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Investigation of Metals in Raw and Roasted Indigenous Coffee Varieties in Ethiopia

BY

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To my Wife Gidamework and my Children Jalalake and Latera

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ABSTRACT

INVESTIGATION OF METALS IN RAW AND ROASTED INDIGENOUS COFFEE VARIETIES IN ETHIOPIA

By Abera Gure

Advisors: Prof. B. S. Chandravanshi and Dr. Taddese Wondimu

The metal contents of raw and roasted coffee varieties that are grown in five different parts of Ethiopia; (Wollega, Sidamo, Harar, Benchi Maji and Kafa zones) have been analyzed. The representative samples of these coffee varieties were collected from Coffee Quality Inspection and Liquoring Center, Addis Ababa, Ethiopia. Different digestion procedures were tested by varying reagent volumes, time of digestion, temperature of digestion, types of reagents and amount of the sample to develop a procedure that consume less reagent volume, short digestion time, low temperature of digestion and smaller mass of the sample. The optimal procedure required 4 hours and consumes 5 mL HNO_3 (70 %) and 1.5 mL $HClO_4$ (70 %) to completely digest 0.5 g of both raw and roasted coffee samples. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples. Recoveries of the spiked samples varired from 85 to 115 % and 84 to 120 % for raw and roasted coffees, respectively. Concentrations of metals; (Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) were analyzed by fame atomic absorption spectrometer employing a four point external calibration curve. The observed metal concentrations in roasted coffee varieties are relatively higher than their corresponding raw varieties. The metals levels observed in both raw and roasted coffee varieties are comparable with their corresponding world wide reported values with the exception of Cr, Cd and Pb that are differing from the reported values. Indeed, the study has revealed the existence of variation of metal composition of coffee beans with the geographical origin in which coffee plants grow.

Key words: Indigenous coffee, Coffee beans, Roasted coffee, Major metals, Trace metals, Toxic metals

ADDIS ABABA UNVERSITY
SCHOOL OF GRADUATE STUDIES

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1. INTRODUCTION

1.1. Origin of Coffee

The origin of coffee is shrouded in myth and great stories. It is commonly believed that the indigenous coffee trees first grew in Ethiopian province "Kafa" and the trees were called "Kafa tree", which may as well the root word for the name of coffee [1-3]. In the tenth century, coffee was considered as a food [3]. The history of coffee begins with a legend that takes place around 600 AD. As to this legend coffee was first discovered by an Ethiopian goatherd, Kaldi, who was living in Kafa province [3, 4].

1.1.1. Coffee Species

Coffee is a member of large *Rubiaceae* family, within which it constitutes the *Coffea genus* that was created by *C. Linnaeus*, in 1737 [5]. On the other hand, *Chealier* (1947) [5] tried to group the species within the *genus coffea* into the following four sections, namily: *Argocoffea, Paracoffea, Mascarocoffea* and *Eucoffea.* The section *Eucoffea*, now more correctly named *coffea*, and *Mascaroffea* includes most of the presently known coffee species [5]. They are further classified in to a number of different species of coffees, based upon their berries size, colour, coffee leave colour, etc [5].

The two main species exploited in the world at present time are *Coffea arabica* and *Coffea conphora (robusta).* They account for at least 99 % of the world's coffee. Of which 70 % is *Coffea arabica* type. Other species of *coffea,* which are less cultivated, are: *Coffea liberica*, *Coffea abeakutyae*, *Coffea dewevrei*, *Coffea congensis*, etc. the different species of *Coffea genus* have very diverse appearances and behaviors [5-7].

Coffea arabica is the species that has been known for longest time and it is also the most widespread throughout the world. It is the most important commercial coffee species, which is cultivated in about 70 % of plantations worldwide [8]. It is originated from Ethiopia, where it grows in very large stands on the high land plateaus (1300 – 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. They have about 100 mm long, 6 to 7 mm width, 3 to 4 mm thick and 0.15 to 0.2 g weight [7].

This species, which is autogamous (self-fertilizing), has relatively uniform characteristics. Nevertheless, it has given rise to a number of varieties (hybrids, mutant, etc), regional types, and cultivars that reflect the influence of environment [7].

1.1.2. Diversity of Coffee in Ethiopia

Ethiopia, as the botanical home of coffee arabica, with an almost fertilizer free environment, produces a number of distinctive regional types of coffee [9]. Coffee grows in Ethiopia, almost in all administrative regions. However, the major coffee growers of the country are, Oromia and Southern regions [9-11]. Even though, the whole coffees cultivated in Ethiopia are *Coffee arabica*, there are wide ranges of variability among coffee cultivars in the country [1, 10]. They can be classified based up on the regions or sub-regions and the sizes of the coffee berries. Some of the varieties of the coffees in the country based up on the area of their production are; Harar coffee, Wollega coffee (includes Nekemt and Gimbi coffee), Jima coffee, Gedeo coffee, Limu coffee, Sidamo coffee, Kaffa coffee, etc. [1, 8]. There are a wide range of variability among coffee types in each coffee growing regions and woredas. Even sometimes, there is a difference among coffee types in a given farmland. Thus, in some coffee growing areas farmers could identify these differences and give the name for the cultivars they use [1, 9, 13]. This variability has been observed due to coffee beans are greatly affected by the areas that they are produced in. The soil, altitude and climate of the coffee growing areas will have a great influence on coffees characteristics from its body (or chemical content) to its flavor to its aroma [13]. Thus, the quality of coffee varies from one region to another and even from one particular farmland (place) to another that exists in the same Woreda or Kebele.

1.1.3. Uses of Coffee

Coffee is one of the most agricultural products in the international trade [3]. It is the second in value, putting into motion approximately 35 billion dollars per year being leaded by petroleum [3, 14]. Coffee represents a commodity of great economic, social and environmental importance to coffee cultivating countries, particularly for developing countries, like Ethiopia [15]. Ethiopia relies greatly on trade of coffee, which generates 60 % of its total export earning [3, 15].

Although, coffee is the most important item in the export market of Ethiopia and the main source of export earnings, Ethiopians are also the leading coffee consumers among all African coffee producers. Almost half of Ethiopia's annual coffee production is consumed domestically [16]. Coffee beans have been used as stimulant beverage in almost all administrative regions of the country. Even in some places in the country, people use it as a special entertainment; they boiled the dried cherry or beans in butter and use it as a ceremonial food for special days, commonly in Wollega [1].

Coffee is also found to be high in health-giving antioxidants. It is considered, as a health drink following a study showing coffee is a surprisingly rich source of anti-cancer agents. A study has found that coffee contributes more antioxidants - which have been linked with fighting heart disease and cancer - to the diet than cranberries, apples or tomatoes. Antioxidants help to rid the body of harmful free radicals, destructive molecules that damage cells and DNA. They have been linked to a number of health benefits, including protection against heart disease and cancer. Studies have associated coffee drinking with a reduced risk of liver and colon cancer, type two diabetes and Parkinson's disease [16].

1.2. Chemical Composition of Coffee Beans

Coffee is the complex mixture of potential “neutriceuticals” [17]. Its chemical composition is determined by a complex interaction of agricultural factors, roasting, blending and brewing. Studies have demonstrated that the chemical composition of coffee beans can discriminate arabica from robusta, country of origin and organic from conventional system of cultivation [18].

The main components of coffee beans have been known over half of the century. As it was stated by an ICS [17], coffee beans constituents, in order of abundance (are: 8 % phenolic polymer (pulp), 6 % polysaccharides, 4 % chlorogenic acids, 3 % minerals, 2 % water, 1 % caffeins, 0.5 % organic acids, 0.3 % sugars, 0.2 % lipids, and 0.1 % aromas. As to Ernesto Illy [20], the aroma of green coffee contains some 250 different volatile molecular species, whereas roasted coffee gives rise to more than 800. On the other hand, some recent studies on the chemical compositions of coffee, has been identified over 600 different substances in green coffee, while roasted coffee contains much larger than the green once. The most important once are; minerals, lipids, caffeine, proteins, fats, carbohydrates and water [19-22]. The chemical composition of coffee varies according to species (arabian or robusta), country origin (Ethiopia, Brazil, Kenya, etc), system of cultivation (organic or conventional) and the way it exist (raw or roasted) [18, 21]. Main coffee components [21] are given in Table 1.

Table 1. Main coffee components [21].

g %/100 g on dry mass	***C. arabica***		***C. 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 – 13.0	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

1.2.1. Mineral composition of raw (green) coffee

In general green coffee contains a large classes of compounds; proteins, carbohydrates, lipids, volatile and non-volatile acids, alkaloids, ash, and water [7]. Green coffee contains a total of 3 – 4.5 % ash [21], this comprises mostly K, Na, Ca, Mg, P and S. Numerous trace elements have also been found, including Fe, Al, Cu, I, F, B, and Mn [7]. As reported by Clarke and Walker [23] and other researchers [21], the ashed mineral content of green coffees averages about 4 % on dry basis, with potassium its main constituent, at about 40 % (expressed as the element K) of this amount.

The metal content of green coffee samples, belonging to the varieties of arabica and robusta coffee has been analyzed using ICP-AES for 11 elements, namely Fe, Zn, P, Mn, Ca, Na, Mg, K, Cu, Sr and Ba [24]. Significant differences have been reported in the metallic content of the two varieties. P, Mn and Cu contents present the largest differences and they are very appropriate to characterize the arabica and robusta coffee varieties. The content of P and Cu is higher for robusta, while arabica coffees have bigger percentage of Mn [24]. The coffee berries and raw coffee beans are shown in Figure 1.

Coffee Berries **Coffee Beans** **Coffee Bean**

Figure 1. Coffee berries and raw coffee beans [9].

1.2.2. Mineral Composition of Roasted Coffee

Roasting is the process by which the green coffee beans that has been produced on a coffee plant is changed into some thing which is nearly ready for consumption [2]. In this process there is a change in the chemical composition of the bean and as well as loss of weight up to 20 % [4]. Specialists place the “roasting zone” in between 185 °C and 240 °C, the optimum temperature being between 150 °C to 230 °C [4]. Roasting temperature and the way the process is conducted have a considerable effect on the quality of coffee. Roasting of coffee can be completed in the range of 5 to 25 minutes depending upon the techniques used [7]. Roasting of coffee gives;

- its taste and aroma
- a change in the chemical composition
- change the size of the bean in a certain way
- loss of weight due to evaporation of water in about 14 – 23 % and loss of volatile substances
- increase in volume of coffee beans

- change of bean colour, of course the color change depends on the intensity and duration of roasting. Roasting can be classified into light (cinnamon or amber), medium and dark (French or Italian) roasting
- Change of bean texture

In general, roasting of green coffee does not change noticeably, the mineral content, but their relative content increases when water and volatile organic compounds disappear [4, 7].

In different studies the metal content of roasted coffee samples have been analyzed for both arabica and robusta coffee types [14, 23, 25, 26]. The composition of Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn have been determined and used as chemical descriptions to differentiate between roasted coffee samples from arabica and robusta coffee varieties. According to the report on roasted coffee varieties present in southeast Brazilian market, there is a difference in metal content among the samples of roasted coffee varieties [25]. These variations in metal compositions among samples roasted coffee can indicate the differences in the factors that influence the cultivation of the coffee plant; such as the type of soil, the use of fertilizers with different chemical compositions and the ambient conditions [25]

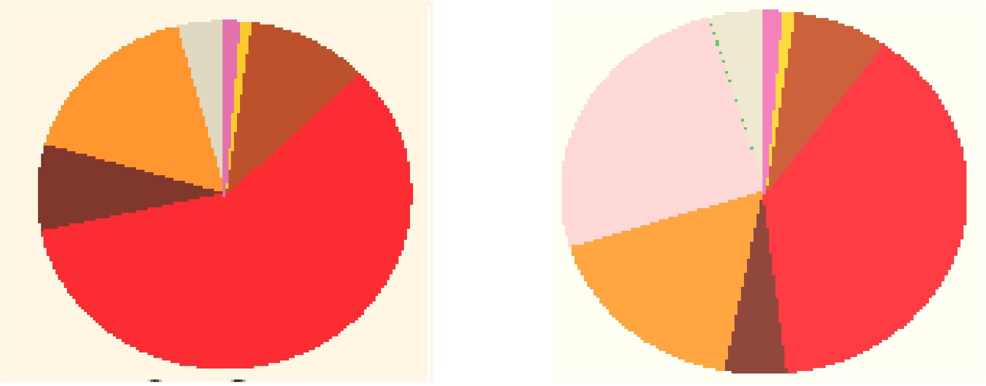
It has been reorganized that the minerals and trace metal compositions of roasted coffee beans are the distorted reflection of the trace mineral composition of the soil and environment in which the plant grew [26]. The minerals bioaccumulations within the coffee beans vary with different trace elements, with different varieties and with different environment in which coffee grows [25, 26]. Concentration of metals in roasted coffee varies with geographic origins in which the plant grows. Roasted coffee beans and roasted ground coffee [22] are shown in Figure 2. Mean concentration of metals in µg/g or ppm, (n = 20), determined in roasted coffee beans, which are grown in different geographic origins [26] are given in Table 2. Chemical composition of raw and roasted arabica coffee (percentage of dry matter) [22] is shown in Figure 3.

Roasted coffee ***Roasted ground coffee***

Figure 2. Roasted coffee bean and roasted ground coffee [22].

Table 2. Mean concentration of metals in μg/g or ppm, (n = 20), determined in roasted coffee beans, which are grown in different geographic origins [26].

Growing regions or countries	**P**	**Zn**	**Mn**	**Fe**	**Mg**	**Al**	**Cu**	**Ca**	**K**	**S**	**Na**
Costa Rica	1920	7.97	23	15	2203	13	18.1	1079	18570	1640	41.5
Colombia	1980	8.01	38	17	2268	19	17.2	1129	19170	1480	40.1
Guatemala	1960	8.03	25	13	2410	8	14.0	1234	19010	1640	28.9
Panama	1740	7.04	26	20	2174	3	16.8	997	18680	1500	9.6
Ethiopia	1860	7.82	21	12	2058	7	13.8	1013	19280	1450	20.1
Kenya	1710	7.15	39	15	2150	4.4	17.8	976	17500	1420	37.3
Sulawesi	2110	7.87	29	21	2347	13	12.5	934	19160	1431	1467
Sumatre	1940	6.51	19	31	2098	36	13.2	1141	19600	1490	10.6



Green Beans

(average water content 8 - 12 %)

Roasted Beans

(average water content 0 - 5 %)

🟪 Caffeine, 🟫 Acids, 🟨 Trigonelline, 🟧 Lipids, 🟩 Volatile aromas, 🟫 Proteins and amino Acids, 🟥 Sugars (mostly polysaccharides), ⬜ Minerals (as oxide ash) and 🟪 Carmelization and condensation products.

Figure 3. Chemical composition of raw and roasted arabica coffee (percentage of dry matter) [22].

1.2.3. Essentiality and Toxicity of Metals in Plants and Animals

Although the minerals composed of only a few percent of the body of animals and green plants, they take a disproportionately large part in the body metabolism for both plants and animals [27]. Numerous investigators have shown that the normal physical activities are not possible in the absence of minerals [27, 28]. Of the mineral elements: P, Ca, K, S, Na and Cl are sometimes called macronutrients, not because they are more important but because they are necessary in somewhat greater amounts than others [27 – 31]. The first five are essential to both plants and animals, and the last two to animals alone [27].

In plants nutrition, mineral nutrients (elements) are classified in to essential and beneficial. The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout (1939) [32]. They concluded that three criteria must be met for an element to be considered essential. These criteria are: (1) a plant must be unable to complete its life cycle in the absence of the mineral element; (2) the function of the element must not be replaceable by another mineral element; and (3) the element must be directly involved in plant metabolism. Beneficial elements are those that can compensate for toxic effects of other elements or may replace mineral nutrients in some other less specific function such as the maintenance of osmotic pressure [32, 33].

On the other hand, from the biological viewpoint, metallic elements that are considered to be mineral nutrients of plants are most commonly classified into four [27-31, 33]; (i) essential macronutrients, (Ca, Mg and K), are those without which plants can not complete their life cycle; (ii) essential micronutrients or trace metals, (Fe, Mn, cu, Zn, Mo, Co, V, Na, and Ga), which are needed by plants in quantities much smaller than the macronutrients and they are usually functioning in plants as components of enzyme systems [28, 33]; (iii) essentiality not demonstrated metals, (Cr, Sr and Ni); and (iv) beneficial metals, (Al, Sr and Rb).

These elements normally enter the plants body through their root from the soil and are transported upward in both xylem and phloem. Plant uses them to build new parts of itself and to carry on a wide range of chemical activities that take place in cell. The amount of these elements in a plant body varies depending upon their presence in air and soil. Their amount or proportion in plants body also depends on many factors; species, age, root distribution of the plant, physical and chemical nature of the soil, proportions and distributions of the elements and the general climatic conditions [28, 30, 32].

Undermost conditions, metallic elements that enter animals are those contained in plants eaten or used as a beverage either directly or indirectly [27, 28]. Thus, the metal content of plants frequently controls the amount of these elements available in different animals bodies. The functions of elements in the animal's body are so diverse, valid statements of principle concerning their functions are rare [31]. According to the grouping used by the food and nutrition board [34], metals are mostly grouped under bone related nutrients (Ca and Mg) and additional trace elements (Cr, Cu, Fe, Mn, Mo, Zn, Ni, and V). On the other hand, heavy metals in foods and beverages are classified into two; based upon their essential and toxic nature. For example Fe, Zn, Cu, Mn, Cr, Co, and V are essential. While Pb, Cd. Ni, As and Hg are toxic at a certain levels [35, 36].

Furthermore, based upon the amount that is required in human nutrition, metallic elements are classified as: (1) bulk mineral elements or macro metals; Ca, Mg, K and Na (Recommended Dietary Allowance RDA > 200 mg/day). (2) Trace minerals or metals; the most important trace metals (RDA < 200 mg/day) are Cr, Cu, Fe, Mn, Mo, Se, V and Zn. They are used in the enzymatic systems and can be harmful when their injection rate is too high [36]. (3) Other metals that have been not definitively established as essential to human nutrition; includes Ni, Rb, Sr, Ti, Te, and W [37, 38]. The metallic elements of interest in this study are K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Pb, Ni and Cd. In the following section general roles played by these elements in both plants and animals are presented.

Potassium, calcium and magnesium are essential macronutrients for both plants and animals [27 –31]. Potassium is absolutely essential for plants and cannot be entirely replaced by any other element [27]. It is necessary for formation of sugars, starches, carbohydrates, protein synthesis and cell division in roots and other parts of the plant. It helps to adjust water balance, improves stem rigidity and cold hardiness, enhances flavor and color on fruit and vegetable crops, increases the oil content of fruits and is important for leafy crops [32]. It can also function to regulate osmotic balance, especially in stomata opening/closing; enzyme activator [33]. Its deficiencies result in low yields, mottled, spotted or curled leaves, scorched or burned look to leaves [32]. Potassium is present in animal body, mostly in the muscles tissue and in blood plasma [27]. It is the principal intercellular cation with no RDA but a minimum requirement of 1600 milligrams per day would be adequate [37].

Potassium is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. Deficiency symptoms include weakness, anorexia, nausea, drowsiness and irrational behavior. Potassium is found in most foods since it is an essential constituent of all living cells. The richest dietary sources are unprocessed beans, nuts, leafy green vegetables and fruit [37]. Calcium is also believed to have several functions in the plant [27]. It activates enzymes, is a structural component of cell walls, influences water movement in cells and is necessary for cell growth and division [27, 32]. Some plants must have calcium to take up nitrogen and other minerals. Its deficiency causes stunting of new growth in stems, flowers and roots [32]. In animals, calcium is the outstanding single constituent of bones and teeth. It is the most abundant mineral in the body with RDA for adults 1200 milligrams [37] or approximately 1 g [27]. It occurs in the bone in the ratio of 3:1 mole $Ca_3(PO_4)_2$ and $CaCO_3$, respectively. Very small fraction of calcium can also serve in heart, muscles, and nerve systems. Ca and PO_4^{3-} are properly utilized in the body by vitamin D [27]. The best natural sources are sea vegetables, low-fat yogurt, skim milk, beans, seeds, nuts, green vegetables, etc. Intakes over 2000 milligrams per day may lead to hypocalcaemia, induce constipation, and inhibit the intestinal absorption of iron, zinc, and other essential minerals [32].

Magnesium is essential for plants and animals. It is relatively more abundant in the parts of plants concerned with vital process, such as seeds and foliages than in storage parts such as stems and roots [27]. It is a critical structural component of the chlorophyll molecule and is necessary for functioning of plant enzymes to produce carbohydrates, sugars and fats. It is used for fruit and nut formation and essential for germination of seeds. Deficient plants appear chlorotic, show yellowing between veins of older leaves; leaves may drop. Magnesium is leached by watering and must be supplied when feeding. In animals magnesium is the essential composition of bone and teeth with RDA for adults of 350 milligrams [32]. It play important role in metabolism of phosphorus, starch and sugars. Many biochemical and physiological processes require magnesium. It is necessary for vitamin C and calcium metabolism. It keeps teeth healthy, brings relief from indigestion and can aid in fighting depression. More than 300 enzymes are known to be activated by magnesium. The best natural sources are whole seeds, nuts, legumes, unmilled grains, green vegetables and bananas. Phytate or fiber may reduce magnesium absorption. Alcohol acts as a diuretic, causing vast quantities to be lost in the urine [32].

Trace minerals such as Fe, Cu, Mn, Zn, Ni, Cr and Co are essential micronutient for both plants and animals [32-33]. They combine with vitamins, form enzymes and are necessary for almost every physiological process. These minerals are found in a broad range of plant and animal foods, as well as in drinking water [39].

Iron is necessary for many enzyme functions and as a catalyst for the synthesis of chlorophyll. It is essential for the young growing parts of plants. Deficiencies may result in pale leaf color of young leaves followed by yellowing of leaves and large veins. Iron is lost by leaching and is held in the lower portions of the soil structure. Under conditions of high pH (alkaline) iron is rendered unavailable to plants. When soils are alkaline, iron may be abundant but unavailable. Applications of an acid nutrient formula containing iron chelates, held in soluble form, should correct the problem [32, 33]. In animals, Iron is a constituent of hemoglobin. Body iron content is regulated by the amount absorbed. The absorption is influenced by body stores and by the amount and type of iron in ingested foods. The RDA for adults is 15 milligrams [39]. It is a vital component of many enzymes; it can promote resistance to disease and prevent fatigue. A deficiency can cause anemia, resulting in impaired concentration, reduced physical performance and work capacity, and decreased immune function. Ascorbic acid is necessary for the proper assimilation of iron. The best natural sources of iron are sea vegetables, clams, cockles, mussels, oysters, yeast, molasses, beans, nuts, seeds and cereals. Tea, coffee, bran and phytates decrease iron absorption. There are no reported cases of toxicity from foods but iron poisoning may occur from ingesting large amounts of medicinal iron supplements [38, 39].

Copper, manganese and zinc function in essential enzyme systems in both plants and animals [31]. Manganese is present in all plants and animals and essential element in nutrition [27]. In plants, it is involved in enzyme activity for photosynthesis, respiration, and nitrogen metabolism. Deficiency in young leaves may show a network of green veins on a light green background similar to an iron deficiency. In the advanced stages the light green parts become white, and leaves are shed. Brownish, black, or grayish spots may appear next to the veins. In neutral or alkaline soils plants often show deficiency symptoms. In highly acid soils, manganese may be available to the extent that it results in toxicity [32, 33]. Manganese is an essential component of numerous enzymes involved in bone formation and in the metabolism of amino acids, lipids, and carbohydrates. Its deficiency has been reported in animals but rarely in human. RDA for manganese is established as 2.3 mg/day for men and 1.8 mg/day for women [34, 40]. A deficiency can cause poor reproductive performance, growth retardation, abnormal formation of bone and cartilage, and an impaired glucose tolerance [38].

Zinc is an essential micronutrient for animals, plants and microorganisms. They accumulate considerable amount of Zn in their system without any damaging effect [31]. It is a component of enzymes or a functional cofactor of a large number of enzymes including auxins (plant growth hormones). It is essential to carbohydrate metabolism; protein synthesis and internodal elongation (stem growth). Deficient plants have mottled leaves with irregular chlorotic areas. Zinc deficiency leads to iron deficiency causing similar symptoms. Deficiency occurs on eroded soils and is least available at a pH range of 5.5 - 7.0. Lowering the pH can render zinc more available to the point of toxicity [32].

The RDA is 15 milligrams per day for men and 12 milligrams per day for women. Recent research suggests that men have a higher need for zinc than do women. Thus, it is appropriate that the RDA is sex-specific for zinc. Zinc is an essential trace element that must be supplied in the diet of human beings so that growth and health can be maintained. It is necessary for protein synthesis and the metabolism of vitamin A; it helps the healing process of internal and external wounds, decreases cholesterol deposits and promotes mental awareness. A deficiency can cause loss of appetite, growth retardation and immunological abnormalities [38, 39].

Nickel has just recently won the status as an essential trace element for plants according to the Agricultural Research Service Plant, Soil and Nutrition Laboratory in Ithaca, New York [32]. It is required for the enzyme unease to break down urea to liberate the nitrogen into a usable form for plants. Nickel is required for iron absorption. Seeds need nickel in order to germinate. Plants grown without additional nickel will gradually reach a deficient level at about the time they mature and begin reproductive growth. If nickel is deficient plants may fail to produce viable seeds. According to some study [39], nickel is now quite firmly established as an essential nutrient, but no Recommended Dietary Allowance (RDA) or Estimated Safe and Adequate Intake (ESADI) has yet been set for nickel**.** Research showed that nickel was to be found in blood and tissues at quite consistent levels, that it is associated with DNA and RNA in amounts that suggest physiological significance, and that it occurs also in blood in amounts that appear biologically meaningful. The danger of nickel toxicity from food appears to be very low, since large amounts of nickel are required to produce any toxic effects through ingestion. In contrast to this, in some literature nickel is grouped with toxic metals; Pb and Cd [35]. Because contact with nickel or nickel salts cause skin irritations and cancer.

Cobalt is required for nitrogen fixation in legumes and in root nodules of nonlegumes. The demand for cobalt is much higher for nitrogen fixation than for ammonium nutrition. Deficient levels could result in nitrogen deficiency symptoms [32]. It is a part of vitamins $B_{12,}$ an essential vitamin in animal nutrition. Its the normal range and deficiency range in plants in ppm dry basis is 0.01 - 0.67 and < 0.01, respectively. While its dietary requirement for animals μg/g dry basis 0.07 - 0.11 and its toxicity level is not mentioned [28].

Chromium is another micronutrient for both plants and animals. In human nutrition the chromium requirement is estimated to be in a range of 50 micrograms to 200 micrograms per day. Trivalent chromium is required for maintaining normal glucose metabolism. Evidence shows that chromium improves glucose tolerance. Diabetes and coronary heart disease are associated with low chromium concentrations in human tissue. The chemical forms of chromium in foods are not known with certainty, but the bioavailability of chromium compounds has been found to be high in brewer's yeast, shellfish, whole wheat bread and mushrooms. An increased incidence of bronchial cancer has been associated with exposure to dusts containing chromate. But the carcinogenicity of certain chromates bears no relevance to the nutritional role of non-toxic trivalent chromium [37].

In contrast to these essential macro and micronutrients, elements like Pb and Cd are toxic. Of course the exact harmful effects of lead on animals are poorly understood. Nearly half the quantity of Pb, which enters the body through food, water, air and beverages, is retained in the system. Some of the toxic effects of lead accumulation in the body are; (1) it combines with the body proteins and inhibits the enzyme systems necessary for the formation of bone marrow, (2) it remains immobilized in bones and gets remobilized during high Ca, metabolism, and (3) children are more susceptible to Pb poisoning, the adult critical dosage is 0.8 μg/L and for children 0.25 μg/L. In mild dosages it causes headache, fatigue and anemia whereas at high dosage it causes nephrite [31].

On the other hand, with the increased use of metals in industry, the daily intake of Cd by human and animals is of the order of 300 – 400 μg/L. however, this situation does not appear to pose serious problem as the retentions of the metal is as low as 2 %. But the toxic effect of Cd pollution will occur if its daily intake increases [41]. The main toxic effects of this element are: hypertension, anemia, retardation of growth, abnormality of spleen or pancreas, heart abnormality, damage of liver and kidney. The metal is also known to increase the bone porosity and inhibit the repair metabolism [31].

1.3. Descriptions of Coffee Varieties Studied

There are large numbers of *C. arabica* varieties that are grown in different part of the country, Ethiopia. Even though, the demarcation between the varieties is not clear and simple, experts or researchers classify Ethiopian coffees based upon the Farmland, Kebele, Woreda or Zones in which they grow [1]. Among the different coffee varieties, which are grown in the country, five coffee varieties, from five different zones, were chosen for the study. The choices of these varieties (zones) were based on different factors: such as their tendency to supply for export, their historical coffee growing tendency, their domestic use, etc.

In general, these zones are the most coffee growing regions for a long period of time. They are also the most coffee suppliers of both unwashed and washed dry coffee beans for domestic use and international market (or export) [10].

1.3.1. Coffee Types

Wollega Coffee. It is cultivated in highland of west Ethiopia and is valued for its flavor and its large bean size. It includes coffee varieties that are grown in two zones of the country (East and West Wollega zones).

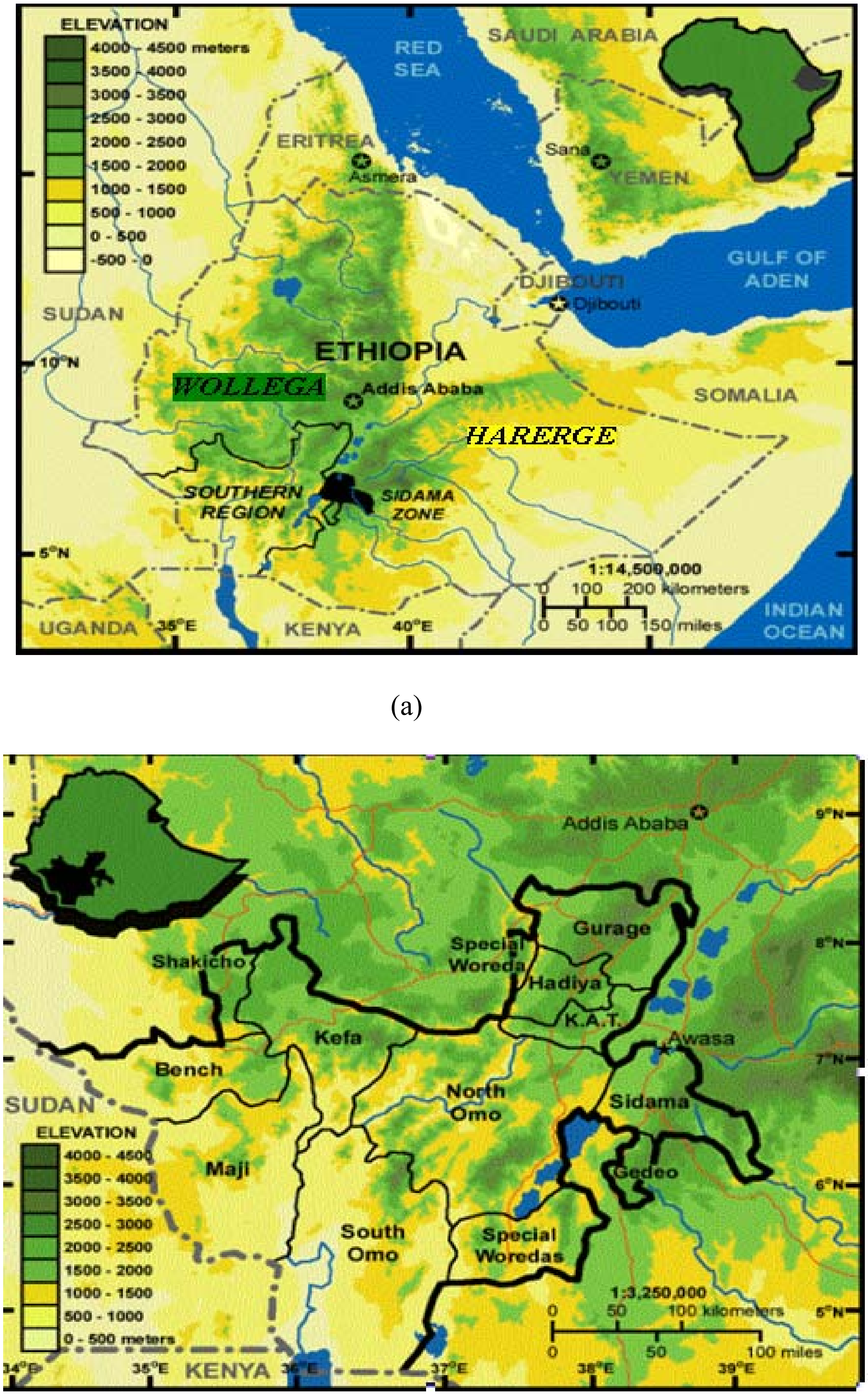
Sidamo and Bench Maji Coffee Types. These are coffee varieties that are grown in the south and south western, high and low lands, part of the country, respectively. Beans of these coffee varieties are medium size and greenish color.

Harar Coffee. It is the most common coffee type that is produced in the Eastern highlands of Ethiopia. It is one of the finest premium coffees in the world and is grown at an altitude range of 2000 - 2750 meters.

Kafa Coffee. It is the coffee type that is grown in southwestern Ethiopia, which is the historical area of coffee origin. Professionals classify this type coffee in to high and low land coffees. The geographic origins where these coffee varieties are growing are shown in Figure 4.

1.3.2. Sample Collection Sites

Coffee samples were collected with the help of Coffee Quality Inspection and Liquoring Center, Addis Ababa, Ethiopia. The choice of site was made on the distance of farm site and availability of different coffee varieties grown in the country. The ways of naming coffee varieties samples studied is the same as the naming that has been given by Coffee Quality Inspection and Liquoring Center.



(a)

(b)

Figure 4. Map of coffee growing area in Ethiopia (a) Ethiopia and (b) Southern region.

1.4. Objectives of the Study

1.4.1. General Objectives

The main objective of this project is to determine the extent of the accumulation of essential and toxic metals in raw and roasted indigenous coffee varieties produced in Ethiopia.

1.4.2. Specific Objectives

1. To collect samples of dry raw coffee bean varieties representing different coffee growing regions, from coffee quality control and liquoring center, Addis Ababa, Ethiopia.
2. To determine selected nutrient composition: both essential (K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr,) and toxic metals (Pb, Ni, Cd,) using flame atomic absorption spectrometer (FAAS).
3. To compare the levels of metals in some *Coffea arabica* varieties cultivated in the different parts of the country.
4. To compare the levels of the metals in raw and roasted indigenous coffee varieties in Ethiopia.
5. To determine the effect of roasting on the metallic composition of coffee varieties.
6. To compare the level of metals in Ethiopian coffee varieties with the level of metals in other countries' coffee (such as Brazil, Nigeria, India, etc…).

2. EXPERIMENTAL

2.1. Instrumentation and Apparatus

Automated moisture measuring apparatus, Dicey-John (Multi-Grain, Germany); Coffee Roasting Machine, PROBAT-WERKE BRZ-4 (Von Gimborn Machinenfabrik GmbH, 1997, Germany); a blending device (Moulinex, France) was used for grinding and homogenizing of both raw and roasted coffee samples; quick-fit round bottom flasks (150 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the powdered raw and roasted coffee samples. **BUCK SCIENTIFIC MODEL 210VGP** (East Norwalk, USA) and **SPECTRAA. 20 PLUS** (Australia) Atomic Absorption Spectrophotometers equipped with deuterium ark background correctors were used for analysis of the analyte metals (K, Ca, Fe, Zn, Cu, Co, Cr, and Cd) and (Mg, Mn, Ni and Pb), respectively.

2.2. Chemicals, Reagents and Standard Solutions

Chemicals and reagents that were used in the analysis are all analytical grades. 70 % HNO_3, (SpectrosoL, BDH, England) and 70 % $HClO_4$ (Analar$^{®,}$ BDH, England) were used for digestion of coffee samples. Lanthanum nitrate hydrate (99.9 %, Aldrich, Muwaukee, USA) was used for releasing calcium and magnesium from the sample. Stock standard solutions of the metals (K, Ca, Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd) (1000 mg/L, PURO-GRAPHIC^{tm} calibration standards, BUCK SCIENTIFIC prepared as nitrates for each element in 2% HNO_3) and for Mg Spectrol$^{®}$ $^{(}$BDH Chemicals Ltd, England), $Mg(NO_3)_2$, standard solution were used for the preparation of calibration curves for the determination of metals in the samples. Deionized water was used for cleaning of glassware and dilution of sample solutions.

2.3. Procedure

2.3.1. Collection and Pretreatment of Coffee Beans

A total of 10 unwashed dry coffee bean samples, 5 for roasted and 5 for raw, were collected from these zones (1 roasted and 1 raw sample from each zone). Names of these zones with their corresponding coffee types and moisture contents of coffee samples collected are given in Table 3.

Table 3. Names of zones with their corresponding coffee types and moisture contents of coffee samples collected.

No.	Name of zone	Coffee type	High/low land	Moisture (%)
1	Wollega	Wollega coffee	High land	8.6
2	Sidamo	Sidamo coffee	High land	9.8
3	Harar (Harargie)	Harar (Harargie) coffee	High land	9.1
4	Kafa	Kafa coffee	High/low land	9.5
5	Bench Maji	Bench Maji coffee	Low land	9.9

2.3.2. Sample Preparation

2.3.2.1. Roasted Coffee

About 100 g of each coffee samples were roasted by using coffee roasting machine, PROBAT-WERKE BRZ-4 (Von Gimborn Machinenfabrik GmbH, 1997, Germany), at the site where samples were collected. All the roasting process was carried out by an expert on coffee roasting from Coffee Quality Inspection and Liquoring Center (Addis Ababa, Ethiopia). Temperatures and the time needed for roasting of coffee samples are given in Table 4.

Table 4. Temperatures and time needed for roasting of coffee samples.

Coffee type	Wollega coffee	Sidamo coffee	Harar coffee	Kafa coffee	Bench Maji coffee
Temp (°C)	180	180	175	160	195
Time (min.)	7	7	6	7	7

All roasted samples were kept in polyethylene plastic bags until they were ground by blending device in the Laboratory.

For pre-concentration of analytes, the procedure developed by Suseela *et al.* [11] was tested for digestion of powdered roasted coffee samples with a mixture of concentrated 70 % HNO_3, 70 % $HClO_4$ and 30 % H_2O_2. Different modifications of this procedure were tested by varying different parameters (Table 7). The optimum procedure which was used in the analysis of roasted coffee samples was carried out as follows: A 0.5 g of powdered roasted coffee samples was added into a round bottom flask (150 mL). To this flask 4.0 mL HNO_3 (70 %) and 1 mL $HClO_4$ (70%) were added and the mixtures were digested on a micro Kjeldahl digestion flask by setting the temperature dial at 9 (approximately 350 °C) for 2 h. Then, after addition of 1.0 mL HNO_3 (70 %) and 0.5 mL $HClO_4$ (70%) the digestion was continued for two more hours. After a total of 4 h digestion, the residue of the digest was allowed to cool by leaving it for about 30 min in an open air. After it was cooled it was cooled about 40 mL of deionized water was added, to dissolve the precipitates formed on cooling and minimize the dissolution of a filter paper by the digest residue while filtrating with Whatman®, (110 mm, dia), filter paper. The filtrate of the sample was diluted to 50 mL with deionized water. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS.

2.3.2.2. Raw Coffee

All raw coffee samples were ground using a blender device in the laboratory. For the preconcentration of analytes, the same optimized digestion procedure as that used for the roasted samples was followed.

2.3.3 Analytical Procedure

2.3.3.1. Determination of metals in the coffee samples

For the determination of metals in both raw and roasted coffee samples, four series of standard metal solutions (Table 5) were prepared by diluting the stock solutions of the metal with deionized water. A blank (deionized water) and standards were run in flame atomic absorption spectrometer (BUCK SCIENTIFIC MODEL 210GP and SPECTRAA. 20 PLUS) and four points of calibration curve were established. Sample solutions were each aspirated into the AAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample. The operating conditions of AAS employed for each analyte are given in Table 6.

Table 5. Series of working standards for determination of metals in raw and roasted coffee. varieties using flame atomic absorption spectrometer

No	Element	Concentration of standards, in mg/L	Correlation Coefficient
1	Ca	1.00, 2.00, 4.00, 8.00	0.96
2	K, Mg	0.50, 1.00, 2.00, 4.00	0.99 (for both)
3	Fe, Zn	0.10, 0.50, 1.00, 5.00	0.94, 0.96, respectively.
4	Mn, Ni, Pb	0.05,0.10, 0.20, 0.40	0.99, 0.98, 0.97, respectively.
5	Cr, Co, Cu	0.01, 0.05, 0.10, 0.50	0.96, 0.98, 0.97, respectively.
6	Cd	0.005, 0.010, 0.020, 0.040	0.95

Table 6. Instrumental operating conditions for determination of metals using flame atomic absorption spectrometer.

Element	Ca	Cd	Cr	Co	Cu	Fe	K	Mg	Mn	Ni	Pb	Zn
λ	422.7	228.9	357.9	240.7	324.8	248.3	766.5	285.2	279.5	232.0	217.0	213.9
DL	0.01	0.005	0.05	0.05	0.02	0.03	0.01	0.001	0.01	0.04	0.10	0.005
SW	0.7	0.7	0.7	0.2	0.7	0.2	0.7	0.5	5.0	0.2	1.0	0.7
LC	2.0	2.0	2.0	4.5	1.5	7.0	2.0	4.0	0.2	4.0	5.0	2.0

λ; Wave length (nm), DL; Detection limit (mg/L), SW; slit width (nm) and LC; Lamp current (mA)

2.3.4. Evaluation of Analytical Figures of Merit

2.3.4.1. Accuracy and precision

Accuracy and precision are probably the most often quoted terms to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and to attempt to establish the probable limits of errors of these values [42, 43]. The analyst will thus be concerned with the question of precision (repeatability of results), that is, the agreement between a set of results for the same quantity; and also with accuracy, that is the difference between the measured value and the true value of the quantity, which is determined [43]. In this study the precision of the results were evaluated by the standard deviation of the results of triplicate samples ($n = 3$), analyzed under the same condition. Standard deviation is a useful parameter in estimating and reporting the probable size of indeterminate errors. On the other hand, the accuracy and validity of the measurement were determined by analyzing spiked samples using BUCK SCIENTIFIC standard solutions.

The procedure of spiking was as follows: for the determination of the validity of the developed optimized procedures used for determination of metals in raw and roasted coffee bean samples, known concentration of standard solutions (that is 100 mg/L of Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn and 10 mg/L of Cd) were prepared. From these solutions based upon the amount that make the concentration the final solution 0.30 mg/L (Ca, K & Mg), 0.20 mg/L (Cr, Co, Cu, Fe, Mn, Ni, Pb and Zn) and 0.02 mg/L (Cd); 0.15, 0.10 and 0.10 mL, respectively, were added to 0.50 g of raw and roasted coffee samples. Then they were digested with the developed digestion procedures for both raw and roasted coffee samples. After diluting the spiked samples to the required volume with deionized water, they were analyzed with the same procedure followed for the analysis of coffee samples. Triplicate samples were prepared and triplicate readings were obtained. The results are given in Table 8.

2.3.4.2. Determination of detection limits

Detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank [44, 45] or the minimum concentration that can be detected by the analytical method with a given certainty [45]. For a measurement, detection limit can be properly estimated from the standard deviation of several blank determinations [46]. There are numerous ways of determining detection limits of a given measurement [44]. 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 [13, 36, 44]. In this study the detection limit of each element was calculated as three times the standard deviation of the blank ($3\sigma_{blank,}$ $n = 5$), which is summarized in Table 9.

2.3.4.3. Determination of limits of quantitation

Limit of quantitation (or limit of determination) is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. An acceptable level of precision is typically 10 to 20 % of relative standard deviation depending upon the concentration level measured. However, in the absence of specified precision, the limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank [44]. Limit of quantitation is the lowest limit for precise quantitative measurements [45]. The quantitation limit of each element was calculated as ten times the standard deviation of the blank ($10\sigma_{blank,}$ $n = 5$). The results are given in Table 9.

3. RESULTS AND DISCUSSION

3.1. Moisture Determination

In reporting concentration of metals in both raw and roasted coffee samples determination of their dry mass were necessary [26]. Their metal compositions are reported based upon their dry masses. 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 using an automated apparatus (section 2.1). The moisture content of the five dry coffee sample varieties varies from 8.6 % to 9.9 % (Table 3 and Figure 5).

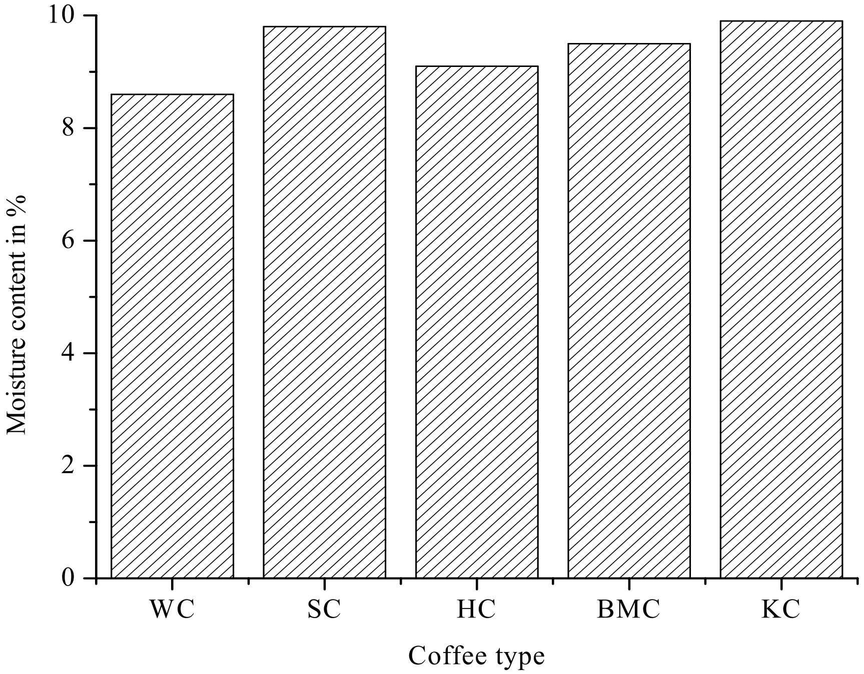


Figure 5. Moisture contents of dry coffee samples. (WC = Wollega coffee, SC = Sidamo coffee, HC = Harar coffee, BMC = Benchi majii coffee and HK = Kafa coffee).

3.2. Site Selection for Collection of Samples

All of the samples were collected from Coffee Liquoring and Inspection Center, Addis Ababa, Ethiopia, which is the only place where all coffee varieties in the country are available. For this study, Coffee Liquoring and Inspection Center, was chosen for the above-mentioned reason and for its closeness or simplicity for transportation. Coffee samples collected from this center are those that are widely used domestically and exported to the different part of the world [3]. The choice of the coffee varieties to be analyzed was based upon many parameters, such as altitude, geographical origin, domestic usage, and amount of their supply for the export. Based on these reasons a total of five coffee varieties from different coffee growing regions were collected to see particularly, the geographical and latitudinal impact on the metal compositions of Ethiopian coffee varieties. For these reasons three coffee varieties that are grown in the different highland parts of the western, southern and eastern part of the country, that is in Wollega, Sidamo and Harar, respectively, were collected for the analysis of their metal contents in both the raw and the corresponding roasted coffee samples. On the other hand, to see the effect of lowland on the metal content of Ethiopian coffee the two highly coffee growing lowlands, Kafa and Benchi Maji coffee varieties that are grown in the southwestern part of the country, were collected. Of course, Kafa coffee can be classified to both high and lowland coffee varieties.

In general, the varieties collected, have been cultivated for a long period of time in comparison to other coffee varieties that have been grown in the different parts (zones) of the country. Thus, the analysis of these types of coffee varieties will indicate the metal composition of the indigenous coffee varieties that are grown in Ethiopia.

3.3. 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, eleven procedures were tested for digestion of both raw and roasted coffee samples (Table 7).

The optimized procedure was selected depending upon:

- clarity of digests
- minimal reflux time/digestion time
- minimal reagent volume consumption
- absence of undigested coffee samples
- simplicity and
- acceptable use of masses of coffee samples.

Based upon these criteria, the optimal digestion procedure chosen was the one that requires 4 h for complete digestion of 0.50 g of coffee powders, (both raw and roasted coffee), with 5 mL HNO_3 (70 %) and 1.5 mL $HClO_4$ (70 %) (Table 7). This procedure was developed with some modification of literature procedure which was used to measure the trace metal content in different brands of coffee products available in Indian market by FAAS [11]. However, the other tested procedures have some limitation to fulfill the stated criteria. They require higher reagent volume, longer digestion time and large masses of samples. Also they result in the formation of turbid digests and colored digest solutions.

Table 7. Procedures Tested for Digestion of Roasted Coffee Samples.

No	**Amount of coffee sample**	**Reagent added**	**Temp. Kjeldahl apparatus (fixed at)**	**Total digestion time (h)**	**Nature of the digest after filtration**
1	1 g	3.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	8	3.0	Turbid
2	1 g	3.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	8	4.5	Clear but pale yellowish color
3	0.5 g	6.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	8	4.5	Clear but pale yellowish color
4	0.5 g	6.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	8	4.5	Clear but pale yellowish color
5	0.5 g	6.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%) 2.0 mL H_2O_2 (30%)	8	4.5	Clear but pale yellowish color
6	0.5 g	5.0 mL HNO_3 (70 %) 1.0 mL $HClO_4$ (70%)	9	3.0	Clear but pale yellowish color
7	0.5 g	5.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	9	4.0	Clear and colorless (Optimum)
8	0.5 g	4.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	9	4.0	Clear and pale yellow
9	0.5 g	9.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	9	4.5	Clear but weak pale yellow color
10	0.5 g	8.0 mL HNO_3 (70 %) 1.5 mL $HClO_4$ (70%)	9	5.5	Clear and colourless
11	0.5 g	6.0 mL HNO_3 (70 %) 1 mL H_2SO_4 (70 %) 1.5 mL $HClO_4$ (70%)	9	5.0	Turbid

NOTE: In all cases the residue of the digest contains white precipitates, which was dissolved upon addition of water.

3.4. Validation of Optimized Procedure

The accuracy of the optimized procedure was evaluated by analyzing the digests of spiked samples for both raw and roasted coffee samples. The recoveries of metals in the spiked coffee samples were 85 % to 115 % and 84 % to 120 % for raw and roasted coffee samples, respectively. The results are given in Table 8 and Figure 6. Generally, good recoveries were obtained for all metals, (particularly in raw coffee samples, for metals like Cd, Mn, Pb and Zn; and in roasted coffee samples for Cr, Mg and Pb). Thus, the optimized procedure has good accuracy.

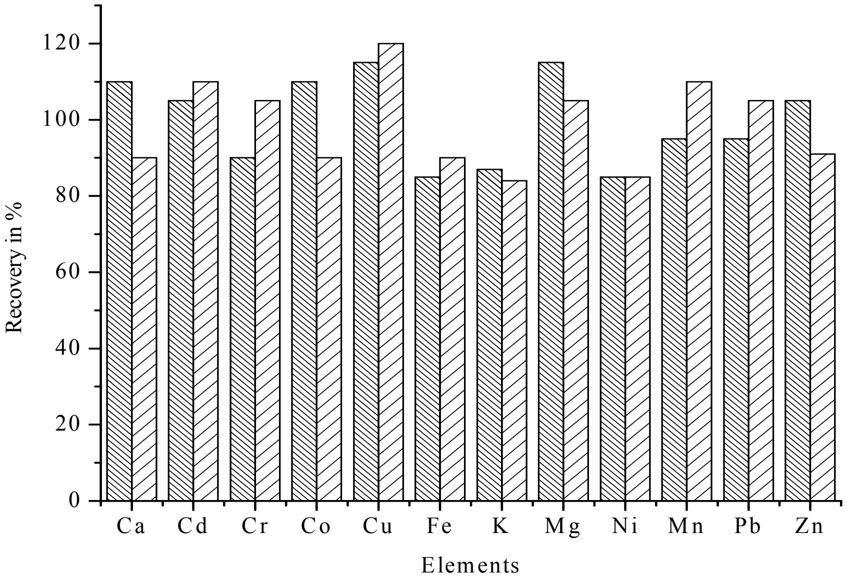


Figure 6. Recoveries of metals in raw and roasted coffee samples. (▩ raw coffee ▨ roasted coffee).

Table 8. Analytical results obtained for validation of the optimized procedure.

Element	**Sample type**	**Amount added (mg/L)**	**Amount found (mg/L)**	**Recovery (%)**
Ca	Raw	0.30	0.33 ± 0.02	110 ± 2.7
	Roasted	0.30	0.27 ± 0.01	90 ± 3.5
Cd	Raw	0.020	0.021 ± 0.002	105 ± 2.6
	Roasted	0.02	0.018 ± 0.002	110 ± 2.6
Cr	Raw	0.20	0.17 ± 0.01	90 ± 3.2
	Roasted	0.20	0.21 ± 0.02	105 ± 3.0
Co	Raw	0.20	0.20 ± 0.01	110 ± 2.5
	Roasted	0.20	0.19 ± 0.01	90 ± 3.5
Cu	Raw	0.20	0.23 ± 0.03	115 ± 2.5
	Roasted	0.20	0.24 ± 0.03	120 ± 3.5
Fe	Raw	0.20	0.17 ± 0.01	85 ± 2.5
	Roasted	0.20	0.18 ± 0.02	90 ± 2.4
K	Raw	0.30	0.26 ± 0.03	87 ± 3.5
	Roasted	0.30	0.24 ± 0.02	84 ± 3.5
Mg	Raw	0.30	0.23 ± 0.02	115 ± 2.3
	Roasted	0.30	0.21 ± 0.01	105 ± 2.5
Ni	Raw	0.20	0.17 ± 0.01	85 ± 2.4
	Roasted	0.20	0.17 ± 0.01	85 ± 2.1
Mn	Raw	0.20	0.19 ± 0.02	95 ± 3.1
	Roasted	0.20	0.22 ± 0.02	110 ± 3.2
Pb	Raw	0.20	0.19 ± 0.01	95 ± 2.8
	Roasted	0.20	0.21 ± 0.01	105 ± 2.3
Zn	Raw	0.20	0.21 ± 0.01	105 ± 2.5
	Roasted	0.20	0.18 ± 0.02	91 ± 3.2

3.5. Determination of Metals

The optimized digestion procedure requires addition of 5.0 mL HNO_3 (70 %) and 1.5 mL $HClO_4$ (70 %) for the mineralization of 0.5 g of raw and 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.

Finally, all the twelve metallic elements in each digested blank were analyzed in triplicate with FAAS using external calibration graphs as described in the experimental section. Then the limits of detection and quantitation were calculated as three and ten times the standard deviation of the blank ($3\sigma_{blank}$ and $10\sigma_{blank,}$ $n = 5$), respectively. The values of limits of detection and quantitation for each element are summarized in Table 9. It is likely that the large volume (6.5 mL) of the mixture of reagents (HNO_3 and $HClO_4$) used in the digestion of coffee samples could have contributed to the high observed method detection and quantitation limits. Despite such high blank values the concentrations of analytes in the samples were above both method detection and quantitation limits, except for Cd and Pb.

Similarly, concentrations of these metals in the digests and diluted solutions of both raw and roasted coffee samples were determined with FAAS employing external calibration graphs. Concentrations of major and trace metallic elements are given in Table 10 and 11, for both raw and roasted coffee samples, respectively.

Table 9. Method Detection and Quantitation Limits, (n = 5, DLM =3σ_{blank} and MQL = 10$\sigma_{blank,}$ in mg/kg), for both raw and roasted coffee samples.

Element	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Ni**	**Pb**	**Zn**
MDL	0.07	0.01	0.07	0.06	0.08	0.04	0.07	0.11	0.02	0.04	0.05	0.10
MQL	0.2	0.04	0.2	0.2	0.3	0.1	0.2	0.4	0.05	0.1	0.2	0.3

Note. DLM = Method detection limit and MQL = Method quantitation limit.

The concentrations of these elements in different coffee types were varied in both raw and roasted coffee samples. The ranges of macro elements (Ca, Ka and Mg) in raw and roasted coffee were found to be (mg/g): 0.12 – 0.71, 6.01 – 9.00, 5.09 – 5.49 and 0.50 – 0.99, 14.31 – 19.40, 5.47 – 6.08, respectively (Table 10). On the other hand, the ranges of the concentration of trace metals, (Cr, Co, Cu, Fe, Mn, Ni and Zn), (Table 11), in raw coffee were found to be (mg/kg)): 0.21 – 0.28, 2.60 – 8.40, 10.50 – 22.90, 26.20 – 43.0, 13.00 – 19.00, 1.0 – 2.50 and 3.80 – 21.10, respectively; and in the corresponding roasted coffee the ranges were found to be (mg/kg): 0.43 – 0.56, 5.80 – 19.30, 13.00 – 27.60, 37.30 – 47.50, 15.00 – 20.00, 1.00 – 3.50 and 6.00 – 30.40, respectively.

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 three raw coffee sample varieties (Wollega, Sidamo and Harar coffee). In the remaining two varieties its concentration was found to be below the detection limit of the method. In the contrary, in the corresponding roasted coffee samples, its concentration was below the detection limit of the method for the all coffee varieties studied. Similarly in all raw and roasted coffee samples the concentration of Pb was found to be below the detection limit, with exception of in Kafa coffee type (for both raw and roasted), in which its concentration was 0.06 and 0.07 mg/kg, respectively and in roasted Benchi maji coffee 0.06 mg/kg. Although nickel was observed in almost all samples, in both raw Benchi maji and Kafa coffee samples its concentration was below the detection limit of the method. Concentrations of major and trace metallic elements are given in Table 10 and 11, for both raw and roasted coffee samples, respectively.

Table 10. Mean concentration (X ± SD, n = 3, mg/g dry weight) and relative standard deviation (RSD %) of major elements in raw and roasted coffee samples.

Element	**X ± SD /RSD**	**WC**		**SC**		**HC**		**BMC**		**KC**	
		RC	**ROC**	**RC**	**ROC**	**RC**	**ROC**	**RC**	**ROC**	**RC**	**ROC**
Ca	**X ±SD**	0.71 ± 0.05	0.79 ± 0.02	0.88 ± 0.01	0.93 ± 0.06	0.90 ± 0.01	1.38 ± 0.02	1.00 ± 0.01	1.05 ± 0.05	1.25 ± 0.07	1.53 ± 0.06
	RSD	7.0	2.5	1.1	6.4	1.1	1.4	1.0	4.8	5.6	4.0
K	**X ±SD**	14.1 ± 0.70	16.1 ± 1.04	14.5 ± 0.30	16.1 ± 0.8	13.9 ± 0.90	14.9 ± 1.1	17.0 ± 1.02	19.4 ± 0.97	13.0 ± 0.10	14.3 ± 1.0
	RSD	5.0	6.5	2.1	4.8	6.5	7.4	6.0	5.0	0.8	7.0
Mg	**X ±SD**	1.67 ± 0.03	1.67 ± 0.03	1.67 ± 0.02	1.75 ± 0.07	1.67 ± 0.02	1.77 ± 0.01	1.69 ± 0.10	1.84 ± 0.07	1.69 ± 0.04	1.89 ± 0.02
	RSD	1.8	1.8	1.2	4.0	1.2	0.6	0.6	3.8	2.4	1.1

Note: WC = Wollega coffee; SC = Sidamo coffee; HC = Harar coffee; BMC = Benchi majii coffee; HK = Kafa coffee; X = Mean; SD = Standard deviation.

Table 11. Mean concentration, (X ± SD, n = 3, mg/g dry weight), and relative standard deviation, (RSD %), of trace elements in raw and roasted coffee samples, (rf = results found with a statement of its uncertainty).

Element	**X ± SD /RSD**	**WC**		**SC**		**HC**		**BMC**		**KC**	
		RC	**ROC**	**RC**	**ROC**	**RC**	**ROC**	**RC**	**ROC**	**RC**	**ROC**
Cd		rf	< 0.01	< 0.01	< 0.01	rf	< 0.01	<0.01	< 0.01	rf	< 0.01
Cr	**X ± SD**	0.21 ± 0.02	0.43 ± 0.03	0.27 ± 0.03	0.55 ± 0.01	0.25 ± 0.01	0.52 ± 0.01	0.26 ± 0.02	0.53 ± 0.02	0.28 ± 0.01	0.56 ± 0.01
	RSD	9.5	7.0	11.1	1.8	4	1.9	7.7	3.8	3.6	1.8
Co	**X ± SD**	6.8 ± 0.02	15.4 ± 0.08	2.6 ± 0.01	5.8 ± 0.03	3.3 ± 0.01	7.5 ± 0.02	3.7± 0.03	8.2 ± 0.03	8.4 ± 0.20	19.3 ± 0.5
	RSD	0.3	0.5	0.4	0.5	0.3	0.3	0.8	0.4	2.4	2.6
Cu	**X ± SD**	18.3 ± 1.3	22.7 ± 1.0	22.9 ± 2.2	27.6 ± 3.0	15.8 ± 1.70	19.6 ± 1.	15.7± 0.6	19.5 ± 1.0	10.5 ± 0.6	13.0 ± 1.0
	RSD	7.1	4.4	9.6	10.9	10.8	5.1	3.8	5.1	5.7	7.7
Fe	**X ± SD**	29.0 ± 1.0	41.0 ± 4.0	26.2 ± 1.5	37.3 ± 1.5	43.0 ± 3.0	46.0 ± 2.0	28.8 ± 2.0	39.9 ± 2.0	44.0 ± 4.0	47.5 ± 5.5
	RSD	3.5	9.8	5.7	4.0	7.0	4.4	6.9	5.0	11.1	11.6
Mn	**X ± SD**	15.0 ± 0.7	15.0 ± 0.7	19.0 ± 1.0	20.0 ± 1.0	13.0 ± 1.0	15.0 ± 0.7	15.0 ± 0.7	17.0 ± 0.7	17.0 ± 0.7	19.0 ± 1.0
	RSD	4.7	4.7	5.3	5.0	7.7	4.7	4.7	4.1	4.1	5.3
Ni	**X ± SD**	2.0 ± 0.01	2.0 ± 0.01	2.0 ± 0.01	2.0 ± 0.01	2.5 ± 0.02	3.5 ± 0.02	< 0.04	1.0 ± 0.01	< 0.04	1.0 ± 0.01
	RSD	0.5	0.5	0.5	0.5	0.8	0.6		1.0		1.0
Pb	**X ± SD**	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
	RSD								16.7	16.7	14.3
Zn	**X ± SD**	12.4 ± 0.05	19.7 ± 0.08	21.1 ± 0.1	30.4 ± 0.5	14.3 ± 0.3	21.3 ± 0.1	8.3 ± 0.5	14.1 ± 0.04	3.8 ± 0.06	6.0 ± 0.05
	RSD	0.4	0.3	0.5	1.6	2.1	0.5	6.0	0.3	1.6	0.8

3.6. Distribution Pattern of Metals in Different Coffee Samples

The distribution and accumulation of metals in coffee beans are a distorted reflection of the mineral composition of the soil and environment in which the coffee plant grows. The soil plant system is highly specific for different, elements, plant species and environmental conditions [28]. Under most conditions, metallic elements that are present in coffee beans must have existed in the rooting zone of the plant, at least in a slightly soluble form. Therefore, the actual metal content of coffee beans (for both raw and roasted) vary considerably according to coffee species, geographic origin, coffee type, the use of fertilizers with different chemical compositions and other characterizing features [23, 27, 28]. Suseela *et al.* [11] and Martin *et al.* [25, 26], have reported that the metal contents of coffee beans is one of the important parameter to differentiate between coffee varieties, arabica and robusta. They have also reported the geographical dependence of the elemental content of coffee beans, which are produced from the same coffee species.

The results of this study showed that the metal contents of coffee beans varied with the geographical origin in which the coffee plant grows. This variation is probably attributed to mineral content of the soil on which the plants grow. Particularly the major elements K varied within the coffee type studied. The highest K content was observed for both raw and roasted Benchi majii coffee and the least K content was in the Kafa coffee for both raw and roasted coffee beans. The higher K content in Benchi majii coffee may probably relate to the contamination of the soil with potassium containing fertilizer(s). Nevertheless, its concentration is not maximum in comparison to other countries coffee composition.

The observed concentrations of Mg were more or less the same in all raw and roasted coffee varieties studied, respectively. Similarly, Ca concentrations were also not significantly varied among the coffee varieties. In both raw and roasted coffee samples the trend in concentration Ca is the same. That is; Kafa > Benchi Maji ≈ Harar > Wollega > Sidamo coffee.

On the anther hand, the variation of concentration in terms of their trace metal composition was also observed, especially, for Co, Cu, Fe, Mn, and Zn. However, the observed change in concentration of major metals Ca and Mg and trace metals; Cd, Cr, Ni, and Pb with the variation of coffee beans geographic origin were insignificant. The variation of metals concentration among the different coffee types studied was summarized in Table 10 and 11.

3.7. Comparison of Metals in Raw and Roasted Coffee Samples

When the concentration of metals in raw and its corresponding roasted coffee beans were compared the roasted coffee beans have relatively higher metal concentrations. The comparative results were presented in Figure 7 for major metals and Figure 8 for trace metals.

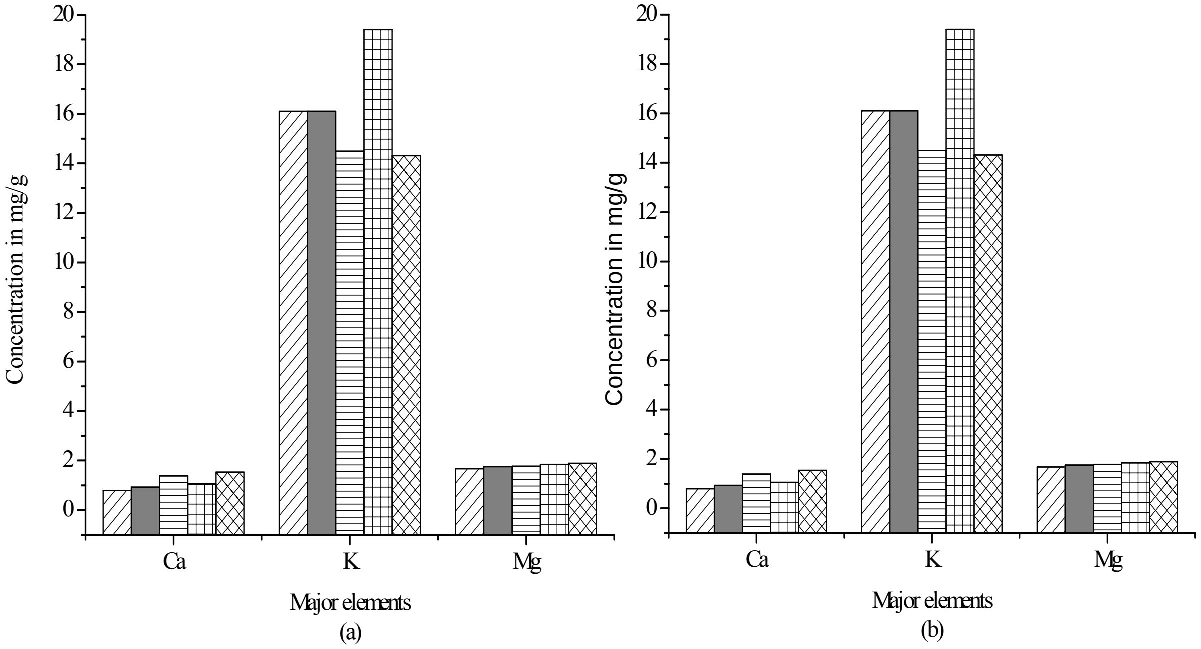
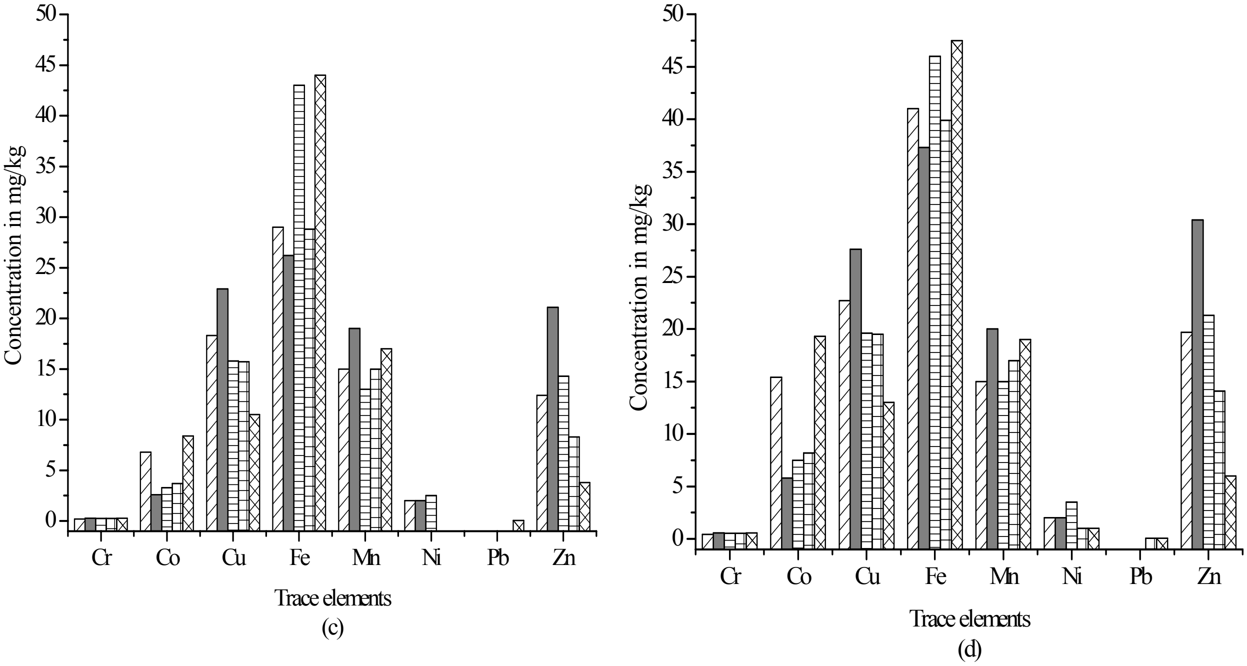


Figure 7. Mean concentrations (n = 3, mg/g, dry weight) of major metals in (a) raw and (b) roasted coffee samples. (Note: For figure (a) and (b) ▨ WC, ■ SC, ☰ HC, ▦ BMC, ▩ KC).

It has been reported in literature that roasting of coffee beans is one of the ways of enriching the concentration of mineral content of coffee because roasting does not change noticeably the mineral content. Instead it increases their relative content by removing water and volatile organic compounds [4, 7]. From the five coffee types studied Benchi majii coffee has relatively highest concentration of K, while Kafa coffee and Harar coffee have the least in concentration of this metal. In both raw and roasted samples the trend in K concentration is the same. That is; Benchi majii > Sidamo ≈ Wollega > Harar ≈ Kafa. However, the concentration of Ca and Mg is more or less the same in all the five coffee samples, except they vary in the raw and roasted samples. In general, in both raw and roasted coffee samples the trend in concentration of major metals is the same. That is; K > Mg > Ca.



(c)

(d)

Figure 8. Mean concentrations, (n = 3, mg/kg, dry weight), of trace metals in (c) raw and (d) roasted coffee samples. (Note: For figure (c) and (d): ▨ WC, ■ SC, ☰ HC, ▦ BMC, ▩ KC).

Of the trace metals, in all coffee samples the concentration of Fe is relatively higher than any other trace element analyzed. But its concentration varies within the coffee varieties analyzed. The highest and the least concentration were observed in Kafa and Sidamo coffee, respectively, for both raw and roasted coffee samples. In general, even though, their concentration is different in almost all raw and roasted samples, the dominant trace metals were Fe, Co, Cu, Mn and Zn. Particularly; Sidamo coffee has relatively higher concentration of Cu, Mn and Zn than the other coffee samples.

The trends of the distribution of the dominant trace metals (Fe, Co, Cu, Mn and Zn) were different in the different coffee varieties studied. Their trends were: for (Cu); Sidamo > Wollega > Harar ≈ Benchi Maji > Kafa coffee, (Fe); Kafa ≈ Harar > Wollega ≈ Benchi Maji > Sidamo coffee, (Co); Kafa > Wollega > Benchi Maji ≈ Harar > Sidamo coffee, (Mn); Sidamo ≈ Kafa > Wollega ≈ Benchi Maji > Harar coffee and Sidamo ≈ Kafa > Benchi Maji > Wollega ≈ Harar coffee in the raw and roasted coffee beans, respectively, and (Zn); Sidamo > Harar > Wollega > Benchi Maji > Kafa coffee. These comparative results were given in Table 11 and Figure 8.

3.8. Comparison of Observed Metals Concentration with the Reported Values

Many researchers have reported the concentration of metals in raw (green) and roasted coffee varieties, which are grown in the different part of the world. Sanatos and Olivira [14], Suseal *et al.* [11], Martin *et al.* [26], Anderson and Smith [27] and Onianwa *et al.* [36] have reported the mineral content of roasted coffee beans that are grown in the different parts of the world; such as Brazil, Colombia Costa Rica, Ivory Cost, Mexico, Nigeria, India, and Uganda. Similarly, Suseal *et al.* [11], and Martin *et al.* [25] have also reported the metal content of raw (green) coffee (from coffee beans) that are grown in different geographic origins. The comparative study of the observed results from this study and reported values are presented in Table 12 and 13 for raw and roasted coffee beans, respectively.

For trace metals; Cd, Co, Ni and Pb, there were no reported literature values to compare their concentrations with the observed result in raw coffee.

Table 12. Comparison of observed metals concentration (mg/kg, dry mass) in raw coffee beans with the reported values.

Element	**Present study**	**Suseal *et al.* [11]**	**Martin *et al.* [25]**
Ca	710 - 1250	869 -1171	990 -1370
Cd	ND	NR	NR
Cr	0.21 - 0.28	0.40 – 1.0	NR
Co	2.6 – 6.8	NR	NR
Cu	11 - 30	12 -20	15 -77
Fe	26 - 43	37 - 57	26 - 55
K	13010 - 17000	14000 -16000	12380 - 182800
Mg	1670 - 1690	1800 - 2000	1720 - 2060
Mn	13 - 19	18 - 59	26 - 39
Ni	2 – 2.5	NR	NR
Pb	< 0.05	NR	NR
Zn	4 - 21	5 - 8	4 - 61

ND: not detected (below detection limit); NR: not reported

Note: Present study, Ethiopian coffee, Suseal *et al.* [11]; reported result of Krival *et al.* on Arabica coffee of different countries (like; Colombia, Costa Rica, Cuba, Mexico, Nicaragua, Panama and Paupa) and Martin *et al.* [25]; Brazil coffee

Table 13. Comparison of observed metals concentration, (mg/kg, dry mass), in roasted coffee beans with the reported values.

Element	**Present study**	**Sanatos and Olivira [14]**	**Suseal** *et al.* **[11]**	**Martin** ***et al.*** **[26]**	**Anderson & Smith [27]**	**Onianwa** ***et al.*** **[36]**
Ca	790 - 1540	1110 - 1890	490 - 791	890 - 1010	934 - 1234	NR
Cd	ND	NR	0.001 - 0.03	NR	NR	0.02 - 0.31
Cr	0.43 – 0.56	ND	0.7 - 0.8	NR	NR	0.89 - 6.98
Co	6 - 19	NR	NR	NR	NR	0.1 - 14
Cu	13 - 28	0.5 - 23	0.4 - 16	13 - 18	13 -18	2 - 9
Fe	37 - 48	14 - 450	16 - 92	50 - 60	12 - 31	6 - 174
K	14310 -19400	32500 - 39800	14000 -29000	14070 - 14660	17500 - 19600	NR
Mg	1870 - 1890	2120 - 4150	2000 - 3100	1730 - 1940	2058 -2347	NR
Mn	15 - 20	4 - 39	7 - 13	27 - 30	19 - 39	NR
Ni	1.0 - 3.5	NR	0.6 - 2.0	NR	NR	0.04 - 2.58
Pb	ND	NR	0.02 - 0.2	NR	NR	0.09 - 0.91
Zn	6 - 30	3 - 15	2 - 9	5 - 29	6 - 8	4 - 14

ND: not detected (below detection limit); NR: not reported.

Note: Present study, Ethiopian coffee, Sanatos and Olivira [14], Brazil coffee, Suseal *et al.* [11]: Indian coffee, Martin *et al.* [26]; Brazil coffee type, Anderson and Smith [27; for different countries coffee, and Onianwa *et al.* [36]; Nigerian coffee.

The results presented in Table 11 and 12 for raw and roasted coffee beans, respectively, the metals content observed are more or less comparable with the reported literature values.

However, relatively lower concentration of K observed in this study in comparison to the reported values. This may probably confirm that Ethiopian coffee is cultivated without the use of fertilizers. In addition, concentration of Cr is lower than the reported results for both raw and roasted samples. The concentrations of Co, Cu, and Ni are slightly higher than the reported values. This might need further analysis to confirm the result. In general, for both raw and roasted, the consistencies of observed results with the reported values give an additional confirmation for the validity of this study.

4. CONCLUSION AND RECOMMENDATION

An efficient digestion procedure was developed and validated through recovery studies. The optimal digestion procedure allows the use of acids with minimum volumes leading to reduced blank values, low detection and quantitation limit. Further more, this condition allowed almost all elements to be analyzed with greater precision and accuracy.

The investigation revealed the dependence of metal accumulation in coffee beans on the geographical origin in which coffee plant grows. Although the data set is relatively small to draw authoritative conclusions about the metals content of raw and roasted Ethiopian coffee beans, the investigation has indicated the presence of macro essential (Ca, K, and Mg) and micro essential (Cr, Co, Cu, Fe, Mn and Zn) elements and provided baseline data for comparison of possible future changes. In addition, this study revealed comparability of the metal component of Ethiopian coffee with coffees of other countries origin. According to this study, the presence of Cd and Pb is negligible in Ethiopian coffee. However, in some literature trace amounts of these metals were reported in other countries coffee varieties.

For this study the samples collected were unwashed dried coffee beans and which were ready for the export. In contrary, there is also washed dried coffee beans, which is highly demanded in the international market because of its more quality. Thus, future studies should be focused on the investigation of metal contents of this type coffee. Moreover, parallel to this; emphases should be placed on the analysis of the soils on which the coffee plant grows. In general, basic and valid research should be conducted on coffee beans to know their nutritional values, since coffee is one of the most common beverage or nutrition to millions of peoples around the world.

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