detection limit of each element is above the instrument detection limit as shown in Table 9.

Table 9. Method detection and quantification limits, (n = 8, MDL = 3 σ blank and MQL = 10 σ blank, in mg/kg), for roasted coffee samples

Elements	K	Mg	Ca	Zn	Mn	Cd	Pb
MDL	0.02	0.09	0.1	0.11	0.11	0.11	0.09
MQL	0.05	0.3	0.4	0.4	0.4	0.4	0.3

*Note: MDL = Method detection limit and MQL = Method quantification limit

4.6. Determination of Metals in Roasted Coffee Samples

The optimized digestion procedure requires addition of 3 mL HNO_3 (70 %), 1 mL $HClO_4$ (70 %), 1 mL H_2O_2 (30%) and 1 mL H_2SO_4 (98%) for the mineralization of 0.5 g of roasted coffee samples. However, this amount acid mixture is large enough to cause high values of the analytes in the blank solution. Because of this reason determination of the limits of detection and quantitation for the developed procedure is necessary. For this reason, a blank solution consisting of the mixture of digestion reagents was digested following the digestion procedure. The digests were diluted to 50 mL with deionized water.

The concentrations of these elements in the three roasted coffee sample types were varied. The ranges of macro elements (K, Mg and Ca) and microelement (Zn) in roasted coffee were found to be (mg/kg): 13.6669 – 15.7860, 7.1473 – 8.3676, 3.6691 – 5.5690 and 0.3987 – 2.3367, respectively (Table 10). On the other hand, the ranges of the concentration of trace metal and nonessential metals, (Mn, Cd and Pb), in the three roasted coffee samples were found to be below detection limit except Cd in Harar C which is found near to detection limit.

Generally in all roasted coffee samples the concentration of macro-elements K > Mg > Ca and from the micro elements Zn was found above detection limit. The concentration of Mn and Pb were found to be below the detection limit. However, for Cd results were found with a statement of its uncertainty (the concentration found was not reliable, but it was not below the detection limit; i.e. it was just around the detection limit) in one of the three samples of roasted Hararghe coffee bean varieties (Harar C). In the remaining two varieties its concentration was found to be below the detection limit of the method.

4.7. Concentration Levels of Selected Metals in Roasted Coffee Samples

The concentrations of macro-elements (K, Mg and Ca), micro-elements (Mn and Zn) and non-essential elements (Cd and Pb) determined in three types of roasted coffee samples summarized in Table 10. Generally, the mean concentration of metals in all roasted coffee samples follows the order: K > Mg > Ca > Zn > Cd, but the micro element Mn and the non-essential metals Pb were found to be below detection limit (not detected using FAAS).

Harar A roasted coffee contained K in highest amount of the macro-elements with concentration 13.6670 ± 1.2 mg/kg followed by Mg (8.3676 ± 0.04 mg/kg) and Ca (5.569 ± 0.2 mg/kg). Ca was found to be present at the lowest concentration of the macro-elements analyzed, among the concentration of trace micro-elements and for Zn (0.3897 ± 0.03 mg/kg), Mn (ND), Cd (ND) and Pb (ND) no significant difference was found.

Harar B roasted coffee contained the macro-element K with concentration 15.78597 ± 1.2 mg/kg was found to be present in the highest level followed by Mg (8.09087 ± 0.3 mg/kg) and Ca (4.2133 ± 0.2 mg/kg), and for Zn, Mn, Cd and Pb no significant difference was found.

Harar C roasted coffee; among the macro-elements, K was found in highest amount with concentration 15.0796 ± 1.2 mg/kg than Mg (7.1473 ± 0.7 mg/kg) and Ca (3.6691 ± 0.4 mg/kg). Harar C roasted coffee also contains Zn with concentration 2.3367 ± 0.2 mg/kg and Cd (0.2282 ± 0.02 mg/kg) while Mn and Pb were found to be below detection limit.

Table 10. Concentration of metals in coffee powder samples (X ± SD, n = 3, g/kg)

Element	Coffee Variety		
	Harar A	Harar B	Harar C
K	13.6670[b]	15.786[a]	15.0796[a]
Mg	8.3676[a]	8.09087[a]	7.1473[b]
Ca	5.569[a]	4.21333[b]	3.669[b]
Mn	ND	ND	ND
Zn	0.38973[a]	0.5459[a]	2.3367[b]
Cd	ND	ND	0.2282
Pb	ND	ND	ND

*Values in the same raw with the same superscript lower case letters are not significantly different (P = 0.05).

From one way ANOVA test for significant difference; for concentrations of Mn, Cd and Pb in the three varieties and Zn in variety A and B no significant difference at p = 0.05, for K and Ca a significant difference at p = 0.05 was observed between variety A and the rest two varieties (B and C) and for Mg and Zn a significant difference at P = 0.05 was observed between variety C and the rest two (A and B) of the three roasted coffee samples. Generally in all roasted coffee samples for the mean concentrations of Cd, Mn and Pb no significant difference were found.

4.8. Comparison of the Metal Contents in Roasted Hararghe Coffee Bean Varieties with Other Reported Values

In different studies the metal content of roasted coffee samples have been analyzed for different Arabica coffee types. The composition of Ca, K, Mg, Zn, Mn, Cd and Pb have been determined in roasted coffee of Bule Hora woreda, Borena Zone, Ethiopia, (Tewodros and Nigus, 2014); Levels of selected essential and nonessential metals in roasted Coffee beans of Yirgacheffe and Sidama, Ethiopia, (Tesfay et al, 2015); commercially available Ethiopian roasted coffee powders and their infusions, (Ashu and Chandravanish, 2011), and roasted beans of Hararghe coffee bean varieties in the present study.

Although various chemical analyses target to similar objective, there may also be a difference in sampling, sample preparation and analytical techniques. Considering all these, the results of the present study were compared to the findings of other authors. Ashu and Chandravanshi (2011) determine the concentrations of metals (K, Mg, Ca, Na, Mn, Fe, Cu, Zn, Co, Pb, Cd) in three brands of commercially available roasted Ethiopian coffee powders (Abyssinia, Alem and Pride). The mean concentration of each metal in the three brands of coffee powder samples was (μg/g): K (14488 ± 467), Mg 1964 ± 78), Ca (945 ± 65), Na (484 ± 12), Fe (52.0 ± 4.0), Mn (23.0 ± 0.9), Cu (14.0 ± 0.6), Zn (15.0 ± 0.8) and Co (1.60 ± 0.05) in which both those results are comparable with the present study except for the microelement Mn which is undetectable in the present study.

The results in the present study the general trend of the metal concentration for macro-elements K > Mg > Ca is in good agreements. Regarding the trace microelements, Zn > Mn in the present study. Similarly, reports by Ashu and Chandravanshi (2011); Tewodros and Nigus

(2014) and Tesfay *et al.,* (2015) shows that toxic elements Cd and Pb were not detectable under their analyses conditions using FAAS which was in a good agreement with the present study. Lead and Cadmium rank among the most toxic of the inorganic contaminants. The main adverse effects of these metals on health are neurological, hematological, endocrinological, cardiovascular, gastrointestinal and hepatic systems also affects growth, reproduction and development, and contains a carcinogenic potential (Moreira, 2004).

In the present study lead was not detected but the study on coffee from Cerrado Mineiro region (Alto Paranaíba – MG, Brazil), by Sabrina *et al.* (2017) shows that in 86 % of analyzed samples of roasted and ground coffee, the element lead was in concentrations above the permitted by the regulations of the European Union (European Commission Regulation, 2008) (0.2 mg/kg). One of the roasted and ground coffee samples, corresponding to 2 % of the samples showed a higher concentration of zinc than the maximum (50 mg/kg) set by the Brazilian legislation. Cr and Pb were higher than the maximum permitted under Brazilian law and Mercosul regulations (Brazil, 2013) (0.5 mg/kg), some containing almost three times the value. Generally, the concentration of the macro and micro-elements in roasted Hararghe coffee bean varieties in the present study were compared with other reported values in table 11.

Table 11. Comparison of metals concentration in the present study with the literature values

Concentration of Elements (μg/g)							Reference
K	Mg	Ca	Mn	Zn	Cd	Pb	
14361−14583	1959−1968	843−1045	22−24	12−19	ND	ND	Ashu and Chandravanish (2011)
13890 –13950	2010– 2030	1490– 1530	16– 18	16 – 19	ND	ND	Tewodros and Nigus (2014)
18563−19610	1943−2030	931−1009	14−18	19 −23	ND	ND	Tesfay et al., (2015)
NR	NR	NR	9.8– 39.8	5.5– 55.8	0.03– 0.1	0.03– 1.6	Sabrina *et.al*., (2017)
13.667-15.786	7.147– 8.368	3.669– 5.569	ND	0.40– 2.34	0.21– 0.24	ND	Present study (μg/g)

*Note: NR = not reported; ND = not detected.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Coffee is one of the most popular and widely consumed beverages in the world, having extensive commercial as well as social importance. It is also one of the most important agricultural products in the international trade. Owing to this, the present study tried to determine the concentration of K, Mg, Ca, Mn, Zn, Cd and Pb in roasted Hararghe coffee bean varieties, collected from ECX Dire Dawa branch. An efficient digestion procedure was optimized and validated through recovery studies. For this study the samples collected were unwashed sun dried coffee beans and were ready for export. The optimum conditions were validated through spiking experiment, from which good percentage recoveries 94 % – 112 %, for roasted coffee samples were obtained.

Concentrations of metals in the three samples of Hararghe coffee bean varieties were compared using one way ANOVA. All samples contained higher amount of macro elements than micro elements. K was the most accumulated metal followed by Mg, Ca, and Zn for all roasted coffee samples. According to this study, in all roasted coffee bean samples the level of toxic element Pb and micro element Mn were found to be below the method detection limits, while Cd was detected at the lowest level in only Harar C coffee variety and not detected in the rest two varieties. The values found are in line with previously reported data on other types of coffee samples. The metal composition of Hararghe coffee bean varieties was found to be similar to that of other reported values in literature.

From the present study one can observe that there are permissible amounts of macro and trace elements in roasted Hararghe coffee bean varieties. It could be suggested that the roasted coffee beans under investigation could be source of dietary minerals and trace metals and could be valuable in complementing available food composition data.

5.2. Recommendation

This study indicated that the toxic metals (Cd and Pb) in the roasted Hararghe coffee bean varieties were below the method detection limit and the concentrations of the detected metals (K, Mg, Ca, Mn, and Zn) were in the range of recommended maximum permissible limits indicating no risk of health drinking the coffee brew of these varieties concerning the above selected metals. Based on the results of this study, the following points are forwarded.

1. Although this study showed elemental composition of roasted Hararghe coffee bean varieties and its daily intake values; It is better if further analysis on extended number of essential and toxic metals is conducted to draw strong conclusion.

2. Parallel to the study of metal content in roasted Hararghe coffee bean varieties further investigations should be done on the harvest, transportation and storage process to avoid metals contamination and maintain the quality of Hararghe coffee.

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7. APPENDIX

Appendix I

Table 1. Anova Table for the Selected Metals Mean Comparison (a and b)

The ANOVA Procedure

Class Level Information

Class Levels Values

SAMPLE 21 (ACa, ACd, AK, AMg, AMn, APb, AZn, BCa, BCd, BK, BMg, BMn, BPb, BZn, CCa, CCd, CK, CMg, CMn, CPb and CZn)

Number of observations 63

Dependent Variable: METAL_CONC_ METAL CONC#

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	1720.054832	86.002742	317.15	<.0001
Error	42	11.389322	0.271174		
Corrected Total	62	1731.444154			

R-Square	Coeff Var	Root MSE	METAL_CONC_ Mean
0.993422	12.85181	0.520744	4.051911

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	20	1720.054832	86.002742	317.15	< 0.0001

a

	METAL CONCENTRATION						
SAMPLE	K	Ca	Mg	Mn	Zn	Cd	Pb
A	13.667[b]	5.5687[e]	8.3676[c]	0[h]	0.3897[h]	0[h]	0[h]
B	15.786[a]	4.2133[f]	8.0909[c]	0[h]	0.5459[h]	0[h]	0[h]
C	15.0796[a]	3.6691[f]	7.1473[d]	0[h]	2.3367[g]	0.2282[h]	0[h]

b

*t Tests (LSD) for METAL_CONC_

* Least Significant Difference or LSD (0.05) $\geq$ 0.8581

*Means with the same letter are not significantly different.

*NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.