Pakistan J. Zool., vol. 46(3), pp. 633-641, 2014.

Vegetables as a Potential Source of Metals and Metalloids for Human Nutrition: A Case Study of *Momordica charantia* Grown in Soil Irrigated with Domestic Sewage Water in Sargodha, Pakistan

Zahara Bibi,[1]* Zafar Iqbal Khan,[1] Kafeel Ahmad,[1] Muhammad Ashraf,[2] Abrar Hussain[3] and Nudrat Aisha Akram[4]

[1]*Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan*
[2]*University College of Agriculture, University of Sargodha, Sargodha, Pakistan*
[3]*Science and Technology Division, University of Education, Township Campus, Lahore, Pakistan*
[4]*Department of Botany, GC University, Faisalabad, Pakistan*

Abstract.- Bitter gourd (*Momordica charantia*) grown around refuse and effluent sites of District Sargodha, Pakistan were analyzed for six metals and metalloids viz., cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), zinc (Zn) and selenium (Se) by atomic absorption spectrophotometric method. Results showed that the concentrations of Co, Ni, Cu, As, Zn and Se at both sites were below the permissible limit. The levels of heavy metal and metalloid (mg/kg dry wt.) in vegetable at site-I were 0.83 for Co, 4.78 for Ni, 20.22 for Cu, 13.35 for As, 52.25 for Zn, 0.78 for Se and 0.874 for Co, 5.63 for Ni, 22.68 for Cu, 13.54 for As, 61.77 for Zn and 0.642 for Se at site-II. Transfer factors in the range of 0.049-10.97 were obtained, with Zn and Cu having the highest concentration factors of 10.97 and 2.46, respectively. The differences in uptake of these heavy metals and metalloids are ascribed to difference in tolerance to these metals by the vegetable species. The contamination factor at site-I was greater as compared to those observed at site-II. Concerning the eating habit of inhabitants, the daily intake of As, Cu, Zn and Ni was above the reference dose. Therefore, the intake of *Momordica charantia* was not safe for consumption in the sampling area. To reduce the health risk effects, it is suggested to treat the industrial wastes properly and phyto-extract the overload of heavy metals and metalloids from polluted sites.

Key words: Metals and metalloids, wastewater, *Momordica charantia*, maximum permissible limits, dietary toxicity.

INTRODUCTION

An assessment is necessary for the regulation, bioremediation and handling of harmful material in the whole ecosystem. Risk assessment of ecosystem is dawning in the field of environmental sciences and for sustainability of overall ecosystem, significant survey is essential in industrialized countries. In Pakistan, the appliance of wastewater is confined in agricultural sub-farms. District Sargodha is the region where municipal wastewater is used to irrigate crops especially vegetables (Ahmad *et al.*, 2013). Under semi-arid and arid conditions, the use of sewage water is advantageous because of its low cost and easy way of conservation and disposal (Mapanda *et al.*, 2005). Wastewater provides necessary nutrients such as nitrogen and phosphorus for cultivation purpose (Nyamangara and Mzezewa, 1999).

Heavy metals and metalloids are abundant in areas where anthropogenic pressure is greater. The presence of metals and metalloids can cause severe problems for all organisms. In agricultural soil, the accumulation of metals badly affects the crop growth and food quality (Msaky and Calvert, 1990; Fergusson, 1990; Ma *et al.*, 1994; Adhikari *et al.*, 2004; Islam *et al.*, 2007) and environmental health, including terrestrial animals and soil fauna and flora. Plant intake these pollutants by absorbing it from surface of plant, which is in direct contact with contaminated soil and polluted air (Al-Jassir *et al.*, 2005; Singh and Kumar, 2006).

Vegetables comprises of essential dietary components by supplying calcium, proteins, vitamins and other nutrients (Thompson and Kelly, 1990). Vegetables resist the pH of the acidic substances produced during the digestion process. In addition to essential elements, vegetables also contain toxic elements. Accumulation of these toxic

* Corresponding author: zahrabibi13@gmail.com, zikhan11@gmail.com
0030-9923/2014/0003-0633 $ 8.00/0

compounds can be a threat for human health (Damek-Poprawa and Sawicka-Kapusta, 2003; Türkdogan *et al*., 2003). Vegetables also absorb metals from air and deposit them on vegetables parts exposed to polluted environment (Zurera-Cosano *et al*., 1989; Sobukola *et al*., 2010). The vegetables grown in polluted soil take up large quantities of heavy metals in their different parts that can cause public health problems (Mahfuz *et al*., 2004; Khan *et al*., 2008; Khanam *et al*., 2011). The awareness has increased among the people at national and international level about the adverse effects and risks caused by heavy metals present in the food additives and as a result, the permissible level of metal has decreased up to some extent in food items (Radwan and Salama, 2006). The cleanup of heavy metals from polluted soil is very expensive. However, the metal removal from contaminated soil by microbial activity has become the most common (Shakoori *et al*., 2004; Rehman *et al*., 2007; Resmi *et al*., 2010).

Momordica charantia commonly known as bitter gourd. Bitter gourd is one of the most popular vegetables in Southeast Asia. It is a member of the Cucurbitaceae family. It is rich in vitamins, minerals, carbohydrates and proteins (Miniraj *et al*., 1993; Desai and Musmade, 1998; Behera *et al*., 2010). Bitter gourd shows significant variation in nutrient contents including calcium, proteins, phosphorous, iron, ascorbic acids and magnesium (Kale *et al*., 1991). Studies in Sargodha, Pakistan focus primarily on Co, Ni, Cu, As, Zn and Se concentration in *Momordica charantia* and rhizospheric soil. In addition, heavy metal and metalloid uptake by the vegetable and the pollution load and transfer factor from soil to edible portion of vegetable as well as health risks due to consumption of the vegetable were assessed. This information may help understand whether or not these municipal wastewater irrigated sites could be fit for cultivation of vegetables particularly the *Momordica charantia*.

MATERIALS AND METHODS

This study was conducted in the vicinity of Sargodha district, Punjab, Pakistan. Sargodha city lies in 32°08′00″ north latitude and 73°7′00″ east longitude. Sargodha is an agricultural city. Its climate is extreme, ranging 35-49°C in summer and in winter 5-23°C. It is almost 30 miles away from motorway and is linked to it through several link roads. Vegetables are grown extensively in this region. A reconnaissance survey was conducted in the peri-urban sites, irrigated with municipal wastewater.The samples of *Momordica charantia* (edible portion) and rhizospheric soil were collected from two sites. Site-I located at 10 km distance and site-II at 25 km distance from Sargodha city. The samples of *Momordica charantia* and rhizospheric soil were collected randomly from wastewater irrigated fields. The metals and metalloids under investigation were cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), zinc (Zn) and selenium (Se).

Samples collection

Samples of *Momordica charantia* (each included more than 03 repetitions) were collected from two sites irrigated with wastewater. To remove dust, vegetable samples were washed with distilled water and air dried and then placed in an oven at 70ºC for consecutive 3 days to remove moisture contents.

Soil samples were collected under the vegetable. The soil was excavated up to 12-15 cm depth by an auger containing all layers (Sanchez, 1976). Firstly, the soil samples were dried in an air and then dried at 72°C in an oven for three consecutive days (Campbell and Planks, 1992) and were pulverized and mashed into powder form by using a grinder and sieved. Prior to analysis, the samples were packed into polythene bags.

Reagents

Ultra pure water was used throughout the work. Hydrochloric acid (90, s.g.119), nitric acid (40, s.g 1.4), hydrogen peroxide (35% w/v) were analytical reagents.

Digestion of soil samples

H_2O_2 and H_2SO_4 reagents were used in 2:1 (8 ml and 4 ml) for digestion of 1g soil sample. The samples were taken in pyrex flasks and placed in a digestion chamber for 30 min until the evaporation of reagents ceased. The flasks were removed from the chamber and added 2 ml of H_2O_2 into it. For further heating, the flasks were again heated in a chamber until colorless end point achieved. The reaction mixture was filtered and to it distilled water was added to make the final volume equal to 50 ml. These samples were stored in plastic bottles until further analysis.

Digestion of vegetable samples

H_2O_2 and H_2SO_4 reagents were used in 2:1(4 ml and 2 ml) for digestion of 1g vegetable sample. Further course of the protocol was same as adopted for soil analysis.

Metal and metalloid analysis

In order to determine accurate values of different metals and metalloids in the soil and vegetable samples, standards of Co, Ni, Cu, As, Zn and Se were prepared to calibrate flame atomic absorption spectrophotometer (FAAS) and graphite furnace atomic absorption spectrophotometer (GFAAS). Ni, Cu and Zn were analyzed by atomic absorption spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp., 1980). Co concentrations were determined using graphite furnace atomic absorption spectrophotometer (AA-6300 and GFAEXi7i, Shimadzu, Japan). A fluorometer was used for selenium (Se) and AAS-GF-HG (Perkin Elmer) for arsenic (As) determination. A standard curve was constructed following Watkinson (1966) using a stock solution of 1,000 mg l^{-1} each.

Quality assurance

For all the metals, accuracy and precision of analyses were assured within ±2% error following precautions proposed by the National Institute of Standard and Technology, Standard Reference Material (SRM 1570). The coefficients of variation of replicate analyses were determined and variations less than 10% considered reliable.

Statistical analysis

Statistical analysis of data was done by SPSS 17. For soil, one-way ANOVA and for vegetable and site, two-way ANOVA was used. Correlations between the vegetable and the soil were also worked out. Significance of means was calculated at probability levels of 0.05, 0.01 and 0.001 (Steel and Torrie, 1980).

Transfer factor for vegetable/soil system

To analyze the accumulating capability of metals from soil to vegetable, a quantitative assessment of the correlation between the concentration of metal contents in the vegetable and in subsequent soils was done by transfer factor (Cui *et al*., 2004).

$$TF = [\text{Metal}]_{\text{vegetable}} / [\text{Metal}]_{\text{soil}}$$

where: TF stands for transfer factor

$[M]_{\text{vegetable}}$ is the concentration of metals in the vegetables (mg/kg)

$[M]_{\text{soil}}$ is the concentration of metals in the soils (mg/kg)

Pollution load index

Pollution load index (PLI) has been evaluated by following at a particular site (Liu *et al*., 2005).

$$\text{PLI} = \text{Metal concentration in investigated soil} \,/\, \text{reference value of the metal in soil}$$

Health risk index

Health risk index was calculated as the ratio of estimated exposure to metal via vegetable and oral references dose (Cui *et al.*, 2004).

$$\text{Health risk index (HRI)} = \text{DIM}/ R_fD \text{ (USEPA, 2002)}$$
$$\text{Daily intake of metal (DIM)} = C_{\text{metal}} \times D_{\text{food intake}} / B_{\text{average weight}}$$

where: C_{metal}, $D_{\text{food intake}}$ and $B_{\text{average weight}}$ represents the heavy metal concentrations in vegetables (mg/kg), daily intake of vegetables (kg/day) and average body weight (kg), respectively. From Integrated Risk Information System (USEPA, 2010), the R_fD values were Cu (0.04), Se (5×10^{-3}), Ni (0.02), As (3×10^{-4}), Co (0.043) and Zn (0.37) mg/kg/day. The average daily metal intake in 0.345 kg of vegetables for adult and the average body weight of 60 kg was considered (Ge, 1992; Wang *et al*., 2005). An index greater than 1 is considered dangerous for human (USEPA, 2002).

RESULTS

According to one-way analysis of variance of data for metals and metalloids in soil, sites had the significant (P<0.01) effect on As concentration while non-significant (P>0.05) effect on Co, Ni, Cu, Zn and Se concentration (Table I). Two-way analysis of variance of data for metals and metalloids in vegetable illustrated highly significant (P<0.001) effect of site on Zn while Ni was significantly (P<0.01) affected but indicated non-significant (P>0.05) effect on Co, Cu, As and Se concentration. Due to site-vegetable interaction, Co, As and Zn concentration was non-significantly (P>0.05) affected while significant (P<0.01) effect on Ni whereas Cu and Se concentration was less significantly (P<0.05) affected by sites (Table II).

Table I.- One-way analysis of variance of metal and metalloid in soil at different sites.

Metal and metalloid	Site
Co	1.171^{ns}
Ni	0.754^{ns}
Cu	0.267^{ns}
As	0.975^{**}
Zn	0.467^{ns}
Se	0.002^{ns}

****** = significant at 0.01 level ns = not significant

Table II.- Two-way analysis of variance of metal and metalloid in vegetable at different sites.

Metal and metalloid	Site	Vegetable	Site × Vegetable
Co	0.006^{ns}	0.06^{*}	0.03^{ns}
Ni	24.39^{***}	10.55^{**}	8.19^{**}
Cu	0.13^{ns}	76.09^{***}	30.07^{*}
As	1.80^{ns}	5.89^{*}	0.75^{ns}
Zn	223.09^{***}	34.19^{ns}	70.60^{ns}
Se	0.08^{ns}	0.007^{ns}	0.37^{*}

, significant at 0.001level; ***, significant at 0.01 level
*****, significant at 0.05 level; ns, not significant

Heavy metals and metalloids in soil

At both sites, the level of metals and metalloids in raw water irrigated soil were found below the maximum permissible limit recorded by the USEPA (1997). The concentration of all heavy metals and metalloids investigated at site-II were lower than those found at site-I except Co and Ni. At site-I, the concentration was Co (17.01), Ni (3.12), Cu (9.49), As (25.34), Zn (6.02), Se (1.82) and at site-II Co (17.63), Ni (3.63), Cu (9.19), As (24.77), Zn (5.63), Se (1.79) mg/kg, respectively (Table III).

Table III.- Concentration (mg/kg) of some metal and metalloid pollutants in soil samples obtained from two different sites of district Sargodha.

Metal and metalloid	Sampling sites (Means ±S.E)		Permissible maximum limit (USEPA, 1997)
	Site-I	Site-II	
Co	17.005±0.31	17.63±0.29	65
Ni	3.12±0.03	3.63±0.02	50
Cu	9.49±0.17	9.19±0.39	50
As	25.34±0.14	24.77±0.03	40
Zn	6.02±0.14	5.63±0.23	200
Se	1.82±0.03	1.795±0.02	3

S.E = Standard error

Heavy metals and metalloids in Momordica charantia

The total heavy metals and metalloids in vegetable (*Momordica charantia*) collected from two sub-urban sites of Sargodha city are presented in Table IV.

Table IV.- Concentration (mg/kg) of some metal and metalloid pollutants in vegetable (*Momordica charantia*) obtained from two different sites of district Sargodha.

Metal and metalloid	Sampling sites (Means ±S.E)		Permissible maximum limit (WHO, 1996)
	Site-I	Site-II	
Co	0.83±0.01	0.87±0.03	1
Ni	4.78±0.023	5.63±0.17	2
Cu	20.22±0.29	22.68±0.17	20
As	13.35±0.26	13.54±0.14	7
Zn	52.25±0.03	61.77±0.16	50
Se	0.78±0.27	0.64±0.01	-

S.E = Standard error

The average concentration of Co in the vegetable (dry wt.) was 0.834 mg/kg at site-I and 0.87 mg/kg at site-II. Co level was lower than the permissible level of 1 mg/kg as reported by the WHO (1996). Vegetable Co concentration at site-I was relatively higher.

Ni concentrations in the vegetable parts average 5.63 mg/kg at site-II and 4.78 mg/kg at site-I, respectively. Higher concentration was observed at site-II than those at site-I. The concentrations of Ni in the vegetable exceeded the permissible limit of 2 mg/kg as recorded by the WHO (1996).

Cu concentration in the vegetable at site-II average 22.68 mg/kg and 20.22 mg/kg at site-I, respectively. Cu concentration at both sites in the vegetable exceeded the permissible limit of 20 mg/kg recommended by the WHO (1996).

As concentration in vegetable (*Momordica charantia*) at site-I average 13.35 mg/kg and 13.54 mg/kg at site-II, respectively. As concentration in the vegetable at both sites were above the permissible limit of 7 mg/kg as given by the WHO (1996).

Zn level in edible parts of *Momordica charantia* at site-I average 52.25 mg/kg and 61.77 mg/kg at site-II, respectively. Zn level in the vegetable exceeded the permissible limit of 50 mg/kg as illustrated by the WHO (1996).

Se concentration in tissues of vegetable averaged 0.78 mg/kg at site-I and 0.64 mg/kg at site-II, respectively and with considerably higher level at site-I comparable to that at site-II.

The average transfer factor of the selected heavy metals and metalloids were calculated as the concentration of metals and metalloids in tissues of vegetable related to concentrations in the soil. At site-I, transfer factor was in the order: TF Zn (8.679) > TF Cu (2.130) > TF Ni (1.53) > TF As (0.526) > TF Se (0.430) > TF Co (0.05) and TF Zn (10.97) > TF Cu (2.46) > TF Ni (1.55) > TF As (0.546) > TF Se (0.357) > TF Co (0.049) at site-II, respectively (Table V).

Table V.- Transfer factor for vegetable/soil system.

Sampling sites	Transfer factor					
	Co	Ni	Cu	As	Zn	Se
Site-I	0.049	1.529	2.130	0.526	8.679	0.430
Site-II	0.049	1.55	2.467	0.546	10.97	0.357

Co (r = -0.612) showed negative and significant correlations while Zn (r = -0.225) and Se (r = -0.410) showed negative and non-significant correlations between the soil and the vegetable. Cu (r = 0.041), As (r = 0.140) and Ni (r = 0.549) correlations were positive and non-significant (Table VI).

Table VI.- Metal and metalloid correlation between soil-vegetable.

Metal and metalloid	Soil-vegetable
Co	-0.612*
Ni	0.549
Cu	0.041
As	0.140
Zn	-0.225
Se	-0.410

*. Correlation is significant at 0.05 probability level (2-tailed)

The severity and variation in pollution level along sites can be calculated by pollution load index. The contamination factor at site-I was greater as compared to those observed at site-II. In soil, the reference values for Co, As and Se were 9.1, 29.0 and 0.7 mg/kg (Dutch Standards, 2000) and for Ni, Cu and Zn were 9.06, 8.39 and 44.19 µg/g dry matter, respectively (Singh *et al.*, 2010). The pollution load index at site-I was in the sequence: Se (2.60) > Co (1.89) > Cu (1.13) > As (0.87) > Ni (0.345) > Zn (0.136) and at site-II: Se (2.56) > Co (1.96) > Cu (1.09) > As (0.85) > Ni (0.401) > Zn (0.127) (Table VII).

Table VII.- Pollution load index (PLI) for metal and metalloid in soil.

Sampling sites	Pollution load index					
	Co	Ni	Cu	As	Zn	Se
Site-I	1.889	0.344	1.131	0.873	0.136	2.60
Site-II	1.958	0.400	1.095	0.854	0.127	2.56

The risk index due to metals and metalloids via *Momordica charantia* ranged from 0.112-259.51. The risk index at site-I was in the order: As (255.87) > Cu (2.906) > Ni (1.374) > Zn (1.001) > Se (0.897) > Co (0.112) and at site-II: As (259.51) > Cu (3.260) > Ni (1.619) > Zn (1.183) > Se (0.738) > Co (0.117). Highest risk index was observed for As and lowest due to Co (Table VIII).

Table VIII.- Health risk index of metal and metalloid via intake of vegetable (*Momordica charantia*) from wastewater irrigated sites.

Sampling sites	Health risk intake (mg/day)					
	Co	Ni	Cu	As	Zn	Se
Site-I	0.111	1.374	2.906	255.87	1.001	0.897
Site-II	0.117	1.619	3.260	259.51	1.183	0.738

DISCUSSION

Non-significant effect of sampling sites was observed on Co, Ni, Cu, Zn and Se concentration whereas reverse was true for As concentration. The higher metal and metalloid concentrations in the vegetable and the soil could be due to excessive use of hazardous chemicals and sewage water on irrigated land. The levels of metal and metalloid in the soil at both sampling areas were significantly lower than the permissible limits. Among all metals and metalloids, the As concentrations were at elevated level, whereas Se concentrations were lower at both sites. Elevated level of Cu in wastewater irrigated soil was investigated in present study whereas the concentration of Zn was within range determined by Khan *et al*. (2013a) in Lahore, Pakistan. Zn concentrations in soil were found to be higher in current study while the result for Ni agrees with the findings of Hussain *et al*. (2006). The Co, Cu and Se levels in the present investigation were lower whereas the values found for Ni were similar to the findings of Khan *et al*. (2013b) in Sargodha, Pakistan while working on status of pollutants in soil and vegetable.

In *Momordica charantia*, sites had significant effect on Ni and Zn while non-significant effect on Co, Cu, As and Se concentrations. In vegetable, Co was in appreciable amount while Ni, Cu, As and Zn were beyond the maximum limit. The variations in concentration of heavy metal concentrations in vegetables of the same site may be attributed to the differences in their physiology and morphology for heavy metal accumulation, uptake, retention and exclusion (Carlton-Smith and Davis, 1983; Kumar *et al*., 2009). The order of metals and metalloids in vegetable (*Momordica charantia*) in this investigation was: Zn>Cu>As>Ni>Co>Se. Zn had highest concentration in vegetable in this investigation. Chao *et al*. (2007) demonstrated that Zn level in vegetables was abundant among all metals in Nanjing city, China. The concentration of Cu and Zn in vegetable was higher in present study as compared to the findings of Mosleh and Alamgrabi (2013) in Jeddah, Saudi Arabia, irrigated with treated wastewater. Concentration of Zn in current study in vegetable was lower whereas the concentration of Cu was higher as compared to the finding of Achakzai *et al*. (2011) in Quetta, Pakistan. Therefore, the consumption of this vegetable grown by irrigating with wastewater may have no hazardous effect on public at time of investigation in Sargodha, Pakistan.

Table V presents the metal transfer from the soil to the *Momordica charantia*. Transfer factor was computed to calculate the ability of vegetable to bioaccumulate metals. Highest transfer factors were obtained for Zn, Ni and Cu. The values of transfer factor were below 1 for Co, As and Se at both sites. One reason for these finding is that some metals may experience antagonistic and synergistic behavior of other metals in nature and are retained less strongly by the soil and are absorbed by the plants (Lokeshwari and Chandrappa, 2006). Chao *et al*. (2007) found highest transfer of Zn from soil to vegetable, indicating that Zn had greater capability of accumulation from soil to vegetable, collected from wastewater irrigated site Nanjing, China. The uptake of metal by plants could be affected by multiple factors like nature of soil, plant species, plant age, climatic factor and soil pH (Alloway and Ayres, 1997; Uwah, 2009).

To investigate the relationship of heavy metals and metalloids in soil and vegetable, Pearson correlation coefficient was conducted (Pentecost, 1999). The Ni, Cu and As correlations were perfectively positive and non-significant among soil and vegetable while reverse was true for Co (Table VI). A strong relationship indicates a balance flow of these metals and metalloids among soil and vegetable. Zn and Se exhibited negative and non-significant correlations between soil and vegetable, which showed that the relationship was far more complicated among soil and vegetable. This indicates the imbalance of Zn, Se and Co in the studied area.

To estimate the contamination status of metal and metalloid in soil, pollution load index was determined. Pollution load index determines whether the soil quality is suitable for agricultural use or vegetable growth. Pollution load index was calculated as metal concentration is sewage irrigated soil with respect to reference value. Pollution load at site-I was greater as compared to that found at site-II (Table VII). Due to anthropogenic inputs, agricultural runoff and industrial activities, the pollution load due to Co, Cu and Se was greater than 1 at both sites. The pollution load due to Co and Se was lower in current study whereas due to Ni, Cu and As was similar to the findings of Khan *et al*. (2013b) in Sargodha, Pakistan while working on the status of pollutants in soil and vegetable. Pollution load index greater than 1 indicates that these metals can cause environmental risk and investigated sites need proper monitoring and necessary steps should be taken to overcome it. The contamination factor due to Ni, As and Zn was less than 1, which indicates that these metals were not involved in causing environmental risk.

Risk index ratio greater than 1 has a deleterious effect on human whereas less than 1 has no obvious effect (USEPA, 2002). At both sites, the risk indices for As, Zn, Ni and Cu were greater than 1, while less than 1 for Se and Co (Table VIII). According to Singh *et al*. (2010) the health risk indices due to Ni, Cu and Zn were 0.39, 0.003 and 0.001 mg/day, which were many times lower than the present study. The results concluded that residents around waste sites were exposed to risk due to Ni, Cu, As and Zn by consuming nearby grown vegetables but no notable risk was observed due to Co and Se.

CONCLUSIONS

The long-term use of wastewater for irrigation resulted in increased heavy metal and metalloid concentrations namely Co, Ni, Cu, As, Zn and Se in vegetable. These metals are then transferred to plants before being transferred to the human body. The higher values of health risk index showed that heavy metal contamination in the site irrigated with wastewater presented a significant threat to human health. To reduce the health risk effects, it is suggested to treat the industrial wastes properly and phyto-extract the overload of heavy metals and metalloids from polluted sites.

ACKNOWLEDGEMENTS

The Higher Education Commission, Pakistan is acknowledge for providing the financial support in the Form of project # 2484/13.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

ACHAKZAI, A.K.K., BAZAI, Z.A. AND KAYANI, S.A., 2011. Accumulation of heavy metals by lettuce (*Lactuca sativa* L.) irrigated with different levels of wastewater of Quetta city. *Pakistan J. Bot.*, **43:** 2953-2960.

ADHIKARI, T., MANNA, M.C., SINGH, M.V. AND WANJARI, R.H., 2004. Bioremediation measure to minimize heavy metals accumulation in soils and crops irrigated with city effluent. *Fd. Agric. Environ.*, **2:** 266-270.

AHMAD, K., KHAN, Z.I., JABEEN, H., ASHRAF, M., SHAHEEN, M. AND RAZA, S.H., 2013. Assessment of heavy metals and metalloids toxicity in buffaloes fed on forages irrigated with domestic wastewater in Bhalwal, Sargodha, Pakistan. *Pakistan J. Zool.*, **45:** 1629-1637.

AL-JASSIR, M.S., SHAKER, A. AND KHALIQ, M.A., 2005. Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabia. *Bull. Environ. Contam. Toxicol.*, **75:** 1020-1027.

ALLOWAY, B.J. AND AYRES, D.C., 1997. *Chemical principles for environmental pollution*. Blackie Academic Professional, pp. 190-220.

BEHERA, T.K., BEHERA, S. AND BHARATHI, L.K., 2010. Bitter gourd: Botany, horticulture, breeding. *Horticul. Rev.*, **37:** 101-141.

CAMPBELL, C.D. AND PLANKS, C.O., 1992. An assessment of the hazards of lead in food. *Regul. Toxicol. Pharmacol*., **16:** 265-277.

CARLTON-SMITH, C.H. AND DAVIS, R.D., 1983. Comparative uptake of heavy metals by forage crops grown on sludge-treated soils. In: *Proceeding of International Conference on heavy metals in the environment*. CEP Consultants Ltd., Edinburgh, UK, pp. 3933-3940.

CHAO, W., XIAO-CHEN, L., LI-MIN, Z., PEI- FANG, W. AND ZHI-YONG, G., 2007. Pb, Cu, Zn and Ni concentrations in vegetables in relation to their extractable fractions in soils in suburban areas of Nanjing, China. *Polish J. environ. Stud*., **16:** 199-207.

CUI, Y.G., ZHU, Y.G., ZHAI, R.H., CHEN, D.Y., HUANG, Y.Z., QUI, Y. AND LIANG, J.Z., 2004. Transfer of metals from near a smelter in Nanning, China. *Environ. Int.*, **30:** 785-791.

DAMEK-POPRAWA, M. AND SAWICKA-KAPUSTA, K., 2003. Damage to liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, **186:** 1-10.

DESAI, U.T. AND MUSMADE, A.M., 1998. *Pumpkins, Squashes and Gourds*. pp. 273-298.

DUTCH STANDARDS, 2000. *Circular on target values and intervention values for soil remediation.*

FERGUSSON, J.E., 1990. *The Heavy elements: Chemistry, environmental impact and health effects.* Pergamon Press; Oxford, pp. 382-399.

GE, K.Y., 1992. *The status of nutrient and meal of Chinese in the 1990s.* Beijing People's Hygiene Press, pp. 415-434.

HUSSAIN, S.I., GHAFOOR, A., AHMAD, S., MURTAZA, G. AND SABIR, M., 2006. Irrigation of crops with raw sewage: Hazard assessment of effluent, soil and vegetables. *Pak. J. agric. Sci.*, **43:** 3-4.

ISLAM, E., YANG, X. AND MAHMOOD, Q., 2007. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *J. Zhejiang Univ. Sci. B.*, **8:** 1-13.

KALE, A.A., CADAKH, S.R. AND ADSULE, R.N., 1991. Physico-chemical characteristics of improved varieties of bitter gourd (*Momordica charantia* L.) Maharashtra. *J. Hort*., **5:** 56-59.

KHAN, S., CAO, Q., ZHENG, Y.M., HUANG, Y.Z. AND ZHU, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut*., **152:** 686-692.

KHAN, A., JAVID, S., MUHMOOD, A., MJEED, T., NIAZ, A. AND MAJEED, A., 2013a. Heavy metal status of soil and vegetables grown on peri-urban area of Lahore district. *Soil Environ*., **32**: 49-54.

KHAN, Z.I., BIBI, Z., AHMAD, K., ASHRAF, M., AKRAM, N.A. AND ARSHAD, F., 2013b. Assessment of metals and metalloids accumulation in wastewater irrigated soil and uptake by pumpkin (*Cucurbita maxima*) at Sargodha, Pakistan. *Asian J. Chem.*, **25:** 15194-15199.

KHANAM, D., RAHMAN, S.H., ISLAM, M.S., AHSAN, M.A., SHAHA, B., AKBOR, M.A., BEG, R.U. AND ADYEL, T.M., 2011. Seasonal implication of heavy metal contamination of surface water around Dhaka Export Processing Zone (DPEZ), Savar, Bangladesh. *J. Sci.*, **34:** 21-35.

KUMAR, A., SHARMA, I.K., SHARMA, A., VARSHNEY, S. AND VERMA, P.S., 2009. Heavy metals contamination of vegetable foodstuffs in Jaipur (India). *Electron. J. Environ. Agric. Fd. Chem*., **8:** 96-101.

LIU, W., ZHAO, J.Z., OUYANG, Z.Y., SODERLUND, L. AND LIU, G.H., 2005. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environ. Int*., **31:** 805-812.

LOKESHWARI, H. AND CHANDRAPPA, G.T., 2006. Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. *Curr. Sci.*, **91:** 622-627.

MA, Q.Y., TRAINA, S.J. AND LOGAN, T.J., 1994. Effect of aqueous Al, Cd, Fe (II), Ni and Zn on Pb immobilization by hydroxyapatite. *Environ. Sci. Technol.*, **28:** 1219-1228.

MAHFUZ, M.A., AHMAD, J.U., SULTANA, M.S., RAHMAN, M.M., GONI, M.A. AND RAHMAN, M.S., 2004. Status of physicochemical properties of wastewater in Bangladesh: A cash studies in Dhalai Beel of DEPZ. *J. environ. Res.*, **2:** 9-15.

MAPANDA, F., MANGWAYANA, E.N., NYAMANGARA, J. AND GILLER, K.E., 2005. The effects of long-term irrigation using wastewater on heavy metal contents of the soil under vegetables in Harare, Zimbabwe. *Agric. Ecosys. Environ.*, **107**: 151-165.

MINIRAJ, N., PRASANNA, K.P. AND PETER, K.V., 1993. Bitter gourd *Mormordica* spp. In: *Genetic improvement of vegetable plants* (eds. C. Kalloo and Bergh). Pergamon Press, Oxford, UK. pp. 239-246.

MOSLEH, Y.Y.I. AND ALMAGRABI, O.A.E., 2013. Heavy metal accumulation in some vegetables irrigated with treated wastewater. *Int. J. Green Herbal Chem.*, **2:** 81-90.

MSAKY, J.J. AND CALVERT, R., 1990. Adsorption behavior of copper and zinc in soils: influence of pH on adsorption characteristics. *Soil Sci*., **150:** 513-522.

NYAMANGARA, J. AND MZEZEWA, J., 1999. The effect of long-term sewage sludge application on Zn, Cu, Ni, and Pb levels in a tropical soil under pasture grass. *Agric. Ecosys. Environ*., **73:** 199-204.

PENTECOST, A., 1999. Analyzing environmental data. *Testing if a relationship occurs between two variables using correlation*, Pearson Education Limited, England, pp. 102-106.

PERKIN-ELMER, NORWALK AND CONNECTICUT, N.O., 1980. Heavy trace metals and macro-nutrients status in herbal plants of Nigeria. *Fd. Chem*., **85:** 67-71.

RADWAN, M.A. AND SALAMA, A.K., 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Fd. Chem. Toxicol.*, **44:** 1273-1278.

REHMAN, A., FAROOQ, H. AND SHAKOORI, A.R., 2007. Copper tolerant yeast, *Candida tropicalis*, isolated from industrial effluents; its potential use in wastewater treatment. *Pakistan J. Zool.*, **39:** 405-412.

RESMI, G., THAMPI, S.G. AND CHANDRAKARAN, S., 2010. *Brevundimonas vesicularis* sp. a novel biosorbent for removal of lead from wastewater. *Int. J. Environ. Res.*, **4:** 281-288.

SANCHEZ, P.A., 1976. *Properties and management of soils in tropics*, John Wiley and Sons, NY. pp. 97-101.

SHAKOORI, A.R. REHMAN, A. AND HAQ, R.U., 2004. Multiple metal resistance in the ciliate protozoan, *Vorticella microstoma*, isolated from industrial effluents and its potential in bioremediation of toxic wastes. *Bull. Environ. Contam. Toxicol*., **72:** 1046-1051.

SINGH, S. AND KUMAR, M., 2006. Heavy metal load of soil, water and vegetables in peri-urban Delhi. *Environ. Monit. Assess.,* **120:** 79-91.

SINGH, A., SHARMA, R.K., AGARWAL, M. AND MARSHALL, F.M., 2010. Health risk assessment of heavy metals via dietary intake of foodstuffs, from waste water irrigated site of dry tropical area of India. *Fd. Chem. Toxicol*., **48:** 611-619.

SOBUKOLA, O.P., ADENIRAN, O.M., ODEDAIRO, A.A. AND KAJIHAUSA, O.E., 2010. Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *Afr. J. Fd. Sci*., **4:** 389-393.

STEEL, R.G.D. AND TORRIE, J.H., 1980. *Principles and procedures of statistics*, 2nd Ed. McGraw Hill Book Co. Inc., New York, pp. 336-354.

THOMPSON, H.C. AND KELLY, W.C., 1990. *Vegetable Crops*. 5th Ed. MacGraw Hill Publishing Company Ltd., New Delhi.

TÜRKDOGAN, M.K., KILICEL, F., KARA, K., TUNCER, I. AND UYGAN, I., 2003. Heavy metals in soil, vegetables and fruit in the endemic upper gastrointestinal cancer region of Turkey. *Environ. Toxicol. Pharmacol.,* **13:** 175-179.

USEPA (US Environmental Protection Agency), 1997. *Exposure factors handbook. Volume II - food ingestion Factors*. EPA/600//P-95/002Fa. Office of Research and Development. Washington, DC, USA.

USEPA (US Environmental Protection Agency), 2002. Region 9, *Preliminary remediation goals*.

USEPA (US Environmental Protection Agency), 2010. *Integrated risk information system*.

UWAH, E.I., 2009. Concentration levels of some heavy metal pollutants in soil, and carrot (*Daucus carota*) obtained in Maiduguri, Nigeria. *J. appl. Sci*., **4:** 76-88.

WANG, X., SATO, T., XING, B. AND TAO, S., 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Sci. Total Environ*., **350:** 28-37.

WATKINSON, J.H., 1966. Semi-automated fluorometric determination of selenium in biological materials with 2,3 diaminonaphthalene. *Anal. Chem*., **38:** 92-97.

WHO, 1996. *Trace elements in human nutrition and health*. World Health Organization. Geneva.

ZURERA-COSANO, G., MORENO-ROJAS, R., SALMERON-EGEA, J. AND POZO-LORA, R., 1989. Heavy metal uptake from greenhouse border soils for edible vegetables. *J. Sci. Fd. Agric.,* **49:** 307-314.

(Received 16 August 2013, revised 16 November 2013)