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ASSESSMENT OF METALS IN ROASTED INDIGENOUS COFFEE VARIETIES OF ETHIOPIA

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ABSTRACT. The metals content (Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) of roasted coffee varieties grown in five different regions of Ethiopia was determined by flame atomic absorption spectrometry. Representative samples were collected from Coffee Quality Inspection and Liquoring Center in the capital city, Addis Ababa, Ethiopia and the metals were extracted by wet digestion. The optimal digestion required 4 hours refluxing at 270 °C on Kjeldhal hot plate with a mixture of 5 mL HNO_3 (70%) and 1.5 mL $HClO_4$ (70%) to completely digest 0.5 g of roasted coffee samples. Recoveries of metals in the spiked samples varied from 90 to 110%. Analysis of variance revealed significant differences in the concentrations of Ca, Co, Cu, Mn, Cr, Ni and Zn with the variation of coffee beans geographic origin. Pearson correlation coefficients indicated high positive correlation among some metals and high negative correlation among others. The amounts of metals that a person can get from two cups of coffee are well below the daily recommended values and drinking two cups of coffee is safe for an adult person and free from the risks of Cd and Pb toxicity.

KEY WORDS: Metals content, Roasted coffee, Indigenous coffee varieties, Ethiopia

INTRODUCTION

It is commonly believed that the indigenous coffee trees first grew in "Kafa" Province in Ethiopia and the trees were called "Kafa tree", which may as well is the root word for the name of coffee [1, 2]. The two main species of coffee exploited in the world at present are *Coffea arabica* and *Coffea conephora (robusta)*. They account for as large as 99% of the world's coffee, of which 70% is *Coffea arabica* type [2]. Other less cultivated species of *Coffea* include *Coffea liberica*, *Coffea abeakutyae*, *Coffea dewevrei*, *Coffea congensis*, etc. Different species of *Coffea genus* have very diverse appearances and behaviors [1, 3-5].

Ethiopia, exclusively cultivates a number of distinctive regional types of *Coffee arabica* [6-8] almost in all the administrative regions of the country. However, the major coffee grower regions are Oromia Regional State and Southern Nations, Nationalities, and Peoples' Region (SNNPR) [2, 3, 6]. There are wide ranges of variability among coffee cultivars in the country and thus, can be classified based on regions or sub-regions of their cultivation and the sizes of coffee berries [7, 8]. Examples of coffee varieties based on areas of their cultivation are; Harar coffee, Wollega coffee, Jimma coffee, Gedeo coffee, Limu coffee, Sidamo coffee, Kaffa coffee, etc. [7, 8]. The soil, altitude and climate of coffee growing areas have a great influence on the variability of coffee from its body (or chemical contents) to its flavor and aroma [9].

Coffee is one of the most important agricultural products in the international trade. Next to petroleum, coffee is the second in value, putting into motion approximately 35 billion dollars per year [10]. It is a commodity of great economic, social and environmental importance to

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coffee cultivating countries, particularly for developing countries, like Ethiopia [1, 7, 10], which generates 60% of its total export earnings [1, 7].

Coffee is the complex mixture of potential "neutriceuticals" [11]. The chemical composition of coffee varies based on species (*arabica* or *robusta*), country of origin (Ethiopia, Brazil, Kenya, etc), system of cultivation (organic or conventional) and its physical form as raw or roasted [12-14]. The most important constituents are minerals, lipids, caffeine, proteins, fats, carbohydrates and water [13, 14].

Roasting is heating process in which the green coffee beans are nearly made ready for grinding and consumption. Roasting greatly increases the chemical complexity of coffee. Green coffee contains about 250 different volatile molecular species, whereas roasted coffee gives rise to more than 800 [13]. The roasting temperature and the way the process is conducted, which may take 5 – 25 minutes, have a considerable effect on the quality of coffee [15, 16].

In several studies, metals content of roasted coffee samples have been analyzed for both arabica and robusta coffee types [10, 13, 17, 18]. The composition of Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn have been used as chemical descriptors to differentiate between roasted arabica and robusta coffee varieties. According to the report on roasted coffee varieties in southeast Brazilian market, there is a difference in metals content among the samples of roasted coffee varieties [17]. These variations in metal compositions among samples of roasted coffee can indicate the differences in the cultivation of the coffee plants; such as the type of soil, the use of fertilizers with different chemical compositions and the ambient conditions [17].

The minerals bioaccumulations within the coffee beans vary with different trace elements, varieties and environment in which coffee grows [17, 18]. The amount of elements in plants, generally, depend on many factors; species, age, root distribution of the plant, physical and chemical nature of the soil, proportions and distributions of elements and the general climatic conditions [20-24]. Under most conditions, metallic elements that enter animals are those contained in plants eaten or used as a beverage either directly or indirectly [19, 25, 26]. Thus, the metal content of edible plants frequently controls the amount of these elements available in different animal bodies.

Some recent studies have reported the mineral contents in roasted coffee. Dos Santos *et al.* [27] have evaluated some metals in Brazilian coffees cultivated during the process of conversion from conventional to organic agriculture. Pohl *et al.* [28] have described different techniques for the determination of the elemental composition of coffee. Nędzarek *et al.* [29] have reported the concentrations of six heavy metals (Mn, Co, Ni, Cr, Ag, Pb) in coffee infusions from eleven samples, roasted and purchased in four countries: Bosnia and Herzegovina, Brazil, Lebanon and Poland. Cuong *et al.* [15] have studied the effect of roasting conditions on concentration of nine elements (K, Mg, Ca, Na, Fe, Cu, Mn, Zn and Pb) of Vietnam robusta coffee.

From the Ethiopian perspective, Ashu and Chandravanshi [30] have reported the concentration of metals (K, Mg, Ca, Na, Mn, Fe, Cu, Zn, Co, Pb, Cd) in three brands of commercially available Ethiopian roasted coffee powders and their infusions. Gebretsadik *et al.* [16] have reported the levels of nine metals (K, Mg, Ca, Na, Mn, Cu, Zn, Cd and Pb) in roasted coffee beans of Yirgacheffe and Sidama, Ethiopia. Mehari *et al.* [31] have characterized the cultivation region of Ethiopian coffee by elemental analysis. These reports clearly indicate that there is limited information on the mineral contents of roasted coffee cultivated in wider areas of Ethiopia.

Therefore the objectives of this study were (i) to determine selected essential nutrients (K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr) and toxic metals (Pb, Ni, Cd) in the roasted indigenous coffee varieties from five different regions of Ethiopia using flame atomic absorption spectrometer (FAAS); (ii) to compare the levels of metals in some *Coffea arabica* varieties cultivated in five different parts of the country; (iii) to compare the level of metals in Ethiopian coffee varieties with the level of metals in the roasted coffee from other countries and (iv) to compare against health regulatory limits of the metals in coffee to provide guideline information on the implication of the consumption of these coffee products.

EXPERIMENTAL

Instruments and apparatus

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 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 roasted coffee samples. Buck Scientific Model 210VGP (East Norwalk, USA) and SPECTRAA 20 PLUS (Australia) Flame Atomic Absorption Spectrometer 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.

Chemicals, reagents and standard solutions

Chemicals and reagents that were used in the analysis were 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%, Sigma Aldrich, Muwaukee, USA) was used for overcoming the loss of Ca and Mg as refractory phosphates and/or sulfates in the flame. Stock standard solutions of K, Ca, Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd (1000 mg element/L, Puro-Graphic™ calibration standards, Buck Scientific prepared as nitrates for each element in 2% HNO_3) and of Mg (1000 mg/L Spectrol® (BDH Chemicals Ltd, England), $Mg(NO_3)_2$, standard solution) were used for the preparation of a series of calibration standards for the determination of metals in the samples. Deionized water was used for cleaning of glassware and dilution of sample solutions.

Description of study area

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 [1, 7, 8]. Wollega coffee is cultivated in highland of west Ethiopia and is valued for its flavor and large bean size. It includes coffee varieties that are grown in three zones of the country Kelem Wollega, East Wollega and West Wollega zones. Sidamo and Bench Maji coffee varieties are grown in the south and south western high and low lands of SNNPR, respectively. Beans of these coffee varieties are medium sized and greenish in color. Harar coffee is the most common coffee type that is produced in the Eastern highlands of the country, in east and west Hararghe zones. It is one of the finest premium coffees in the world and is grown at an altitude of 2000 - 2750 meters. Kafa coffee is also grown in SNNPR of Ethiopia, which is the historical origin of coffee. The choice of these varieties and growing zones was based on different factors such as propensity to supply for export, their historical coffee growing tradition, 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 for domestic use and international markets [1, 32].

Sample collection

Coffee samples were collected from the Coffee Quality Inspection and Liquoring Center, Addis Ababa, Ethiopia. The choice of site was based on availability of the different coffee varieties. A total of five green coffee varieties were collected (100 g composite sample from a multiple subsites). Names of these coffee types and altitude are given in Table 1.

Table 1. Names of coffee types, elevation, moisture content and roasting conditions.

S. No.	Coffee variety	Elevation	Mosture (%)	Roasting temp. (^{o}C)	Roasting time (min)
1	Wollega coffee	High land	8.6	180	7
2	Sidamo coffee	High land	9.8	180	7
3	Harar coffee	High land	9.1	175	6
4	Kafa coffee	High/low land	9.5	160	7
5	Bench Maji coffee	Low land	9.9	195	7

Pretreatment of coffee samples

About 100 g of each coffee sample was roasted using coffee roasting machine at sample collection site. All the roasting process was carried out by an expert coffee roaster from Coffee Quality Inspection and Liquoring Center (Addis Ababa, Ethiopia). The roasting conditions are given in Table 1. All the roasted coffee samples were cooled to room temperature, packaged in polyethylene plastic bags, transported to the laboratory and stored until they were ground by blending device.

Digestion of roasted coffee samples

The method proposed by Suseela *et al.* [33] was tested for digestion of the ground roasted coffee samples using a mixture of 70% HNO_3, 70% $HClO_4$ and 30% H_2O_2. Different attempts were under taken in order to develop another simple alternative procedure that require shorter reflux time/digestion time as well as reduced types and volumes of the reagents. Accordingly, series of procedures involving changes of reagents volume, reagents composition and digestion temperature and digestion time were investigated and the optimal conditions were established. The results for tested modifications of the open-vessel digestion procedure are given in Table 2.

The optimum procedure used in the analysis of the 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 mixture digested on a micro Kjeldahl digestion flask at 270 ^{o}C for 2 h. After addition of 1.0 mL HNO_3 (70%) and 0.5 mL $HClO_4$ (70%), the digestion was continued for additional 2 h. The residue obtained after a 4 h digestion period was allowed to cool down for 30 min in an open air. About 40 mL of deionized water was added to dissolve the residue and to also minimize the dissolution of the filter media while filtrating. The filtrate was diluted to 50 mL with deionized water. The diluted samples were kept in the refrigerator below 4 ^{o}C until analysis by FAAS in about 2-4 weeks.

Table 2. Procedures tested for digestion of roasted coffee samples.

No.	Sample mass (g)	Reagents added 70% of HNO_3, $HClO_4$, and 30% H_2O_2	Digestion temp. (^oC)	digestion time (h)	Appearance of filtered digests
1	1.0	3.0 mL HNO_3, 1.5 mL $HClO_4$	240	3.0	Turbid
2	1.0	3.0 mL HNO_3 1.5 mL $HClO_4$	240	4.5	Clear but pale yellowish
3	0.5	6.0 mL HNO_3 1.5 mL $HClO_4$	240	4.5	Clear but pale yellowish
4	0.5	6.0 mL HNO_3 1.5 mL $HClO_4$ 2.0 mL H_2O_2	240	4.5	Clear but pale yellowish
5	0.5	5.0 mL HNO_3 1.0 mL $HClO_4$	270	3.0	Clear but pale yellowish
6	**0.5**	**5.0 mL HNO_3 1.5 mL $HClO_4$**	**270**	**4.0**	**Clear and colorless (Optimum)**
7	0.5	4.0 mL HNO_3 1.5 mL $HClO_4$	270	4.0	Clear and pale yellow
8	0.5	9.0 mL HNO_3 1.5 mL $HClO_4$	270	4.5	Clear but weakly pale yellow
9	0.5	8.0 mL HNO_3 1.5 mL $HClO_4$	270	5.5	Clear and colorless
10	0.5	6.0 mL HNO_3 1 mL H_2SO_4 1.5 mL $HClO_4$	270	5.0	Turbid

Note: The bold font indicates the optimum condition. In all cases the residue of the digest contains white precipitates, which was dissolved upon addition of water.

Determination of metals in the coffee samples

For the determination of metals in the roasted coffee 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 FAAS 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 FAAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample.

Accuracy and precision

In this study, the precision of the results was evaluated by the standard deviation of the results of triplicate samples (n = 3) analyzed under the same conditions [34]. The accuracy and validity of the measurement were determined by analyzing spiked samples using standard solutions [34].

The procedure of spiking was as follows: for the determination of the validity of the developed optimized procedures used for determination of metals in the 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, suitable amounts that would make the final solution concentration of 0.30 mg/L (Ca, K and 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 roasted coffee samples. The resulting mixtures were digested with the optimum digestion procedure for the roasted coffee samples. After diluting the spiked samples to the required volume (i.e., 50 mL) with deionized water, the metals content was analyzed by FAAS. Triplicate samples were prepared and triplicate readings were obtained.

Determination of method detection and quantitation limits

A general accepted definition of method detection limit (MDL) is the concentration that gives a signal three times the standard deviation of the blank or background signal [9, 34, 35]. In this study the MDL of each element was calculated as three times the standard deviation of the blank ($3\sigma_{blank,}$ n = 5). Method quantitation limit (MQL) is the lowest limit for precise quantitative measurements [36]. The MQL is the same as the concentration that gives a signal 10 times the standard deviation of the blank [34]. The MQL of each element was calculated as ten times the standard deviation of the blank ($10\sigma_{blank,}$ n = 5).

Data analysis

Microsoft office Excel 2007 was utilized for the construction of calibration curves and data analysis. Statistical software SPSS 20 was used for one way analysis of variance (ANOVA) to compare the mean values of the metals among various coffee samples at $p = 0.05$. The degree of positive or negative correlation between the metals was assessed using Pearson correlation coefficient.

RESULTS AND DISCUSSION

Optimization of digestion procedure

A total of ten procedures were tested for digestion of roasted coffee samples by varying reagent volume, reagent composition, digestion temperature and digestion time. The optimized procedure was selected based on: clarity of digests, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested sample particles, simplicity and acceptable use of masses of coffee samples.

Based on these criteria, the optimal digestion procedure required 4 h for complete digestion of 0.50 g of coffee powders with 5 mL HNO_3 (70%) and 1.5 mL $HClO_4$ (70%) at 270 ^oC. This procedure was developed with some modifications of the literature procedure [33] used to measure the trace metal content in different brands of coffee products in Indian market by FAAS. However, the other tested procedures have some limitation to fulfill the stated criteria. All other procedures that required higher reagent volumes, took longer digestion time, accommodate smaller sample mass or produced turbid or colored digests were rejected.

Validation of optimized procedure

The accuracy of the optimized procedure was evaluated by analyzing the digests of spiked samples of roasted coffee. The recoveries of metals in the spiked coffee samples were 90% to 110% which are within the acceptable range (100 ± 10%). Thus the optimized procedure was found to have good accuracy.

Determination of the metals

The concentrations of major and trace metallic elements in the roasted coffee samples are given in Table 3.

Table 3. Mean concentration (X ± SD, n = 3, mg/kg dry weight) of major metals in the roasted coffee samples.

Metal	Wollega coffee	Sidamo coffee	Harar coffee	Bench Maji coffee	Kafa coffee
K	16100 ± 1040^{a}	16100 ± 800^{a}	14900 ± 1100^{a}	19400 ± 970^{a}	14300 ± 100^{a}
Mg	1670 ± 30^{b}	1750 ± 70^{b}	1770 ± 10^{b}	1840 ± 70^{b}	1890 ± 20^{b}
Ca	790 ± 20^{c}	930 ± 60^{d}	1380 ± 20^{e}	1050 ± 50^{f}	1530 ± 60^{g}
Cd	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cr	0.43 ± 0.030^{h}	0.55 ± 0.01^{i}	0.52 ± 0.01^{i}	0.53 ± 0.02^{i}	0.56 ± 0.01^{i}
Co	15.40 ± 0.08^{j}	5.80 ± 0.03^{k}	7.50 ± 0.02^{l}	8.20 ± 0.03^{l}	19.30 ± 0.50^{m}
Cu	22.70 ± 1.00^{n}	27.60 ± 3.00^{o}	19.60 ± 1.00^{p}	19.50 ± 1.00^{p}	13.00 ± 1.00^{q}
Fe	41.00 ± 4.00^{r}	37.30 ± 1.50^{r}	46.00 ± 2.00^{r}	39.90 ± 2.00^{r}	47.50 ± 5.50^{r}
Mn	15.00 ± 0.70^{s}	20.00 ± 1.00^{t}	15.00 ± 0.70^{s}	17.00 ± 0.70^{s}	19.00 ± 1.00^{t}
Ni	2.00 ± 0.01^{u}	2.00 ± 0.01^{u}	3.50 ± 0.02^{v}	1.00 ± 0.01^{w}	1.00 ± 0.01^{w}
Pb	< 0.05	< 0.05	< 0.05	0.06 ± 0.01	0.07 ± 0.01
Zn	19.70 ± 0.08^{x}	30.40 ± 0.50^{y}	21.30 ± 0.10^{x}	14.10 ± 0.04^{x}	6.00 ± 0.05^{z}

The same letter indicated that the values were not significantly different at p = 0.05, according to Duncan's multiple range test.

The concentrations of these elements in different coffee types were varied in the roasted coffee samples. The ranges of macro elements (Ca, K and Mg) were found to be: 1530 ± 60 - 790 ± 20, 14300 ± 1000 - 19400 ± 970, 16700 ± 30 - 1890 ± 20 in mg/kg, respectively (Table 3). On the other hand, the ranges of the concentration of trace metals, (Cr, Co, Cu, Fe, Mn, Ni and Zn) (Table 3) found to be: 0.43 ± 0.03 - 0.56 ± 0.01, 5.80 ± 0.03 - 19.3 ± 0.50, 13.0 ± 1.00 - 27.60 ± 1.00, 37.3 ± 1.50 - 47.5 ± 5.50, 15.0 ± 0.70 - 20.0 ± 1.00, 1.00 ± 0.01 - 3.50 ± 0.02 and 6.00 ± 0.05 - 30.40 ± 0.50 in mg/kg, respectively. Cd concentration was below the MDL for the all coffee varieties studied. Similarly, Pb was also found to be below the MDL, with exception of Bench Maji and Kafa coffee in which its concentration was 0.06 and 0.07 mg/kg, respectively.

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 [31]. The soil plant system is highly specific for different elements, plant species and environmental conditions [22, 24, 26]. Under most conditions, metallic elements 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 vary considerably according to coffee species, geographic origin, coffee type, the use of fertilizers with different chemical compositions and other characterizing features [13, 19]. Suseela *et al.* [33] and Martin *et al.* [17, 18] have reported that the metal contents of coffee beans are 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.

One way analysis of variances (ANOVA) at 95% confidence level was used to compare the mean values of metals in the five varieties of coffee samples. As shown in Table 3, the one way ANOVA study revealed that there were no significant differences between the five coffee bean samples in terms of their content of K, Mg and Fe. However, the studied coffee samples exhibited significant differences in the concentrations of Ca, Co, Cu, Fe, Mn, Cr, Ni and Zn with the variation of coffee beans geographic origin.

Pearson correlation

To assess the correlation between the metals' concentrations in the roasted coffee samples, the Pearson correlation coefficient (r) was used [34]. As can be seen in Table 4, the values of Pearson correlation coefficient indicated that there are weak, moderate, and strong positive and/or negative correlations between the metals. The weak negative and weak positive correlation indicates that the availability or absence of one metal has less effect on the other. But, the observed high positive correlation for Mg with Co; Ca with Co and Fe; Cr with Mn; as well as Co with Fe might be due to their common anthropogenic or natural sources. The high negative correlation observed for Mg and Cu, Ni and Zn; Ca with Cu; Co with Cu and Zn; and Cu with Fe may indicate different sources of the metals.

Table 4. Pearson correlation matrices for metals in roasted coffee samples (n = 5).

	K	Mg	Ca	Cr	Co	Cu	Fe	Mn	Ni	Zn
K	1.00									
Mg	0.01	1.00								
Ca	-0.69	0.57	1.00							
Cr	-0.36	0.42	0.12	1.00						
Co	-0.47	0.87	0.78	0.63	1.00					
Cu	0.28	-0.88	-0.87	-0.16	-0.86	1.00				
Fe	-0.66	0.52	0.99	0.02	0.71	-0.86	1.00			
Mn	-0.09	0.12	-0.31	0.91	0.25	0.25	-0.41	1.00		
Ni	-0.40	-0.74	0.10	-0.62	-0.51	0.32	0.17	-0.59	1.00	
Zn	0.07	-0.97	-0.70	-0.25	-0.86	0.96	-0.68	0.10	0.57	1.00

Comparison of observed metals concentration with the reported values

Many researchers have reported the concentration of metals in the roasted coffee varieties, which are grown in the different part of the world. For instance, the mineral content of roasted coffee beans that are grown in the different parts of the world; such as Brazil [10, 18], different countries including Colombia, Costa Rica, Ivory Cost, Mexico, and Uganda [19], Nigeria [35], and India [33] have been reported. The comparative study of the observed results from this study and reported values are presented in Table 5.

Table 5. Comparison of metals concentration, (mg/kg, dry mass) in Ethiopian roasted coffee beans with the reported values.

Metal	Ethiopian coffee (present study)	Brazilian coffee [10]	Indian coffee [33]	Brazilian coffee [18]	Different countries coffee [19]	Nigerian coffee [35]
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	13-8	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.

The results presented in Table 5 shows that the metals content observed in the roasted coffee beans in this study are more or less comparable with the reported literature values. However, relatively lower concentration of K is 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 in the roasted samples. The concentrations of Co, Cu, and Ni are slightly higher than the reported values. In general, the consistencies of observed results with the reported values give an additional confirmation for the validity of this study.

Daily intake of metals from roasted coffee

Daily intake of metals from the roasted coffee was calculated based on the assumption that an adult person consumes an average of 5 g dry roasted coffee (two cups of coffee) per day and is given in Table 6.

The amounts of all the metals that a person can assimilate from two cups of coffee are well below the daily recommended values. This indicates that consumption of 5 g of the roasted coffee, which is equivalent to two cups of coffee is safe for an adult person. It also indicates that drinking two cups of coffee daily can be a good source of the essential metals but not sufficient enough to meet the daily requirement. Therefore, a consumer must get supplementary intake of essential metals from other sources. Since the levels of Cd and Pb in the roasted coffee samples are almost negligible, it is possible that consumers may be free from the risks of Cd and Pb as a result of drinking two cups of coffee prepared from the roasted coffee per day.

Table 6. Comparison of daily intake of metals from the roasted coffee with recommended daily intake and tolerable upper limit of daily intake of metals [37, 38].

Metal	(mg/kg) from roasted coffee	(mg) from 5 g roasted coffee	Daily recommended intake	Tolerable upper limit
Ca	790-1530	3.95-7.65	1000-1200 mg	2500 mg/day
Mg	1670-1890	8.35-9.45	320-420 mg	750 mg/day
K	14300-19400	71.5-97.0	4700 mg	ND*
Cr	0.43-0.56	0.00215-0.0028	25-35 µg	120 µg/day
Cu	13.0-27.6	0.065-0.138	0.9-2 mg	10 mg/day
Fe	37.3-47.5	0.1865-0.2375	10-15 mg	45 mg/day
Mn	15.0-20.0	0.075-0.100	1.8-2.3 mg	11 mg/day
Ni	1.00-2.00	0.005-0.010	70-170 µg/kg*	1 mg/day
Zn	6.00-30.40	0.030-0.152	10-15 mg	40 mg/day
Cd	< 0.01	<0.0005	ND	7 µg/kg bw/week
Pb	< 0.05-0.07	<0.00025-0.00035	0.02-3 µg/kg bw	25 µg /kg bw/day
Co	5.80-19.30	0.029-0.0965	5-40 g /day*	0.25 mg/day

*Indicates the estimated daily intake, bw = body weight, ND = not determined.

CONCLUSION

An efficient procedure for digestion of roasted coffee was developed and validated through recovery studies, which reduced blank levels through use of minimum acid volumes, and resulted in low detection and quantitation limits, and created analytical conditions with greater precision and accuracy. Although, the data set is relatively small to draw authoritative conclusions about the metals content of 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. Except for K, Mg and Fe, the metals exhibited variations with the geographic origin of coffee samples. The observed positive correlations of Mg with Co; Ca with Co and Fe; Cr with Mn; as well as Co with Fe, could be due to common anthropogenic or natural sources. On the other hand, negative correlation observed for Mg and Cu, Ni and Zn; Ca with Cu; Co with Cu and Zn; and Cu with Fe might be indicative of a different source of these metals. Negligible amounts of Cd and Pb are present in Ethiopian coffee relative to coffee varieties from some other countries. The study revealed that drinking two cups of coffee per day is safe for an adult person and free from the risks of Cd and Pb toxicity.

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