Analytical Chemistry: An Indian Journal

Analytical Chemistry
ISSN: 0974-7419

Research Article|Vol21 Iss 3

Levels of Essential and Non-Essential Metals in Coffee Beans and Soils of the Three Major Coffee Producing Woredas of Gedeo Zone

Wondimeneh Dubale*

Department of Chemistry, School of Graduate Studies, Dilla University, Dilla, Ethiopia

***Corresponding author**: Wondimeneh Dubale, Department of Chemistry, School of Graduate Studies, Dilla University, Dilla, Ethiopia, E-Mail: chemobose@yahoo.com

Received: February 20, 2021; **Accepted:** February 29, 2021; **Published:** March 29, 2021

Abstract

The levels of nine essential (K, Ca, Mg, Mn, Cu, Zn, Co, Ni and Cr) and two non-essential (Cd and Pb) metals were determined in coffee beans (from farmer's farms and washing industries) and soil samples using flame atomic absorption spectroscopy. The samples were collected from the three major coffee producing woredas of Gedeo zone, namely Kochere, Yirgacheffee and Wonago. Effective optimum microwave digestion procedure was developed for coffee bean samples. The efficiency of the optimized procedure was validated by spiking experiment and the percentage recoveries for entire metals lie within the range of 90%-106%. Soil samples were digested with slight modification of EPA 3050B acid digestion method. In all coffee bean samples the concentrations of macroelements were higher than microelements. Among the macroelements, K was determined to be the highest followed by Mg and Ca. On the other hand, among the trace microelements, Cu quantified in the maximum level followed by Mn, Zn, Co, Ni and Cr, respectively. Toxic metals Pb and Cd were not detected in coffee beans of Gedeo zone. Like coffee beans, soil samples also contained highest amount of macroelements than microelements with maximum and minimum concentration of Mg and Cd, respectively. Pb was below method detection limit in all soil samples. The concentrations of metals in coffee beans from farmer's farms and washing industries were not significantly different at 95% confidence level within a single woreda. The levels of heavy metals in coffee bean samples were found to be below the maximum permissible limits set by FAO/WHO for different food items and herbal plants.

Keywords: Coffee bean; Essential metals; Non-Essential metals; FAAS; Microwave digestion

Introduction

The right way to put samples in the chromatographic tank is also necessary if it's not put in the right way it can affect results. Coffee is one of the most popular and widely consumed beverages in the world, having extensive commercial as well as social importance [1,2]. It is also one of the most important agricultural products in the international trade [3], putting into motion approximately US$ 35 billion per year and being surpassed only by petroleum [1]. In addition to its wide consumption in beverage form, lately its use as an ingredient in some food processing industries is increasing day by day. For instance, it is used as a flavoring to various pastries, ice-creams, chocolate, etc [4].

There are many different species of coffee in the world; however two main species are specially cultivated for commercial production around the world. These are commonly known as *Coffea arabica* and *Coffea canephora* [5]. Coffea arabica, known as Arabica coffee, accounts for 75%-80% of the world's production and over 90% of the market. *Coffea canephora*, known as Robusta coffee, accounts for about 20% and differs from the Arabica coffees in its taste [3,6]. Coffea arabica is the only species occurring in Ethiopia and is geographically isolated from the rest of the Coffea species [7].

Coffee is produced in more than 70 countries. For the countries, it is the major source of foreign currency earnings as well as a significant proportion of tax income and gross domestic product; furthermore, it supports the livelihoods of millions of rural families [8]. The coffee sub-sector play very great role in Ethiopian economy and it is also important in terms of providing income for a large number of households: it is estimated that between 7.5 and 8 million households depend on coffee for a considerable share of their income and provides jobs for many more people in coffee-related activities (e.g. coffee processing, transporting or

Citation: Dubale W. Levels of Essential and Non-Essential Metals in Coffee Beans and Soils of the Three Major Coffee Producing Woredas of Gedeo Zone. Anal Chem Ind J. 2021;21(3):156

marketing). It is estimated that the sub-sector impacts on approximately 15% of the population, and around 20% of the land area [9].

Living organisms (including plants, animals and microorganisms) store and transport metals so as to get appropriate concentration for later uses in physiological reactions as well as a means of protection against the toxic effects of the metals [10]. Mineral elements play critical role in building body tissue and regulating numerous physiological processes. They are thus essential constituents of enzymes and hormones; regulate a variety of physiological processes, and are required for the growth and maintenance of tissues and bones [11].

Gedeo zone is known throughout the world by its coffee, especially Yirgacheffee coffee beans which have special taste and aroma. In the international market, Yirgacheffee coffee beans have a trademark name with the rights owned by Ethiopia. As a result, costs and consumptions of Yirgacheffee coffee beans were greatly increased over the past few years. However, a recent literature survey revealed that no report was found on the levels of essential and non-essential metals in coffee beans (from farmer's farms and coffee washing industries) of Gedeo zone. Hence, it was worthwhile investigating the levels of metals in coffee beans of the zone. Furthermore, the results of the current findings would provide information about the levels of metals in the soils of the zone.

The objectives of this study were: (i) to determine the level of essential (K, Ca, Mg, Cu, Mn, Zn, Co, Ni and Cr) and non-essential (Cd and Pb) metals in coffee beans from farmer's farms and coffee washing industries of the three woredas, (ii) to compare the levels of metals in coffee beans from farmer's farms and coffee washing industries within each woreda and (iii) to correlate the concentration of metals in the supportive soil under coffee plant with that of coffee bean.

Materials and Methods

Equipments and apparatus

Stainless steel soil sampling auger (Oakfield Apparatus Company, Oakfield, USA), ceramic mortar and pestle, electric motor grinder (Retsch, GmbH & Co. KG Type ZM 1, Hann 1, Germany), digital analytical balance (ADAM, Model AFP-110L, England) with ± 0.0001 precision, round bottom flask (100 mL) fitted with a reflux condenser, borosilicate volumetric flasks (25, 50 and 100 mL), pipettes (Pyrex, USA), micropipettes (Dragonmed, 1-10 L, 100-1000 L, Shangai, China), microwave digester (Buck Scientific Model BMS 1, USA) and flame atomic absorption spectrophotometers (Buck Scientific Model 210VGP, USA) equipped with deuterium arc back ground correctors and hollow cathode lamps with air-acetylene flame were used in this study.

Reagents and chemicals

Chemicals and reagents used for the analysis were all analytical grade. The reagents and chemicals used in this study were: HNO_3 (68%-70%), H_2O_2 (30%), HCl (37%) (UNI-CHEM® chemical reagent, China), 0.1% LaCl3.H2O (UNI-CHEM® chemical reagent, China), stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mg, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, Pd and Cd (Buck Scientific Puro-Graphic, USA).

Apparatus cleaning

Apparatus such as volumetric flasks, measuring cylinders, digestion flasks and all other necessary apparatus used for the experiment were washed with detergents and tap water, rinsed with deionized water, soaked in 2% nitric acid for 24 hrs, rinsed with deionized water five times, dried in oven (Model N50C, England) and kept in dust free place until analysis begins.

Sample collection

Coffee beans (from farmer's farms and coffee washing industries) and soil samples were collected from the three woredas during the main coffee harvesting season. Coffee bean samples were collected as follows: from a single woreda eight kebeles were selected based on their coffee producing capacity. Again from a single kebele eight farmers were selected based on their coffee producing capacity. Then after, from a single farmer farm a minimum of five coffee plants were used for sampling. Finally the whole samples were homogenized to form one representative coffee bean sample of a single woreda.

On the other hand, coffee washing industries were selected based on their washing capacity per a day and the number of farmers who provides their coffee cherry to the industries. For the current work, from a single woreda five washing industries were selected and coffee bean samples were collected. Then after, the whole samples were homogenized to form one representative sample for each woreda.

Soil samples were systematically collected from five different sites under each sampled coffee plant at 75 cm radius and 30 cm depth. Since the investigation was concerned with possible uptake of essential and non essential metals by coffee plants, then samples were collected from the whole area that the root system of the plant penetrates. Finally, the samples were collected into non reacting polyethylene bags and thoroughly mixed to form one composite sample for each woreda and were taken to the laboratory.

Preparation of coffee bean samples

For sampling coffee beans, red cherries during their main harvesting time were carefully selected and collected. To make pulping and grading easier, only ripe red cherries were collected, (i.e. the entire cherry after harvest was first cleaned to separate the unripe, overripe and damaged cherries and to remove dirt, soil, twigs and leaves). Clean and washed bags were

used to collect the beans. The collected coffee cherries were dried in full sun on a hard, flat and clean surface such as raised tables. Drying was used to remove moisture from the coffee bean in a slow continuous process as it takes up to 4 weeks before the cherries were dried to the optimum moisture content, depending on the weather conditions. The coffee beans were then removed from the dried husk. After grinding the dried coffee beans, 50 g was used for analysis. Finally, the powdered coffee bean samples were kept in polyethylene plastic bags until time of analysis. Coffee bean samples from washing industries were also prepared in the same way as the beans from farmer's farms.

Preparation of soil samples

Soil samples were systematically collected from eight different sites in each kebele at 75 cm canopy radius of coffee plant in 30 cm depth using stainless steel soil sampling auger. Since the investigation was concerned with possible uptake of essential and non-essential metals by coffee plants, then samples were collected from the whole area that the root system of the plant penetrates. After removal of visible pieces of plant residues, the soil samples were air dried and homogenized. The dried soil samples were ground and sieved by using 2 mm nylon sieves. The total amount of soil samples collected from a single woreda provided over 500 g of sieved soil, of which 50 g was used for chemical analysis. Before chemical analysis, the sieved soil sample was further dried in an oven at 50°C for one and half hour to make its moisture content uniform. Finally the samples were stored in sealed polythene containers until analysis.

Optimization of digestion conditions

In order to get clear and colorless solution of coffee digest which is suitable for FAAS analysis different microwave digestion procedures were attempted. Major variables were volume of HNO_3 and H_2O_2; microwave digestion temperature and time. A total of 20 trails were made by varying the above three parameters one after the other. The optimum digestion procedure was selected depending upon: the clarity of digests (solution without any residue and suspended matter), minimal reagent volume, minimal microwave digestion time and temperature. The developed optimum digestion conditions for coffee bean samples were given in **TABLE 1**.

TABLE 1. **Optimum microwave digestion conditions of coffee bean samples.**

Step	**1**	**2**	**3**
Temperature (°C)	145	210	50
Time (min)	5	10	10
Power (W)	85	90	0

Digestion of coffee beans

0.3 g of coffee bean powder was directly weighed in a PTFE digestion vessel, 7 mL of HNO_3 conc. and 2 mL of H_2O_2 conc. were added and the vessels were placed in a fume hood for 10 min for pre-digestion before they were placed on the turntable of the microwave system. Finally, the sample was digested at the optimum conditions. The digestion was carried out in triplicate for each bulk sample. Digestion of a reagent blank was also performed with the same procedure in parallel with the digestion of the samples keeping all digestion parameters the same. Six blanks were digested for coffee beans samples. The digest was allowed to cool at room temperature. 0.1% $LaCl_3.7H_2O$ was added to the digested solution to eliminate the chemical interference of Ca and Mg ions and the solution was then filled to the mark (25 mL) with deionized water. The solutions were stored in the refrigerator until analysis.

Digestion of soil samples

For digestion of soil samples the EPA 3050B [12] method was applied with a very slight modification. The procedure used for the digestion of soil sample was as follows: initially 1:1 ratio of HNO_3 and H_2O (deionized) was prepared. Of which 10 mL was added in a digestion vessel containing 500 mg of the dried and sieved soil sample. The sample was heated to 110°C and refluxed for 25 minutes. Then it was allowed to cool and 5 mL of conc. HNO_3 was added and refluxed for 120 minutes. After 2 hours, due to the appearance of brown fumes, the digestion was repeated by addition of 5 mL conc. HNO_3 twice within 10 minutes interval until no brown fumes were given off by the sample indicating the complete reaction with HNO_3. After the digestion of sample with conc. HNO_3 was completed and the sample has cooled, 3 mL of water (deionized) and 5 mL of 30% H_2O_2 was added. The sample was heated for 90 minutes until excessively vigorous effervescence was disappeared. After cooling, 10 mL conc. HCl was added to the sample and heated at 95°C for 25 minutes. Finally, after completion of digestion with HNO_3, H_2O_2 and HCl, the digestate was allowed to cool, filtered through Whatman No. 42 filter paper and the resulting clear light yellow solution was made up to 50 mL with deionized water. Reagent blanks were also prepared and digested with the same procedure as that of soil sample. All the solutions were stored in tightly capped polyethylene bottles and stored in a refrigerator until analysis.

Calibration procedure and determination of metals

Calibration curves were prepared to determine the concentration of the metals in coffee bean and soil sample solutions. For each metals the calibration curves were made from diluted solutions prepared from stock standard solutions containing 1000

mg/L, in 2% HNO_3, of the metals K, Ca, Mg, Mn, Zn, Cu, Co, Cr, Ni, Cd and Pb. Determination of the metals in coffee bean and soil samples was made by FAAS. To avoid loss through ionization, the concentration of K was determined by emission mode of the instrument. Three replicate determinations were carried out for each metal and the same analytical procedure was employed for determination of elements in blank solutions.

Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice [13]. Since certified standard reference materials were not available, spiking method was used to validate the method. The spiking was performed in four flasks for coffee bean samples. 0.3 g of coffee bean powder sample was taken in four different flasks. To the first flask 200 µL of 1000 mg/L Ca and 300 µL of 1000 mg/L Mg were spiked. 1500 µL of the same concentration of K as in the first flask was spiked to the second flask. The third flask was spiked with 25 µL of 10 mg/L Cu, Mn and Zn. In the fourth flask 15 µL of 10 mg/L of Ni, Co and Cr was spiked. All the spiked samples were digested in triplicate following the optimal digestion procedure developed for coffee bean samples.

Method detection limit (LOD)

Method detection limit is the lowest concentration of an analyte that can be identified, measured and reported with 99% confidence level that the analyte concentration is greater than zero [14]. In this experiment, after digestion of six blank solutions of coffee beans and six blank solutions of soil samples, triplicate reading was obtained for each sample. Then the method detection limit of each element was calculated as three times the standard deviation of the blank (3δ blank, where δ = standard deviation of the blanks).

Statistical analysis

In order to test whether there was a significant difference in metal contents between coffee beans from farmer's farms and washing industries t-tests were performed. Additionally, one way ANOVA was used to check whether there was a significant difference in metal contents within coffee bean and soil samples of the three woredas. Finally, correlation of metals within coffee beans and the supportive soil under coffee plantation was performed. All mathematical and statistical computations were made using Excel 2007 and origin 8 software.

Results and Discussion

Analytical figures of merit

Instrument operating conditions (slit width, lamp current, wavelength and others) were adjusted for maximum signal intensity according to the manufacturer guide for each metal. The instrument operating conditions, method detection limits and correlation coefficients of the calibration curves for the determination of metals in coffee bean and soil samples by FAAS are given in **TABLE 2**.

TABLE 2. **Instrument operating conditions, method detection limits and correlation coefficients of calibration curves for the determination of metals in the samples by FAAS.**

No.	Elements	Instrument operating conditions				MDL (mg/kg) for		Correlation Coefficients (r)
		Wavelength (nm)	Slit width (nm)	Lamp current (mA)	IDL (mg/L)	Coffee bean samples	Soil samples	
1	Mg	285.2	0.7	1	0.001	0.38	0.36	0.9995
2	K	766.5	0.7	-	0.01	0.35	0.38	0.9999
3	Ca	422.7	0.7	2	0.01	0.4	0.35	0.9994
4	Cr	357.9	0.7	2	0.05	0.09	0.1	0.9996
5	Mn	279.5	0.7	3	0.01	0.31	0.33	0.9995
6	Co	240.7	0.2	4.5	0.05	0.06	0.09	0.9992
7	Ni	232	0.2	7	0.04	0.2	0.1	0.9997
8	Cu	324.7	0.7	1.5	0.02	0.33	0.25	0.9999
9	Zn	213.9	0.7	2	0.005	0.17	0.18	0.9998
10	Pd	233.3	0.7	2	0.1	0.2	0.17	0.999

11	Cd	22.8	0.2	7	0.005	0.04	0.05	0.9994

IDL=Instrumental detection limit, MDL=Method Detection Limit

As can be seen from the **TABLE 2**, the method detection limits for all the metals were greater than the instrumental detection limits which clearly indicate that the method is applicable for the determination of metals at trace levels. On the other hand, the correlation coefficients of the calibration curves for the entire metals were greater than or equal to 0.999 which assured the linearity of instrumental response for individual analytes. The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis and calculating the relative standard deviations for each metal. The values of relative standard deviations (%RSD) are less than 10% for all mean concentrations of metals in each sample. Therefore, the precision of results obtained by the current method is very good. In this experiment the efficiency of the optimized procedure was checked by spiking experiment. The spiking was performed in four flasks for coffee bean samples. The spiked samples were then digested using the optimized procedure and calculated the percent recoveries. As given in **TABLE 3**, the recoveries results for metals in coffee bean samples lie within the range of 90%-106 %. This indicates that contamination was not a problem in the digestion procedure and the recovery results were within the acceptable range. Accordingly, the result shows the validity and reliability of the proposed methods of digestion for coffee bean metal content analysis.

TABLE 3. **Recovery test results for the optimized procedure of coffee bean sample.**

No.	Elements	[a]Conc. in sample (µg/g)	Amount added (µg/g)	[a]Conc. in spiked sample (µg/g)	[b]Recovery (%)
1	Mg	1833.00 ± 39.51	600	2395.67 ± 18.58	93.78 ± 5.00
2	K	15042.80 ± 53.30	2500	17315.33 ± 51.25	90.92 ± 3.05
3	Ca	1037.33 ± 23.03	400	1460.00 ± 15.62	105.67 ± 2.18
4	Cr	1.92 ± 0.17	3	4.82 ± 0.21	96.56 ± 1.02
5	Mn	21.13 ± 1.14	5	26.05 ± 1.20	98.27 ± 4.01
6	Co	2.47 ± 0.17	3	5.18 ± 0.04	90.33 ± 4.81
7	Ni	2.43 ± 0.14	3	5.41 ± 0.02	99.33 ± 4.48
8	Cu	23.39 ± 1.27	5	28.28 ± 1.16	97.93 ± 2.70
9	Zn	12.75 ± 1.01	5	17.50 ± 1.11	95.00 ± 6.12

[a]Concentration values are average of three analyzed samples ± standard deviation.
[b]Recovery values are mean ± standard deviation.

Distribution pattern of metals in coffee bean samples

In both coffee samples, among macroelements, K was the most abundant metal followed by Mg and Ca. The highest levels of K in the coffee beans is probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissues and trans-located from old plant tissues to new plant tissues [15]. The other probable reason for higher concentration of K, Mg and Ca is that, the elements are among the major elements required by plants. Among the micro elements determined, Cu was the most accumulated microelement followed by Mn and Zn in coffee bean samples of the three woredas. Higher Cu levels in the beans may be attributed to the higher availability of this micronutrient in the supportive soils under coffee plantation. The trends of variation for the other trace metals is Co>Ni>Cr in coffee beans of the three woredas. The level of Cr was the least among all the metals. The toxic metals Pb and Cd were not detected in coffee beans of the three woredas. Since there is no environmental pollution due to industrial activities in Gedeo zone, the concentrations of the toxic metals are expected to be very low in coffee beans and soils of the zone. In addition, the low levels of the toxic metals might be also an evidence for the absence of the use some commercial fertilizers and herbicides for coffee plantation in the zone. Furthermore, Pb and Cd have no nutritional value for human beings; their low concentration was appreciable. Therefore, coffee beans of Gedeo zone will not cause any health risk due to these toxic elements for the consumers.

In coffee beans from Kochere farmer's farms, K (14631.30 ± 46.70 µg/g) was found to be in highest concentration among the macroelements. In addition, Mg and Ca were also found in appreciable amount with a concentration of 1748.3 ± 32.48 µg/g and 1252.93 ± 30.17 µg/g, respectively. Relative to macroelements, smaller amount of trace metals were detected in coffee beans of the woreda. Of which Cu (28.54 ± 0.88 µg/g) was present in higher concentration than the other microelements. Likewise the levels of Mn (23.60 ± 1.30 µg/g) and Zn (10.40 ± 0.96 µg/g) were higher than that of Co (2.86 ± 0.25 µg/g) and Ni (1.66 ± 0.08 µg/g). Cr was detected at the lowest concentration of all metals with amount of 1.04 ± 0.08 µg/g. As can be

shown in **TABLE 4**, similar to Kochere woreda, almost the same distribution pattern of metals was observed in farmer's coffee beans from Yirgacheffee and Wonago woredas.

TABLE 4. **Average concentration (mean ± SD) of metals in coffee bean samples from farmer's farms.**

No.	Elements	Kochere	RSD (%)	Yirgacheffee	RSD (%)	Wonago	RSD (%)
1	Mg	1748.23 ± 32.48	2.18	1833.00 ± 39.51	2.16	1758.87 ± 18.21	1.04
2	K	14631.30 ± 46.70	2.08	15042.8 ± 53.29	0.35	14861.1 ± 38.20	0.26
3	Ca	1252.93 ± 30.17	2.4	1037.33± 23.02	2.22	1132.60 ± 23.14	2.04
4	Cr	1.04 ± 0.08	7.69	1.92 ± 0.17	9.04	1.08 ± 0.08	7.69
5	Mn	23.60 ± 1.30	5.53	21.14 ± 1.4	6.62	17.25 ± 0.72	4.17
6	Cu	28.54 ± 0.88	3.1	23.39 ± 1.27	5.43	26.76 ± 0.86	3.22
7	Zn	10.40 ± 0.96	9.1	12.75 ± 1.01	7.93	8.74 ± 0.73	8.35
8	Co	2.86 ± 0.25	8.75	2.47 ± 0.16	6.67	2.49 ± 0.19	7.66
9	Ni	1.66 ± 0.08	4.53	2.43 ± 0.14	5.81	2.34 ± 0.11	4.7
10	Pb	ND	-	ND	-	ND	-
11	Cd	ND	-	ND	-	ND	-

Like coffee beans from farmer's farms, the beans from Yirgacheffee washing industries also contains the highest amount of K (14979.60 ± 50.15 µg/g), followed by Mg (1876.67 ± 21.13 µg/g) and Ca (1089.67 ± 39.00 µg/g). Similarly, Cu (23.08 ± 0.99 µg/g) was the most accumulated trace metal followed by Mn (19.93 ± 1.57 µg/g) and Zn (13.04 ± 0.87 µg/g) in the beans of the woreda. The concentrations of the other essential trace metals detected in coffee beans were Co (2.31 ± 0.12 µg/g), Ni (2.32 ± 0.17 µg/g) and Cr (1.90 ± 0.13 µg/g). The result indicates that the concentrations Cr (1.91 ± 0.13 µg/g) was the least to be quantified out of all detected metals. As can be shown in **TABLE 5**, the distribution of metals in coffee beans from all washing industries of three woredas follows the same distribution pattern.

TABLE 5. **Average concentration (mean ± SD) of metals in coffee bean samples washing industries.**

No.	Elements	Kochere	RSD %	Yirgacheffee	RSD %	Wonago	RSD %
1	Mg	1759.05 ± 21.16	1.2	1876.67 ± 21.13	1.21	1742.74 ± 23.02	1.32
2	K	14601.80 ± 35.33	0.24	14979.60 ± 50.15	0.33	14850.9 ± 45.56	0.31
3	Ca	1269.91 ± 21.30	1.68	1089.67 ± 39.00	3.58	1144.58 ± 27.90	2.44
4	Cr	0.97 ± 0.09	8.92	1.90 ± 0.13	6.57	0.94 ± 0.07	7.52
5	Mn	22.60 ± 0.88	3.89	19.93 ± 1.57	7.87	17.15 ± 0.46	2.67
6	Cu	28.16 ± 0.68	2.43	23.08 ± 0.99	4.3	26.82 ± 0.98	3.67
7	Zn	10.87 ± 0.92	8.44	13.04 ± 0.87	6.68	9.41 ± 0.31	3.32
8	Co	2.75 ± 0.14	5.09	2.31 ± 0.12	5.28	2.38 ± 0.17	7
9	Ni	1.56 ± 0.12	7.47	2.32 ± 0.17	7.19	2.12 ± 0.11	5.19
10	Pb	ND	-	ND	-	ND	-
11	Cd	ND	-	ND	-	ND	-

The significant deference in the mean concentration of metals within coffee beans from farmer's farms and washing industries was tested using t-tests. The result indicated that, at 95% confidence level, except Cr and Zn in Kochere woreda, Ca and Mn in Yirgacheffee woreda and Cr and Ni in Wonago woreda, all other quantified metals are not significantly different in coffee beans

from farmer's farms and washing industries. The insignificant difference of the metals could be attributed to the similarity of soil type in which the coffee plants grow, the similarity of geographical location and the common climatic conditions that the coffee beans shares. Furthermore, insignificant difference for most metals indicates that coffee beans are not affected by contamination in washing industries.

One way ANOVA was used to check whether there is a significant difference or not in metals concentration within the coffee beans of three woredas. At 95% confidence level, except Co the mean concentration of Mn, Zn Cu, Ni, Cr, K, Ca and Mg are significantly different in farmer's coffee beans of three woredas. Likewise, all the analyzed metals mean concentrations show a significant difference in coffee beans from washing industries of the three woredas. The probable reasons for the significant difference of metals concentration within coffee bean samples may be due to the difference in ages (due to age difference between coffee beans) and verities of sampled coffee plants [16] and also due to the significant difference in mineral composition within soils of each woreda.

Distribution pattern of metals in soil samples

In all soil samples, among the macroelements Mg was found at the highest concentration followed by Ca and K. Likewise, out of the microelements Mn was found to a higher extent followed by Cu and Zn. The other trace metals were ranked as Co>Ni>Cr>Cd. The toxic metal Pb was found to be below the detection limit in soil samples of three woredas. Generally, the concentration of metals in soil samples almost follows the same trend as that of coffee beans samples of the zone, i.e. higher amount of macroelements than microelements.

Wonago woreda soil sample analysis shows that, Mg (2153.06 ± 34.49 μg/g) was the largest of all the detected metals. Ca and K were found in larger amount next to Mg with values of 1248.82 ± 48.30 μg/g and 711.24 ± 57.13 μg/g, respectively. Out of the analyzed microelements, Mn (337.10 ± 28.36 μg/g) was found to be in larger amount followed by Cu (70.98 ± 2.18 μg/g) and Zn (34.60 ± 1.98 μg/g). Likewise, the concentrations of Co, Ni and Cr were found to be 11.66 ± 0.78 μg/g, 7.30 ± 0.32 μg/g and 5.55 ± 0.41 μg/g, respectively. The level of Cd (3.49± 57.13 μg/g) was the least among all tested metals. The toxic heavy metal, Pb, was found to be below the method detection limit. In general the concentration pattern of metals in Wonago soil was ranked as Mg>Ca>K>Mn>Cu>Zn>Co>Ni>Cr>Cd. As can be shown in **TABLE 6**, the distribution of metals in soil samples of the three woredas follows the same pattern.

TABLE 6. **Average concentration (mean ± SD) of metals in soil samples.**

No.	Elements	Kochere	RSD%	Yirgacheffee	RSD%	Wonago	RSD%
1	Mg	2236.06 ± 53.77	2.4	2216.6 ± 39.62	1.79	2153.06 ± 34.49	1.7
2	K	815.91 ± 25.77	3.16	905.43 ± 36.37	3.8	711.24 ± 57.13	8.03
3	Ca	1167.77 ± 24.09	2.06	1313.91 ± 30.27	2.3	1248.82 ± 48.30	3.87
4	Cr	5.01 ± 0.21	4.12	5.63 ± 0.26	4.55	5.55 ± 0.41	7.34
5	Mn	412.94 ± 16.08	3.9	385.88 ± 21.87	5.67	337.10 ± 28.36	8.41
6	Cu	61.89 ± 3.76	6.09	76.9 ± 1.34	1.74	70.98 ± 2.18	3.07
7	Zn	47.14 ± 2.51	5.31	45.92 ± 3.22	7.01	34.60 ± 1.98	5.75
8	Co	10.05 ± 0.63	6.22	8.59 ± 0.32	3.7	11.66 ± 0.78	6.65
9	Ni	6.45 ± 0.48	7.52	8.33 ± 0.55	6.55	7.30 ± 0.32	4.38
10	Cd	3.11 ± 0.15	4.81	2.59 ± 0.21	8.29	3.49 ± 0.26	7.47
11	Pb	ND	-	ND	-	ND	-

ND=Not detected

There exist statistically significant differences (at 95 % confidence level) in mean concentration of the metals except Mg and Cr within soil samples of the three woredas. The source for this significant difference may be arises from the variation in pH, moisture content and chemical compositions of the soils of three woredas [17].

Comparison of the concentration of metals in coffee beans with literature values

Even if there is a difference in sample preparation and analysis techniques, results obtained in the present study were compared with the values reported by different researchers. Many authors reported the concentration of essential and non essential metals in raw and roasted coffee beans, which are grown in different part of the world including Ethiopia. The comparison for the concentration of metals in coffee beans obtained in the current study with reported values is presented in **TABLE 7**.

TABLE 7**. Comparison for the quantified metals concentration (µg/g) in coffee beans with literature values.**

Metals	Present study		Martin *et al.* [18] (Brazil)	Anderson *et al.*[19] (Costarica)	Suseela *et al.* [20] (India)	Abera Gura *et al.* (Ethiopia) [21]
	CBFF	CBWI				
Mg	1748-1833	1743-1877	1720 - 2060	2203	2000-3100	1670-1690
K	14631-15043	14602-14980	12280-18280	18570	14000-29000	13010-17000
Ca	1037-1253	1090-1270	990-1370	1079	869-1171	710-1250
Cr	1.04-1.92	0.94-1.90	NR	NR	0.4-1.00	0.21-0.28
Mn	17.25-23.60	17.15-22.60	26-39	38	7-13	13-19
Cu	23.39-28.54	23.08-28.16	15-77	18.1	0.4-16	Nov-30
Zn	8.74-12.75	9.41-13.04	Apr-61	7.97	2-9	Apr-21
Co	2.47-2.86	2.31-2.75	NR	NR	NR	2.6-6.8
Ni	1.66-2.43	1.56-2.32	NR	NR	NR	2.0-2.5
Cd	ND	ND	NR	NR	NR	ND
Pb	ND	ND	NR	NR	NR	ND

ND: not detected (below detection limit); NR: not reported CBFF=Coffee beans from farmer's farms CBWI=Coffee beans from washing industries

Results reported by different researchers from different countries are more or less consistent with the findings of the present work. However, the concentrations of Cr and Mn are slightly higher than the reported values.

Maximum permissible limits of metals

Concentrations of traces heavy metals in plants were highly essential for good health of animals and human beings, but the metals should be within permissible limits as recommended by FAO/WHO and other standard providing bodies. Concentrations higher or lower than the recommended limits have adverse effects on health [22,23]. Literature information's were not found for maximum permissible limits of heavy metals in coffee bean. Therefore comparison was made with standards set for other food items and herbal plants.

TABLE 8. **Comparison of current results for coffee beans and coffee leaves with maximum permissible limits set by FAO/WHO, different organizations and countries.**

Metals	Present study		MPL (µg/g)	Type of plant	The MPL is set by	Reference
	CBFF	CBWI				
Cu	23-29	23-28	40	In foods	FAO/WHO, 1993	28
			20	MP	China, 2005	27
			150	MP	Singapore, 2005	27
Zn	9-13	9-13	50	Grain	USDA, 2000	24, 26
			100	Beans	USDA, 200	
			27.4	Edible plants	FAO/WHO, 1984	27
Cd	ND	ND	0.05	Leafy vegetables and fresh herbs	Walker, 1988	24, 25, 26
			0.3	MP	China, 2005	27
Pb	ND	ND	0.3	Cereals and legumes	CAC, 2000	27, 28
Cr	1-2	1-2	2	MP	Canada, 2005	28
Co	2.5-3	2-3	0.2	MP	Not justified	29
Ni	1.7-2.4	1.6-2.3	1.5	MP	Not justified	29
Mn	17-24	17-23	no MPL	MP	WHO	29

MP=Medicinal Plant, MPL=Maximum permissible limits, ND=Not detected, CBFF=Coffee beans from farmer's farms CBWI=Coffee beans from washing industries

The levels of metals found in coffee bean samples were below the maximum permissible limit according to the international standards for heavy metals. From this it can be inferred that currently there is no health risk associated with heavy metals during the consumption of coffee beans of Gedeo zone.

Correlation of Metals in Coffee Bean and Soil Samples

Correlation coefficients between soil and coffee bean samples were calculated for each metal separately. As seen in **TABLE 9**, there are positive correlations between soil and coffee beans for most metals content in the investigated region. This relation was not statically significant for K, but was significant for Mg, Ca, Cr, Mn, Zn and Co. Positive relationships for metal content between soil and plants are expected results, because plants take nutritional elements from the soil *via* their roots [24]. Furthermore, it verifies that the dependence of metal concentration in the coffee beans on the amount metals under supportive soil of coffee plant.

TABLE 9. **Pearson correlation coefficient for metals in coffee beans with soil samples.**

Metals	Mg	K	Ca	Cr	Mn	Cu	Zn	Co	Ni
r	0.8811	0.3997	0.7929	0.6309	0.9994	-0.9561	0.7577	0.7512	-0.1954
where, r is the Pearson correlation coefficient									

Conclusions

This study determined levels of essential and non-essential metals in coffee beans (from farmer's farms and washing industries) and soil samples. The result showed that all samples contains highest amount of macroelements than microelements. In both coffee beans levels of metals decreased in the order of: K>Mg>Ca>Cu>Mn>Zn>Co>Ni>Cr and in soil samples they decreased in the order of: Mg>Ca>K>Mn>Cu>Zn>Co>Ni>Cr>Cd. However, in coffee beans, the toxic metals (Pb and Cd) were not determined and the levels of trace heavy metals were below the maximum permissible limits set by FAO/WHO and CAC for different food items. Therefore, there is no health risk associated with toxic and trace heavy metals during the consumption of coffee beans of Gedeo zone. Statistical comparison of the concentrations of metals within coffee beans from farmer's farms and washing industries reveled that, there is no significant difference in the concentrations of metals within a single woreda. In contrast, there is significant difference in the concentrations of metals within the three woredas. Furthermore, statistical analysis showed that for most metals there was significantly strong positive correlation between the concentration of metals in coffee bean and supportive soil under coffee plantation. This may suggest that the dependence of metal concentration in the coffee beans on the level of metals under supportive soil of coffee plantation.

Acknowledgements

The authors express their gratitude to the Department of Chemistry, Hawassa University, Ethiopia, for providing the laboratory facilities. Wondimeneh Dubale is thankful to entire farmers who willingly allow their coffee plants and farms for sampling. Furthermore, his sincere thanks go to the owners of coffee washing industries of all woredas who provide him industrially washed coffee bean samples.

References

1. Grembecka M, Malinowska E, Szefer P. Differentiation of market coffee and its infusions in view of their mineral composition. Sci Total Environ. 2007;383(1-3):59-69.
2. Xiaofeng Y, Zhijun B, Jian Z, et al. Coffee consumption and risk of cancers: A meta-analysis of cohort studies. BMC Cancer. 2011;11(1):96.
3. dos Santos EJ, de Oliveira E. Determination of mineral nutrients and toxic elements in Brazilian soluble coffee by ICP-AES. J Food Compos Anal. 2001;14(5):523-31.
4. Li J, Petra S, Chang-Claude, et al. Coffee consumption modifies risk of estrogen receptor negative breast cancer. Breast Cancer Res. 2011;13(3):1-9.
5. Image analysis for Ethiopian coffee classification
6. Variations in Coffee Processing and Their Impact on Quality and Consistency
7. Kufa T. Environmental Sustainability and Coffee Diversity in Africa. ICO World Coffee Conference. 2010;26-8.
8. Tornincasa P, Furlan M, Pallavicini A, et al. Coffee species and varietal identification. Tools for Identifying Biodiversity: Progress and Problems. 2010;307-13.

9. Gebreselassie S and Ludit E. Agricultural Commercialization in Coffee Growing Areas of Ethiopia. Future Agriculture. 2008;2-4.
10. Kitata RB, Chandravanshi BS. Concentration levels of major and trace metals in onion (*Allium cepa* L.) and irrigation water around Meki Town and Lake Ziway, Ethiopia. Bulletin of the Chemical Society of Ethiopia. 2012;26(1):27-42.
11. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: A Review. Afr J Food Sci. 2010;4(5):200-222.
12. Epa US. Method 3050B. Acid digestion of sediments, sludges, and soils. Revision. 1996;2:1-12.
13. Ajay S, Rohit S. Validation of analytical procedures: a comparison of ICH vs Pharmacopoiea (USP) and FDA. Int Res J Pharm. 2012;3(6):39-42.
14. Forsyth RK. Quality Assurance Program. Sierra Analytica. 2008;21-2.
15. Weis JS, Weis P. Metal Uptake, Transport and Release by Wetland Plants: Implications for Phytoremediation and Restoration. Environ Int. 2004;30(5):685-700.
16. Gebretsadik DW, Chandravanshi BS. Levels of Metals in Commercially Available Ethiopian Black Teas and Their Infusions. Bull Chem Soc Ethiop. 2010:24(3):339-49.
17. Ghehsareh AM, Samadi N. Effect of soil acidification on growth indices and microelements uptake by greenhouse cucumber. Afr J Agric Res. 2012;7(11):1659-65.
18. Anderson KA, Smith BW. Chemical profiling to differentiate geographic growing origins of coffee. J Agric Food Chem. 2002;50(7):2068-75.
19. Suseela B, Bhalke S, Vinod Kumar A. Daily intake of trace metals through coffee consumption in India. Food Addit Contam. 2001;18(2):115-20.
20. Martın MJ, Pablos F, González AG. Characterization of green coffee varieties according to their metal content. Anal Chim Acta. 1998;358(2):177-83.
21. Investigation of Metals in Raw and Roasted Indigenous Coffee Varieties in Ethiopia.
22. Yahaya MI, Ezeh GC, Musa YF. Analysis of Heavy Metals Concentration in Road Sides Soil in Yauri, Nigeria, African Journal of Pure and Applied Chemistry. 2010;4(3):22-30.
23. Leung AO, Duzgoren-Aydin NS, Cheung KC, Wong MH. Heavy metals concentrations of surface dust from e-waste recycling and its human health implications in southeast China. Environ Sci Technol. 2008;42(7):2674-80.
24. Onder S, Dursun S, Gezgin S, Demirbas A. Determination of Heavy Metal Pollution in Grass and Soil of City Centre Green Areas (Konya, Turkey). Pol J Environ Stud. 2007;16(1):150-52.