

Physicochemical parameters and toxic heavy metals concentration in coffee

Running title: Toxic Heavy Metals in Coffee

Abstracts:

Background and Objective

Coffee is 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 is 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 coffee 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).

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, and Na were 24.873 ± 6.76 , 6.670 ± 4.88 , 235.985 ± 100.05 , 407.024 ± 226.56 , 8.637 ± 10.14 , 0.014 ± 0.01 , 333.865 ± 247.35 , 0.271 ± 0.22 , 0.939 ± 0.36 respectively. Toxic metals concentration were as Al, Cd, Cu, Ni, Pb, Si, Zn were 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 1.853 ± 1.66 respectively. These concentration values were high compared to threshold limit values (TLVs) of metals.

26 **Conclusion**

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

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

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32 **Introduction**

33 Coffee originates from the plant *Camellia sinensis*, a tree that may grow up to 52 feet in height unless
34 cultivated.[1] Tea plants require significant rainfall of 50 inches a year and grow in acidic soil.
35 Contaminants may vary in the soil, air, or water in which the plants are grown. Acidic soil may result in
36 excess available aluminum and fluoride [1]. An acid or alkali soil pH also enhances leaching of toxic
37 heavy metals from the soil [2]. Increasing pH with soluble calcium would reduce the absorption of
38 fluoride [1]. Environmental pollutants such as fluoride and aluminum have been found in tea in part due
39 to the tea plants absorption and deposition and concentration of these compounds in the leaves [3]. The
40 drinking of more than 5 liters of tea per week may result in dental or skeletal fluorosis [4]. Mercury, lead,
41 arsenic, and cadmium as well as other toxic elements have been found in tea leaves as described in the
42 literature [5, 6]. Lead, arsenic, and cadmium have also been found in brewed black tea [7]. These soil and
43 air contaminants may be directly related to the use of coal fired power plants.[8]

44 There is an abundance of literature demonstrating the adverse health effects of various heavy metal and
45 metalloid elements on the human organism. By numerous mechanisms, including endocrine disruption
46 [10], cytotoxicity [11], mitochondrial dysfunction [12], and oxidative stress [13- 14], a spectrum of toxic
47 elements is able to disturb cellular and metabolic homeostasis and induce clinical illness. The literature is
48 replete with many common disease processes such as carcinogenesis [15], insulin resistance [16],
49 neurodegeneration [17], and immune dysregulation [18-19]. Rather than isolated incidents of single
50 exposures, it is apparent that toxic metal contact is a widespread phenomenon [20] with many potential

51 sources including tainted food and drink, contaminated skin products, and contaminated air. Many toxic
52 metals such as cadmium and lead have very long half-lives and thus are classified as persistent toxicants
53 [21]. As some toxic elements appear to persist because of enterohepatic recycling [22, 23], even smaller
54 levels of exposure can bioaccumulate and effect long-term harm.

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

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

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

69 **4. Material and Methods**

70 **Sampling method and Study setting**

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

74 **Sample Preparation (Experiment)**

75 After collection of Samples were collected by using stratified random methods. All the samples were in
76 the form of powder. First sample was dried before the measurement of metals. Standard solutions were
77 prepared according to the Shimadzu Perkin Elmer Pure Atomic Spectroscopy Standards guidelines (NIST
78 traceable CRM, Perkin Elmer Corporation, USA and Merck □ Germany). Working standard solutions
79 were prepared by diluting the stock solution with 0.1 M nitric acid for checking the linearity. The final
80 residue was dissolved in 0.1 M HNO₃ solution and make up to 50 ml.

81 The glassware and polyethylene containers used for analysis. First washed with tap water, then soaked
82 overnight in 6N HNO₃ solution and rinsed several times with ultrapure water to eliminate absorbance due
83 to detergent. Accurately weighed (1 g) plant samples were transferred into a silica crucible and kept in a
84 muffle furnace for ashing at 450

85 **Analytical Procedure for coffee:**

86 One gram (1gm) coffee samples were digested using 12cm³ of a mixture 5ml v/v) of concentrated HCl
87 and HNO₃ acids. Analar grade reagents were used for the preparation of the standard solutions of these
88 metals using their nitrate salts (Ca, K, Na Mg, Mn, Pb, Cu, Fe, Na, K and Zn) The diluted digests were
89 analyzed by using Inductively Coupled Plasma (ICP-OES) was used for Mg, Mn, Ca, Pb, Cu, Fe and Zn.
90 The metal concentrations in the coffee samples were read from standard curves by extrapolation. Also,
91 the soluble samples of coffees were diluted and determine the physic and chemical parameters and
92 compare between the two types of samples according to trace elements and physical constituents so the
93 determination of physic- chemical characteristics and parameters of preserving teas and coffees in two
94 steps and these parameters which are used for soluble coffees according to its high degree of solubility
95 such as

96 1- The physical parameters: such as pH, Conductivity, TDS, and temperature.

97 2- The chemical parameters: Ammonia, Nitrate, Nitrite, Sulfate, Sulfide, and Phosphate,
 98 So, the determination of physic- chemical characteristics and parameters of preserving coffees in two
 99 steps and these parameters which are used for soluble coffees according to its high degree of solubility
 100 such as physic and chemical analysis for samples solutions according to the following table:

101 **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)	

Table 1 Physical and Chemical Properties of Coffee Samples(n=13)	102
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103 **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	ICP-OES and AAS	

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105 **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

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108 **5. Results:**

109 **A- Physical and chemical analysis:**

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

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

125 **B- Heavy metals:**

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

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

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

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➤ 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

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

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➤ Concentration levels of toxic metals: silver was ranged between 0 and 2.423 mg/kg, aluminum was ranged between 0.82 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)

149 **Discussion:**

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

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

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

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

174 Toxic heavy metals such as Al, As, Cd, Cr, Ni, Pb, Sb, and As also found high in the study results such as
175 Al concentration (mg/kg) the maximum concentration was 31.769, As concentration (mg/kg) the
176 maximum concentration was 0.107 Cd concentration (gm/kg) the maximum concentration was 0.0119, Cr
177 concentration (mg/kg) the maximum concentration was 1.1997. These results also consistent with other
178 study results in which the concentration these toxic metals were found high.[39]

179 **7. Conclusion:**

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

183 **Declaration**

184 **Ethical approval and Consent to Participate**

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

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

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

193 **Consent for Publication**

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

196 **Availability of Data and Material**

197 Data is confidential and not shared

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8. References:

1. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger (*Zingiber officinale*) cultivated in Ethiopia. *Springer Plus*, 2015; 4, 127.
2. Hiroyuki, K.T., Kazunobu, S., Takeji, T. (2011). Analysis of Iodinelike (Chlorine) Flavor-causing Components in Brazilian Coffee with Rio Flavor. *Food Sci. Technol. Res* 17(4): 347 – 352.
3. Ostrowska J., Stankiewicz A., Skrzydlewska E., Antioxidant properties of green tea. *Bromotol. Toxicol. Chem.* 2001; 2: 131.
4. Chandra S., De Mejia Gonzalez E., Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agric. Food Chem.* 2004; 52: 3583-3589.
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. C., Zhao J., Cholesterollowering effect of a theaflavin-enriched green tea extract: a randomized controlled trial. *Arch. Intern. Med.* 2003; 163: 1448- 1453.
6. Hasegawa R., Chujo T., Sai-Kato K., Umemura T., Tanimura A., Kurokawa Y., Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. *Food Chem. Toxicol.* 1995; 33: 961-970.
7. Fujiki H. Green tea: health benefits as cancer preventive for humans. *Chem. Rec.* 2005; 5: 119-132.
8. Bettuzzi S., Brausi M., Rizzi F., Castagnetti G., Peracchia G., Corti A., Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with highgrade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res.* 2006; 66: 1234-1240.
9. Seenivasan S., Manikandan N., Muraleedharan N.N., Selvasundaram R., Heavy metal content of black teas from south India, *Food Control.* 2008; 19: 746-749.
10. E. Álvarez-Ayuso, A. Giménez, and J. C. Ballesteros, “Fluoride accumulation by plants grown in acid soils amended with flue gas desulphurisation gypsum,” *Journal of Hazardous Materials*, 2011;192(3);1659–1666.

- 228 11. Z. Tan and G. Xiao, "Leaching characteristics of fly ash from Chinese medical waste
229 incineration," *Waste Management and Research*, vol. 30, no. 3, pp. 285–294, 2012.
- 230 12. M. Fujimaki Hayacibara, C. S. Queiroz, C. P. Machado Tabchoury, and J. Aparecido Cury,
231 "Fluoride and aluminum in teas and tea-based beverages," *Revista de Saude Publica*,
232 2004;38(1),100–105.
- 233 13. S.-C. C. Lung, H.-W. Cheng, and C. B. Fu, "Potential exposure and risk of fluoride intakes from
234 tea drinks produced in Taiwan," *Journal of Exposure Science and Environmental Epidemiology*,
235 2008;18(2),158–166.
- 236 14. X.-P. Wang, Y.-J. Ma, and Y.-C. Xu, "Studies on contents of arsenic, selenium, mercury and
237 bismuth in tea samples collected from different regions by atomic fluorescence spectrometry,"
238 *Guang Pu Xue Yu Guang Pu Fen Xi*, 2008;28(7)1653–1657
- 239 15. W.-Y. Han, F.-J. Zhao, Y.-Z. Shi, L.-F. Ma, and J.-Y. Ruan, "Scale and causes of lead
240 contamination in Chinese tea," *Environmental Pollution*,2006;139(1);125–132.
- 241 16. Shekoohiyan, M. Ghoochani, A. Mohagheghian, A. H. Mahvi, M. Yunesian, and S. Nazmara,
242 "Determination of lead, cadmium and arsenic in infusion tea cultivated in north of Iran," *Iranian*
243 *Journal of Environmental Health Science & Engineering*.2012;9,37-42.
- 244 17. D. Tang, T.-Y. Li, J. J. Liu et al., "Effects of prenatal exposure to coal-burning pollutants on
245 children's development in China," *Environmental Health Perspectives*, 2008;116(5)674–679.
- 246 18. M. E. Sears and S. J. Genuis, "Environmental determinants of chronic disease and medical
247 approaches: recognition, avoidance, supportive therapy, and detoxification," *Journal of*
248 *Environmental and Public Health*, 2012;3,15.
- 249 19. T. I. Lidsky and J. S. Schneider, "Lead neurotoxicity in children: basic mechanisms and clinical
250 correlates," *Brain*, 2003;126(1);5–19.
- 251 20. S. J. Genuis, G. Schwalfenberg, A. K. Siy, and I. Rodushkin, "Toxic element contamination of
252 natural health products and pharmaceutical preparations," *PLoS One*, 2012;7(11).
- 253 21. M. A. Rahman, B. Rahman, and N. Ahmed, "High blood manganese in iron-deficient children in
254 Karachi," *Public Health Nutrition*, 2013;16(9),1677–1683
- 255 22. F. M. Crinella, "Does soy-based infant formula cause ADHD? Update and public policy
256 considerations," *Expert Review of Neurotherapeutics*,2012;12,(4)395–407.
- 257 23. Klag MJ, Wang NY, Meoni LA, et al. Coffee intake and risk of hypertension. The Johns Hopkins
258 Precursors Study. *Arch Intern Med* 2002;162:657–62.
- 259 24. Ramato Ashu and Bhagwan Singh Chandravanshi," concentration levels of metals in
260 commercially available ethiopian roasted coffee powders and their infusions" *Bull. Chem. Soc.*
261 *Ethiop.* 2011, 25(1), 11-24.

- 262 25. Grembecka, M.; Malinowska, E.; Szefer, P. " Differentiation of market coffee and its infusions in
263 view of their mineral composition" *Sci. Total Environ.* 2007, 383, 59.
- 264 26. Santos, E.E.; Lauria, D.C.; Porto da Silveira, C.L. " Assessment of daily intake of trace elements
265 due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city" *Sci. Total Environ.*
266 2004, 327, 69.
- 267 27. Nędzarek, A.; Tórz, A.; Karakiewicz, B.; Clark, J.S.; Laszczyńska, M.; Kaleta, A.; Adler, G.
268 Concentrations of heavy metals (Mn, Co, Ni, Cr, Ag, Pb) in coffee. *Acta Biochim. Polonica*,
269 2013, 60, 623–627.
- 270 28. Dos Santos, J.S.; Dos Santos, M.L.P.; Conti, M.M.; Dos Santos, S.N.; De Oliveira, E.
271 Evaluation of some metals in Brazilian coffees cultivated during the process of conversion
272 from conventional to organic agriculture. *Food Chem.* 2009;115,1405–1410.
273
- 274 29. Offsetdrukkerij Haveka B.V., Alblasserdam" coffee and cardiovascular risk; an epidemiological
275 study. Oms lag Pieter-Jan Kersbergen2004. [Online] Available on www.who.int/coffee. access on
276 20/02/2019
- 277 30. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger
278 (*Zingiber officinale*) cultivated in Ethiopia. *Springer Plus.* 2015; 4 127.
- 279 31. Ayele, E.; Urga, K.; Chandravanshi, B.S. Effect of cooking temperature on mineral content
280 and antinutritional factors of yam and taro grown in southern Ethiopia. *Int. J. Food Eng.*
281 2015, 11, 371–382.
- 282 32. Weldegebriel, Y.; Chandravanshi, B.S.; Wondimu, T. Concentration levels of metals in
283 vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. *Ecotoxicol.*
284 *Environ. Saf.* 2012, 77, 57–63.
285
- 286 33. 13. Illy, E. The complexity of coffee. *Sci. Am.* 2002, 286, 86–91.
- 287 34. Anderson, K.A.; Smith, B.W. Chemical profiling to differentiate geographic growing origins
288 of coffee. *J. Agric. Food Chem.* 2002, 50, 2068–2075.
289
- 290 35. Suseela, B.; Bhalke, S.; Kumar, A.V.; Tripathi, R.M.; Sastry, V.N. Daily intake of trace
291 metals through coffee consumption in India. *Food Addit. Contam.* 2001, 18, 115–120.
- 292 36. Gebretsadik, A.T.; Berhanu, T.; Kefarge, B. Levels of selected essential and nonessential
293 metals in roasted coffee beans of Yirgacheffe and Sidama, Ethiopia. *Am. J. Environ. Protect.*
294 2015,4,188–192.
295

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

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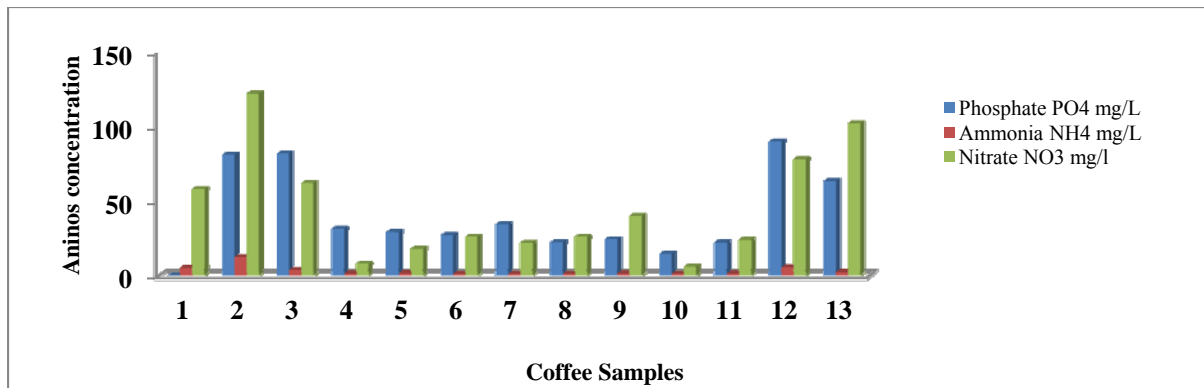
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319 **Figure (1) Average Level concentrations of phosphate, ammonia and nitrate between different coffee samples**

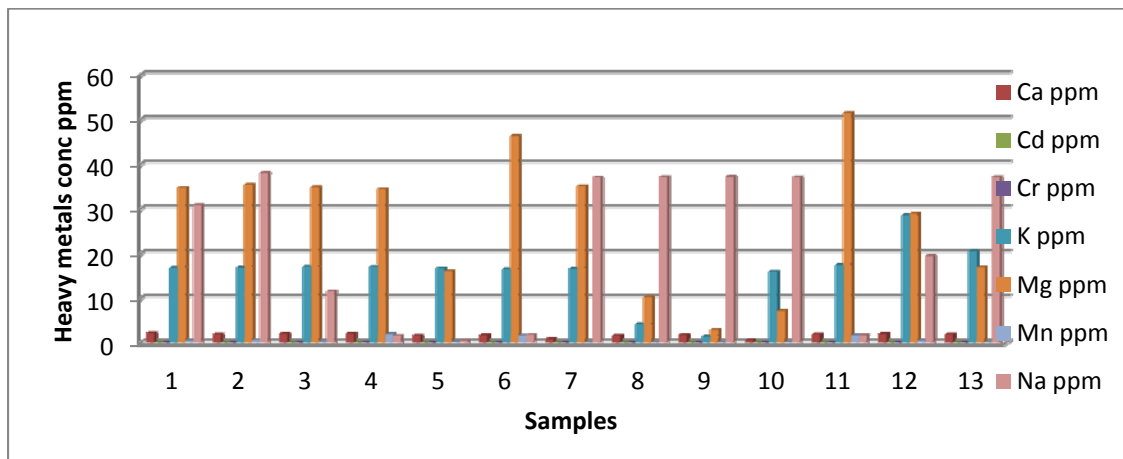
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326 **Figure 2 Non toxic heavy metals concentrations in different coffees samples**

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