*Pak. J. Bot.*, 47(1): 217-224, 2015.

# BIOACCUMULATION OF HEAVY METALS AND METALLOIDS IN LUFFA (*LUFFA CYLINDRICA* L.) IRRIGATED WITH DOMESTIC WASTEWATER IN JHANG, PAKISTAN: A PROSPECT FOR HUMAN NUTRITION

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**Abstract**

In the present study, 12 heavy metals (Cr, Mn, Ni, Cd, Co, Cu, Pb, Zn, Fe, Se, As, and Mo) were assessed in a potential vegetable *Luffa cylindrica*. The vegetable was collected randomly from two different sites located at Jhang, Punjab Pakistan. The analyses of variance of data collected from soil showed non-significant effect on Se, Zn, As, Cr, Ni, Mo and Pb while significant effect on Fe, Co, Mn, Cu and Cd metals. Concentrations of all 12 heavy metals in the soil samples were low at sampling site-I as compared to those at site-II except Ni. These concentrations were found below the safe limits except that of Cd. At site-I, the concentrations recorded for different heavy metals were: As > Fe > Pb > Mn > Cd > Co > Cu > Mo > Zn > Ni > Se > Cr while at site-II were: As > Fe > Mn > Pb > Co > Cd > Cu > Mo > Zn > Ni > Se > Cr. Enrichment co-efficient of Cr was higher which showed that root of luffa plant accumulated more Cr concentration from the contaminated soil. The order of enrichment co-efficient was recorded at site-I as: Cr > Zn > Mn > Cu > Fe > Ni > Mo > Pb > As > Se > Co > Cd, and at site-II Cr > Zn > Mn > Ni > Cu > Fe > Mo > Pb > Se > As > Co > Cd. The transfer co-efficient of Mn was higher which indicates that more contents of Mn were transferred from roots to upper edible part. The order of transfer co-efficient at site-I was: Ni > Se > Mo > Cr > Zn > Fe > Mn > Cd > Pb > As > Cu > Co and at site-II was Mn > Zn > As > Fe > Pb > Se > Cd > Co > Mo > Cu > Ni > Cr. Correlation analysis showed that Mn, Se, Co, Cd, Ni, Mo and Pb had positive non-significant correlation, whereas a negative and non-significant correlation for Zn, As, Fe and Cr. The order of pollution load index at site-I was Cd > Mo > Se > Pb > Cu > Co > As > Fe > Mn > Ni > Zn > Cr and at site-II: Cd > Mo > Se > Pb > Cu > Co > As > Fe > Mn > Ni > Zn > Cr. Overall, at both sites, lowest concentration of Cr and highest of As were observed which need substantial awareness. Health risk index depends on soil characteristics, chemical composition, rate of consumption and type of a vegetable. In the present study, the order of health risk index due to these heavy metals at site-I was as As > Mo > Mn > Pb > Ni > Cd > Zn > Se > Fe > Co > Cr > Cu and at site-II as As > Mn > Mo > Pb > Cd > Zn > Ni > Se > Fe > Co > Cr > Cu.

**Keywords:** Metals and metalloids, Wastewater, *Luffa cylindrica*, Maximum permissible limits, Dietary toxicity.

## Introduction

Contamination of soil is mainly due to industrial and anthropogenic activities (Mclaughlin *et al*., 1999; Grigalavièiene *et al*., 2005; Qadir *et al.,* 2013). Soil pollution is due to misuse of soil such as poor agricultural practices, and industrial and municipal wastes (Bachaver, 1973; Qadir *et al*., 2013). Chemical fertilizers and herbicides are also one of the causes of soil pollution (Demirezen & Aksoy, 2004). The occurrence of heavy metals in an ecosystem is of great biological importance due to their poisoning at high concentration and accumulation in food chain due to non-biodegradability. Some heavy metals and metalloids like mercury, cadmium, lead, chromium, and silver are obliquely spread and are toxic even at low concentrations (Aekola *et al*., 2008; Qadir *et al*., 2013). The accumulation of heavy metals in agricultural land is due to the wastewater irrigation and soil contamination which may be as a result of environmental pollution (Mapanda *et al*., 2005). One of the main reasons of soil contamination is that if the irrigation is long lasting then heavy metals and metalloids build-up in soil and food crops (Khan *et al*., 2008). Heavy metals and metalloids are accumulated in edible and non-edible parts of vegetables which may cause health disorders both in humans and animals as there is no well-established mechanism to eliminate them from the living body (Alam *et al*., 2003; Arora *et al*., 2008).

Metals like copper, lead, cadmium and mercury are reported to be exceptionally toxic because these may cause environmental hazards (Hseu, 2004; Sharma *et al*., 2009). Some metals and metalloids are essential for humans as they play an important role in biological systems such as manganese, copper, iron and zinc, but their excessive intake may cause toxic effects (Kaur & Goyal, 2011; Sipter *et al*., 2008). Wastewater carries toxic heavy metals and metalloids such as Mn, Cu, Cr, Pb, Zn, Ni and Cd in surface soil which create problems for sage utilization of agricultural soil (Yadav *et al*., 2002; Luo *et al*., 2012). Concentration of metals and metalloids in vegetables depends on soil texture as well as nature and type of plants (Kabata & Pendias, 1984). Evidence regarding heavy metals is also available in Pakistan. For example, a survey study was conducted by Khan *et al*., (2013) to explore the levels of heavy metals in vegetables growing in sewage water in district Lahore, Pakistan. Likewise, Ehsan *et al*., (2011) also explored the heavy metal concentration in sewage water in Peshawar and suggested that sewage water contains high concentrations of Cu, Mn, Pb and Cd in that area.

Luffa (*Luffa cylindrica)* commonly known as tori in the Asian region is a tropical plant. It is grown in temperate region because it requires warm temperature. Luffa plant is purgative, emetic, and useful in asthma and splenic enlargement. It is mainly used for hemorrhoids, rheumatism and chest pain. The fruit of luffa is anthelmentic, laxative and leprosy. This plant has climbing green stems (Obon & Aluyor, 2009). It contains steroids, alkaloids, saponin, flavonoid, glycosides and tannins (Singh *et al*., 2010a, b). Chemical composition of *Luffa cylindrical* included cellulose, hemicelluloses and lignin. The fruit of the luffa plants is soft and cylindrical in shape (Mazali & Alves, 2005). Keeping in view the importance of *Luffa cylindrical* and heavy metals' contamination in Pakistan, the aim of this study was to determine the enrichment factor and translocation of heavy metals in agricultural soil irrigated with sewage water and their intake into vegetables. In addition to find-out the pollution load and health risk index in Jhang, Pakistan.

## Materials and Methods

**Study area:** The current investigation was conducted in Jhang, Pakistan. The temperature of this region is hot, with considerable variation between winter and summer. Jhang District is mainly a rural land and *Luffa cylindrical* has been grown on a large commercial scale in this region to fulfill the demand of the consumers of this region. The main metals and metalloids under research were molybdenum (Mo), iron (Fe), chromium (Cr), lead (Pb), zinc (Zn), selenium (Se), cadmium (Cd), arsenic (As), copper (Cu), cobalt (Co), nickel (Ni) and manganese (Mn).

**Soil:** Complex soil samples of 1 kg each (mixture of 5 sub-samples) were collected from both sampling sites. At the depth of 20 cm, the soil sub-samples were collected randomly from each site wherein the vegetable was grown. Soil samples were first air-dried and then dried in an oven for 48 h at 70°C; thereafter the samples were pulverized and firmed into fine particles with the help of an electric grinder. Soil fine particles were passed through a 2 mm sieve to remove hard materials. For further analyses, these soil samples were stored in plastic bags.

**Vegetable:** At the depth of 15-20 cm diameter, vegetable samples were collected indiscriminately from both sites and then separated from root and collected the edible parts. The vegetable samples were washed thoroughly with distilled water and oven-dried for 6 days at 70°C, weighed and ground in a grinder and then passed through a 1mm mesh sieve to remove impurities prior to analysis following Campbell & Planks (1992).

**Digestion of soil and vegetable samples:** About one gram soil or vegetable sample was added to a flask, then poured 4 ml $H_2SO_4$ and 8 ml $H_2O_2$ into it. The samples were digested by placing them in digestion chambers until evaporation stopped. After removing from the digestion chamber, 2 ml of $H_2O_2$ were added and again placed them in the chamber for further heating, till the samples became colorless. The digested samples were taken-out from the chamber and filtered using the Whatman filter paper # 42 and made the volume up to 50 ml by adding double distilled water, marked and stored in plastic bottles.

**Analysis of metal elements:** After digestion, the vegetable and soil samples were subjected to metal analyses by using an atomic absorption spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp, 1980). Mo and Co concentration were determined using graphite furnace atomic absorption spectrophotometer. Se concentrations in samples were determined by the fluorometric method of Watkinson (1966) and arsenic (As) concentration was determined by AAS-GF-HG (Perkin Elmer).

**Statistical analysis:** For statistical analysis the SPSS software version 17 was used. One-way analysis of variance was applied on soil samples and for vegetable samples, a two-way analysis of variance with interaction was used. Correlation between soil and vegetable samples of both sites was also worked out. Significance of means was tested at 0.01, 0.001 and 0.05 probability levels as suggested by Steel & Torrie (1980).

**Transfer co-efficient for edible/root system (TC):** Transfer co-efficient was calculated to assess the accumulation of metals from root to edible portion (Cho *et al*., 2007).

$$TC = \frac{\text{Metal concentration in edible part}}{\text{Metal concentration in root tissue}}$$

where: TC is the transfer coefficient for edible/root system

**Enrichment coefficient (EC) for vegetable/soil system:** In order to assess the transferring capability of heavy metals from soils to vegetable tissues, a quantitative assessment between metal contents in vegetables and in corresponding soils was done by determining the enrichment coefficients for the vegetable/soil system.

Enrichment coefficient was considered to evaluate the transfer of metals from soils to vegetables, and it was worked out using the following formula suggested by Antonidias & Alloway (2001).

$$EC = \frac{\text{Metal concentration in vegetable}}{\text{Metal concentration in soil}}$$

where: EC for enrichment coefficient for vegetable/soil system;

**Pollution load index (PLI):** Metal concentrations have been evaluated at particular sites by pollution load index (Liu *et al*., 2005).

$$PLI = \frac{\text{Metal concentration in investigated soil}}{\text{Reference value of the metal in soil}}$$

**Health risk index (HRI):** The ratio of health risk index is estimated by vegetable sample and oral reference dose and it was calculated following Cui *et al*., (2004). The values of $R_fD$ for different metals in 5 mg/kg/day given as Cd (0.001), Cr (1.5), Mn (0.041), Fe (0.70), Co (0.043), Ni

(0.02), Cu (0.04), As ($3\times10^{-4}$), Se ($5\times10^{-3}$), Mo (0.009) and Zn (0.3) were taken from the Integrated Risk Information System (USEPA, 2010). The value of $R_fD$ for Pb (0.0035 mg/kg/day) was taken from the study of WHO (1993). An index value more than 1 is not healthful to humans (USEPA, 2002). The average daily intake of metals in 0.345 kg of vegetable per day for adult residents was used as reported earlier (Ge, 1992; Wang *et al*., 2005). Daily intake was calculated as follows:

$$\text{Daily intake of metal (DIM)} = C_{metal} \times D_{food\ intake} / B_{average\ weight}$$

where $C_{metal}$, $B_{average\ weight}$ and $D_{food\ intake}$ represent the metal contents in vegetable (mg/kg) and average body weight (kg) and daily intake of vegetable (kg/day/person), respectively.

## Results

Analysis of variance of soil data showed a non-significant effect of sites on Zn, Se, As, Cr, Ni, Mo and Pb concentrations while a significant ($p$<0.01) effect was observed for Mn, Co, Fe, Cd and Cu concentrations (Table 1).The results of the analysis of variance for interaction between the sites and vegetable showed highly significant effect ($p$<0.001) of site on Zn while the sites had a significant effect on Mn, Cr and Ni but they showed a non-significant effect on Cd, Cu, Mo, Pb, Se, As, Co and Fe concentrations. Due to site-vegetable interaction, the concentration of heavy metals and metalloids was non-significantly affected by the sites (Table 2).

At both sites, the levels of metals and metalloids in raw water irrigated soil from where the vegetable (*Luffa cylindrica*) samples were taken, was found below the maximum permissible limits (USEPA, 1997). The concentrations of all heavy metals and metalloids investigated were lower at site-I than those at site-II (Table 3). At site-I the concentrations of the following metals were: Mn (14.30), Zn (4.09), Se (1.46), As (30.42), Co (12.12), Fe (20.50), Cd (13.03), Cr (0.13), Ni (2.56), Cu (11.18), Mo (9.00), Pb (15.55) and at site-II, Mn (17.73), Zn (4.56), Se (1.61), As (30.77), Co (15.75), Fe (24.59), Cd (15.59), Cr (0.16), Ni (2.31), Cu (14.36), Mo (9.70), Pb (15.97) mg/kg, respectively.

Average Mn concentration in the vegetable parts was 68.9 mg/kg at site-II and 54.76 mg/kg at site-I, respectively. Higher concentration was observed at site-II than that at site-I. The concentration of Mn in the vegetable tissues exceeded the permissible level of 30 mg/kg (WHO, 1996). Zn level in the vegetable tissues averaged 63.6 mg/kg at site-II and 54.08 mg/kg at site-I (Table 4). The level of Zn in the vegetable tissue exceeded the permissible level (50 mg/kg) as suggested by WHO (1996).

The mean Se concentration in the vegetable tissues was found to be 0.574 mg/kg at site-II and 0.629 mg/kg. Average arsenic (As) concentration in this vegetable was recorded as 11.85 mg/kg at site-I and 11.6 mg/kg at site-II. Arsenic concentration in the vegetable tissues at both sites of sampling was above the permissible level of 7 mg/kg (WHO, 1996). The mean cobalt (Co) concentration in *Luffa cylindrica* tissue was 0.597 mg/kg at site-II and 0.414 mg/kg at site-I. While, in all samples, Co level was within the permissible level of 1 mg/kg dry wt. (WHO, 1996). The vegetable Co concentration was however higher at site-II than that at site-I (Table 4).

**Table 1. One-way analysis of variance in soil at two different sites.**

| Metals and metalloids | Sites |
|---|---|
| Mn | $35.103^{**}$ |
| Zn | $0.661^{ns}$ |
| Se | $0.069^{ns}$ |
| As | $0.378^{ns}$ |
| Co | $39.640^{***}$ |
| Fe | $50.041^{*}$ |
| Cd | $19.753^{***}$ |
| Cr | $0.002^{ns}$ |
| Ni | $0.190^{ns}$ |
| Cu | $30.245^{***}$ |
| Mo | $1.478^{ns}$ |
| Pb | $0.528^{ns}$ |

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively; ns = Non-significant

**Table 2. Two-way analysis of variance of metal elements in *Luffa cylindrica* collected from two different sites.**

| Metals | Site | Vegetable | Site × vegetable |
|---|---|---|---|
| Mn | $419.5^{*}$ | $442.4^{*}$ | $205.8^{ns}$ |
| Zn | $480.7^{***}$ | $237.8^{**}$ | $2.221^{ns}$ |
| Se | $0.031^{ns}$ | $0.023^{ns}$ | $0.097^{ns}$ |
| As | $5.388^{ns}$ | $0.199^{ns}$ | $2.883^{ns}$ |
| Co | $0.201^{ns}$ | $0.111^{ns}$ | $3.750E\text{-}9^{ns}$ |
| Fe | $50.937^{ns}$ | $23.558^{ns}$ | $26.734^{ns}$ |
| Cd | $0.015^{ns}$ | $0.002^{ns}$ | $0.006^{ns}$ |
| Cr | $3.640^{*}$ | $16.413^{***}$ | $92.147^{***}$ |
| Ni | $11.471^{*}$ | $5.762^{ns}$ | $33.069^{**}$ |
| Cu | $21.561^{ns}$ | $170.512^{**}$ | $35.986^{ns}$ |
| Mo | $1.711^{ns}$ | $0.038^{ns}$ | $52.606^{*}$ |
| Pb | $1.569^{ns}$ | $0.071^{ns}$ | $0.042^{ns}$ |

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively; ns = Non-significant

Average Fe concentration in the vegetable tissue was 40.28 mg/kg at site-II and 39.4 mg/kg at site-I, respectively. The vegetable Fe concentration at both sites did not exceed the permissible level of 1000 mg/kg (WHO, 1996). Average Cd level in the edible parts of *Luffa cylindrica* was found to be 0.315 mg/kg at site-I and 0.31 mg/kg at site-II of sampling. The Cd level in the vegetable tissue did not exceed the permissible level of 0.5-2.0 mg/kg (Codex and Alimentarius, 2001). The average concentration of Cr in the vegetable dry tissues from site-I was 12.33 mg/kg and from site-II 9.19 mg/kg, respectively. While, in all samples, Cr level was lower than the permissible limit of 50 mg/kg dry wt. (WHO, 1996). Vegetable Cr concentration was, however, higher at site-I than that at site-II and also below the tolerable limit, 23 mg/kg (Weigert, 1991) (Table 4).

**Table 3. Concentration of metals and metalloids pollutants (mg/kg dry wt.) in soil obtained from two different sites of District Jhang, Pakistan.**

| Metals and metalloids | Sampling sites | | Permissible maximum limit (mg/kg) |
|---|---|---|---|
| | Site-I (means ±S.E) (mg/kg) | Site-II (means ±S.E) (mg/kg) | |
| Mn | 14.30 ± 0.94 | 17.73 ± 0.33 | 80 |
| Zn | 4.09 ± 0.24 | 4.56 ± 0.24 | 200 |
| Se | 1.46 ± 0.22 | 1.61 ± 0.19 | 3 |
| As | 30.42 ± 1.94 | 30.77 ± 0.66 | 40 |
| Co | 12.12 ± 0.63 | 15.75 ± 0.53 | 65 |
| Fe | 20.50 ± 1.22 | 24.59 ± 1.34 | 21000 |
| Cd | 13.03 ± 0.23 | 15.59 ± 0.36 | 3 |
| Cr | 0.13 ± 0.02 | 0.16 ± 0.02 | 400 |
| Ni | 2.56 ± 0.23 | 2.31 ± 0.27 | 50 |
| Cu | 11.18 ± 0.23 | 14.36 ± 0.28 | 50 |
| Mo | 9.00 ± 0.23 | 9.70 ± 0.51 | 40 |
| Pb | 15.55 ± 0.37 | 15.97 ± 0.30 | 300 |

**Table 4. Concentration of metals and metalloids pollutants (mg/kg dry wt.) in *Luffa cylindrica* obtained from two different sites of District Jhang, Pakistan.**

| Metals and metalloids | Sampling sites | | Permissible maximum limit |
|---|---|---|---|
| | Site-I (means ± S.E) | Site-II (means ± S.E) | |
| Mn | 54.76 ± 1.86 | 68.9 ± 6.80 | 30 |
| Zn | 54.08 ± 2.037 | 63.6 ± 1.98 | 50 |
| Se | 0.629 ± 0.162 | 0.574 ± 0.157 | _ |
| As | 11.85 ± 1.80 | 11.6 ± 1.45 | 7 |
| Co | 0.414 ± 0.042 | 0.597 ± 0.156 | 1 |
| Fe | 39.4 ± 2.05 | 40.28 ± 2.16 | 1000 |
| Cd | 0.315 ± 0.019 | 0.31 ± 0.01 | 0.5 |
| Cr | 12.33 ± 0.55 | 9.19 ± 0.24 | 50 |
| Ni | 6.94 ± 0.75 | 3.21 ± 0.20 | 2 |
| Cu | 18.87 ± 1.74 | 18.32 ± 2.06 | 20 |
| Mo | 18.33 ± 2.06 | 10.51 ± 1.31 | 5 |
| Pb | 2.34 ± 0.22 | 1.74 ± 0.20 | 10 |

The mean nickel concentration in *Luffa cylindrica* parts was found to be 6.94 mg/kg at site-I and 3.21 mg/kg at site-II, respectively. The concentration of Ni in the vegetable tissues exceeded the permissible level of 2 mg/kg (WHO, 1996). Cu concentration in *Luffa cylindrica* tissues was 18.87 mg/kg on an average at site-I and 18.32 mg/kg at site-II respectively. Cu concentration at both sites in the vegetable exceeded the permissible level of 20 mg/kg (WHO, 1996) (Table 4).

Mean vegetable Mo concentration was 18.33 mg/kg at site-I and 10.51 mg/kg at site-II, respectively. However, Mo concentration in the vegetable tissues at both sampling sites was above the permissible level of 5 mg/kg (WHO, 1996). Mean Pb concentration in the vegetable was recorded to be 2.34 mg/kg at site-I and 1.74 mg/kg at site-II, respectively. The Pb concentration in the vegetable at both sampling sites did not exceed the permissible level, 3-10 mg/kg (Codex & Alimentarius, 2001) (Table 4).

The average enrichment coefficient of the selected heavy metals and metalloids at site- I and at site-II ranged from 0.02-92.2 mg/kg depending upon the metal concentration (Table 5). Transfer factor was in the following order: Cr (74.51) > Zn (11.83) > Mn (3.638) > Cu (1.81) > Fe (1.72) > Ni (1.41) > Mo (1.23) > Pb (1.51) > As (0.406) > Se (0.30) > Co (0.04) > Cd (0.02) at site-I and at site-II was: Cr (92.2) > Zn (12.44) > Mn (3.07) > Ni (1.98) > Cu (1.81) Fe (1.64) > Mo (1.39) > Pb (1.21) > Se (0.39) > As (0.34) > Co (0.04) > Cd (0.02), respectively.

Transfer coefficient was used to determine the transfer of metal contents from root to edible portion. The transfer coefficient values recorded for Cd, Cr, Ni, Cu, Mo, Pb, Mn, Zn, Se, As, Co and Fe were 1.04, 1.22, 1.92, 0.86, 1.25, 0.98, 1.05, 1.11, 1.42, 0.95, 0.75 and 1.11 mg/kg at site-I and 0.86, 0.62, 0.70, 0.70, 0.77, 0.90, 1.26, 1.12, 0.89, 1.08, 0.81 and 0.996 mg/kg at site-II, respectively (Table 6). As compared to site-II, the transfer coefficients for all metals at site-I were greater. The order of transfer coefficients at site-I was: Ni > Se > Mo > Cr > Zn > Fe > Mn > Cd > Pb > As > Cu > Co, whereas at site-II Mn > Zn > As > Fe > Pb > Se > Cd > Co > Mo > Cu > Ni > Cr.

**Table 5. Enrichment co-efficient of metal elements in *Luffa cylindrica* from soil to root.**

| Study area | Metals | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mn | Zn | Se | As | Co | Fe | Cd | Cr | Ni | Cu | Mo | Pb |
| Site-I | 3.638 | 11.83 | 0.30 | 0.406 | 0.04 | 1.72 | 0.02 | 74.51 | 1.41 | 1.81 | 1.23 | 0.151 |
| Site-II | 3.07 | 12.44 | 0.39 | 0.34 | 0.04 | 1.64 | 0.02 | 92.2 | 1.98 | 1.81 | 1.39 | 0.21 |

**Table 6. Transfer coefficient of each metal elements in *Luffa cylindrica* from root to vegetable.**

| Study area | Metal elements | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mn | Zn | Se | As | Co | Fe | Cd | Cr | Ni | Cu | Mo | Pb |
| Site-I | 1.05 | 1.11 | 1.42 | 0.95 | 0.75 | 1.11 | 0.02 | 74.51 | 1.41 | 1.81 | 1.23 | 0.151 |
| Site-II | 1.26 | 1.12 | 0.89 | 1.08 | 0.81 | 0.99 | 0.02 | 92.2 | 1.98 | 1.81 | 1.39 | 0.21 |

Zn (*r* = -0.132), As (*r*= -0.151), Fe (*r*= -0.010), Cr (*r*= -0.396) and Cu (*r* = -0.168) showed negative and non-significant correlations between the soil and vegetable, while Mn (*r* = 0.255), Se (*r* = 0.280), Co (*r*= 0.333), Cd (*r* = 0.429), Ni (*r*= 0.399), Mo (0.111) and Pb (*r* = 0.201) showed positive and non-significant correlations between the soil and vegetable (Table 7).

Pollution severity and its variation along the sites was determined using the pollution load index. The contamination factor at site-II was greater as compared to that observed at site-I (Table 8). The reference value of Mn was 46.75 mg/kg which was higher than the present value (Singh *et al*., 2010b). The reference values of Co, As, Mo and Se in soil were 9.1, 29.0, 3.0 and 0.7 mg/kg (Dutch Standards, 2000). The Fe concentration was lower in the present study than the reference value of 56.90 mg/kg (Dosumu *et al*., 2005). The reference values of Ni, Cu, Zn, Cd, Pb and Cr in soil were 9.06, 8.39, 44.19, 1.49, 8.15 and 9.07 µg/g (Singh *et al.*, 2010a). The pollution load index at site-I was in the following sequence: Cd (8.744) > Mo (3.00) > Se (2.085) > Pb (1.907) > Cu (1.337) > Co (1.331) > As (1.048) > Fe (0.365) > Mn (0.305) > Zn (0.092) > Ni (0.282) > Cr (0.014) and at site-II: Cd (10.463) > Mo (3.023) > Se (2.30) > Pb (1.959) > Co (1.730) > Cu (1.717) > As (1.061) > Fe (0.438) > Mn (0.379) > Ni (0.254) > Zn (0.103) > Cr (0.017).

The health risk index of metal and metalloid contents through ingestion of *Luffa cylindrica* ranged from 0.0078-22.712 mg/day (Table 9). The risk index at site-I was in the order: As (22.712) > Mo (11.7108) > Mn (7.679) > Zn (1.036) > Pb (3.8442) > Ni (1.995) > Cd (1.811) > Se (0.723) > Fe (0.3236) > Co (0.0553) > Cr (0.0472) > Cu (0.0081) and at site-II was in the order: As (22.233) > Mn (9.662) > Zn (1.219) > Mo (6.7147) > Pb (2.8585) > Cd (1.812) > Ni (0.9228) > Se (0.6601) > Fe (0.3308) > Cr (0.352) > Co (0.0798) > Cu (0.0078). Highest risk index was observed for As and lowest due to Cu.

**Table 7. Correlation between soil and vegetable with respect to each metal element in *Luffa cylindrical*.**

| Metal elements | Soil-vegetable (*r*) |
|---|---|
| Mn | 0.255 |
| Zn | -0.132 |
| Se | 0.280 |
| As | -0.151 |
| Co | 0.333 |
| Fe | -.100 |
| Cd | 0.429 |
| Cr | -0.396 |
| Ni | 0.399 |
| Cu | -0.168 |
| Mo | 0.111 |
| Pb | 0.201 |

**Table 8. Pollution load index (Contamination factor) for metals (mg/kg) in soil of *Luffa cylindrical*.**

| Study area | Metal elements | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mn | Zn | Se | As | Co | Fe | Cd | Cr | Ni | Cu | Mo | Pb |
| Site-I | | | | | | | | | | | | |
| Site-II | | | | | | | | | | | | |

**Table 9. Health risk intake (HRI) of metal elements via intake of *Luffa cylindrica* by waste water irrigated sites.**

| Study area | Metal elements | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mn | Zn | Se | As | Co | Fe | Cd | Cr | Ni | Cu | Mo | Pb |
| Site-I | 0.30 | 0.09 | 2.08 | 1.04 | 1.33 | 0.365 | 8.744 | 0.01 | 0.28 | 1.337 | 3.00 | 1.90 |
| Site-II | 0.38 | 0.10 | 2.30 | 1.06 | 1.73 | 0.438 | 10.46 | 0.02 | 0.25 | 1.717 | 3.02 | 1.96 |

## Discussion

Significant differences between the two sites were found in terms of soil Mn, Co, Fe, Cd and Cu contents, whereas the reverse was true for Zn, Se, As, Cr, Ni, Mo and Pb in soil. Total concentration of heavy metals in the soil irrigated with wastewater from where the samples of vegetable (*Luffa cylindrica*) were taken from two different sites was found lower than the maximum permissible limit except Cd (USEPA, 1997). These differences could be ascribed to variation in agricultural practices and other environmental factors prevalent on the two sites. The levels of heavy metals in soil were within the recommended ranges and these metal levels could be beneficial for proper functioning of biological systems (Ward, 1995) whereas Cd level was well over the recommended range. The concentration of Fe, Mn and Pb was higher while the level of Cr was many-fold lower at both sites. The low concentration of soil Cr in the present study may be due to its transfer to aerial parts of plant. The soil Cd level was higher while Cu level lower in the present investigation than those recorded by Murtaza *et al*., (2003). The soil Pb concentration was higher whereas soil Mn in the present investigation corresponded with findings of Hussain *et al*., (2006). The concentrations of all selected metals and metalloids were lower than the permissible level. Zn concentration in the soil was established to be higher in the present study while the results for Fe were parallel to the findings of Uboh *et al*., (2011). The soil Co level was greater in the current research than those investigated by Hanaa *et al*., (2000).

For *Luffa cylindrica,* both sites had a significant effect on Mn, Co and Fe, while a non-significant effect on Zn, As and Se concentrations. The concentration of Mn in vegetable tissues exceeded the permissible level of 30 mg/kg (WHO, 1996). The level of Zn in vegetable tissue exceeded the permissible level of 50 mg/kg (WHO, 1996). As concentration in vegetable tissues at both sampling sites were above the permissible level of 7 mg/kg (WHO, 1996). Vegetable Co concentration was, however, higher at site-II than that at site-I. The vegetable Fe concentration at both sites did not exceed the permissible level of 1000 mg/kg (WHO, 1996). This could be due to abundance of metal in soil or its importance for plant growth (Baker & Brooks, 1989). It can also be due to different soil characteristics such as organic contents, acidity and ability of plant root to penetrate where the toxic metals are present (Okoronkwo *et al*., 2006). Zn has the highest concentration in the vegetable in this investigation. Chao *et al*., (2007) reported that Zn was the most abundant among other metals in vegetables grown in wastewater irrigated site in Nanjing city, China. It was reported that the level of Zn was lower in the present study than the values recorded by Tulonen*et al*., (2006). The Co level was higher in the present research than those recorded by Murtaza *et al*., (2003) in some vegetables at Faisalabad, Pakistan. The amount of availability of Mn in soil is influenced by pH, organic matter content, moisture and soil aeration. Mn availability increases as soil pH decreases. Aerial deposition of these metals could be another factor of pollution (Yoon *et al*., 2006).

The Cd level in vegetable tissue did not exceed the permissible level of 0.5-2 mg/kg (Codex & Alimentarius, 2001). In all samples, Cr level was lower than the permissible limit of 50 mg/kg dry wt. (WHO, 1996). Vegetable Cr concentration was, however, higher at site-I than that at site-II and also below the tolerable limits, 23 mg/kg (Weigert, 1991). The concentration of Ni in the vegetable tissue exceeded the permissible level of 2 mg/kg (WHO, 1996). Copper (Cu) concentration at both sites in the vegetable exceeded the permissible level of 20 mg/kg (WHO, 1996). Mo concentration in the vegetable tissues at both sites of sampling was above the permissible level of 5 mg/kg (WHO, 1996). The Pb concentration in the vegetable at both sites of sampling area did not exceed the permissible level, 3-10 mg/kg (Codex & Alimentarius, 2001). These might be attributed to the possible pollution of sampling area due to excessive amount of chemicals, fertilizers, herbicides and other agrochemicals as well as use of wastewater in irrigation, the respective soil and environmental factor pertinent in the sampling area (Uboh *et al*., 2011). Low level of Cd recorded in this investigation could be due to the reason that this metal is non-essential for plant growth and metabolism (Yoon *et al*., 2006). The metal ranges found in the soil and vegetable in this investigation were lower than those reported elsewhere for soil and vegetable (Radwan *et al*., 2006; Ebong *et al*., 2007). It was reported that Pb and Cd levels were higher while that of Mo was lower in the present study than those recorded by Murtaza *et al*., (2010) in Faisalabad, Pakistan. Cd, Pb, Cr and Ni levels were lower in the present research than the values recorded by Radwan *et al*., (2006) in *Luffa cylindrica*. The levels of Cd and Cr were higher in the present study in the vegetable tissues than the findings of Murtaza *et al*., (2003) in Faisalabad, Pakistan.

The enrichment coefficient was computed for the metals and metalloids to quantify the differences in metal bioavailability and identify efficiency of the vegetable (*Luffa cylindrica*) to accumulate metal. Due to bioaccumulation, the heavy metals and metalloids in wastewater irrigated soils were transferred to the vegetable. The transfer factor values were below 1.0 for Se, As, Co, Cu and Zn obtained from both sites of sampling. The uptake of metals by a vegetable can be affected by edaphic factors, climatic factors and plant age (Alloway & Ayris, 1997). Chao *et al*., (2007) found highest transfer of Zn from soil to vegetable, indicating that Zn has greater capability of accumulation from soil to vegetable collected from wastewater irrigated site Nanjing, China. Most of the results indicated that the plants with transfer coefficient greater than 1.0 have capability of translocation metals easily from root to aerial parts of plants. Due to efficient transport system, the transfer coefficient greater than 1.0 indicates efficient metal transport from root to shoot (Duruibe *et al.,* 2007). The heavy metal concentration in vegetable varies from site to site. This may be due to translocation of metal in different parts of plants or uptake capacity of vegetable through root system (Yoon *et al.,* 2006; Vousta *et al.,* 1996). The order of enrichment coefficient was recorded at site-I as: Cr > Zn > Mn > Cu > Fe > Ni > Mo > Pb > As > Se > Co > Cd, and at site-II: Cr > Zn > Mn > Ni > Cu > Fe > Mo > Pb > Se > As > Co > Cd.

In the current study, Zn, As, Fe and Cr showed a negative and non-significant correlation between the soil and the vegetable, while positive non-significant results were for Mn, Se, Co, Cd, Ni, Mo and Pb between the soil and the vegetable. Relationship of soil and vegetable metals was established by using the Pearson correlation coefficient (Pentecost, 1999). A strong relationship indicates a balance flow of these metals between the soil and the vegetable. Cr showed a negative correlation between the soil and the vegetable. This indicates the imbalance of Cr in the study area. The positive correlation indicates a strong association between soil and vegetables, whereas a negative correlation means that there was a weak relationship between the soil and the vegetable.

The pollution load index indicates whether the quality of soil is suitable for vegetable growth and agricultural use. To estimate the contamination status of metals and metalloids in soil, pollution load index was determined. Pollution load index was calculated as metal concentration in sewage irrigated soil with respect to the reference value. Pollution load at site-I was greater as compared to that found at site-II. Due to anthropogenic inputs, agricultural runoff and industrial activities, the pollution load due to Se, As and Co was greater than 1.0 at both sites. Pollution load index greater than 1.0 indicates that these metals can cause environmental risk and the investigated sites need proper monitoring. Necessary steps should be taken to overcome it. There is, in general, a decrease in PLI values downstream indicating dilution and dispersion of metal content with increasing distance from the source areas. In general, a decrease in PLI values downstream indicates dilution and dispersion of metal content with increasing distance from the source areas. The order of pollution load index at site-I was: Cd > Mo > Se > Pb > Cu > Co > As > Fe > Mn > Ni > Zn > Cr, and at site-II: Cd > Mo > Se > Pb > Cu > Co > As > Fe > Mn > Ni > Zn > Cr.

The health risk index of metal contents (mg/day) through ingestion of *Luffa cylindrica* ranged from 0.0078-11.711 mg/day. The order of health risk index due to these heavy metals at site-I was: As > Mo > Mn > Pb > Ni > Cd > Zn > Se > Fe > Co > Cr > Cu, and at site-II: As > Mn > Mo > Pb > Cd > Zn > Ni > Se > Fe > Co > Cr > Cu. Highest risk index was observed for As and lowest for Cu. At site-I, the health risk index for Cd, Ni, Mo, Pb, Mn, Zn and As was greater than 1.0, while that for Cr, Cu, Se, Co and Fe was less than 1.0. At both sites the health risk index for Cd, Mo and Pb was greater than 1.0, while that for Cr, Ni and Cu was less than 1.0. If the health risk index is less than 1.0, it means no obvious risk is involved. An index greater than 1.0 is considered as not safe for human health (USEPA, 2002). Daily intake of Cd and Pb via *Luffa cylindrica* was higher, while the intake of Cu was lower in the present study than those recorded by Uboh *et al*., (2011). The health risk assessment is dependent on physical characteristics of soil, its chemical composition, vegetable type and rate of consumption of a vegetable. To avoid the metal accumulation in food chain, standard monitoring of metal contents in vegetables is necessary.

**Acknowledgements**

The Higher Education Commission, Pakistan is acknowledged for providing the financial support through a research project # 2484/13 to the first and second authors. The authors thank all the supporters of this project and the referees for their constructive comments.

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(Received for publication 9 December 2013)