

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

### **Concentrations of toxic heavy metals in coffee**

**Running title: Toxic Heavy Metals in Coffee**

#### **Abstracts:**

##### **Background and Objective**

Coffees one of the common drinks in Middle Eastern countries including Saudi Arabia due to its desirable aroma, taste and putative positive physiological functions. The concentration of metals commonly present in coffee powder. The presence of heavy metal concentration in different brands of coffee powder available in Saudi Arabia market has been analyzed.

##### **Methods**

Thirteen different coffees brands were selected in different markets, all assessment was carried out in advanced instruments such as Inductively Coupled Plasma and Atomic Absorption Spectroscopy. There are 14 metals which divide into seven non toxic and seven toxic metals were analyzed. Non toxic metals are magnesium (Mg), calcium (Ca), Potassium (K), sodium (Na), phosphorus (P), iron (Fe), and toxic metals are arsenic (Ar), zinc (Zn),chromium (Cr), nickel (Ni), lead (Pb) , antimony and cadmium (Cd) ) theses metals were direct and indirect effect on the human and environmental health.

##### **Results**

The mean and standard deviation of non-toxic and toxic metals concentration in different samples of coffee were as follows: Ca, Fe, K, Mg, Mn, Mo, Na were  $24.873\pm 6.76$ ,  $6.670\pm 4.88$ ,  $235.985\pm 100.05$ ,  $407.024\pm 226.56$ ,  $8.637\pm 10.14$ ,  $0.014\pm 0.01$ ,  $333.865\pm 247.35$ ,  $0.271\pm 0.22$ ,  $0.939\pm 0.36$  respectively. Toxic metals concentration were as Al, Cd, Cu, Ni, Pb, Si, Zn were

25 11.040±10.03, 0.802 ±2.52, 2.436±3.02, 0.072±0.110, 7.571±9.266, 23.480±27.32 and  
26 1.853±1.66 respectively. These concentration values were high compared to TLVs of metals.

## 27 **Conclusion**

28 It was concluded from the study that coffee powder had high concentration of heavy toxic metals  
29 which is the major public health problem. Thus, quality control for food safety recommended  
30 during production of coffee.

31 **Keywords; Coffee; daily intake; concentration; metals, toxic**

32

## 33 **Introduction**

34 Coffee originates from the plant *Camellia sinensis*, a tree that may grow up to 52 feet in height unless  
35 cultivated.[1] Tea plants require significant rainfall of 50 inches a year and grow in acidic soil.

36 Contaminants may vary in the soil, air, or water in which the plants are grown. Acidic soil may result in  
37 excess available aluminum and fluoride [1]. An acid or alkali soil pH also enhances leaching of toxic  
38 heavy metals from the soil [2]. Increasing pH with soluble calcium would reduce the absorption of  
39 fluoride [1]. Environmental pollutants such as fluoride and aluminum have been found in tea in part due  
40 to the tea plants absorption and deposition and concentration of these compounds in the leaves [3]. The  
41 drinking of more than 5 liters of tea per week may result in dental or skeletal fluorosis [4]. Mercury, lead,  
42 arsenic, and cadmium as well as other toxic elements have been found in tea leaves as described in the  
43 literature [5, 6]. Lead, arsenic, and cadmium have also been found in brewed black tea [7]. These soil and  
44 air contaminants may be directly related to the use of coal fired power plants.[8]

45 There is an abundance of literature demonstrating the adverse health effects of various heavy metal and  
46 metalloid elements on the human organism. By numerous mechanisms, including endocrine disruption  
47 [10], cytotoxicity [11], mitochondrial dysfunction [12], and oxidative stress [13- 14], a spectrum of toxic  
48 elements is able to disturb cellular and metabolic homeostasis and induce clinical illness. The literature is  
49 replete with many common disease processes such as carcinogenesis [15], insulin resistance [16],

50 neurodegeneration [17], and immune dysregulation [18-19]. Rather than isolated incidents of single  
51 exposures, it is apparent that toxic metal contact is a widespread phenomenon [20] with many potential  
52 sources including tainted food and drink, contaminated skin products, and contaminated air. Many toxic  
53 metals such as cadmium and lead have very long half-lives and thus are classified as persistent toxicants  
54 [21]. As some toxic elements appear to persist because of enterohepatic recycling [22, 23], even smaller  
55 levels of exposure can bioaccumulate and effect long-term harm.

56 The toxic elements such as lead, mercury, aluminum, and cadmium. The extremely low levels of lead  
57 accepted in Proposition 65 during the prenatal period come from our knowledge of the accumulation in  
58 the brain and resultant impairment of cognitive development [24-25].

59 Most evidence on the relation between coffee and blood pressure stems from cross-sectional studies. This  
60 evidence, however, is inconsistent. Some of these studies showed a positive relation (27), no relation (28),  
61 or even an inverse relation (29). Such cross-sectional studies have important limitations with respect to  
62 causal inference.

63 In Saudi Arabia, different brands of coffee available and coffee is one of the most common drink in the  
64 population. All the coffee beans were imported from different countries except the Arabic coffee. There is  
65 no previous study to determine the concentration of heavy metals in different brands of coffee which is  
66 the knowledge gap, this study results will help the food administration authority to check the all brands of  
67 coffee for heavy metals concentration and it also help the awareness among community for health  
68 conscious regarding coffee consumptions. The objective of study to determine the concentration of heavy  
69 metals present in different brands of coffee in Saudi Arabia market.

## 70 **4. Material and Methods**

### 71 **Sampling method and Study setting**

72 Coffees samples were taken from different markets in city of Damamm and Khobar and analyzed for  
73 heavy metal content using Inductive Couple Plasma OES. There are total 13 different types of coffees  
74 with different colors were selected through random sampling method.

### 75 **Sample Preparation (Experiment)**

76 After collection of Samples were collected by using stratified random methods. All the samples were in  
77 the form of powder. First sample was dried before the measurement of metals. Standard solutions were  
78 prepared according to the Shimadzu Perkin Elmer Pure Atomic Spectroscopy Standards guidelines (NIST  
79 traceable CRM, Perkin Elmer Corporation, USA and Merck □ Germany). Working standard solutions  
80 were prepared by diluting the stock solution with 0.1 M nitric acid for checking the linearity. The final  
81 residue was dissolved in 0.1 M HNO<sub>3</sub> solution and make up to 50 ml.

82 The glassware and polyethylene containers used for analysis. First washed with tap water, then soaked  
83 overnight in 6N HNO<sub>3</sub> solution and rinsed several times with ultrapure water to eliminate absorbance due  
84 to detergent. Accurately weighed (1 g) plant samples were transferred into a silica crucible and kept in a  
85 muffle furnace for ashing at 450

### 86 **Analytical Procedure for coffee:**

87 One gram (1gm) coffee samples were digested using 12cm<sup>3</sup> of a mixture 5ml v/v) of concentrated HCl  
88 and HNO<sub>3</sub> acids. Analar grade reagents were used for the preparation of the standard solutions of these  
89 metals using their nitrate salts (Ca, K, Na Mg, Mn, Pb, Cu, Fe, Na, K and Zn) The diluted digests were  
90 analyzed by using Inductively Coupled Plasma (ICP-OES) was used for Mg, Mn, Ca, Pb, Cu, Fe and Zn.  
91 The metal concentrations in the coffee samples were read from standard curves by extrapolation. Also,  
92 the soluble samples of coffees were diluted and determine the physic and chemical parameters and  
93 compare between the two types of samples according to trace elements and physical constituents so the  
94 determination of physic- chemical characteristics and parameters of preserving teas and coffees in two

95 steps and these parameters which are used for soluble coffees according to its high degree of solubility  
 96 such as

97 1- The physical parameters: such as pH, Conductivity, TDS, and temperature.

98 2- The chemical parameters: Ammonia, Nitrate, Nitrite, Sulfate, Sulfide, and Phosphate,

99 So, the determination of physic- chemical characteristics and parameters of preserving coffees in two  
 100 steps and these parameters which are used for soluble coffees according to its high degree of solubility  
 101 such as physic and chemical analysis for samples solutions according to the following table:

102 **1- The physical parameters:**

No	Parameters	Unit	Instrument	References
1	pH	-----	pH meter (electrode method)	Standard Method for the Examination of water and wastewater
2	Conductivity	ms/cm	Conductivity meter (electrode method)	
3	TDS	mg/L	Conductivity meter (electrode method)	
4	Temperature	°T	pH meter (electrode method)	

103

104 **2- The chemical parameters:**

No	Parameters	Unit	Instrument	References
1	Ammonia	mg/L	Spectrophotometer (HACH)	Standard Method for the Examination of water and wastewater
2	Nitrate	mg/L		
3	Nitrite	mg/L		
4	Sulfate	mg/L		
5	Sulfide	mg/L		
6	Phosphate	mg/L		
7	Total trace elements	ppm		

105

106 **Toxic Limit and Safe intake of heavy metals**

<u>Heavy Metal</u>	<u>Toxic Limit</u>	<u>Recommended intake/Safe intake</u>
<u>Arsenic</u>	3 mg/day	15 - 25 µg/day
<u>Cadmium</u>	200 µg/kg	15 -50 µg/day
<u>Lead</u>	> 500 µg/L	20 - 280 µg/day
<u>Zinc</u>	150 µg/day	15µg/day

107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136

**5. Results:**

**A- Physical and chemical analysis:**

The mean values of samples as Ph 4.68 with SD0.57, conductivity 0.85, TDS 419.92, temperature 19.58 C, sulfate concentration 25, phosphate 40.3, ammonia 3.07, Nitrate 45.53.(Table 1)

**B- Heavy metals:**

**Nontoxic heavy metals concentrations in different coffee samples**

- Concentration levels of non-toxic metals: The average level concentration of calcium was ranged between 8.94 and 32.09 mg/kg, Iron was ranged between 1.27 and 14.35mg/kg, potassium was ranged between 21.31 and 306.71mg/kg, magnesium was ranged between 43.18 and 767.62mg/kg, manganese was ranged between 0.702 and 24.35 mg/kg, sodium was ranged between 6.84 and 556.5 mg/kg, copper was ranged between 0.133and 4.06 mg/kg, and zinc was ranged between 0.153 and 3.83 mg/kg, (Table 2)

**Toxic heavy metals concentrations in different coffees samples**

- Concentration levels of toxic metals: silver was ranged between 0 and 2.423 mg/kg, aluminum was ranged between0.82 and 31.76 mg/kg, arsenic was ranged between 0 and 0.107 mg/kg, cadmium was ranged between 0 and 0.011, chromium was ranged between 0.0225 and 1.19, nickel was ranged between 0 and 0.258, lead was ranged between 0 and 21.45 mg/kg and antimony was ranged between 0.0086 and 0.133 mg/kg (Table 3)

**Discussion:**

Results of the study found that toxic heavy metal concentration were high in different brands of Coffee .these metals were hazards to various health effects on human body.

137 The different metals were found high concentration in different samples of coffee. The reason for this  
138 high concentration is that the soil where coffee plant grow and environmental conditions which effect the  
139 concentration. [30-32]. There are different factors which effect the concentration such as fertilizer used  
140 with different chemical compositions, coffee species and fertilized land where crop were grow. [33-34].  
141 Previous studies found that the metal concentrations in coffee beans are important indicator to  
142 differentiated between different coffee variety.[35-36]

143 The pH of a coffee has been found to correlate with the perceived acidity in coffee and that is resulted in  
144 correlation between pH values and type of coffees. The pH values were ranged between 3.81 to 5.42 it is  
145 highly acidic in some samples that may lead to affecting on digestion of the food and performance of  
146 stomach. This result is consistent with the previous study [37]which showed that pH values ranges  
147 between 2-4 which is acidic in nature that affected the digestion problem and may lead to stomach cancer.  
148 It is well known throughout the coffees industry that decaffeinated coffee is more acidic than regular  
149 coffee due to the fact that decaffeinated coffee is made from Robusta beans. Robusta beans have a higher  
150 concentration of caffeine and more acidic than other beans. This is problematic for people with health  
151 problems such as acid reflux, GERDS and ulcers making them susceptible to detrimental effect of high  
152 levels of acidity also we found that there are variation in concentration of total dissolved solids.

153  
154 According to the correlation between the heavy metals and types of coffees in the study results shows that  
155 some heavy metals were high concentrations of metals such as Ca, Fe, K, Mg, Mn and Na. The  
156 maximum concentrations of Ca was 32.09, Fe concentration (mg/kg) the maximum was 14.357, the third  
157 metals K concentration (mg/kg) the maximum concentration was 427.84. These results were consistent  
158 with other previous studies results which showed that these metals concentrations were high.[38]

159 Toxic heavy metals such as Al, As, Cd, Cr, Ni, Pb, Sb, and As also found high in the study results such as  
160 Al concentration (mg/kg) the maximum concentration was 31.769, As concentration (mg/kg) the  
161 maximum concentration was 0.107 Cd concentration (gm/kg) the maximum concentration was 0.0119, Cr

162 concentration (mg/kg) the maximum concentration was 1.1997. These results also consistent with other  
163 study results in which the concentration these toxic metals were found high.[39]

## 164 **7. Conclusion:**

165 The study result found that significant concentration of toxic heavy metals present in all samples  
166 of coffee which are hazardous to human health. There is need to develop the health promotion  
167 programme for awareness among community.

## 168 **Declaration**

### 169 **Ethical approval and Consent to Participate**

170 Study was approved by the hospital ethical committee with reference no is 287659 and consent  
171 of participate was obtained from the study participant.

172 All procedures performed in studies involving human participants were in accordance with the  
173 ethical standards of the institutional and/or national research committee and with the 1964  
174 Helsinki declaration and its later amendments or comparable ethical standards. Research involve  
175 human participants, research approved from ethical review committee from hospital,  
176 confidentiality of data has maintained,

177 **Informed consent** inform consent was obtained from each participant

### 178 **Consent for Publication**

179 Informed written consent was received for publication of the manuscript and figure. Authors give  
180 permission to journal for publication

### 181 **Availability of Data and Material**

182 Data is confidential and not shared

183

184

185



186 **8. References:**

- 187 1. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger  
188 (*Zingiber officinale*) cultivated in Ethiopia. *Springer Plus*, 2015, 4, Article No. 127. DOI:  
189 10.1186/s40064-015-0899-5
- 190 2. Hiroyuki, K.T., Kazunobu, S., Takeji, T. (2011). Analysis of Iodinelike (Chlorine) Flavor-causing  
191 Components in Brazilian Coffee with Rio Flavor. *Food Sci. Technol. Res* 17(4): 347 – 352.
- 192 3. Ostrowska J., Stankiewicz A., Skrzydlewska E., Antioxidant properties of green tea. *Bromotol.*  
193 *Toxicol. Chem.* 2001; 2: 131.
- 194 4. Chandra S., De Mejia Gonzalez E., Polyphenolic compounds, antioxidant capacity, and quinone  
195 reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex*  
196 *paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agric. Food Chem.* 2004; 52: 3583-3589.
- 197 5. Maron D. J., Lu G. P., Cai N. S., Wu Z. G., Li Y. H., Chen H., Zhu J. Q., Jin X., J., Wouters B.  
198 C., Zhao J., Cholesterol-lowering effect of a theaflavin-enriched green tea extract: a randomized  
199 controlled trial. *Arch. Intern. Med.* 2003; 163: 1448- 1453.
- 200 6. Hasegawa R., Chujo T., Sai-Kato K., Umemura T., Tanimura A., Kurokawa Y., Preventive  
201 effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with  
202 2-nitropropane. *Food Chem. Toxicol.* 1995; 33: 961-970.
- 203 7. Fujiki H. Green tea: health benefits as cancer preventive for humans. *Chem. Rec.* 2005; 5:  
204 119-132.
- 205 8. Bettuzzi S., Brausi M., Rizzi F., Castagnetti G., Peracchia G., Corti A., Chemoprevention of  
206 human prostate cancer by oral administration of green tea catechins in volunteers with highgrade  
207 prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study.  
208 *Cancer Res.* 2006; 66: 1234-1240.
- 209 9. Seenivasan S., Manikandan N., Muraleedharan N.N., Selvasundaram R., Heavy metal content of  
210 black teas from south India, *Food Control.* 2008; 19: 746-749.
- 211 10. E. Álvarez-Ayuso, A. Giménez, and J. C. Ballesteros, “Fluoride accumulation by plants grown in  
212 acid soils amended with flue gas desulphurisation gypsum,” *Journal of Hazardous Materials*, vol.  
213 192, no. 3, pp. 1659–1666, 2011.
- 214 11. Z. Tan and G. Xiao, “Leaching characteristics of fly ash from Chinese medical waste  
215 incineration,” *Waste Management and Research*, vol. 30, no. 3, pp. 285–294, 2012.
- 216 12. M. Fujimaki Hayacibara, C. S. Queiroz, C. P. Machado Tabchoury, and J. Aparecido Cury,  
217 “Fluoride and aluminum in teas and tea-based beverages,” *Revista de Saude Publica*, vol. 38, no.  
218 1, pp. 100–105, 2004.

- 219 13. S.-C. C. Lung, H.-W. Cheng, and C. B. Fu, "Potential exposure and risk of fluoride intakes from  
220 tea drinks produced in Taiwan," *Journal of Exposure Science and Environmental Epidemiology*,  
221 vol. 18, no. 2, pp. 158–166, 2008.
- 222 14. X.-P. Wang, Y.-J. Ma, and Y.-C. Xu, "Studies on contents of arsenic, selenium, mercury and  
223 bismuth in tea samples collected from different regions by atomic fluorescence spectrometry,"  
224 *Guang Pu Xue Yu Guang Pu Fen Xi*, vol. 28, no. 7, pp. 1653–1657, 2008.
- 225 15. W.-Y. Han, F.-J. Zhao, Y.-Z. Shi, L.-F. Ma, and J.-Y. Ruan, "Scale and causes of lead  
226 contamination in Chinese tea," *Environmental Pollution*, vol. 139, no. 1, pp. 125–132, 2006. .
- 227 16. Shekoohiyan, M. Ghoochani, A. Mohagheghian, A. H. Mahvi, M. Yunesian, and S. Nazmara,  
228 "Determination of lead, cadmium and arsenic in infusion tea cultivated in north of Iran," *Iranian*  
229 *Journal of Environmental Health Science & Engineering*, vol. 9, article 37, 2012.
- 230 17. D. Tang, T.-Y. Li, J. J. Liu et al., "Effects of prenatal exposure to coal-burning pollutants on  
231 children's development in China," *Environmental Health Perspectives*, vol. 116, no. 5, pp. 674–  
232 679, 2008.
- 233 18. M. E. Sears and S. J. Genus, "Environmental determinants of chronic disease and medical  
234 approaches: recognition, avoidance, supportive therapy, and detoxification," *Journal of*  
235 *Environmental and Public Health*, vol. 2012, Article ID 356798, 15 pages, 2012.
- 236 19. T. I. Lidsky and J. S. Schneider, "Lead neurotoxicity in children: basic mechanisms and clinical  
237 correlates," *Brain*, vol. 126, no. 1, pp. 5–19, 2003.
- 238 20. S. J. Genus, G. Schwalfenberg, A. K. Siy, and I. Rodushkin, "Toxic element contamination of  
239 natural health products and pharmaceutical preparations," *PLoS One*, vol. 7, no. 11, Article ID  
240 e49676, 2012.
- 241 21. M. A. Rahman, B. Rahman, and N. Ahmed, "High blood manganese in iron-deficient children in  
242 Karachi," *Public Health Nutrition*, vol. 16, no. 9, pp. 1677–1683, 2013. View at Publisher · View  
243 at Google Scholar
- 244 22. F. M. Crinella, "Does soy-based infant formula cause ADHD? Update and public policy  
245 considerations," *Expert Review of Neurotherapeutics*, vol. 12, no. 4, pp. 395–407, 2012. View at  
246 Publisher ·
- 247 23. Klag MJ, Wang NY, Meoni LA, et al. Coffee intake and risk of hypertension. The Johns Hopkins  
248 Precursors Study. *Arch Intern Med* 2002;162:657–62.
- 249 24. Ramato Ashu and Bhagwan Singh Chandravanshi," CONCENTRATION LEVELS OF METALS  
250 IN COMMERCIALY AVAILABLE ETHIOPIAN ROASTED COFFEE POWDERS AND  
251 THEIR INFUSIONS" *Bull. Chem. Soc. Ethiop.* 2011, 25(1), 11-24.

- 252 25. Grembecka, M.; Malinowska, E.; Szefer, P. " Differentiation of market coffee and its infusions in  
253 view of their mineral composition" *Sci. Total Environ.* 2007, 383, 59.
- 254 26. Santos, E.E.; Lauria, D.C.; Porto da Silveira, C.L. " Assessment of daily intake of trace elements  
255 due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city" *Sci. Total Environ.*  
256 2004, 327, 69.
- 257 27. Nędzarek, A.; Tórz, A.; Karakiewicz, B.; Clark, J.S.; Laszczyńska, M.; Kaleta, A.; Adler, G.  
258 Concentrations of heavy metals (Mn, Co, Ni, Cr, Ag, Pb) in coffee. *Acta Biochim. Polonica*,  
259 2013, 60, 623–627.
- 260 28. Dos Santos, J.S.; Dos Santos, M.L.P.; Conti, M.M.; Dos Santos, S.N.; De Oliveira, E.  
261 Evaluation of some metals in Brazilian coffees cultivated during the process of conversion  
262 from conventional to organic agriculture. *Food Chem.* 2009, 115, 1405–1410.  
263
- 264 29. Offsetdrukkerij Haveka B.V., Alblasserdam" COFFEE AND CARDIOVASCULAR RISK; AN  
265 EPIDEMIOLOGICAL STUDY. Oms lag Pieter-Jan Kersbergen2004.
- 266 30. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger  
267 (*Zingiber officinale*) cultivated in Ethiopia. *Springer Plus*, 2015, 4, Article No. 127. DOI:  
268 10.1186/s40064-015-0899-5.
- 269 31. Ayele, E.; Urga, K.; Chandravanshi, B.S. Effect of cooking temperature on mineral content  
270 and antinutritional factors of yam and taro grown in southern Ethiopia. *Int. J. Food Eng.*  
271 2015, 11, 371–382.
- 272 32. Weldegebriel, Y.; Chandravanshi, B.S.; Wondimu, T. Concentration levels of metals in  
273 vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. *Ecotoxicol.*  
274 *Environ. Saf.* 2012, 77, 57–63.  
275
- 276 33. 13. Illy, E. The complexity of coffee. *Sci. Am.* 2002, 286, 86–91.
- 277 34. Anderson, K.A.; Smith, B.W. Chemical profiling to differentiate geographic growing origins  
278 of coffee. *J. Agric. Food Chem.* 2002, 50, 2068–2075.  
279
- 280 35. Suseela, B.; Bhalke, S.; Kumar, A.V.; Tripathi, R.M.; Sastry, V.N. Daily intake of trace  
281 metals through coffee consumption in India. *Food Addit. Contam.* 2001, 18, 115–120.
- 282 36. Gebretsadik, A.T.; Berhanu, T.; Kefarge, B. Levels of selected essential and nonessential  
283 metals in roasted coffee beans of Yirgacheffe and Sidama, Ethiopia. *Am. J. Environ. Protect.*  
284 2015, 4, 188–192.  
285

- 286 37. Ashu, R.; Chandravanshi, B.S. Concentration levels of metals in commercially available  
287 Ethiopian roasted coffee powders and their infusions. *Bull. Chem. Soc. Ethiop.* 2011, 25, 11–  
288 24.
- 289
- 290 38. Horžić D., Komes D., Belščak A., Kovačević Ganić K., Iveković D., Karlović D. (2009): The  
291 composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chemistry*,  
292 115: 441–448. Rapić V. (1994):
- 293 39. Postupci priprave I, izolacije organskih spojeva, Školska knjiga, Zagreb. Re R., Pellegrini N.,  
294 Proteggente A., et al.. Antioxidant activity applying an improved ABTS radical cation  
295 decolourisation assay. *Free Radical Biology & Medicine*, 2000, 26: 1231–1237.
- 296
- 297
- 298
- 299
- 300
- 301
- 302
- 303
- 304
- 305
- 306
- 307
- 308
- 309
- 310
- 311
- 312
- 313

314  
 315  
 316  
 317  
 318  
 319  
 320

**Tables**

Table 1 Physical and Chemical Properties of Coffee Samples(n=13)		
S.no	Parameter	Mean ±SD
<b>Physical Properties</b>		
1	PH	4.68±0.57
2	Conductivity ms/cm	0.85±0.36
3	TDS mg/L	419.92±177.35
4	Temperature °C	19.58±0.09
5	Color CU	6856.54±2999.06
6	Turbidity NTU	32.20±27.09
7	Degree of color	Deep/Faint
<b>Chemical Properties</b>		
1	Sulfate SO <sub>4</sub> mg/L	25±5.08
2	Sulfide mg/L	1.20±1.42
3	Phosphate PO <sub>4</sub> mg/L	40.13±28.10
4	Ammonia NH <sub>4</sub> mg/L	3.07±3.20
5	Nitrate NO <sub>3</sub> mg/l	45.53±36.58
6	Nitrite NO <sub>2</sub> mg/L	0.23±0.255

333  
 334  
 335  
 336  
 337  
 338

339  
340  
341  
342  
343  
344  
345  
346  
347

Table 2 Concentration of Non Toxic Metal Concentration in Coffee Samples(n=13)

Metals	Mean and SD	Range
Ca	24.873±6.76	6.76-32.09
Fe	6.670±4.88	0.82-14.35
K	235.985±100.05	21.31-427.84
Mg	407.024±226.56	43.18-767.62
Mn	8.637±10.14	0.48-28.69
Mo	0.0143±0.01	0-0.04
Na	333.865±247.35	6.84-564.74
Se	0.271±0.22	0-574
V	0.939±0.36	0.340-1.60

348  
349  
350  
351  
352  
353  
354  
355

356  
357  
358  
359  
360

<b>Metals</b>	<b>Mean and SD mg/kg</b>	<b>Range</b>
<b>Al</b>	11.040±10.03	0.87-31.76
<b>Cd</b>	0.802 ±2.52	0-8.01
<b>Cu</b>	2.436±3.02	0.133-10
<b>Ni</b>	0.072±0.1100	0-0.258
<b>Pb</b>	7.571±9.266	0-23.88
<b>Si</b>	23.480±27.32	0.52-88.83
<b>Sr</b>	9.093±14.39	0-33.78
<b>V</b>	0.751±0.42	0.34-1.60
<b>Zn</b>	1.853±1.66	0.003-4.59

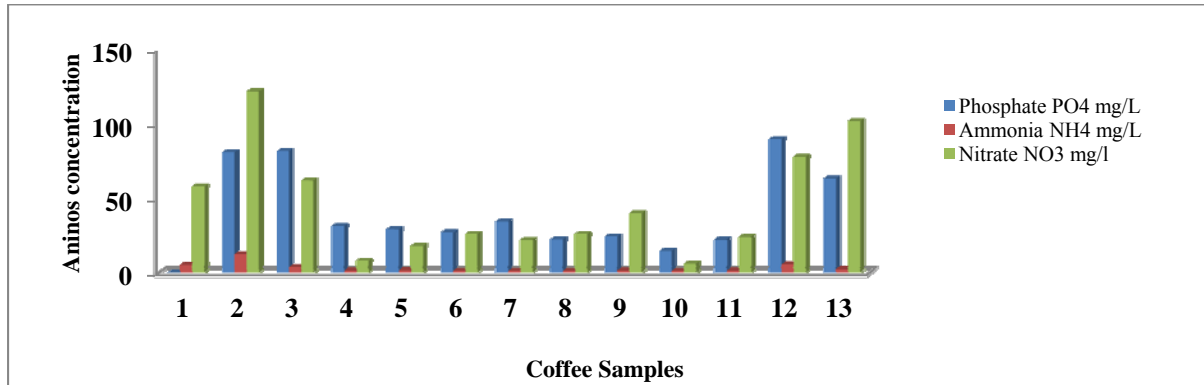
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373

374

375

376 **Figure (1) Average Level concentrations of phosphate, ammonia and nitrate between different coffee samples**

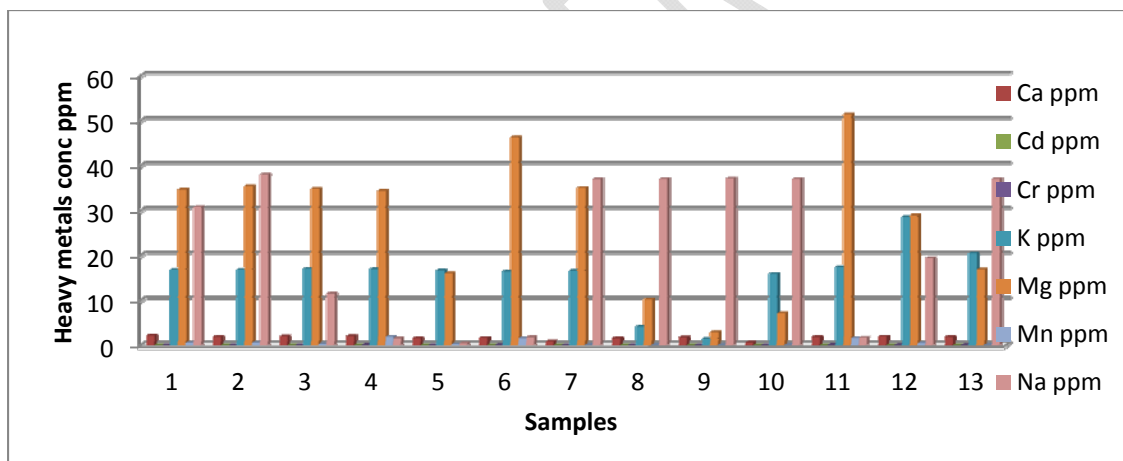
377



378

379

380



381

382

383 **Figure 2 Non toxic heavy metals concentrations in different coffees samples**

384

385

386

387



388

389

390

391

392

UNDER PEER REVIEW