Determination of Essentials and Toxic Metals in Raw and Roasted Coffee in Bule Hora Woreda, Borena Zone, Ethiopia

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ABSTRACT

The determination of mineral nutrients and toxic elements, K, Mg, Ca, Mn, Cu, Zn, Cd, Pb, and Cr were made from raw and roasted coffee that are grown in Bule Hora Worda, by Flame Atomic Absorption Spectrophotometer (FAAS), after sample preparation in Microwave system with HNO_3 and H_2O_2 reagents, the accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples. Recoveries of the spiked samples varied from 81% to 111% and 85% to 107% for raw and roasted coffee respectively. The observed metal concentrations in roasted coffee varieties are relatively higher than their corresponding raw varieties. In all coffee beans sample K (10.77 ± 1.78 mg/g) > Mg (1.675 ± 0.057 mg/g) > Ca (0.922 ± 0.240 mg/g) for raw and K (13.922 ± 3.157 mg/g) > Mg (2.015 ± 0.06 mg/g) > Ca (1.515 ± 0.019 mg/g) for roasted among the macro elements; similarly for raw coffee Mn (0.015 ± 0.003 mg/g) > Cu (0.013 ± 0.005 mg/g) > Zn (0.010 ± 0.002 mg/g) however, Mn (0.018 ± 0.004 mg/g)> Zn (0.017 ± 0.005 mg/g) > Cu (0.014 ± 0.004 mg/g) for roasted coffee among the trace microelements. Toxic metals Pb, Cr and Cd were not detected in all coffee beans samples. The study confirmed that concentrations for most metals were comparable with their corresponding values reported in literature. All essential elements were assessed for their daily intake with the dietary reference intakes (DRI). The concentrations of the metals were also compared with recommended maximum permissible limits and found to be in a good agreement indicating no exposure risk of using the coffee bean under the current situation.

Keywords: Coffee, Acid digestion, Metals, FAAS

1. INTRODUCTION

Coffee is the most popular beverage all over the globe its consumption is progressively increasing particularly in the western countries and U.S.A due to its distinct taste and aroma. It ranks second after petroleum in international trade to earn foreign exchange in many agriculture oriented countries (Butt et al., 2011). Today coffee is grown in a multitude of countries around the world. Whether it is Asia or Africa, Central or South America, the islands of the Caribbean or Pacific, all can trace their heritage to the trees in the ancient coffee forests on the Ethiopian plateau (Kufa et al., 2011).

Coffee belongs to the genus Coffea, in the Rubiaceae family. There are about 103 species of genus Coffea, all exclusively restricted to the tropical forests of Africa, Madagascar and islands of the Indian Ocean (Mascarene Islands) (Kufa). Among the hundreds of species only two species namely Arabica (*coffee arabica*) and robusta (*coffee canephora*) are under commercial cultivation (Butt et al., 2011; Tornincasa, P et al., 2010) *Coffea arabica* (~75 % of the world's production), robusta (~25 %) (Pohl et al., 2013). It is the single most important crop in the Ethiopian economy as it contributes over 60% of the national foreign exchange earnings, 30% of government direct revenue, and subsistence earnings of about 25% of the population (Ashu, and Chandravanshi, 2011).

Ethiopia is one of the eight regions in the world considered to have a strikingly high level of diversity in cultivated crop plants (Gole et al., 2011). Coffee production in Ethiopia is a long standing tradition. Ethiopia is where Coffea arabica, the coffee plant, originates. The plant is now grown in various parts of the world; Ethiopia itself accounts for around 3% of the global coffee market (http://en.wikipedia.org/wiki/Coffee_production_in_Ethiopia). Coffee consumption varies widely according to geographical location. The highest consumption has been observed in Northern Europe (Finland; 12.0 kg per capita/year) whereas in Southern Europe the highest consumption is in Bosnia and Herzegovina (6.1 kg per capita/year) in Brazil 5.8 kg; in Lebanon 4.8 kg and in Poland 2.4 kg (Nędzarek et al., 2013). In Ethiopia the consumption of coffee per capita per year is 1.3 kg

(http://en.wikipedia.org/wiki/List_of_countries_by_coffee_consumption_per_capital)

The chemical composition of coffee varies according to species (Arabian or robusta), country origin (Ethiopia, Brazil, Kenya, etc), system of cultivation (organic or conventional) and the way it exist (raw or roasted). Main coffee components are given in Table 1 (Abera., 2006; Jokanović et al., 2012).

Table 1. Chemical Composition of Coffee (Abera., 2006).

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

The presence of toxic metals in the body systems is highly significant for they are capable of causing serious health problems through interfering with normal biological functioning (Mukesh et al., 2008). Inorganic or aggregated forms of chemical substances (metalloids, heavy metals etc.) in feed and food represent a severe risk for their long term toxicological effects (Aniello et al., 2006; Manahan., 2005). These metals are widely found in nature, particularly in various mineral deposits and soils, meaning that they are available to be taken up by plants and animals that serve as food sources for humans (Nardi et al., 2009).

Contamination of foods by heavy metals has become an inevitable challenge these days. A number of serious health problems can develop as a result of excessive uptake of dietary heavy metals. Furthermore, the consumption of heavy metal contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behaviors, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Singh et al., 2010). The entrance of essential and toxic metals in food chain particularly due to environmental pollution is certain and has well documented health impact to human beings. Thus the present study is aimed to determine extent and accumulation of essential and toxic metals in raw and roasted coffee to indicate their health risks and toxic effects. So far we didn't come across metal ion study

conducted in coffee sample of Bule Hora Woreda.

MATERIALS AND METHODS

Equipment and Apparatus

Polyethylene plastic bags were used to store the sample. Grinding machine (IKA-WERKE GMBH and COKG, German) was used for grinding and homogenizing of both raw and roasted coffee samples. Teflon digestion vessel (60 mL) was used for digestion of coffee samples in microwave digestion (Model BMS-1, Buck Scientific, German), fume hood was used for protection (spraying of gases). Borosilicate volumetric flasks (25, 50 and 100 mL) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA); micropipettes (Dragonmed, 10-100µL, 100-1000 µL, Shangai, China) were used during measuring different quantities of volumes of acid reagents and metal standard solutions. Deionizer (type 04/05, Italy) was used to remove ions from water. Metals' concentration determination was done by flame atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and hollow cathode lamps of Ca, Mg, K, Cd, Cr, Cu, Pb, Mn, and Zn with air-acetylene flame.

Chemicals and Reagents

Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals Mg, K, Ca, Cu, Mn, Zn Cr, Cd and Pb, (Buck Scientific Puro-Graphictm) were used to prepare intermidate and working standared of each metal. Deionized water (chemically pure: 2µs/cm and below conductivity) was used for sample preparation, dilution, and rinsing apparatus prior to analysis. Nitric acid (70% Spectrosol, BDH, England) and hydrogen peroxide (30%, Riedel- de Haen) of analytical reagent quality were used for digesting coffee samples. Strong oxidizing solution of a mixture of 99.5% potassium dichromate and 98% sulfuric acid were also used for cleaning by soaking the apparatus for about 24 hours. All apparatus were rinsed with deionized water and dried before use. The apparatus were also soaked in HNO_3 and rinsed with deionized water before the next analysis.

Sample Collection and preparation

The raw Coffee samples were collected from eight kebele (Buda Megeda, Bordaye, Ebela, Kilenso Resa, Kilenso Mekoninsa, Sorela Wacho, Artu Merema, Hobudie Madona) of Bule Hora woreda using a grap sampling method. The collected grap samples were combined to form a composite sample to

become the representative sample of the bulk. After collecting the sample About 100 g raw coffee samples were ground using a blender device in the laboratory and stored in polyethylene plastic bottle. About 100 g of coffee samples were roasted by using coffee roasting machine; all roasted samples were ground by blending device and stored in polyethylene plastic bottle. For the pre-concentration of analytes, an optimization digestion procedure was used for both raw and roasted coffee.

Microwave Digestion of Coffee Samples

Exactly 0.3 gram of dried and homogenized coffee samples were weighed (analytical digital balance, ADAM®) and quantitatively transferred into 60 mL Teflon digestion vessel, a mixture of 3 mL of concentrated HNO_3 (68%) and 3 mL of H_2O_2 (30%) were added for raw coffee and a mixture of 5 mL of concentrated HNO_3 (68%) and 3 mL of H_2O_2 (30%) were added for roasted coffee all shacked samples were placed in the fume hood for 15 minutes prior to digestion to avoid foam formation. Then the predigested coffee sample and reagents in the digestion vessels were closed and heated on microwave oven (BMS-1). After digestion of coffee samples, to avoid spraying which cause sample lose, the digestion vessel was cooled to room temperature for about 20 minutes in a fume hood and opened carefully wearing eye, hand, and body protection since a large amount of gas is produced during the digestion process. The cooled coffee sample solutions were transferred to 25 mL volumetric flask and the volumes were filled with double distilled deionised water up to the mark. The digested samples were then kept in a refrigerator until analyzed by FAAS. Triplicate digestions were carried out for each sample.

Digestion of the Blanks

Estimation of the metal concentration of the blank is important for the determination of the detection limit of the analytical method used during the study. For the analysis of the coffee samples 8 reagent blank samples were prepared for raw coffee and 8 reagent blank samples were prepared for roasted coffee. All the digested samples were stored in refrigerator until analysis using FAAS.

3.7. Instrument Operating Conditions

For the determination of metals in coffee samples, 10 mg/L (intermediate standard solution) in 100 mL volumetric flask was prepared from 1000 mg/L stock solution of FAAS. Five 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 (Buck Scientific Model 210 VGP, USA) and five points of calibration curve were established. Sample solutions were each aspirated into the AAS instrument and concentration of the metals was recorded. Three replicate determinations were carried out on each sample. The operating conditions of AAS employed for each analyte are given in (Table 2).

Table 2. Instrumental operating conditions for determination of metals using FAAS

No.	Parameter	Wavelength (nm)	Slit width (nm)	Lump current (mA)	Instrumental detection limit (mg/L)	Sample Energy(eV)
1	Cd	228.9	0.7	2.0	0.01	3. 173
2	Cr	357.9	0.7	2.0	0.04	3.567
3	Cu	324.7	0.7	1.5	0.005	3.884
4	Mn	279.5	0.7	3.0	0.03	3.937
5	Mg	285.2	0.7	1.0	0.005	3.857
6	Pb	217	0.7	3.0	0.04	2.470
7	Zn	213.9	0.7	2.0	0.005	3.148
8	k	766.5	0.7	2.0	0.01	1.509
9	Ca	422.7	0.7	2.0	0.05	3.876

3.8. Instrument Calibration

The range of linearity of concentration vs. absorbance curve is of great importance in determining the elemental concentration of both raw and roasted coffee samples and ensures the accuracy of the Atomic Absorption Spectrophotometer and to establish that results of the determination were true and reliable (Dean., 2003).Standard aqueous solutions of different elements were used to calibrate the FAAS machine.

Recovery Test

For the determination of the validity of the developed optimized procedures used for determination of metals in coffee samples, known concentration of standard solutions (that is 10 mg/L of each K, Mg, Ca Zn, Mn, Cu Cr, Cd, and Pb) were prepared. From

these solutions based upon the amount that make the concentration the final solution 0.1 mg/L (for Cr, Cd, and Pb), 0.05 mg/L (for Zn, Mn and Cu), 0.3 mg/L (for K, Mg and Ca) were prepared from each 10 mg/L of intermediate standard solution. From each final solution of the standards, 500 µL (for K, Mg and Ca) and 250 µL(for Pb, Cd, Cr, Zn, Cu, and Mn) were added to 0.3 g of both raw and roasted coffee samples and digested with the same procedures developed microwave digestion for the coffee samples. After diluting the spiked samples to the required volume with deionized water, they were analyzed with the same procedure followed for the analysis of coffee samples. Triplicate samples were prepared and triplicate readings were obtained. Spike recoveries were calculated according to the following formula: (Harvey., 2000).

$$\% \text{ Recovery} = \left(\frac{F - I}{A}\right) * 100$$

Where,

F = spiked sample

I = unspiked portions

A = is the concentration added to the spiked portion

Method Detection Limit (MDL)

Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99 % confidence that the analyte concentration is greater than zero. In other words, it is the lowest analyte concentration that can be distinguished from fluctuations in a blank, which usually correspond to average of the blank signal plus three times standard deviation of the blank (Tolla., 2006; Dean., 2003).

Mathematically given by: $MDL = Y_o + 3\delta$

Where, Y_o = Average of the blank signal

δ = Standard deviation of the blanks

Data Analysis

The statistical analysis was conducted using statistical package of microcal origin 6.1. and Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

For the evaluation of the total concentration of elements using atomic spectrometry methods, samples of coffee require to be solubilize to reduce matrix effects originating from organic compounds and releasing elements in the form of their simple ions. The optimum digestion procedure chosen was the one that requires 25 minute for complete digestion of 0.3 g of raw coffee with 3 mL HNO_3 (68 %) and 3 mL H_2O_2 (30 %). In the case of roasted coffee 0.3 g with 5 mL HNO_3 (68 %) and 3 mL H_2O_2 (30 %) were selected depending

upon: clarity of digests, minimal digestion time, and minimum reagent volume consumption, absence of undigested coffee samples, simplicity and low heating temperature. The reliability of the method used was validated by studying the recovery of the particular metals. The samples and the added standard solutions were subjected to similar analytical procedure as all other samples. Figure (1) the recoveries of the detected metals (Ca, Mg, K, Mn, Zn and Cu) in the spiked samples for raw coffee lies within the range 81 – 111 % with RSD (0.2 – 4.5 %) and for roasted coffee 85– 107 % with RSD (0.1 – 4.9 %) which are within the accepted range. Generally, good recoveries were obtained for metals which were detected in the present study. Thus, the optimized procedure has good accuracy and precision. The correlation coefficients (R-values) ranges from 0.996 – 0.999 from the calibration curves (Appendix I). Thus, the obtained calibration curves were fairly linear, which assured that linearity of instrumental response for individual analyte.

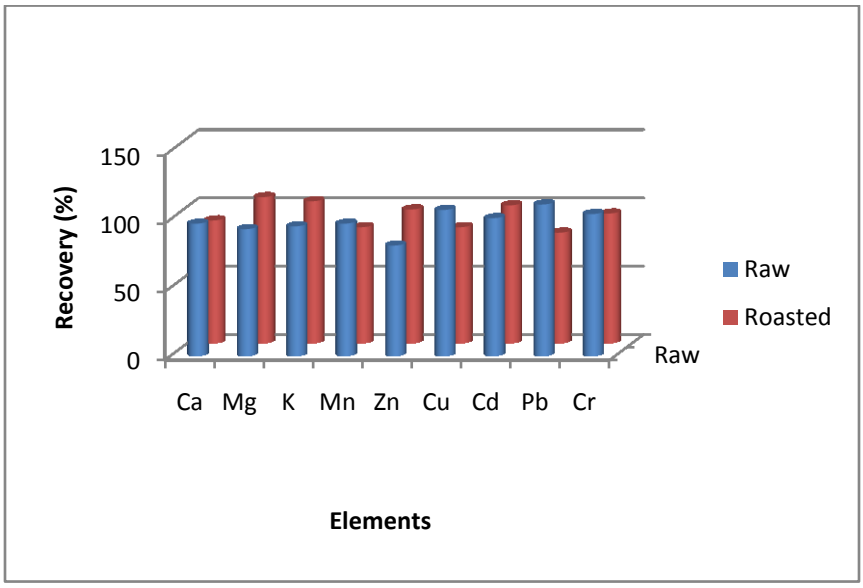


Figure 1. Recoveries of metals in raw and roasted coffee

Eight blank samples or reagents were digested following the same procedure as the coffee samples and each of the samples were analyzed for metal concentrations of Cd, Cr, Ca, Cu, Mn, Mg, K, Pb and Zn Table 3 by FAAS. The standard deviations for each element were calculated from the eight blank measurements to determine method detection limit of the instrument. the method detection limit is less than the results of real sample for detected metals and greater than those of not detected metals as

can be seen from Table 4, the method detection limit of each element is above the instrument detection limit as shown in Table 3.

Table 3. Method detection limits and instrumental detection limit for the determination of metals in coffee (raw and roasted).

Elements	Ca	Mg	K	Zn	Cu	Mn	Cr	Pb	Cd
*MDL(mg/L) for raw coffee	1.205	0.2569	0.1078	0.0564	0.1078	0.0570	0.0618	0.0430	0.0174
*MDL(mg/L) for roasted coffee	1.040	0.1942	0.1135	0.3813	0.1135	0.0693	0,0430	0.0591	0.0154
**IDL(mg/L)	0.05	0.005	0.01	0.005	0.005	0.03	0.04	0.04	0.005

*MDL = Method Detection Limit **IDL = Instrument Detection Limit

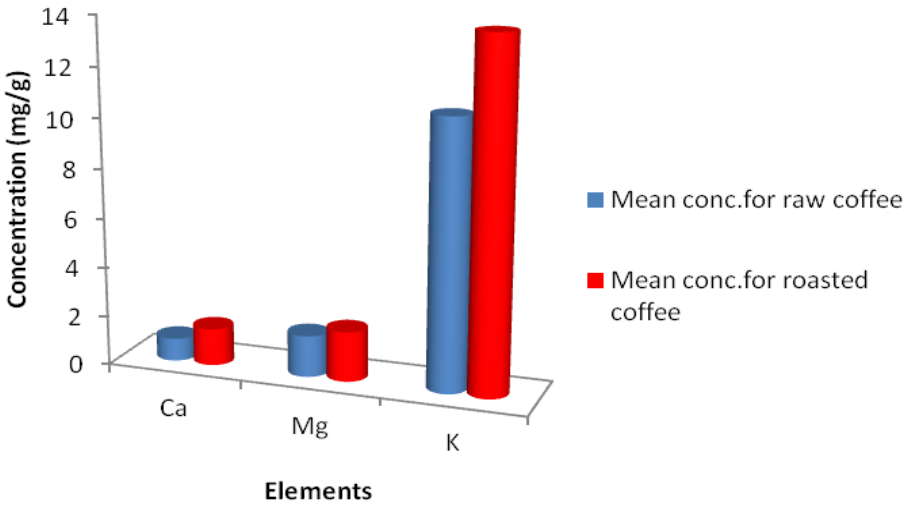
Table 4. Mean metal concentration (Mean ± SD (mg/g) in both raw and roasted coffee.

Elements	Cd	Pd	Cr	Cu	Mn	Zn	Ca	K	Mg
raw coffee	ND	ND	ND	0.013 ± 0.005	0.015± 0.003	0.010 ± 0.002	0.922 ± 0.240	10.77 ± 1.78	1.675 ± 0.057
roasted coffee	ND	ND	ND	0.014 ± 0.004	0.018 ± 0.004	0.017 ± 0.005	1.515 ± 0.019	13.922 ± 3.157	2.015 ± 0.06

ND=Not detected

Among the nine elements analyzed, six elements (Ca, K, Mg Cu, Zn and Mn) were detected and three elements (Cr, Pb, and Cd) were not detected as shown in Table 4; they were below method detection limit. From the detected elements, K (10.77 ± 1.78 mg/g) was the highest and zinc (0.010 ± 0.002 mg/g) was the lowest in raw coffee and for roasted coffee the highest concentration were obtained for K (13.92 ± 3.15 mg/g) and lowest for Copper (0.014 ± 0.004 mg/g). When the concentration of metals in raw and its corresponding roasted coffee beans were compared the roasted coffee beans have relatively higher metal concentrations. The comparative results were presented in Figure 2a for major metals and Figure 2b for trace metals. It has been reported in literature that roasting of coffee beans is one of the ways of enriching the concentration of mineral content of coffee because roasting does not change noticeably the mineral content. Instead it increases their relative content by removing water and

volatile organic compounds (Abera., 2006; Jokanović et al., 2012).



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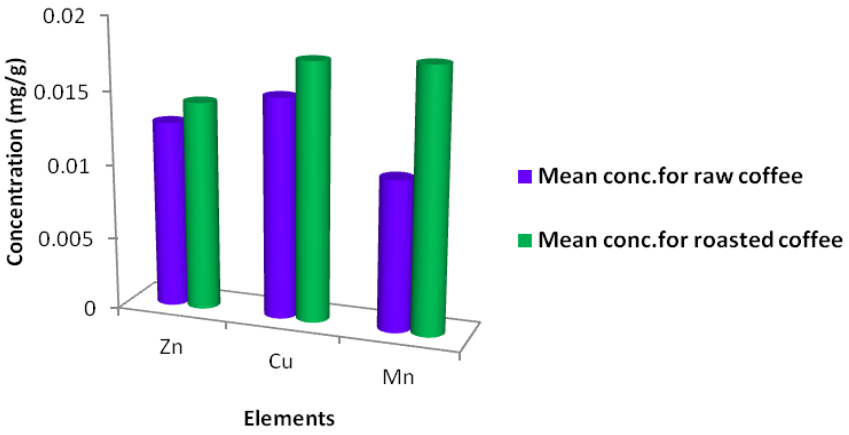

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Figure 2. Mean concentration of detected metals major (*a*) and trace (*b*) element

Table 5. Concentration of metals in raw and roasted coffee for the selected kebele: Mean concentration, ($\bar{x}$ ± SD, n = 3, mg/g dry weight), and relative standard deviation, (RSD %), in raw and roasted Coffee

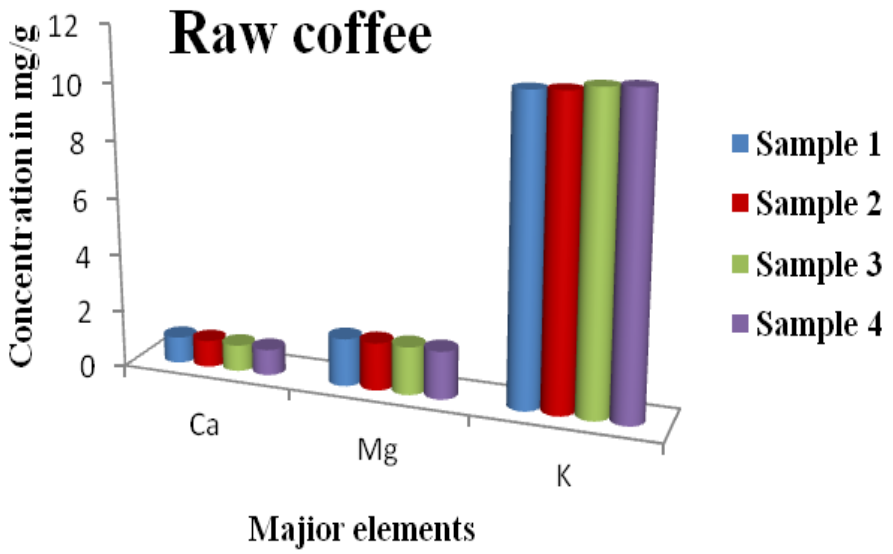
element	$\bar{x}$ ± SD RSD	Study ites							
		A		B		C		D	
		RC	ROC	RC	ROC	RC	ROC	RC	ROC
Ca	$\bar{x}$ ± SD	0.92 ± 0.49	1.53 ± 0.04	0.94 ± 0.04	1.54 ± 0.08	0.92 ± 0.31	1.50 ± 0.03	0.91 ± 0.12	1.49 ± 0.02
	RSD	4.5	0.45	0.38	0.47	2.9	0.19	1.06	0.12
Mg	$\bar{x}$ ± SD	1.67 ± 0.04	2.01 ± 0.11	1.67 ± 0.09	2.02 ± 0.06	1.68 ± 0.05	2.01 ± 0.05	1.68 ± 0.05	2.02 ± 0.02
	RSD	0.18	0.46	0.43	0.23	0.24	0.20	0.24	0.10
K	$\bar{x}$ ± SD	10.62 ± 3.62	13.89 ± 3.69	10.67± 2.93	13.93 ± 3.99	10.86 ± 0.56	13.92 ± 3.72	10.93 ± 0.10	13.95 ± 1.23
	RSD	2.84	2.22	2.29	2.39	0.43	2.23	0.08	0.73
Cu	$\bar{x}$ ± SD	0.012 ± 0.006	0.014 ± 0.007	0.013 ± 0.004	0.015 ± 0.002	0.013±0.004	0.014 ± 0.004	0.013± 0.004	0.014±0.004
	RSD	4.043	3.945	2.484	0.867	2.500	2.321	2.330	2.335
Zn	$\bar{x}$ ± SD	0.011 ± 0.002	0.018 ± 0.011	0.010 ± 0.001	0.017 ± 0.006	0.010 ± 0.001	0.016 ± 0.002	0.010 ±0.002	0.016 ±0.002
	RSD	1.564	4.939	0.814	3.013	0.807	0.982	1.236	0.759
Mn	$\bar{x}$ ± SD	0.016 ± 0.005	0.018 ± 0.003	0.017 ± 0.003	0.019 ± 0.001	0.014 ± 0.002	0.017 ±0.003	0.013± 0.003	0.016 ± 0.009
	RSD	2.651	1.226	1.313	0.449	0.927	1.270	1.638	4.883
Cd	$\bar{x}$ ± SD	< 0.0174	< 0.0154	< 0.0174	< 0.0154	< 0.0174	< 0.0154	< 0.0174	< 0.0154
	RSD								
Pb	$\bar{x}$ ± SD	< 0.0430	< 0.0591	< 0.0430	< 0.0591	< 0.0430	< 0.0591	< 0.0430	< 0.0591
	RSD								
Cr	$\bar{x}$ ± SD	< 0.0618	< 0.0430	< 0.0618	< 0.0430	< 0.0618	< 0.0430	< 0.0618	< 0.0430
	RSD								

A= Ebela and Kilenso Mekoninsa B= Sorela Wacho and Kilenso Resa C= Buda Megeda and Bordaye D= Artu Merema and Hobudie Madon

In coffee beans from all sampling site (Buda Megeda, Bordaye, Ebela, Kilenso Resa, Kilenso Mekoninsa, Sorela Wacho, Artu Merema and Hobudie Madona), potassium was found as the highest concentration among the macro elements in raw (10.77 ± 1.78 mg/g) and roasted (13.922 ± 3.1575 mg/g) coffee. In addition, Mg and Ca were also found in appreciable amount with a concentration of 1.675 ± 32.48 mg/g and 0.9225 ± 0.24 mg/g in raw coffee and 2.015 ± 0.06 mg/g, and 1.515 ± 0.01875 in roasted coffee respectively. This result is in agreement with the result shown in Pawel et al., 2013; Martin et al 1998; Santos and Oliveira., 2001. According to the results in the ANOVA analyses for the concentration of potassium the p-value (> 0.05) indicates that the mean values for K, concentration is not significantly different at 95 % confidence level in the entire study site. Therefore, the ANOVA analysis indicates that the mean concentration of potassium in all sampling site are similar. But, the mean concentration of Ca and Mg in all sampling site are significantly different. The probable reasons for the significant difference of metals concentration within coffee bean samples may be due to the difference in ages between coffee beans and verities of sampled coffee plants (Santos; Oliveira 2001) and also due to the significant difference in mineral composition within soils of each kebele. Relative to macro elements, the concentration of trace metals were detected in both raw and roasted coffee beans from the selected kebeles. Of which Mn (0.015± 0.00325mg/g) in raw coffee and (0.0175 ± 0.004 mg/g) in roasted coffee was present in higher concentration than the other microelements. Likewise the levels of Cu (0.01275 ± 0.0045mg/g) were higher in raw coffee than Zn (0.01025± 0.0015 mg/g) which is in agreement with the result of Pawel et al., 2013 and Martin et al 1998. In roasted coffee the concentrations of Zn (0.01675± 0.00525 mg/g) were higher than that of Cu (0.01425 ± 0.00425 mg/g) the obtained result were in a good agreement with Martin et al., 1999 and Onianwa et al., 1999. In coffee beans of the selected study site, the toxic heavy metals Cr, Pb and Cd were found to be below the method detection limits similar to the result obtained by Martin et al 1998 in raw coffee and by Anderson and Smith., 2002 in roasted coffee.

According to the results in the ANOVA analyses for the concentration of trace metals copper, the p-value (> 0.05) indicates that the mean values for trace metals

concentration is not significantly different at 95 % confidence level in all the study site within each kebele. 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. But, According to the results in the ANOVA analyses for the concentration Zn and Mn in all sampling site are significantly different. Generally, as can be seen from Figure 3 and 4, macro elements (K, Ca, and Mg) were present at higher concentration than trace elements (Mn, Zn, Cu,) in all coffee bean samples of the selected study sites. All coffee beans contain K in highest amount among the macro elements followed by Mg and Ca in each kebeles. 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 (Regassa., 2007). The other probable reason for higher concentration of K, Mg and Ca is that, if the soil which has been used for cultivating the coffee plants is highly fertilized with manure and organic residues and then the soil may be high in available potassium, calcium and magnesium. Consequently, high amount of the metals are accumulated in coffee beans. The highest concentration of macro elements in the beans indicates that, coffee beans are a good phytoaccumulator of these mineral nutrients. Among the analyzed trace metals, Mn was the most accumulated microelement followed by Cu and Zn in raw coffee bean and Zn and Cu in roasted coffee samples of the selected coffee sites. Higher Mn levels in the beans may be attributed to the higher availability of this micronutrient heavy metal in the supportive soils under coffee plantation. Therefore due to the active transfer of Mn^{2+} across the soil root interface and moderate content of Mn^{2+} in the supportive soil under coffee plantation, Mn might be readily taken up by the plant and accumulated in the beans as compared to the other trace heavy metals. Relatively there is slightly lower concentration of copper and zinc than manganese in each sample site probably because of plants to transfer the metal (Cu and Zn) across the root in the soil lower than manganese and there is lower availability of this micronutrient heavy metal in the supportive soils under coffee plantation compared to manganese.



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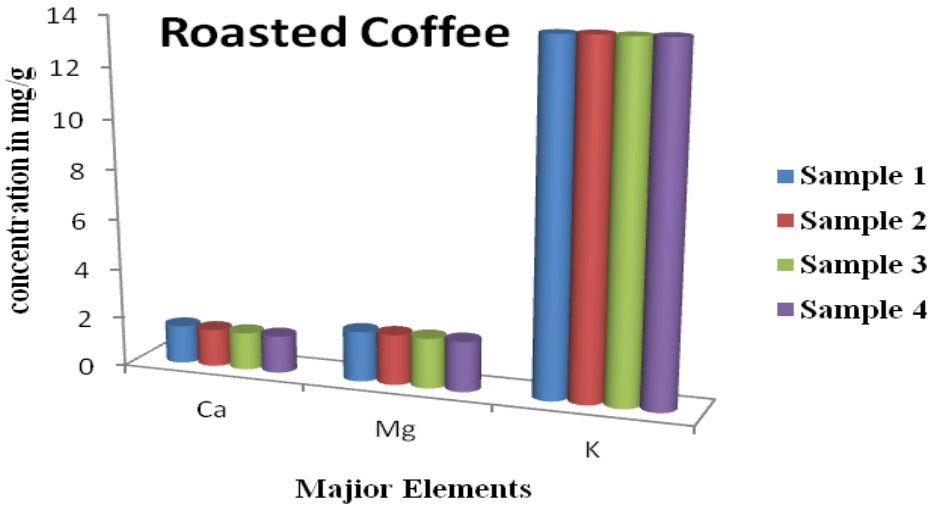

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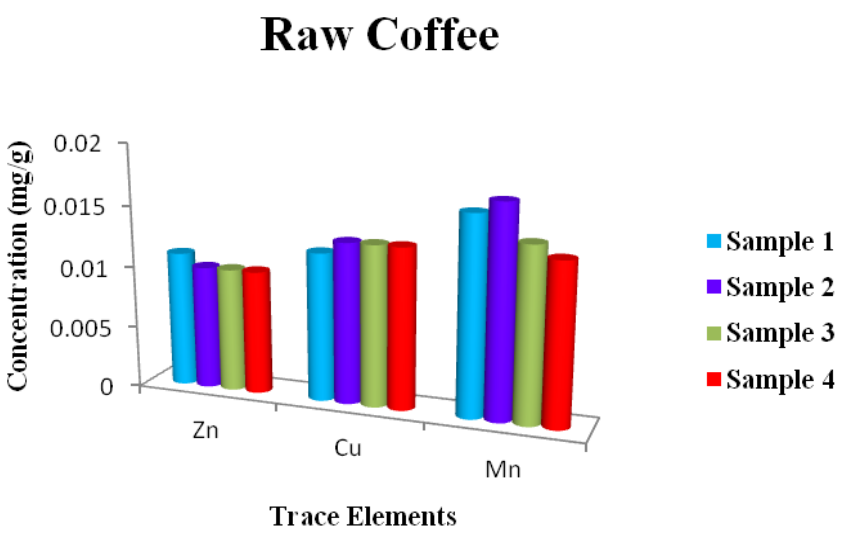
Figure 3. Mean concentrations (n = 3, mg/g, dry weight) of major metals in (*a*) raw and (*b*) roasted coffee samples.



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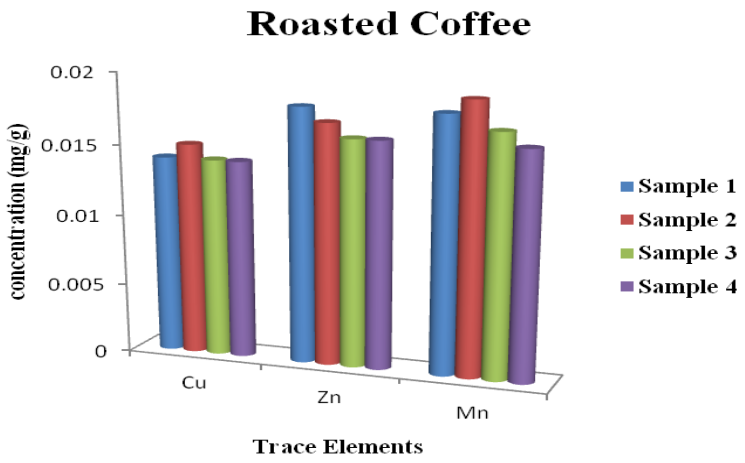

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Figure 4. Mean concentrations, (n = 3, mg/kg, dry weight), of trace metals in (c) raw and (d) coffee samples

Comparison of the Concentration of Metals in Coffee Beans with Literature Values

The obtained results in the present study were compared with the values reported by different researchers in the literature even if there is a difference in sample preparation and analysis techniques. 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. Santos; Oliveira., 2001; Martin et al., 1999; Martin et al., 1999; Anderson and Smith., 2002; Onianwa et al., 1999 have reported the mineral content of roasted coffee beans that are grown in the different parts of the world; such as Brazil, Colombia Costa Rica, Ivory Cost, Mexico, Nigeria, India, and Uganda. Similarly, Abera, G. 2006; Martin et al.m, 1989 have also reported the metal content of raw (green) coffee (from coffee beans) that are grown in different geographical origins. The comparison of the observed results from this study and reported values are presented in Table 6 and 7 for raw and roasted coffee beans, respectively.

Table 6.Comparison of metals concentration (mg/kg, dry weight) in raw coffee with the literature values

Element	**Present study (µg/g)**	**Abera Gure (µg/g)**	**Pawel et al (µg/g)**	**Martin et al [1998]**
Ca	910 - 940	710 - 1250	790-1870	930-1370
Mg	1670 -1680	1670 - 1690	140-2090	1720-2060
K	10620-10930	13010 - 17000	12100-21400	12110-18280
Cu	12 - 14	11 - 30	7.2-76.9	15-77
Zn	10 - 11	4 - 21	3.6-61.3	4-61
Mn	13 - 17	13 - 19	13.4-57.7	16-50
Cr	ND	0.21 - 0.28	<0.08-1.01	NR
Cd	ND	ND	0.70-0.75	NR
Pd	ND	< 0.05	<0.01	NR

Note: Present study, Ethiopian coffee, Abera, G. 2006; reported result on Arabica coffee of Ethiopian coffee growing in different part of the country (Wollega, Sidamo, Harar, Kafa and Benchi Maji) and Martin et al.m, 1989 Brazil coffee.

Table 72. Comparison of metals concentration (mg/kg, dry weight) in roasted coffee with the literature values

Element	**Present study (µg/g)**	**Abera Guru (µg/g)**	**Martin et al.**	**Onianwa et al.**	**Sanatos & Olivira**	**Anderson & Smith**
Ca	1490 - 1530	790 - 1540	880-1030	NR	1060-1890	934 - 1234
Mg	2010 - 2030	1870 - 1890	1730 - 1940	NR	2120-4150	2058-2410
K	13890-13950	14310 -19400	12730-14660	NR	32500-51700	17500- 19600
Cu	13 - 15	13 - 28	11.2-17.8	2.13-9.14	0.51-2.33	12.5-18.1
Zn	16 - 18	6 - 30	5.06-36.9	3.73-14.0	3.17-15.17	6.51-8.03
Mn	16 - 19	15 - 20	13.2-44.6	NR	3.62-38.85	19 - 39
Cr	ND	ND	NR	0.89-6.98	ND	NR
Cd	ND	0.43 – 0.56	NR	0.02-0.31	NR	NR
Pd	ND	ND	NR	0.09-0.91	NR	NR

Note: Present study, Ethiopian coffee, Santos; Oliveira., 2001; Brazil coffee, Abera, G. 2006; Ethiopian coffee, Martin et al., 1999; Brazil coffee type, Anderson and Smith., 2002; for different countries coffee, and Onianwa et al., 1999; Nigerian coffee.

Results reported by different researchers from different countries for raw and roasted coffee beans as shown in Table 6 and 7 are more or less comparable with the findings of the present work. However, relatively lower concentration of K observed in this study in comparison to the reported values, this may probably confirm that coffee in the study area cultivated without the use of fertilizers. In general, for both coffee bean samples, the consistencies of the present results with the reported literature values give an additional confirmation for the validity of this study.

Maximum Allowable 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 (Yahaya et al 2010; Leung et al 2008). It is also known that in addition to the development of various diseases, inequilibrium concentration of heavy metals in human body affects general health, growth and social behavior of human beings (Nergus., 2005).

Table 8.Comparison of current results for coffee beans with maximum permissible limits set by FAO/WHO, different organizations.

metal	Present study		MPL (µg/g)	Type of plant	The MPL is set by	Reference
	Raw	Roasted				
K	10620-10930	13890-13950	1–5%	In plant	FAO, 2006	FAO. 2006.
Ca	910-940	1490-1530	0.2–1.0%	In plant	FAO, 2006	FAO. 2006.
Mg	1670-1680	2010-2030	0.1–0.4%	In plant	FAO, 2006	FAO. 2006.
Cu	12 - 14	13 - 15	40	In foods	FAO/WHO, 1993	Aroraa et al., 2008
			30	In coffee	The Brazilian Food Legislation, 1988	Santos; Oliveira., 2001
			40	Plants (Gesho)	(FAO/WHO, 1995)	Nagari and Abebaw., 2013
Zn	10 - 11	16 - 18	27.4	Edible plants	FAO/WHO,1984	Jabeen et al., 2010
			30	In coffee		Gogoasa et al.,2013
			50	Grain	USDA, 2000	Salama and Radwan.,

			100	Beans	USDA, 2000	*2005.* ;Chukwujindu and Iwegbue.,2012
			50	In coffee	The Brazilian Food Legislation, 1988	Santos; Oliveira., 2001
Mn	13 - 17	16 - 19	500	Plants (Gesho)	(Council of US, 2002)	Nagari and Abebaw., 2013
Cd	ND	ND	0.05	Leafy vegetables and fresh herbs	Walker,1988	Salama and Radwan., *2005.* ; Chukwujindu and Iwegbue.,2012.; and Blagojević et al., 2009
			1.00	In coffee	The Brazilian Food Legislation, 1988	Santos; Oliveira., 2001
			0.01	In coffee		Gogoasa et al.,2013
Pb	ND	ND	0.03	Cereals and legumes	CAC,2000	Aroraa et al., 2008; Jabeen et al., 2010
			1.00	In coffee	The Brazilian Food Legislation, 1988	Santos and Oliveira., 2001
			1.00	In coffee		Gogoasa et al.,2013
Cr	ND	ND	2.0	Medicinal plants	Canada, 2005	Aroraa et al., 2008
			0.10	In coffee	The Brazilian Food Legislation, 1988	Santos; Oliveira., 2001

MPL= Maximum permissible limits, ND = Not detected
ppm = parts per million = mg/kg = µg/g; 10,000 ppm = 1 percent (FAO. 2006).

Literature information's were not found for maximum permissible limits of heavy metals in coffee beans. Therefore comparison was made with standards set for other food items and herbal plants. As shown from Table 8 the mean concentration of Potassium for raw (10620 - 10930 mg kg^{-1}) and roasted (13890 - 13950 mg kg^{-1}), Magnesium for raw (1670 - 1680 mg kg^{-1}) and roasted (2010 - 2030 mg kg^{-1}), Calcium for raw (910 - 940 mg kg^{-1}) and roasted (1490 -1530 mg kg^{-1}) coffee sample were found in the sample site, which were still below the FAO maximum permissible limit for K 1-5%, for Mg 0.1–0.4% and for Ca 0.2–1.0% in plant dry mater (FAO, 2006), indicating no exposure risk to take macro elements to human being. The levels of metals found in all 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 from Bule Hora woreda.

Recommended Daily Intake of Metals

In order to observe the health risk of any pollutant, it is very important to estimate the level of exposure, by detecting the routes of exposure to the target organisms. There are several possible path-ways of exposure to humans but amongst them the food chain is the most important pathway (Aroraa et al., 2008).The daily intake of metals depends on both the concentration and the amount of food consumed. The daily dietary intake of coffee for an average finland population is 12kg in brazil it is 5.8kg in Norway 9.9 kg per capital per year. The reported values of daily coffee Consumption in Germany and Canada are respectively 15.06g and 17.80g. However, in Ethiopia the daily consumption of coffee is very low. It is considered to be 3.56 g/day which is calculated from 1.3 Kg per capita per year (http://en.wikipedia.org/wiki/List_of_countries_by_coffee_consumption_per_capital). The value given per year is changed to per day to know the approximate daily intake in Ethiopia. Assuming a value of 3.56g of coffee consumption per day, the daily intake of the detected metals from roasted coffee are depicted in Table 9 The last column shows the Recommended Dietary intake (RDI) as set by different international organization.

Table 9. Recommended daily intake of metal in coffee sample

Element in coffee	Concentration in roasted coffee (mg /g)	Daily intake (mg /day) for roasted	Recommended daily intake (mg/day)	References
Calcium	1.505	5.360273973	1000	http://lenntech.com/recommended-daly-intake.htm.
Magnesium	2.017	7.183835616	350	http://lenntech.com/recommended-daly-intake.htm.
Potassium	13.922	49.58520548	3500	http://lenntech.com/recommended-daly-intake.htm.
Copper	0.014	0.049863014	2	http://lenntech.com/recommended-daly-intake.htm.
Manganese	0.018	0.064109589	5	Ogabiela et al., 2011
Zinc	0.017	0.060547945	15	Nagari and Abebaw., 2013

Conclusion and recommendation

Coffee is one of the most popular and widely consumed beverages in the world, having extensive commercial as well as social importance. It is also one of the most important agricultural products in the international trade. Owing to this, the present study tried to determine the concentration of K, Mg, Ca, Mn, Cu, Zn, Cr, Cd and Pb in raw and roasted coffee, collected from different kebeles of Bule Hora woreda. A digestion procedure was developed and validated through recovery studies. The optimum conditions were validated through spiking experiment, from which good percentage recoveries 81% – 111 % and 85% – 107%, for raw and roasted coffee samples respectively.

Concentrations of metals in the samples of different kebeles were compared using one way ANOVA. All samples contained higher amount of macro elements than microelements both in raw and roasted coffee. K was the most accumulated metal followed by Mg, Ca, Mn, Cu, Zn for raw and Mg, Ca, Mn, Zn, Cu for roasted coffee respectively. According to this study, the presence of Cd, Cr and Pb were not detected. The results obtained in this research were compared with some of the international guidelines; all the parameters determined were below the guideline. The results of this study were also compared with the results of other countries' coffee in the world. Except some outlined results reported from different countries, the metal composition of Bule Hora woreda coffee is more or less similar to that of other countries. From this it can be inferred that currently there is no health risk associated with heavy metals macro elements during the consumption of coffee beans from Bule Hora woreda.

Therefore, to draw strong conclusion, parallel to the study of metal content in raw and roasted coffee further investigations are needed on the physical and chemical property of soil, the metal content of soil, water for irrigation and fertilizers. From the present study one can observe that there are permissible amounts of macro and trace elements in raw and roasted coffee. So, the coffee cultivated in Bule Hora woreda is not affected by metal contaminants. Therefore, more advertisements and awareness should be made by concerned bodies to increase

and spread the recognition and consumption of coffee beans of Bule Hora woreda in national and international coffee market.

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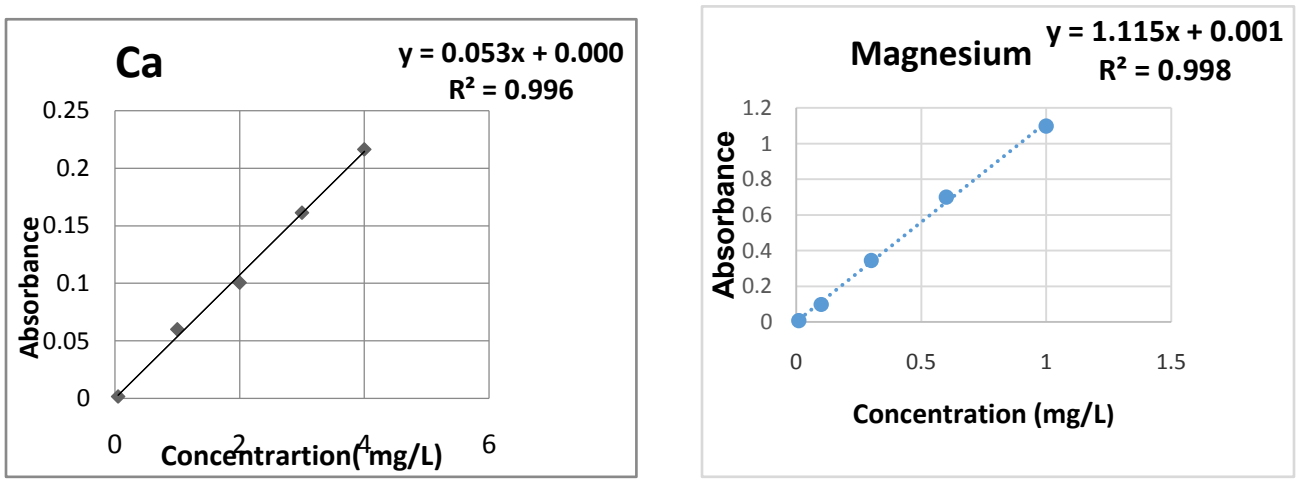
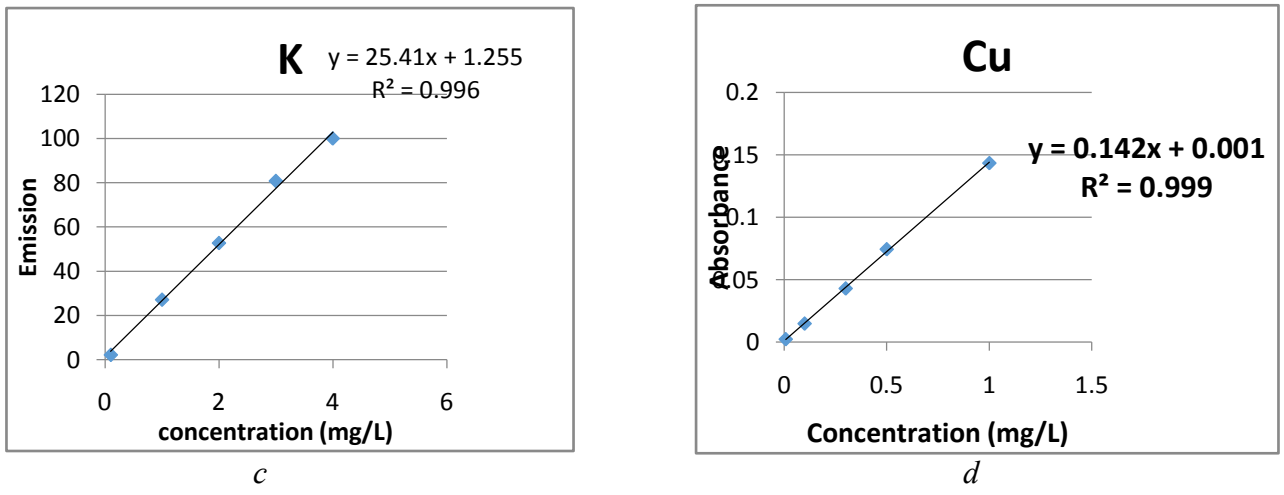
Appendix I

Calibration Curves of Standard Solution



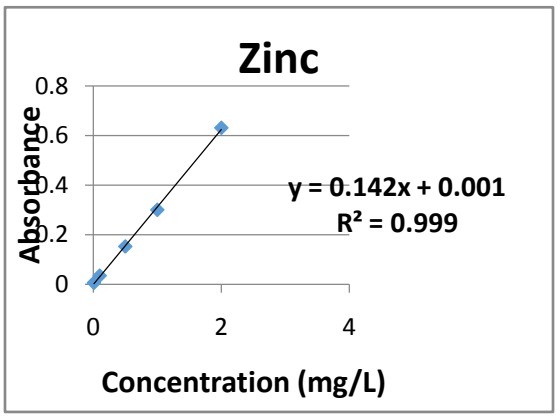
a b

Figure. 1. Calibration Curves of standard solution for Ca (*a*) and Mg (*b*)

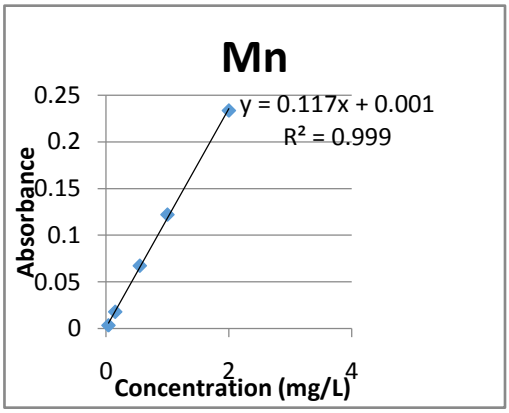


c *d*

Figure. 2. Calibration Curves of standard solution for K (*c*) and Cu (*d*)

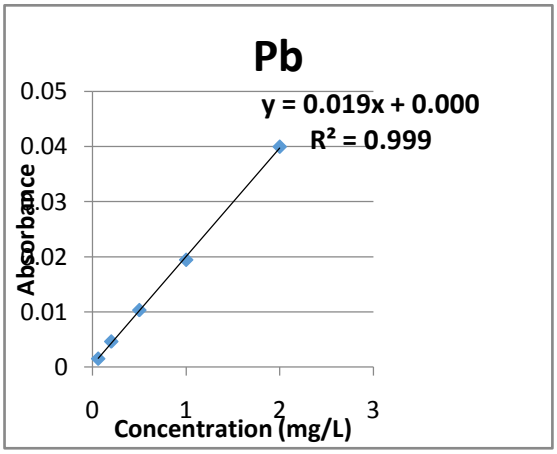


e

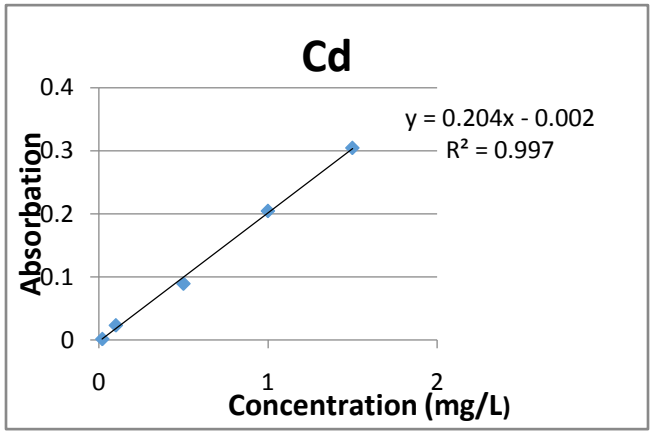


f

Figure. 3. Calibration Curves of standard solution for Zn (e) and Mn (f)



h



g

Figure. 4. Calibration Curves of standard solution for Pd (h) and Cd (g)