Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2015**, 4(6), 309-315

Distribution of some heavy and essential metals Cd, Pb, Cu, Fe and Zn in Mango fruit (Mangifera Indica L.) cultivated in Different Regions of Pakistan

Nusrat Jalbani*, Shahid Bhutto, Suhail Rahujo and Farooq Ahmed

Pakistan Council Scientific and Industrial Research Laboratories Complex, Karachi, Pakistan.

Abstract: In the present study, the concentrations of Cd, Pb, Cu, Zn and Fe from 50 mango samples (Mangifera indica L.) were detected by electro-thermal/flame atomic absorption spectrometry (ETAAS/FAAS). In this study three varieties such as Dusahri, Langra and Chaunsa were collected from different regions (Multan, Sadiqabad, Rahimyar Khan and Mirpurkhas) of Pakistan. The effect of different varieties and environmental conditions on metal accumulation was also investigated. The aim of this study is to compare the level of essential and toxic metal in different regions and correlate the differences observed in metal accumulation to environmental conditions. The accuracy of the conventional acid digestion (CAD) method was checked by analyzing Certified Reference Materials (CRM) i.e. fortified water (TMDA-70), apple leaves (1515) and standard addition technique. The limit of detections (LODs) of the method were found to be 0.113, 2.0, 22.7, 3.85 and 3.05 µg L^{-1} for Pb, Cd, Cu, Fe, and Zn, respectively.

Keywords: Mangifera indica; Heavy metals; atomic absorption specterometry; Pakistan regions.

Introduction

Fruits are an important fraction of human diet since they contain polysaccharides, sugars, vitamins, minerals, macro and micronutrients which provide wonderful taste and excellent health properties.[1] Mango (Mangifera indica) is the national fruit of Pakistan[2] and is named as "King of the Fruits". It is commonly cultivated in many tropical and sub-tropical regions[2, 3]. Heavy metals are naturally present in environment by natural sources and also from anthropogenic activity or contamination during industrial processes, preservation and cooking[4-10]. Heavy metals are becoming part of the active components of pesticides thus, the continued use of fertilizer and pesticides was identified as the primary pathway from cultivated areas into agricultural soil [11, 12]. Under various conditions, heavy metals are potentially toxic to human health and to the environment even at low concentrations when ingested over a long time period[13-15]. The determination of metal ions at trace level by flame/electrothermal atomic absorption spectrometry (FAAS/ETAAS) has several advantages such as high selectivity, speed and fairly low operational cost. Direct determination of trace elements at extremely low concentration is often very difficult due to the insufficient sensitivity of the methods and the matrix interferences[16, 17].

Pakistan is the 5th largest mango producer with a production capacity of nearly one million tons per year, contributing a share of 7.6% in the world market. Multan, Rahim Yar Khan and Mir Pur Khas are the main mango producing regions of Pakistan. The aim of the present study is to examine the level of toxic elements in mango fruit grown in three major regions of Pakistan and to evaluate most commonly cultivated mango export varieties (Dusahri, Chaunsa, Ratol and Langra) for their nutritional and safety status. We examined the dry matter level of toxic elements in mango fruit, produced from the agricultural land and in order to evaluate the potential corresponding health risk. We then, determined the concentrations of toxic metal contamination and essential metals in mango fruits available in Pakistan super markets and assess how the metal contamination of fruits might have impacted food safety standards. This data will help to provide the status of toxic metals pollution in Pakistan and also to assure food safety and protect the end user health.

Materials and methods

Reagents and glassware

Chemicals and reagents were used of high purity analytical grades (Merck, Darmstadt, Germany). Nitric acid (65%) and hydrogen peroxide

**Corresponding author: Nusrat Jalbani*
E-mail address: nusratjalbani_21@yahoo.com
DOI: http://dx.doi.org/

(30%) used were obtained from (Merck, Darmstadt, Germany). Stock standard solutions (1000 µg mL^{-1}) of studied metal ions were purchased from Merck (Darmstadt, Germany). While working standards of corresponding metal ions were prepared freshly on daily basis. The laboratory glass wares were kept overnight in 10% (v/v HNO_3) and washed with water and rinse with distilled water before use. After washing and rinsing, the glasswares were dried in an oven at 80 ^{0}C for 1 h.

Instrumentation

Atomic absorption spectrometer of Hitachi Ltd. (Model 8000 Z) was used, Electro-thermal atomic absorption spectrometer (ETAAS) was used for determination of Pb, Cd, and flame atomic absorption spectrometer (FAAS) was used for determination of Cu, Fe and Zn. Calibration curves were as following, (10-30 µg L^{-1}) for Pb and (0.5-2 µg L^{-1}) for Cd, while for essential elements (0–1.2 µg mL^{-1}) for Cu, Fe and Zn. Instrumental parameters are listed in Table 1

Table 1. Measurement Conditions for Atomic Absorption Spectrometry (AAS)

Measurement Conditions for electro-thermal Atomization Atomic Absorption Spectrometry						
Operating Parameters	Cd			**Pb**		
Lamp current (mA)	7.5			**7.5**		
Wavelength	228.8			**283.3**		
Slit width (nm)	1.3			**1.3**		
Cuvette	Tube			**Tube**		
Temperature Programming						
Drying	80	120	30 Sec	80	120	**30 Sec**
Ashing	300	300	30	400	400	**30**
Atomization	1500	1500	10	200	200	**10**
Cleaning	1800	1800	3.0	2400	2400	**3.0**
Common Parameters						
Sample volume	**10µL analyte**					
Backgrund Correction	**D^2 Lamp**					
Carrier gas Argon	**200 mL/mint**					
Measurement conditions for flame atomic absorption spectrometer						
Elements	Wavelength (nm)	Slit width (nm)	Lamp Current (mA)	Oxidant Pressure	**Fuel**	
Cu	324.8	0.7	30	1.6	**Air-acetylene**	
Fe	372	0.2	5.0	1.6	**Air-acetylene**	
Zn	**213.9**	**0.7**	**30**	**1.6**	**Air-acetylene**	

Table 2 show the Linearity range of concentration versus absorbance.

Table 2. The range of linearity of concentration versus absorbance graph

Metals	**Correlation Coefficients**
Cd	**$Y=0.0059X+0.0017$, $R^2= 0.9996$**
Pb	**$Y=0.0004X+0.0001$, $R^2= 0.9993$**
Cu	**$Y=0.0144X+0.0021$, $R^2= 0.9985$**
Fe	**$Y=0.0285X+0.0003$, $R^2= 0.9989$**
Zn	**$Y=0.0373X+0.0002$, $R^2= 0.9998$**

Sampling

50 mango samples were collected from different regions of Pakistan (Multan, Sadiqabad, Rahimyar Khan and Mirpurkhas). From each selected regions (n=12) about three varieties of mango samples (Chaunsa, Dushari and Langra) were randomly collected in the year of 2010-2011. Mango fruit were well blended and homogenized then properly labeled and kept separately in a plastic bag. Fortified water (TMDA-70) and apple leaves (15151) were used as certified reference material (CRM).

Sample preparation

Wet acid digestion method

Duplicate of 10-15 gram of mango fruit (fresh weight) samples were weighed into 250 mL of conical flask. Fifty mango samples were analyzed. 20 to 40 mL of HNO_3 (65%) and 5 mL of H_2O_2 were added to the samples heating on hot plate at 90 ^{0}C and then mixture was evaporated to dryness. After evaporation and cooling the samples were filtered through Whatman 42 filter paper and then collected into volumetric flasks and diluted with distilled water. Blanks were also treated in the same manner as samples and both were subjected into ETASS/FAAS for determination of the above mentioned metal ions.

Statistical analysis

Basic statistics on analytical data (mean values with standard deviation) were carried out with Microsoft Excel 2003.

Results and Discussion

The present work was focused on establishing trace elemental levels in different region of three types of samples in order to find ecological and environmental relationship between trace elements. Mango fruit is a summer seasonal commodity growing on agricultural land containing high levels of Cu, Fe, and Zn, with an important role in human nutrition. The analyzed samples were categorized on the basis of variety and region. The mean results of three varieties for Pb, Cd, Cu, Fe, and Zn obtained by proposed procedure of metal ions determination are given in Table 3. The results indicated low levels of Pb (5.28±0.28) in Shujabad, of Cd (2.02±0.11) in Tandoallah Yar, of Fe (0.842±0.02) in Multan whereas, lower level of Cu (0.697±0.04) and Zn (1.01±0.05) were found in Muzfar Garih and Sadiqabad regions (Table 3).

Table 3. Elemental contents in mango fruits of different region of Pakistan (Fresh wt) (n=12)

Regions	Cd[a]	Pb[a]	Cu[b]	Fe[b]	Zn[b]
Qitalpur	3.99±0.12	5.78±0.32	1.56±0.05	0.885±0.06	**1.27±0.07**
Tandoallah Yar	2.02±0.11	9.75±0.46	1.78±0.07	1.23±0.08	**1.86±0.09**
Muzfr Garh	2.89±0.21	8.46±0.49	0.697±0.04	1.17±0.06	**1.74±0.08**
Khanewal	2.57±0.22	12.51±0.73	1.63±0.01	1.32±0.07	**1.83±0.11**
Hydrabad	2.07±0.11	12.1±0.77	1.82±0.5	1.25±0.08	**1.86±0.12**
Multan	2.45±0.09	9.53±0.61	0.898±0.09	0.842±0.02	**2.72±0.18**
Shujabad	2.32±0.08	5.28±0.28	0.887±0.07	2.68±0.12	**1.79±0.19**
Sadiqabad	3.38±0.23	11.2±0.64	1.15±0.09	2.35±0.15	**1.01±0.05**
Rahim Yar Khan	5.73±0.33	8.08±0.38	0.953±0.04	2.71±0.18	**1.25±0.06**
Mirpurkhas	**2.37±.11**	**9.33±0.42**	**0.821±0.03**	**2.19±0.12**	**1.63±0.08**

[a]µg Kg^{-1} , [b]mg Kg^{-1}

In the view of results, no significant difference was observed among the varieties of different regions at 95% confidence level as indicated in Table 4 where three essential metals Cu, Fe, and Zn were found at low level in the variety of Dushari in most of the regions.

Table 4. Distribution of selected trace and toxic elements in mangoes varieties grown in Pakistan

Regions		Cd[a]	Pb[a]	Cu[b]	Fe[b]	Zn[b]
Qitalpur	A	2.32±0.15	6.12±0.46	1.75±0.013	0.78±0.065	**1.17±0.055**
	B	3.73±0.21	5.22±0.46	1.61±0.13	1.47±0.02	**1.32±0.120**
	C	5.92±0.36	6.01±0.56	1.32±0.045	0.405±0.021	**1.31±0.055**
Tando allah yar	A	1.90±0.086	9.81±0.55	2.01±0.05	1.33±0.065	**3.02±0.083**
	B	2.11±0.16	9.75±0.62	1.98±0.12	1.49±0.06	**1.3±0.086**
	C	2.05±0.06	9.71±0.66	1.71±0.055	0.865±0.055	**1.27±0.052**
Muzfr garh	A	1.78±0.091	8.41±0.51	0.44±0.023	1.58±0.036	**2.06±0.076**
	B	0.912±0.06	8.50±0.51	1.38±0.02	1.12±0.05	**1.92±0.072**
	C	6.00±0.11	8.48±0.51	0.27±0.01	0.805±0.061	**1.24±0.051**
Khanewal	A	1.56±0.085	13.0±0.73	1.57±0.051	1.36±0.025	**2.70±0.085**
	B	1.75±0.03	12.5±0.91	1.95±0.06	1.635±0.04	**1.65±0.045**
	C	4.40±0.12	12.03±0.68	1.29±0.052	0.956±0.073	**1.33±0.046**
Hydrabad	A	1.17±0.071	12.6±0.61	1.95±0.075	1.63±0.045	**1.45±0.042**

	B	1.23±0.04	11.5±0.82	1.67±0.045	1.49±0.03	**2.89±0.076**
	C	3.83±0.23	12.4±0.71	1.49±0.061	0.63±0.042	**1.25±0.048**
Multan	A	2.11±0.13	9.56±0.62	1.049±0.079	1.07±0.078	**3.01±0.081**
	B	1.63±0.07	9.48±0.43	1.124±0.02	0.615±0.02	**3.25±0.160**
	C	3.61±0.13	9.55±0.58	0.522±0.034	0.84±0.056	**1.92±0.078**
Shujabad	A	2.08±0.11	5.20±0.23	0.8±0.065	3.0±0.125	**2.07±0.078**
	B	2.33±0.06	5.50±0.23	1.2±0.03	1.65±0.071	**1.78±0.032**
	C	2.56±0.15	5.15±0.45	0.66±0.043	3.45±0.25	**1.53±0.036**
Sadiqabad	A	3.44±0.25	13.2±0.76	0.678±0.044	2.56±0.16	**1.48±0.048**
	B	2.62±0.22	10.1±0.78	1.3±0.05	2.66±0.15	**1.14±0.043**
	C	4.08±0.27	9.78±0.61	1.48±0.086	1.85±0.18	**0.413±0.011**
Rahim yar Khan	A	3.09±0.25	8.67±0.57	0.826±0.067	2.16±0.11	**1.23±0.041**
	B	2.41±0.21	8.12±0.31	0.629±0.04	3.19±0.26	**1.07±0.036**
	C	11.70±0.21	7.45±0.72	1.403±0.076	2.75±0.16	**1.46±0.034**
Mirpurkhas	A	0.73±0.03	11.3±0.73	0.557±0.034	2.11±0.12	**1.60±0.073**
	B	2.15±0.19	8.14±0.33	1.31±0.05	1.75±0.13	**1.74±0.025**
	C	**4.24±0.11**	**8.56±0.53**	**0.598±0.033**	**2.71±0.17**	**1.55±0.038**

A = Langra, B = Chonsa, C = Dusheri. [a]µg Kg^{-1} [b]mg Kg^{-1}

The level of Cu in mango fruits ranged from 0.697 to 1.82 mg Kg^{-1}(Table 3). This value was not significantly different between the different regions under study. It is noteworthy that higher level of Cu was observed in the city of Hyderabad while low level was found in Muzfar Garh. An elevated level of Fe (2.71±0.18) and Zn (2.72±0.18) mg Kg^{-1} was found in Rahim Yar Khan and Multan respectively (Table 3). Zinc is an essential element for proper metabolism. The significant differences were found among the different regions of Pakistan when comparing the obtained values at a confidence interval of 95%. In this work the lower level of Fe, Zn, and Cu were found in Multan, Sadiqabad and Muzfr Garh (Table 3). On contrary, the concentration of Cd was higher in Dushari variety and of Pb was higher in most of the studied regions[18]. However, in eight samples level of Pb was found higher in Langra (L) variety while 2nd and 3rd highest level of Pb was obtained in chaunsa (B) and Dushari (C), accordingly (Table 4). The Pb content of mango fruit collected from different regions was found to be within the permissible limit based on EU regulations (Table 7). The highest average concentration of Cd was 5.73±0.33 mg Kg^{-1} in the Rahim Yar Khan and the level of Pb was 12.51±0.73 µg Kg^{-1} in Khanewal (Tabel 3). The concentration of Cd 5.73 µg Kg^{-1} was found at maximum level in all selected variety, while the levels of Cd and Pb were relatively lower in Multan with 3.81±0.21 µg Kg^{-1} and 4.06±0.32 µg Kg^{-1} respectively. From the results it was clear that the Dushari and Langra contained higher concentration of Cd and Pb as compared to variety of chounsa.

Discussion

Fruits are considered key factor for providing nutritional basis in human diet and as well as for their bio-functional components[19]. Hence, the objective of our study was to evaluate the heavy metals present in three varieties of Mango fruit from different regions of Pakistan. Macro and trace elements play a significant role in maintaining health in humans. Heavy metals i.e. Cd and Pb are regularly present in foods and considered dangerous elements even at low contamination[20-21]. These metals are concentrated particularly in kidneys, liver, blood forming organs and lungs. They most frequently result in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme Inhibitions[22], [23]. These heavy metals may replace the essential nutrients in human body adjoining their sites to the vital organs. Their ingestion could lead to severe liver damage, symptoms of chronic toxicity in kidney, pancreas or Alzheimer etc. diseases[24-26]. Nutrients are important and beneficial components for human health[27, 28]. The maximum permitted level of Fe for food is 15mg Kg^{-1} according to WHO[29], the concentration of essential and non-essential elements in mango fruit are found within acceptable limits except Cd [30, 31]. These results indicated that there are common anthropogenic contamination sources regarding heavy metals.

Analytical figure of merit

Under the optimized conditions (Table 1), the analytical performance of the CAD was evaluated. The linear range was 10-30, 0.5-2.0 µg L^{-1} for Pb and Cd, whereas 0.2, 2.0 µg mL^{-1} for Cu, Fe and Zn respectively with the correlation coefficient (R) of 0.998 and 0.9999. The limit of detection (LOD) was calculated as equivalent of three times of standard deviation of blank readings, the calculated LODs are: 0.113, 2.0, 22.7, 3.85, and 3.05 µg L^{-1} for Pb, Cd, Cu, Fe, and Zn respectively.

Validation of proposed methodology

In order to validate the methodology, the accuracy of the proposed method was evaluated by the analysis of CRM; fortified water (TMDA-70) apple leaves (1515) and standard addition recovery/test method to determine trace and toxic metals in mango fruit. The results are given in Tables 5 and 6. The proposed procedure was applied on four replicates of each samples as discussed in ‘‘digestion procedure’’ section. A recovery of the spiked metals

is close to (97.9-102%) which show quantitative recovery of the understudied metals as seen in Table 5. A good agreement exists between the results of the proposed method and can be applied successfully to real samples.

Table 5. The results of certified reference material (CRM), TMDA 70 (Fortified water) µg L^{-1} and Apple leaves (NIST-1515), mg Kg^{-1}.

	Fortified water (TMDA 70)			**Apple leaves (NIST-1515)**		
Analyte	Certified	CAD	% Recovery	Certified	CAD	% Recovery
Cd	145	144±5.3	99.3	0.013	0.0131±0.001	101
Pb	444	445±12	100	0.47	0.46±0.02	97.9
Cu	398	399±9.8	100	5.64	5.63±0.35	99.8
Fe	368	367±11	99.7	83	84±3.21	101
Zn	477	476±18	99.8	12.5	12.8±0.55	102

Table 6. The results of standard addition/recovery for Cd, Pb, Cu, Fe and Zn determination in mango fruit samples (Multan) (*n*=6).

Added	**CAD** $\bar{x} \pm ts/\sqrt{n}$ [a]	**% Recovery**
Cd[a]		
0.0	3.81±0.18	**99.5**
0.5	4.29±0.13	**99.8**
1	4.8±0.18	**99.7**
2	5.79±0.19	**99.5**
Pb[a]		
0.0	3.45±0.15	**99.7**
10	23.43±0.97	**99.9**
20	33.4±0.81	**99.8**
30	13.41±0.78	**99.7**
Cu[b]		
0.0	2.21±.09	**98.9**
0.4	2.58±0.09	**98.9**
0.6	2.78±0.078	**98.7**
0.8	2.97±0.016	**98.9**
Fe[b]		
0.0	3.01±.0.16	**99.4**
0.5	3.49±0.15	**99.3**
1.0	3.98±0.23	**99.2**
2.0	4.97±0.19	**99.4**
Zn[b]		
0.0	2.21±0.12	**99.2**
0.4	2.59±0.12	**98.9**
0.6	2.78±0.16	**99.3**
0.8	**2.99±0.15**	**99.2**

[a]Average value ± confidence interval (P = 0.05) µg L^{-1}, [b]Average value ± confidence interval (P = 0.05) µg mL^{-1}

Applications

In order to evaluate the accuracy of the CAD method, certified reference materials fortified water (TMDA-70) apple leaves (1515) were analyzed and the results are presented in Table 4. Different mango fruit samples were subjected to the CAD procedure for determination of concentrations of Pb, Cd, Cu, Fe and Zn respectively. The results are given in Table 6. The daily intake of all studied metals were calculated on the basis of consumption of a minimum 300 gm/person/day of mango and compared with permissible limits by WHO/RDA as represented in Table 7.

Table 7. Concentration of trace and toxic elements in mango fruit (fresh weight) grown on agricultural land of different regions of Pakistan

Metals	Permissible level ($mgKg^{-1}$)	daily consumption/person/day (mg/person/day)
Cd	0.05[32]	**0.015**
Pb	0.10[32]	**0.03**
Cu	5.0[33]	**1.5**
Fe	5.0[33]	**1.5**
Zn	**5.0[33]**	**1.5**

Average daily consumption rate of fruits per person (fresh weight) = 300 g

In the present study it is important to remark that the concentration of all studied macro and micronutrient were found within permissible limits except Cd.

Conclusion

Pakistan is an agricultural country and production of mango fruits is an important part of this sector. Trace and toxic metals may get into natural water and soil and thus into fruit or plant through polluted air. This can result in the contamination of food chain causing serious problems in human health which is directly affected by ingestion of contaminated food and water.

The uptake of elemental contents Cd, Pb, Fe, Cu and Zn by three varieties of Mango growing in different regions of Pakistan was studied. The variations in uptake of elemental contents by different varieties were evaluated to check higher tolerance limit for toxic elements present in mango fruit. In view of these results, it was concluded that the variety of Mirpur Khas is the best because the trace and toxic metals were found within safe limits as compared to other regions.

References

1- S. Akhtar, N. Safina, M.T. Sultan, S. Mahmood, M. Nasir and A. Ahmad, *Pak. J. Bot.* **2010,** *42(4)*, 2691-2702.

2- M. B. Usman, Fatima, M. M. Khan and M. I. Chaudhry, *Pak. J. Agri. Sci.* **2003**, 40 (3-4).

3- F. A. Jam, S. Mehmood and Z. Ahmad. Academy of Contemporary Research Journal V II (I), 10-15, ISSN: 2305-865X, 2013.

4- O. Mumzuroglu, F. Karatas and H. Geekil, *Food Chem*. **2003**, *83*, 205-212.

5- S.G. Ozcan, N. Satiroglu and M. Soylak, *J Food Chem. Toxicol.* **2010,** *48*, 2401-2406.

6- M. Tuzen, E. Sesli and M. Soylak, *Food Cont.* **2007a,** *18*, 806-810.

7- M. Tuzen, S. Silici, D. Mendil and M. Soylak, *Food Chem*, **2007b.** *103*, 325-330.

8- S. Singh, M. Zacharias, S. Kalpana and S. Mishra. *J. Environ. Chem. Ecotoxicol.* **2012**, 4(10), 170-177.

9- A. Duran, M. Tuzen and M. Soylak, *Int. J. Food Sci. Nutr.* **2008,** *59*, 581-589.

10- M. Tuzen, M. Soylak, D. Citak, H.S. Ferreira, M.G.A. Korn and M.A. Bezerra, *J. Hazard. Mater.* **2009**, *162*, 1041-1045.

11- I. Narin, M. Soylak, K. Kayakirilmaz, L. Elci, and M. Dogan, *Anal. Lett.* **2003**, *36*, 641-658.

12- J.E.V. Nunez, N.M.B.A. Sobrinho and N. Mazur, *Ci. Rural.* **2006**, *36*, 113-119.

13- Z. Parveen, M.I. Khuhro and N. Rafiq, *Bull. Environ. Contam. Toxicol.* **2003,** *71*, 1260-1264.

14- S. Baytak and A.R. Turker, *Talanta*, **2005**, *65*, 938–945.

15- P.C. Aleixo, D.S. Junior, A.C. Tomazelli, I.A. Rufini, H. Berndt and F. J. Krug, *Anal. Chim. Acta.* **2004**, *512*, 329-337.

16- M.K. Jamali, T.G. Kazi, M.B. Arain, H.I. Afridi, N. Jalbani and G.A. Kandhro, *J. Hazard. Mater.* **2008**, 158, 644-651.

17- N. Jalbani, F. Ahmed, T.G. Kazi, U. Rashid, A.B. Munshi and A. Kandhro. *Food Chem. Toxicol.* **2010,** *48*, 2737-2740.

18- D. Petit, F. Claeys, C. Sykes and Y. Noefnet, *J. Physique. IV France*, **2003**, *107* (1053), 1053 - 1056.

19- M.A.M. Sajib, S Jahan, MZ Islam, TA Khan and B.K. Saha, *Int. Food Res. J.* **2014,** *21*(2), 609-615.

20- X. Bi ,L. Ren, M. Gong, Y. He, L. Wang, Z. Ma. The Global J. Soil Sci. 2010, 155 (1-2), 115-120.

21- A.0. Igwegbe, H.M. Belhaj, T.M. Hassan and A.S. Gibali, *J. Food Saf.* **1992.** 13, 7-18.
22- N. Jalbani, T.G. Kazi, N. Kazi, M.K. Jamali and M.B. Arain, *Biol.Trace Elem. Res.* **2008**, 1-12.
23- S. I. Khan, A.K. M. Ahmed, M. Yunus, M. Rahman, S. K. Hore, M. Vahter and M.A. Wahed. *J. Health Popul. Nutr.* **2010**, 28(6), 578-584.
24- M. Yaman, *Anal. Biochem.* **2005.** *339* (1), 1-8.
25- M. Tuzen and M. Soylak, *J. Hazard. Mater.* **2009**, *164*(1), 1428-132.
26- Tu. Zhifeng, He. Qun, X. Chang, Hu. Zheng, Ru. Gao, L. Zhang and Li. Zhenhua, *Anal. Chim. Acta,* **2009,** *649* (2), 252-257.
27- A. Waheed, M. Jaffar and K. Masud, *Nutr. food Sci.* **2003**, *33*, 6.
28- E. Zahir, I.I. Naqvi and S. Mohi Uddin, *J. Basic Appl. Sci.* **2009**, *5* (2), 47-52.
29- WHO. Evaluation of certain food additives and contaminants: 46th Report of the joint FAO/ WHO expert committee on food additives, WHO. Technical report series No. **1997,** *868,* pp i-viii.).
30- G. Herrick and T. Friedland, *Water Air. Soil Poll.* **1990**, *53*, 151-157.
31- FAO/WHO, Toxicological evaluationof certainfoodadditives and contaminants. Forty-first report of the, Joint FAO/WHOExpert CommitteeonFoodAdditives (WHO Technical Report Series, No. 837, WHO, Geneva, **1993**.
32. Commission Regulation (EC) No 629/2008. Amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, **2008.**
33. A. S. M. Hassan, T. A. Abd-El-Rahman, A. S. Marzouk. *Int. J. Food Sci. Nutr. Eng.* 2**014**, 4(3), 66-72.