Metals in green coffee beans from major coffee-growing regions of Ethiopia

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A R T I C L E   I N F O

A B S T R A C T

The metal contents of green coffee beans cultivated in major coffee growing zones of Ethiopia (Wollega, Sidamo, Harar, Bench Maji and Kafa) have been determined in representative samples of the five coffee varieties collected from Coffee Quality Inspection and Liquoring Center located in Addis Ababa, the capital city of Ethiopia. Different sample preparation procedures were tested by varying reagent volumes and types, digestion time, digestion temperature and amount of the sample to decompose the green coffee beans and solubilize the metals. The optimal procedure required 4 h to completely digest 0.5 g of green coffee beans with 5 mL HNO_3 (70%) and 1.5 mL $HClO_4$ (70%) at 270 ᵒC. Concentrations of metals (Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) were determined by flame atomic absorption spectrometer employing a four point external calibration curve. The accuracy of the optimized procedure was evaluated by analyzing sample digests spiked with standard solutions. Recoveries of the spiked samples varied from 90 to 110% in green coffee beans. The metals levels observed in green coffee beans are comparable to literature reports. Cd was not detected in any of the five samples while Pb was detected at trace level in only one of the five samples. This indicated that the Ethiopian green coffee beans are free from the toxic metals. The Pearson correlation coefficients indicated strong to medium positive or negative correlation among the metals in the green coffee beans. The analysis of variance results at 95% confidence level suggested that there were significant difference in the concentrations of all the metals except K between the five sampling areas. Thus, this study has revealed variation of metal composition of green coffee beans with the geographical origin of the coffee verities. The variation in composition among coffee sample might be due the differences in mineral contents of the corresponding soils.

Capsule Summary: The metal contents of green coffee beans cultivated in major coffee growing zones of Ethiopia were determined by flame atomic absorption spectrophotometry. This study indicated that the Ethiopian green coffee beans are free from the toxic metals and variation of metal composition of green coffee beans with the geographical origin.

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INTRODUCTION

It is widely believed that the indigenous coffee trees first grew in Ethiopian province of Kafa and the trees were called "Kafa tree", where the word "coffee" derived from (Research-Extension Task Force of Jimma Research Center, 1996). Coffee is a member of large *Rubiaceae* family, within which it constitutes the *Coffea genus* (Clifford and Willson, 1987). They are further classified in to a number of different species of coffees, based upon their berries size, color, coffee leave color, etc (Clifford and Willson, 1987).

The two main coffee species exploited in the world today are *Coffea arabica* and *Coffea conephora (robusta)*. They account for about 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 (Wellman, 1961; Clifford and Willson, 1987; Coste, 1992). *Coffea arabica* has been known for longest time and it is also the most widespread species. It is the most important commercial coffee species, which is cultivated in about 70% of plantations worldwide (Raina et al., 1998). This ubiquitous species, which originated from and grows in very large stands on the high land plateaus of Ethiopia (1300–1900 m). The size and shape of beans of this type of coffee differ depending upon the varieties, environmental conditions and cropping practices (Coste, 1992).

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 (Exporters - Coffee Cleaners and Graders, 1999; Suseela et al., 2001). Even though, the whole coffees cultivated in Ethiopia are *Coffea arabica* wide variabilities are noted among coffee cultivars in the country (Research-Extension Task Force of Jimma Research Center, 1996; Exporters - Coffee Cleaners and Graders, 1999). The cultivars can be classified based up on the regions or sub-regions and the sizes of the coffee berries and widely identified as Harar coffee, Wollega coffee (includes Nekemt and Gimbi coffee), Jimma coffee, Gedeo coffee, Limu coffee, Sidamo coffee, Kaffa coffee, etc. (Research-Extension Task Force of Jimma Research Center, 1996; Raina et al., 1998). There are a wide range of variability among coffee types in each coffee growing region and woredas (the equivalent of districts). The soil, altitude and climate of the coffee growing areas will have a great influence on coffee characteristics from appearance to chemical content, flavor to aroma (Shalander, 2000).

Coffee is one of the most important agricultural products in the international trade. After crude oil, coffee is the second most valuable commodity; it is worth over 100 billion dollars (Global coffee industry facts, 2017). Coffee represents a commodity of great economic, social and environmental importance to coffee cultivating countries, particularly for developing countries like Ethiopia

(Commission for Environmental Cooperation, 1999). Ethiopia relies greatly on trade of coffee, which generates 60% of its total export earnings (Commission for Environmental Cooperation, 1999).

Coffee is high in health-giving antioxidants and is rich source of anti-cancer agents. Coffee is a complex mixture of potential "nutraceuticals" (Gutu, 1998). Its chemical composition is determined by a complex interaction of agricultural factors and process such as roasting, blending and brewing. Studies have demonstrated that the chemical composition of coffee beans can discriminate the arabica variety from robusta, the country of origin and organic from conventional system of cultivation (ICS Research, 2001).

Coffee contains many classes of compounds such as proteins, carbohydrates, lipids, volatile and non-volatile acids, alkaloids, ash, and water (Coste, 1992). Green coffee contains a total of 3–4.5% ash (Ratnayake et al., 1993), which comprises mostly of K, Na, Ca, Mg, P and S. Numerous trace elements have also been found, including Fe, Al, Cu, I, F, B, and Mn (Coste, 1992). As reported by various researchers (Clarke and Walker, 1974; Ratnayake et al., 1993), the mineral content of green coffee averages about 4% on dry weight basis, with potassium as its main constituent, at about 40% (expressed as the element K).

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 (Gilbert, 1948). Numerous investigators have shown that the normal physical activities are not possible in the absence of minerals (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975). 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 (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975). The first five are essential to both plants and animals, and the last two to animals alone (Gilbert, 1948).

The amount of mineral elements in a plant body varies depending upon their presence in air and soil and other factors such as 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 (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975; Banerji, 2005).

Under most conditions, metallic elements that enter animals are those contained in plants (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975; Banerji, 2005). According to the food and nutrition board, 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 certain levels (Dickinson, 2002). Furthermore, based upon the amount that is required in human nutrition, metallic elements are classified as: (1) bulk mineral elements or macro metals; Ca,

Table 1: Names of zones with their corresponding coffee types and moisture contents

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

Table 2: Procedures for the digestion of green coffee beans samples using a Kjeldahl apparatus

No	Sample mass (g)	Reagent added	Temp. (^{o}C)	Total digestion time (h)	Appearance of the digest
1	1	3.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	240	3.0	Turbid
2	1	3.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	240	4.5	Clear but pale yellowish color
3	0.5	6.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	240	4.5	Clear but pale yellowish color
4	0.5	6.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	240	4.5	Clear but pale yellowish color
5	0.5	6.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%) 2.0 mL H$_2$O$_2$ (30%)	240	4.5	Clear but pale yellowish color
6	0.5	5.0 mL HNO$_3$ (70%) 1.0 mL HClO$_4$ (70%)	270	3.0	Clear but pale yellowish color
7	0.5	5.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	270	4.0	Clear and colorless (Optimum)
8	0.5	4.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	270	4.0	Clear and pale yellow
9	0.5	9.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	270	4.5	Clear but weak pale yellow color
10	0.5	8.0 mL HNO$_3$ (70%) 1.5 mL HClO$_4$ (70%)	270	5.5	Clear and colourless
11	0.5	6.0 mL HNO$_3$ (70%) 1 mL H$_2$SO$_4$ (70%) 1.5 mL HClO$_4$ (70%)	270	5.0	Turbid

Table 3: Analytical results obtained for validation of the optimized procedure

Metal	Amount added (mg/L)	Amount found (mg/L)	Recovery (%)
Ca	0.30	0.33±0.02	110±2.7
Cd	0.020	0.021±0.002	105±2.6
Cr	0.20	0.17±0.01	90±3.2
Co	0.20	0.20±0.01	110±2.5
Cu	0.20	0.23±0.03	115±2.5
Fe	0.20	0.17±0.01	85±2.5
K	0.30	0.26±0.03	87±3.5
Mg	0.30	0.23±0.02	115±2.3
Ni	0.20	0.17±0.01	85±2.4
Mn	0.20	0.19±0.02	95±3.1
Pb	0.20	0.19±0.01	95±2.8
Zn	0.20	0.21±0.01	105±2.5

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 (Onianwa et al., 1999); (3) other metals that have been not definitively

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established as essential to human nutrition, which includes Ni, Rb, Sr, Ti, Te, and W (Santos et al., 2004).

The metallic elements of interest in this study are K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Pb, Ni and Cd. Trace minerals such as Fe, Cu, Mn, Zn, Ni, Cr and Co are essential micronutruient for both plants and animals (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975; Banerji, 2005). These elements combine with vitamins, form enzymes and are necessary for physiological processes. These minerals are found in a broad range of plants and in animal foods, as well as in drinking water (Gilbert, 1948; Galston, 1968; Hewuitt and Smith, 1975; Banerji, 2005). However, few studies report the levels of metallic elements in green coffee beans cultivated in some selected areas in Ethiopia. Getachew and Worku (2014) have determined the levels of essential and toxic metals (K, Mg, Ca, Mn, Cu, Zn, Cd, Pb, and Cr) in the green coffee beans and roasted coffee from Borena Zone, Ethiopia. Mehari et al. (2016) have characterized the cultivation region of Ethiopian coffee based on the contents of Ba, Ca, Cu, Fe, Mg, Mn, P, Si, K, and S. Green coffee is believed to offer a variety of health benefits such as controlling obesity, diabetes, high blood pressure, Alzheimer's disease, and bacterial infections.

Coffee roasting process is known to reduce some chemical constituents while forming others. Considering the production, consumption and its popularity, comprehensive study of the metallic content of green coffee beans from major growing zones of Ethiopia appears to be necessary. The main objective of this study was to: (i) determine the accumulation of essential (K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr) and toxic metals (Pb, Ni, Cd) in the green coffee beans of indigenous coffee varieties from five major coffee growing zones of Ethiopia, (ii) assess inter-regional variability of metals in some *Coffea arabica* varieties cultivated in Ethiopia, and (iii) to compare the level of metals in Ethiopian coffee varieties with those of other countries.

MATERIAL AND METHODS

Instrumentation and apparatus

Automated moisture measuring apparatus (Dicey-John, Germany), a blending device (Moulinex, France) was used for grinding and homogenizing of green coffee beans samples; quick-fit round bottom flasks (150 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the powdered green coffee beans samples. Buck Scientific Model 210VGP (East Norwalk, USA) and Spectraa 20 Plus (Australia) Atomic Absorption Spectrophotometers equipped with deuterium arc background correctors were used for analysis of the metals (K, Ca, Fe, Zn, Cu, Co, Cr, and Cd) and (Mg, Mn, Ni and Pb), respectively.

Chemicals, reagents and standard solutions

Chemicals and reagents used in this study were all analytical grade. Nitric acid (70% HNO_3, SpectrosoL) and perchloric acid (70% $HClO_4$, Analar®) were used for digestion of coffee samples. Lanthanum nitrate hydrate (99.9%, $La(NO_3)_3.xH_2O$,

Aldrich) 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-Graphictm calibration standards (Buck Scientific) prepared as nitrates for each element in 2% HNO3) and for Mg, $Mg(NO3)_2$, standard solution (Spectrol®) 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.

Sampling site selection and collection of coffee verities

There are large numbers of C. *arabica* varieties that are grown in different parts of 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 (Research-Extension Task Force of Jimma Research Center, 1996). Among the different varieties, five coffee varieties from five different zones were chosen for the study. The choice of these varieties was based on different factors, but most notably, adequate supply for export, their historical coffee growing experience, and rate of domestic consumption. In general, these selected zones are historically the most coffee growing for long periods of time, and are also the most suppliers of both unwashed and washed dry coffee beans for domestic use and international markets (Exporters - Coffee Cleaners and Graders, 1999).

The five coffee varieties collected in this study are named after the corresponding farming zones: Wollega, Sidamo, Bench Maji, Harar, and Kafa. Wollega coffee is cultivated in highland of west Ethiopia and is valued for its flavor and its large bean size. Sidamo and Bench Maji coffee varieties are grown in the south and south western high and low lands of the country, respectively.

Beans of these coffee varieties are medium size and greenish in color. Harar coffee 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 of 2000-2750 meters above sea level. Kafa coffee is grown in southwestern Ethiopia, which is historically the origin of coffee. The geographic origins of these coffee varieties are shown in Figure 1.

Coffee samples were collected with the technical guidance of personnel at the Coffee Quality Inspection and Liquoring Center, Addis Ababa, Ethiopia. A total of 5 unwashed dry coffee bean samples, one sample from each zone, were collected from the five selected zones. Names of these zones with their corresponding coffee types and moisture contents of the coffee samples (collected from different zones Wollega, Sidamo, Harar (Harargie), Kafa and Bench Maji) are given in Table 1.

Sample preparation

All the green coffee beans samples were ground using a blending device in the laboratory. For extraction of analytes, the procedure developed by Suseela et al. (2001) was tested for digestion of powdered green coffee beans samples.

Fig. 1: Map of Ethiopia showing the sampling sites (Wollega, Harar, Kafa, Bench Maji and Sidamo)

Digestion of was done using 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 2). The optimum procedure which was used in the analysis of green coffee beans samples was carried out as follows: A 0.5 g of powdered green coffee beans samples was added into a round bottom flask (150 mL). To this flask 4.0 mL HNO3 (70%) and 1 mL $HClO_4$ (70%) were added and the mixtures were digested on a micro Kjeldahl digestion flask by setting the temperature 270 °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 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.

Determination of metals in the coffee samples

For the determination of metals in green coffee beans samples, four series of standard metal solutions 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 and four points of calibration curve were established for each metal.

The correlation coefficients of the calibration curves were very good (>0.999). 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.

Accuracy and precision

Accuracy and precision are probably the most often quoted terms to express the extent of errors in analytical results. Precision was evaluated by the standard deviation of the results of triplicate samples (n = 3) analyzed under the same condition. On the other hand, the accuracy and validity of the measurement were determined by analyzing samples spiked with standard solutions.

Spiking for the determination of the validity of the developed optimized procedures used for determination of metals in green coffee bean samples was done by adding 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) to samples. From these solutions based upon the amount that would make 0.30 mg/L (Ca, K and Mg), 0.20 mg/L (Cr, Co, Cu, Fe, Mn, Ni, Pb and Zn) in the final solution and 0.02 mg/L (Cd); 0.15, 0.10 and 0.10 mL, respectively, were added to 0.50 g of green coffee beans samples. Then they were digested with the developed digestion procedures for green coffee beans 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 3.

Table 4: Detection and quantitation limits, (n = 5, DLM = $3\sigma_{blank}$ and MQL = $10\sigma_{blank}$, in mg/kg), for green coffee beans samples

Metal	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

Table 5: Mean concentration (X ± SD, n = 3, mg/kg dry weight) of elements in green coffee beans from five major coffee growing regions

Metal	Concentration in green coffee beans from region				
	Wollega	Sidamo	Harar	Bench Maji	Kafa
Ca	710 ± 50	880 ± 10	900 ± 10	1000 ± 10	1250 ± 70
K	14100 ± 700	14500 ± 300	13900 ± 900	17000 ± 1020	13000 ± 100
Mg	1670 ± 30	1670 ± 20	1670 ± 20	1690 ± 0.10	1690 ±40
Cr	0.21 ± 0.02	0.27 ± 0.02	0.25 ± 0.01	0.26 ± 0.02	0.28 ± 0.01
Co	6.8 ± 0.02	2.6 ± 0.01	3.3 ± 0.01	3.7± 0.03	8.4 ± 0.20
Cu	18.3 ± 1.3	22.9 ± 2.2	15.8 ± 1.50	15.7± 0.6	10.5 ± 0.6
Fe	29.0 ± 1.0	26.2 ± 1.5	43.0 ± 3.0	28.8 ± 2.0	44.0 ± 4.0
Mn	15.0 ± 0.7	19.0 ± 1.0	13.0 ± 1.0	15.0 ± 0.7	17.0 ± 0.7
Ni	2.0 ± 0.01	2.0 ± 0.01	2.5 ± 0.02	< 0.04	< 0.04
Zn	12.4 ± 0.05	21.1 ± 0.1	14.3 ± 0.3	8.3 ± 0.5	3.8 ± 0.06
Cd	< 0.01	< 0.01	< 0.01	<0.01	< 0.01
Pb	< 0.05	< 0.05	< 0.05	< 0.05	0.06 ± 0.005

Table 6: Pearson correlation coefficients of metals in the green coffee beans

Metal	Correlation between metals									
	Ca	K	Mg	Cr	Co	Cu	Fe	Mn	Ni	Zn
Ca	1.000									
K	-0.167	1.000								
Mg	0.814	0.304	1.000							
Cr	0.827	-0.025	0.541	1.000						
Co	0.390	-0.535	0.397	-0.111	1.000					
Cu	-0.750	0.248	-0.717	-0.277	-0.670	1.000				
Fe	0.594	-0.610	0.234	0.311	0.410	-0.785	1.000			
Mn	0.236	-0.131	0.080	0.503	0.033	0.351	-0.384	1.000		
Ni	-0.766	-0.318	-0.986	-0.507	-0.430	0.633	-0.107	-0.200	1.000	
Zn	-0.677	0.039	-0.832	-0.166	-0.738	0.934	-0.516	0.261	0.794	1.000

Determination of detection and quantitation limits

There are numerous ways of determining detection limits of a given measurement (Bassett et al., 1984; Christian, 2004; Miller and Miller, 2005). A generally accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or the background (Bassett et al., 1984; Christian, 2004; Miller and Miller, 2005). In this study, the detection limit of each element was calculated as three times the standard deviation of the blank (3σblank, n = 5), which is summarized in Table 3.

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. In the absence of specified precision, which is typically set at 10 to 20% of relative standard deviation, the limit of quantitation is the same as the concentration that gives a signal 10 times the standard deviation of the blank (Bassett et al., 1984; Christian, 2004; Miller and Miller, 2005). The quantitation limit of each element was calculated as ten times the standard deviation of the blank (10σblank, n = 5). The results are given in Table 3.

Table 7: ANOVA between and within green coffee beans samples at 95% confidence level

Metal	Source of variation	Degree of freedom	$F_{calculated}$	p-value	$F_{critical}$
Ca	Between samples	4	76.7	0.000	3.48
	within samples	10			
	Total	14			
K	Between samples	4	18.9	0.000	3.48
	within samples	10			
	Total	14			
Mg	Between samples	4	0.529	0.720	3.48
	within samples	10			
	Total	14			
Cr	Between samples	4	7.82	0.000	3.48
	within samples	10			
	Total	14			
Co	Between samples	4	2280	0.000	3.48
	within samples	10			
	Total	14			
Cu	Between samples	4	32.1	0.000	3.48
	within samples	10			
	Total	14			
Fe	Between samples	4	34.2	0.000	3.48
	within samples	10			
	Total	14			
Mn	Between samples	4	22.5	0.000	3.48
	within samples	10			
	Total	14			
Ni	Between samples	2	1250	0.000	5.14
	within samples	6			
	Total	10			
Zn	Between samples	4	1782	0.000	3.48
	within samples	10			
	Total	14			

RESULTS AND DISCUSSION

Moisture determination

In reporting concentration of metals in green coffee beans samples determination of their dry mass were necessary (Martin et al., 1999). 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. The moisture content of the five dry coffee sample varieties varies from 8.6% to 9.9% (Table 1).

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 green coffee beans samples (Table 2). 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 green coffee beans powders, with 5 mL HNO_3 (70%) and 1.5 mL $HClO_4$ (70%) (Table 2). 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 (Suseela et al., 2001). 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.

Validation of optimized procedure

The accuracy of the optimized procedure was evaluated by analyzing the digests of spiked green coffee beans samples. The recoveries of metals in the spiked green coffee beans

Table 8: Comparison of observed metals concentration (mg/kg, dry mass) in green coffee beans with the reported values

Metal	Present study	Martin et al. (1998)	Suseela et al. (2001)	Getachew and Worku (2014)	Mehari et al. (2016)
Ca	710-1250	990-1370	869-1171	910-940	801-1460
Cd	ND	NR	NR	<17.4	NR
Cr	0.21-0.28	NR	0.40-1.0	<61.8	NR
Co	2.6-8.4	NR	NR	NR	NR
Cu	11-23	15-77	12-20	12-13	11.2-26.2
Fe	26-44	26-55	37-57	NR	21.0-66.0
K	13010-17000	12380-182800	14000-16000	10620-10930	14100-19500
Mg	1670-1690	1720-2060	1800-2000	1670-1680	1350-1810
Mn	13-19	26-39	18-59	13-17	20.0-49.0
Ni	<0.04-2.5	NR	NR	NR	NR
Pb	< 0.05	NR	NR	<43.0	NR
Zn	4-21	4-61	5-8	10-11	NR

ND: not detected (below detection limit); NR: not reported.
Note: Present study, Ethiopian coffee. Suseela et al. (2001) reported result on Arabica coffee of different countries (like; Colombia, Costa Rica, Cuba, Mexico, Nicaragua, Panama and Paupa) and Martin et al. (1998) reported on Brazilian coffee. Getachew and Worku (2014) and Mehari et al. (2016) have reported results on Ethiopian coffee.

samples were 90% to 110%. The results are given in Table 3. These recoveries were within the acceptable range (100 ± 10%) (Christian, 2004; Miller and Miller, 2005). Thus, the optimized procedure has good accuracy.

Determination of detection and quantitation limits and concentrations of metals in samples

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 green coffee beans samples. For the determination of the detection and quantitation limits, a blank solution consisting of the mixture of digestion reagents was digested following the digestion procedure and the resulting diluted to 50 mL with deionized water.

Finally, all the twelve 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 4. 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 of green coffee beans samples were determined with FAAS employing external calibration graphs. Concentrations of major and trace metallic elements are given in Table 5.

The concentrations of these elements in different coffee types were varied in the green coffee beans samples. The ranges of macro elements in were found to be (mg/g): Ca 710–1250, K 13010–17000 and Mg 1670–1690. The ranges of the concentration of trace metals were found to be (mg/kg): Cr 0.21–0.28, Co 2.60–8.40, Cu 10.50–22.90, Fe 26.2–44.0, Mn 13.0–19.0, Ni <0.040–2.50 and Zn 3.80–21.10, respectively. The concentration of Cd was found to be below the detection limit of the method in all the five coffee varieties studied. Similarly in all the green coffee beans samples the concentration of Pb was found to be below the detection limit, with exception of in Kafa coffee type in which its concentration was 0.06 mg/kg. Although nickel was observed in almost all samples, in Bench Maji and Kafa coffee

samples its concentration was below the detection limit of the method. Concentrations of major and trace elements are given in Table 5.

Distribution pattern of metals in different coffee samples

The distribution and accumulation of metals in green 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 (Gilbert, 1948). Under most conditions, metallic elements that are present in green 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 green coffee beans vary considerably according to coffee species, geographic origin, coffee type, the use of fertilizers with different chemical compositions and other characterizing features (Gilbert, 1948; Illy, 2002; Anderson, 2002). Suseela et al. (2001) and Martin et al. (1998, 1999) have reported that the metal contents of green 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 green coffee beans, which are produced from the same coffee species.

The results of this study showed that the metal contents of green 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. The major elements K varied within the coffee type studied but not significantly. The highest K content was observed in Bench Maji coffee and the least K content was in the Kafa coffee. Nevertheless, its concentration is comparable to other countries coffee composition.

The observed concentrations of Mg were more or less the same in all the coffee varieties studied. Similarly, Ca concentrations were also not significantly varied among the coffee varieties. In all the green coffee beans samples the trend in concentration Ca is the same. That is; Kafa > Bench 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 green coffee beans geographic origin were relatively less pronounced. The variation of metals concentration among the different coffee types studied was summarized in Table 5.

Pearson correlation of metals in green coffee beans samples

To correlate the effect of one metal concentration on the concentration of the other metal in the green coffee beans samples, the Pearson correlation matrices using correlation coefficient (r) for the samples were used (Miller and Miller, 2005). The correlation coefficients are given in Table 6. It was found that there is high positive correlation of Ca with (Mg and Cr), Cu with Zn and Ni with Zn and moderate

positive correlation of Ca with (Co and Fe) Mg with (Cr and Co), Cr with Mn and Co with Fe, which may arise from common anthropogenic or natural sources as well as from similarity in chemical properties. It was also found that there is high negative correlation of Ca with (Cu, Ni and Zn) Mg with (Cu, Ni and Zn), Co with Zn and Cu with Fe and moderate negative correlation of K with (Co, Fe and Ni), Cr with Ni, Co with (Cu and Ni) and Fe with (Mn and Zn), which indicate that higher absorption of a metal may affect the absorption of negatively correlated metals in the coffee plant. The other metals have showed weak negative or positive correlation indicating that the presence or absence of one metal affect in lesser extent to the other metals.

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a widely used statistical method to compare group means. The one-way ANOVA can compare the mean of more than two groups of samples. ANOVA uses the F-statistic to compare whether the differences between sample means are significant or not (Miller and Miller 2005). During the processes of sample preparation and analysis of green coffee beans, random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, to determine whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc., or other variations in cultivation procedures). The ANOVA results (Table 7) showed that there exist statistically significant differences at 95% confidence level in mean concentrations of all the metals except K which is expected from variation in experimental procedure. The source for this significant difference between sample means may be the difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by the coffee plant. For K the difference between samples mean is not significant. The variation among results of K in five green coffee samples should not be attributed to anything more than random error in the analytical procedure.

Comparison of observed metals concentration with the reported values

Many researchers have reported the concentration of metals in green coffee beans, which are grown in the different part of the world. Suseela et al. (2001) and Martin et al. (1998) have also reported the metal content of green 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 8. For trace metals; Cd, Co, Ni and Pb, there were no reported literature values to compare their concentrations with the observed result in green coffee beans.

The results presented in Table 8 indicate that the metals content observed are more or less comparable with the reported literature values. However, relatively lower

concentration of K was 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 the green coffee beans samples. The concentrations of Co, Cu, and Ni are slightly higher than the reported values. Getachew and Worku (2014) have reported much higher concentration of Cd, Pb and Cr in the Ethiopian green coffee beans. Such higher concentrations have not been reported in green coffee beans from any country. It appears that there might be a mistake their calculations or there is something wrong in their analysis. This might need further analysis to confirm the result. In general, for green coffee beans, the consistencies of observed results of present study with the reported values give an additional confirmation for the validity of this study.

CONCLUSIONS

An efficient procedure for the digestion of Ethiopian green coffee varieties was developed and validated through recovery studies. The optimal digestion procedure was suitable for the digestion of the five green coffee varieties, allowed the use of minimum volumes of acids leading to reduced blank values, low detection and quantitation limits and greater precision and accuracy. This study revealed the dependence of metal accumulation in Ethiopian green coffee beans on the geographical origin of the varieties. This study further indicated the occurrence of macro essential (Ca, K, and Mg) and micro essential (Cr, Co, Cu, Fe, Mn, Ni and Zn) elements at adequate quantities and provided baseline data useful for the study of future trends. In addition, this study revealed comparability of the metal content of the Ethiopian green coffee verities with coffees of other countries. Cd was not detected in any of the five samples while Pb was detected at the very trace level in only one of the five samples compared to trace amounts reported for coffee varieties from other countries. The Pearson correlation coefficients indicated strong to medium positive or negative correlation among the metals in the green coffee beans. The analysis of variances results at 95% confidence level suggested that there were significant differences in the mean concentrations of all the metals except K among the five sampling areas which could be attributed to differences in mineral contents of the corresponding soils or pH of soils which predict the extent of mineral absorption by the coffee plant.

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REFERENCES

Anderson, K.A., Smith, B.W., 2002. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agriculture and Food Chemistry 50, 2068-2075.

Banerji, S.K., 2005. Environmental Chemistry, 2nd ed. Prentice – Hall of India: New Delhi.

Bassett, J., Denny, R.C., Jeffery, G.H., Mendham, J., 1984. Vogel's Text Book of Quantitative Inorganic Chemistry, 5th ed. Longman Group Ltd: England.

Christian, G.D., 2004. Analytical Chemistry, 2nd ed. John Wiley and Sons, Inc: USA.

Clarke, R.J., Walker, L.J. 1974. Potassium and other mineral contents of green, roasted and instant coffee. Journal of Science of Food and Agriculture 25, 1389-1404.

Clifford, M.N., Willson, R.C., 1987. Coffee Botany, Biochemistry and Production of Beans and Beverages. Croom Helm Publisher: New York.

Commission for Environmental Cooperation, 1999. Measuring Consumer Interest in Mexican Shade-grown Coffee: An Assessment of the Canadian, Mexican and US Markets. Published by the Communications and Public Outreach Department of the CEC Secretariat: Canada.

Coste, R., 1992. Coffee, The Plant and The Product. MacMillan: London.

Dickinson, A., 2002. Recommended Intakes of Vitamins and Essential Minerals. Council for Responsible Nutrition: Online Publication.

Exporters - Coffee Cleaners and Graders, 1999. Coffee Specialties for Roasters From: Ethiopia. MOPLACO Trading Co. Ltd: Ethiopia.

Galston, A.W., 1968. The Green Plant. Prentice-Hall, Inc.: New York.

Getachew, T., Worku, N., 2014. Determination of essentials and toxic metals in raw and roasted coffee in Bule Hora Woreda, Borena Zone, Ethiopia. International Journal of Research 1, 1386-1411.

Gilbert, F.A., 1948. Mineral Nutrition for Plants and Animals. University – Oklahoma Press Normau: USA.

Global Coffee Facts & Statistics of 2014-2015. Website: http://www.wevio.com/global-coffee-industry-facts-statistics-of-2014-2015/. Accessed on 19 March 2017.

Gutu, S.Z., 1998. Policy Options for Ethiopia's Coffee Exports. Montclair State University: New Jersey.

Hewuitt, E.J., Smith, T.A., 1975. Plant Mineral Nutrition. Fletcher and Son Ltd.: London.

ICS Research, 2001. Chemical Composition of Coffee, Institute for Coffee Studies. Vanderbilt University Medical Center: Nashville Tennessee, U.S.

Illy, E., 2002. The complexity of coffee. Scientific Amemican 286, 86-91.

Martin, M.J., Pablos, F., Gonzalez, A.G., 1998. Characterization of green coffee varieties according to their metal content. Analytica Chimica Acta 358, 177-183.

Martin, M.J., Pablos, F., Gonzalez, A.G., 1999. Characterization of arabica and robusta roasted coffee varieties and mixture resolution according to their metal content. Food Chemistry 66, 365-370.

Mehari, B., Redi-Abshiro, M., Chandravanshi, B.S., Combrinck, S., McCrindle, R., 2016. Characterization of the cultivation region of Ethiopian coffee by elemental analysis. Analytical Letters 49, 2474-2489.

Miller, J.N., Miller, J.C., 2005. Statistics and Chemometrics for Analytical Chemistry, 5th ed. Pearson Practice Hall: England.

Onianwa, P.C., Adetola, I.G., Iwegbue, C.M.A., Ojo, M.F., Tella, O.O., 1999. Trace heavy metal composition of some Nigerian beverages and food drinks, Food Chemistry 66, 275-279.

Raina, S.N., Mukai, Y., Yamamoto, M., 1998. In situ hybridization identifies the diploid progenitor species of *Coffea arabica* (Rubiaceae). Theoretical and Applied Genetics 98, 1204-1209.

Ratnayake, W.M.N., Hollywood, R., O'Grady, E., Starvic, B., 1993. Lipid content and composition of coffee brews prepared by different methods, Food and Chemical Toxicology 31, 263-269.

Research-Extension Task Force of Jimma Research Center, 1996. Recommended Production Technologies for Coffee and Associated Crops. Institute of Agricultural Research: Addis Ababa.

Santos, E.E., Lauria, D.C., Porto da Silveria, C.L., 2004. Assessement of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeerio City. Science of the Total Environment, 329, 69-79.

Shalander, K., Francis, B., Pazo, E.A., Taye, K., Africa, Z., Julius, O., 2000. Research and Development Options for Enhancing Income and Sustainability of Farming Systems in Kafa-Sheka Zone of Ethiopia. International Centre for Development Oriented Research in Agriculture: Wageningen, The Netherlands.

Suseela, B., Bhalke, S., Kumar, A.V., Tripathi, R.M., Sastry, V.N., 2001. Daily intake of trace metals through coffee consumption in India. Food Additives and Contaminants 18, 115-120.

Wellman, F.L., 1961. Coffee: Botany, Cultivation and Utilization. Interscience Publisher: New York.