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Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan

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Abstract Soil pollution with heavy metals due to discharge of untreated urban and industrial wastewater is a major threat to ecological integrity and human well-being. The presenting study aimed to determine human health risks associated via food chain contamination of heavy metals routing from irrigation of urban and industrial wastewater. Irrigated water, soil and vegetables were analyzed for Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+}; transfer factor (TF), daily intake of metals (DIM) and health risk index (HRI) were also calculated. Cr^{2+}, Pb^{2+} and Cd^{2+} in vegetables cultivated by wastewater exceeded the permissible limits (European Union, 2002) while TF was lower for all metals except Co^{2+} and HRI was found to be maximum for *Spinacia oleracea* (2.42 mg/kg) and *Brassica campestris* (2.22 mg/kg) cultivated by wastewater. *S. oleracea*, *B. campestris*, *Coriandrum sativum* posed a severe health risk with respect to Cd and Mn.

1. Introduction

World's urban communities are increasing faster than global population as the urbanization progresses in the least-developed regions (UN-HABITAT, 2004). Urban development caused momentous alteration to the environment by increasing the waste material accumulation through anthropogenic activities (Chen, 2007). Urban expansion is promoting a concern for farmers to use contaminated land for food crop's production (Nabulo, 2009). In urban and peri urban areas, land contamination with toxic metals is common as a result of industrial and municipal activity. Wastewater irrigation to increase the yield of food crops (vegetables) is the principal source of contamination in urban agricultural lands (Qadir et al., 2000). These effluents are rich in toxic metals and are a chief contributor to metals loading in waste irrigated and amended soils (Singh et al., 2004; Mapanda et al., 2005).

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Soil contaminated with metals is a primary route of toxic element exposure to humans. Toxic metals can enter the human body by consumption of contaminated food crops, water or inhalation of dust (Cambra et al., 1999). It has been estimated that more than 70% of dietary intake of cadmium is contributed via food chain (Wagner, 1993). Vegetables grown on contaminated land may accumulate toxic metals. Prolonged consumption of contaminated foodstuff may lead to the unceasing accumulation of toxic metals in the liver and kidney of humans resulting in the disturbance of biochemical processes, such as, liver, kidney, cardiovascular, nervous and bone disorders (WHO, 1992; Jarup, 2003).

Health risk assessment of heavy metals in contaminated vegetables is being carried out in developed countries (Milacic and Kralj, 2003); however, little is explored in developing countries (Lock and de Zeeuw, 2001). In Pakistan very few published data on heavy metal contamination in vegetables is available (Jan et al., 2010; Khan et al., 2010; Jamali et al., 2009; Akbar et al., 2009). Environmental abetment practice is almost missing due to the lack of environmental management and un-operational environmental pollution laws.

Wastewater irrigation is a common practice in urban and peri-urban areas of district Lahore. Lahore is the capital of Punjab province, Pakistan and is lying along 31.34° N and 74.22° E. The total area of Lahore district is 1772 km^2 and it is 217 m above sea level. The business mart of Pakistan, Lahore is a well suited site for industries. There are various types of industries situated in and around the city. In Lahore metropolitan area 1121 unplanned industries are sited, which include 642 steel re-rolling factories and foundries discharging iron scrap, lead, cadmium and hazardous chemicals, 36 textile industries and 295 other industries like leather tanneries, electroplating miles, pigment factories, etc. (Saleemi, 1990). These industries discharge their wastewater containing various types of hazardous chemicals and toxic metals in the twelve drainages of the city managed by Water and Sanitation authority (WASA). After receiving a huge flux of wastewater and municipal wastes these drainages pass from urban area to sub urban areas and drain into the river Ravi. Cultivation of vegetables with the industrial and sewage effluents of these drains is a common practice in the coinciding agricultural land along the 35 km stretch between the Lahore ring road and the river Ravi. Local farmers across these drainages use surface wastewater to irrigate their agricultural fields for cultivation of vegetables. A large quantity of various vegetables is sold in the supply market of the city. It has been reported that serious health problems can develop as a result of accumulation of dietary heavy metal uptake through food crops irrigated with contaminated wastewater (Saleemi, 1990). Thereby, this study was conducted to assess the heavy metal concentration in soils, resulted uptake by the vegetables and eminent transfer to the food chain which assist in evaluating the related health hazards linked with it.

2. Material and methods

2.1. Site description

Agricultural land to the north and east of Lahore city along river Ravi and Jallo town was selected as the study area (Fig. 1). Two main zones were selected on the basis of industrial wastewater irrigation and ground water irrigation. Ground water from deep bore well is used for irrigation at the ground water irrigated zone, designated as GWZ and it is located near the Wagha (north east of city) and Jallo (east of the city), whereas wastewater from urban drains was used for irrigation at the wastewater irrigated zone; designated as WWZ and it is located between the Lahore Ring Road and the river Ravi along the stretch of 35 km. Each zone was further subdivided into five sites.

2.2. Water sampling

Water samples that were used for irrigation practices were collected from each site in pre cleaned high-density polyethylene bottles. These bottles were rinsed earlier with a metal-free soap and then soaked in 10% HNO_3 overnight, and finally washed with deionized water (Chary et al., 2008). Samples were brought to the Ecotoxicology and Environmental biology laboratory, Quaid-I-Azam University, Islamabad, Pakistan and stored at 4 °C.

2.3. Soil sampling

Soil from agricultural land was collected by digging a monolith of $10 \times 10 \times 15$ size by using a plastic scooper. Non soil particles e.g. stones, wooden pieces, rocks, gravels, organic debris were removed from soil. Soil was oven dried and this dried soil was sieved through a 2 mm sieve and stored in the labeled polythene sampling bags (Lei et al., 2008).

2.4. Plant sampling

A diversity of vegetables are grown in the study area; *Solanum tuberosum* L., *Brassica oleracea capitata*, *Brassica oleracea*, *Brassica campestris* L., *Brassica rapa* L., *Raphanus sativus* L., *Spinacia oleracea* L., *Beta vulgaris* L., *Allium sativum* L., *Daucus carota* L., *Coriandrum sativum* L. were collected from each site of the sampling zone in 3–5 replicates and stored in labeled polythene sampling bags and brought to the Ecotoxicology and Environmental biology laboratory, Quaid-I-Azam University, Islamabad, Pakistan, where they were harvested in edible and non-edible parts, finally washed with tap water to remove any kind of deposition like soil particles. Edible parts of vegetables were then oven dried and ground into powdered form for making the plant digests (Jamali et al., 2009). A complete description of vegetables collected from the study area is given in Table 1.

2.5. Digestion of samples

1 g soil and vegetable samples were digested by 15 ml tri acid mixture i.e. HNO_3, $HClO_4{\cdot}H_2SO_4$ at 5:1:1 ratio at 100 °C until the transparent solution appeared. Water samples were filtered through Whatman No. 42 filter papers and 50 ml of filtrate was stored at 4 °C after adjusting it at pH 2. To determine the suspended metals filter paper was cut into small pieces, digested in HNO_3 and HCl in 3:1 ratio at 180 °C for 15 min (USEPA Method: 3005A). Volume of each digest was adjusted to 50 ml by adding distilled water and stored for further analysis.

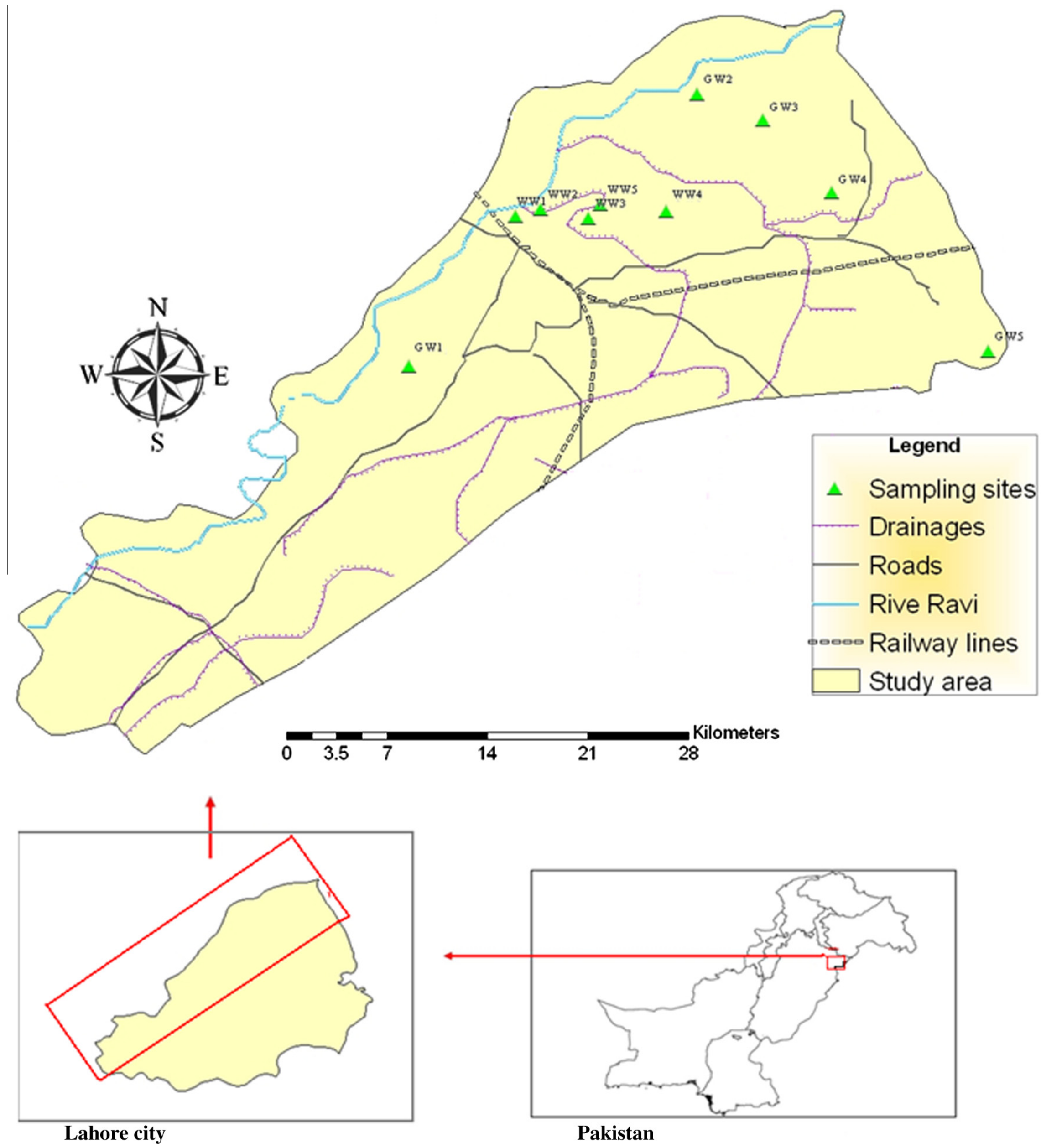

Figure 1 Location map of study area, Lahore, Pakistan.

Table 1 Description of vegetables examined in this study.

Plant species	Family	English name	Vernacular name	Part used
Solanum tuberosum L.	Solanaceae	Potato	Aalo	Tubers
Brassica oleracea capitata	Brassicaceae	Cabbage	Band Gobi	Leaves
Brassica oleracea	Brassicaceae	Cauliflower	Phool Gobi	Fruiting flower
Brassica campestris L.	Brassicaceae	Brassica	Sarsun	Leaves and tender
Brassica rapa L.	Brassicaceae	Turnip	Shaljum	Underground stem
Raphanus sativus L.	Brassicaceae	Radish	Mooli	Underground stem
Spinacia oleracea L.	Amaranthaceae	Spinach	Paalak	Leaves
Beta vulgaris L.	Amaranthaceae	Beet/chard	Chqndr	Underground stem
Allium sativum L.	Amaryllidaceae	Garlic	Thoom	Bulb
Daucus carota L.	Apiaceae	Carrot	Gajr	Underground stem
Coriandrum sativum L.	Apiaceae	Coriander	Dhania	Leaves

2.6. Heavy metal analysis

Concentrations of heavy metals were determined by a flame atomic absorption spectrophotometer (Varian FAAS-240) in the Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan.

2.7. Quality control analysis

Chemicals were purchased from MERCK Chemicals Germany and used for the sample preparation. Double deionized water was used for solution preparation and glassware was washed with 10% HNO_3. Standards were prepared for each metal from their stock solution to calibrate the instrument. Precision and accuracy of analysis were checked through repeated analysis against NIST Standard reference Material 1570A for vegetables, RM 1643E for water and SRM 2709 for soil for heavy metals.

2.8. Data analysis

2.8.1. Transfer factor (TF)

Soil to plant metal transfer was computed as transfer factor (TF), which was calculated by using the equation

$$TF = C_{Plant}/C_{Soil}$$

where, C_{Plant} is the concentration of heavy metals in plants and C_{Soil} is the concentration of heavy metals in soil.

2.8.2. Daily intake of metals (DIM)

Daily intake of vegetables in adult was calculated by data obtained during the survey though a questionnaire. DIM was calculated by the following equation

$$DIM = C_{metal} \times C_{factor} \times D_{food\ intake}/B_{average\ weight}$$

(Chary et al., 2008)

where, C_{metal}, C_{factor}, $D_{food\ intake}$ and $B_{average\ weight}$ represent the heavy metal concentrations in plants (mg kg^{-1}), conversion factor (0.085), daily intake of vegetables and average body weight, respectively. The average vegetable intake was calculated by conducting a survey where about 200 people (males and females) having an average body weight of 47 kg were asked for their daily intake of particular vegetables from sampling sites.

2.8.3. Health risk index (HRI)

To assess the human health risk of heavy metals, it is necessary to calculate the level of human exposure to that metal by tracing the route of exposure of pollutant to human body. There subsist many exposures routes for heavy metals that depend upon a contaminated media of soil and vegetables on the recipients. Receptor population use the vegetables enriched with higher concentration of heavy metals which enters the human body leading to health risks (Khan et al., 2008). In the present research work vegetables grown at the wastewater were collected from the study area and their metal concentration was used to calculate the health risk index (HRI). The health risk index of the present research work was compared with the one reported by Khan et al. (2010) and Jan et al. (2010). Results of HRI were found to be lower than those of Khan et al. (2010) and Jan et al. (2010). Value of HRI depends upon the daily intake of metals (DIM) and oral reference dose (Rf_D). Rf_D is an estimated per day exposure of metal to the human body that has no hazardous effect during life time (US-EPA IRIS, 2006).

The health risk index for Cr, Ni, Cu, Pb, Cd, Mn and Zn by consumption of contaminated vegetables was calculated by following equation

$$HRI = DIM/R_{fd}$$

(Jan et al., 2010)

where DIM represents the daily intake of metals and R_{fd} represents reference oral dose. R_{fd} value for Cr, Ni, Cu, Pb, Cd, Mn and Zn is 1.5, 0.02, 0.04, 0.004, 0.001, 0.033 and 0.30 (mg/kg bw/day) respectively (US-EPA IRIS, 2006).

2.9. Statistical analysis

The data were statistically analyzed by SPSS software version 12 and Statistical version 5.5. One way ANOVA was applied for evaluating the significant difference between heavy metal concentration in vegetables grown in wastewater and ground water.

3. Results

3.1. Concentration of heavy metals in water and soil samples

Heavy metal concentration in irrigated water at WWZ and GWZ is presented in Table 2. Concentration of Cu was found to be maximum i.e. 18.15 mg/L in water samples of WWZ, followed by the concentration of Ni^{2+}, Cr^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} compared with the Indian standards (Awashthi, 2000) while concentration of Zn^{2+} was in the range of permissible limits (Awashthi, 2000). Compared with the GWZ; except Cu^{2+}, concentration of heavy metals in water samples were in the range of Indian permissible limits (Awashthi, 2000). Soil concentration of heavy metals varied among the sites of WWZ and GWZ. Concentration of heavy metals of soil obtained from different sites of WWZ was found to be significantly high as compared to the soil obtained from various sites of GWZ (Table 2). However, the concentration of heavy metals in WWZ was below the safe limits set by EU (European standard 2002) and Indian standards (Awashthi, 2000) except Cd that was 3.15 mg/kg. Concentration of metals among different sites of GWZ was found to be in safe limit.

3.2. Concentration of heavy metals in vegetables

Concentration of heavy metals in vegetables on a dry weight basis grown in wastewater and ground water along with the permissible limits set by European Union (2002) and Indian standards (Awashthi, 2000) is presented in Table 3.

Results of the one way ANOVA revealed that the heavy metal concentration was significantly higher in vegetables grown at WWZ than those grown at GWZ. Concentration of Cr^{2+}, Pb^{2+} and Cd^{2+} from vegetables of WWZ exceeded the permissible limits (European Union, 2002) but was in the range of Indian safe limits (Awashthi, 2000). From GWZ vegetables the concentration of Cr^{2+} exceeded the EU safe limits (European Union, 2002) in all vegetables and Cd^{2+} concentration also exceeded the safe limits except in *S. tuberosum*, *B. oleracea*, and *D. carota*.

Table 2 Mean values of heavy metal concentration in waste water and tube well water (mg/L) and soil (mg/kg) irrigated with waste water and tube well water.

Heavy metals	Soil of wastewater		Soil of tube well water		Standard		Wastewater		Tube well water		Standard
	Range	Value	Range	Value	EU[a]	Ind. St.[b]	Range	Value	Range	Value	Ind. St.[b]
Cr	11.37–33.39	21.01 ± 11.3	9.27–21.39	14.01 ± 6.4	100	NA[c]	0.19–0.54	0.33 ± 0.18	0.03–0.441	0.17 ± 0.23	0.05
Co	4.28–13.39	8.04 ± 4.7	1.39–6.28	3.65 ± 2.4	50	60–110	0.023–0.12	0.064 ± 0.05	0.012–0.054	0.029 ± 0.02	0.05
Ni	14.03–40.28	28.83 ± 13.4	9.99–22.08	14.5 ± 6.61	50	75–150	0.91–5.94	2.88 ± 2.67	0.39–1.77	0.93 ± 0.73	1.4
Cu	11.28–43.53	28.74 ± 15.55	6.34–26.39	15.28 ± 10.19	100	135–270	9.28–32.69	18.15 ± 12.7	2.47–11.04	6.27 ± 4.35	0.05
Pb	8.38–21.40	15.38 ± 6.55	3.39–6.34	7.40 ± 4.54	100	250–500	0.26–0.70	0.43 ± 0.22	0.04–0.49	0.21 ± 0.24	0.1
Cd	1.29–5.18	3.15 ± 9.1	0.16–2.23	1.17 ± 1.03	3	3–6	0.18–0.37	0.19 ± 0.17	0.004–0.2	0.01 ± 0.008	0.01
Zn	29.12–83.28	50.84 ± 28.6	23.27–45	34.13 ± 10.8	300	300–600	0.34–1.39	0.83 ± 0.52	0.11–0.27	0.17 ± 0.08	5.0
Mn	20.48–64.19	39.01 ± 22.59	9.07 = 36.28	21.24 ± 13.82	2000	NA[c]	0.19–1.13	0.63 ± 0.48	0.07–0.21	0.13 ± 0.07	0.1

± Standard deviation.
[a] European Union Standards European Union (2006).
[b] Indian standards Awashthi (2000).
[c] Not available.

Cr^{2+} concentration among the vegetables grown at WWZ was maximum in *A. sativum* i.e. 4.1 mg/kg (dry weight) followed by *B. oleracea capitata* (3.06 mg/kg), *B. campestris* (2.62 mg/kg), *S. oleracea* (2.42 mg/kg) *C. sativum* (2.32 mg/kg), *B. oleracea* (2.15 mg/kg), *D. carota* (1.92 mg/kg), *S. tuberosum* (1.60 mg/kg) etc. The maximum Pb concentration in WWZ vegetables was found in *S. oleracea* i.e. 2.90 mg/kg and the minimum concentration was found in *A. sativum* i.e. 0.38 mg/kg while Cd^{2+} was maximum in *S. oleracea*. Among heavy metal concentration in vegetables grown at WWZ the trend appeared as Cr > Pb > Cd > Co > Ni > Cu > Zn > Mn while in vegetables grown at GWZ the trend appeared as Cr > Cd > Pb > Co > Ni > Mn > Zn.

3.3. Transfer factor of metals from soil to vegetables

Table 4 summarizes the metal transfer factor in vegetables from the study area. TF for vegetables grown on WWZ ranges from 0.06–0.14, 5.52–6.74, 0.10–0.35, 0.14–0.20, 0.025–0.18, 0.059–0.59, 0.17–1.50, 0.54–1.17 for Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} respectively. Co^{2+} TF was the highest for *C. sativum* (6.74), followed by the *S. oleracea* (6.37), *B. rapa* (6.22), *S. tuberosum* (6.22), *B. vulgaris* (6.0), *A. sativum* (5.99), *D. carota* (5.90), *B. campestris* (5.67), *B. oleracea* (5.62), *B. oleracea capitata* (5.52). Cr^{2+} TF was lowest for all the vegetables compared to other metals. Trend of metal TF from soil to vegetables grown at WWZ was in the order of $Co^{2} > Zn^{2} > Mn^{2+} > Ni^{2+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Cr^{2+}$. Metal TF for vegetables grown at GWZ for Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} ranged from 0.06–0.1, 1.080–2.087, 0.08–0.30, 0.02–0.19, 0.02–0.057, 0.06–0.31, 0.08–0.38 and 0.11–0.5 respectively. The highest TF (2.087) for Co^{2+} was found in *S. oleracea*. Trend of metal TF for vegetables grown at GWZ was in the order of $Co^{2+} > Zn^{2+} > Ni^{2+} > Mn^{2+} > Cd^{2+} > Pb^{2+} > Cu^{2+} > Cr^{2+}$.

3.4. DIM and HRI of heavy metals

Values of DIM calculated for adults (average age 47 years), are presented in Table 5. These data revealed that the values of daily intake of metal were high for vegetables grown at WWZ as compared to the vegetables grown at GWZ.

In case of vegetables grown at WWZ, DIM was found to be the highest for Co^{2+} followed by the Zn^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{2+}, Pb^{2+} and Cd^{2+}. Daily intake of metal for Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} ranged from 0.014–0.0043, 0.050–0.061, 0.0031–0.0103, 0.0043–0.0060, 0.0005–0.003, 0.010–0.08 and 0.02–0.048 respectively. The trend for DIM in vegetables grown at GWZ was in the order of $Co^{2+} > Mn^{2+} > Zn^{2+} > Ni^{2+} > Cu^{2+} > Cr^{2+} > Pb^{2+} > Cd^{2+}$ and the values of DIM ranged from 0.00095–0.00147, 0.0046–0.0087, 0.0013–0.0045, 0.0005–0.0019, 0.00021–0.00043, 0.000094–0.00044, 0.001–0.014 and 0.003–0.01 for Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} respectively.

The Health risk index for heavy metals by consumption of vegetables grown on WWZ and GWZ for adults was calculated and values are given in Table 6. The maximum HRI was found for *S. oleracea* (2.42) and *B. campestris* (2.22) grown at WWZ. HRI of Cr^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} ranging from 0.0009–0.0029, 0.19–0.52, 0.11–0.15,

Table 3 Comparison of mean values ±standard deviation of heavy metal concentration in vegetables grown at WWZ and GWZ.

Vegetables	Value	Cr[a]	Cr	Co[a]	Co	Ni[a]	Ni	Cu[a]	Cu	Pb[a]	Pb	Cd[a]	Cd	Mn[a]	Mn	Zn[a]	Zn
Solanum tuberosum L.	M ± S.D.	1.60 ± 0.9	1.29 ± 0.64	53.18 ± 2.38	6.04 ± 2.08	6.04 ± 6.2	3.37 ± 1.56	4.16 ± 2.7	1.3 ± 0.74	0.49 ± 0.27	0.26 ± 0.1	0.21 ± 0.13	0.17 ± 0.19	17.77 ± 11.4	5.97 ± 5.12	21.77 ± 18.32	5.29 ± 2.4
	Range	0.88–2.61	0.76–1.98	52.97–57.21	4.37–8.38	1.29–19.02	–	1.09–6.12	0.8–2.3	0.19–0.63	0.17–0.37	0.08–0.35	0.01–0.39	10.43–30.91	1.94–11.74	10.35–42.81	2.91–7.89
Brassica oleracea capitata	M ± S.D.	3.06 ± 1.06	1.33 ± 0.87	47.2 ± 14.3	7.04 ± 3.4	5.046 ± 5.72	3.04 ± 2	4.92 ± 3.11	0.89 ± 0.35	2.86 ± 1.22	0.265 ± 1.10	0.27 ± 0.09	0.27 ± 0.13	17.26 ± 11.3.	11.18 ± 7.4	37.76 ± 16	10.61 ± 5.6
	Range	2–4.019	0.35–2.80	32.8–67.09	3.27–9.88	0.96–14.67	0.9–5.03	1.63–7.84	0.56–1.45	1.88–4.23	0.01–0.67	0.17–0.36	0.02–0.03	9.29–30.2	6.031–19.69	21.98–53.91	6.93–17.09
Brassica oleracea	M ± S.D.	2.15 ± 1.01	0.99 ± 0.80	47.9 ± 15	4.40 ± 2.03	2.95 ± 2.791	2.95 ± 1.74	4.49 ± 2.1	0.74 ± 0.77	1.66 ± 1.22	0.317 ± 0.27	0.23 ± 0.069	0.19 ± 0.23	9.06 ± 2.32	5.67 ± 2.26	35.44 ± 16.51	4.40 ± 3.01
	Range	1.15–4.15	0.20–1.98	30.12–71.41	2.18–6.16	0.76–8.91	1.92–4.97	2.1–6.09	0.12–1.87	0.91–3.07	0.01–0.38	0.18–0.31	0.01–0.46	6.57–11.18	3.76–8.18	20.98–55.91	1.11–7.02
Brassica campestris L.	M ± S.D.	2.62 ± 1.73	0.98 ± 0.45	48.45 ± 12	7.22 ± 4	6.43 ± 8.41	2.86 ± 1.71	5.42–4.2	1.85 ± 1.54	2.77 ± 1.49	0.2 ± 0.163	1.08 ± 1.71	0.37 ± 0.22	69.9 ± 11.6	9.33 ± 5.5	47.10 ± 15	8.4 ± 1
	Range	1.37–4.87	0.36–1.89	39.06–67.98	2.91–10.58	1.00–21.91	1.78–4.83	1.84–10.17	0.3–1.32	1.12–4.03	0.19–0.88	0.32–1.73	0.11–0.53	57.84–81.01	5.37–15.69	35.43–63.91	4.63–12.71
Brassica rapa L.	M ± S.D.	1.44 ± 2	0.91 ± 0.34	53.2 ± 20	4.30 ± 3	5.76 ± 12.09	2.98 ± 1.81	4.90 ± 1.8	0.7 ± 0.34	0.77 ± 0.55	0.41 ± 0.32	0.36 ± 0.26	0.27 ± 0.06	19.5 ± 5.8	4.97 ± 2.5	28.53 ± 7	2.49 ± 1.3
	Range	0.29–3.02	0.54–1.76	23.98–76.13	1.08–6.97	1.02–32.51	1.91–5.09	2.87–6.5	0.36–1.21	0.19–1.29	0.03–1.30	0.20–0.67	0.21–0.33	14.47–25.83	1.34–6.31	21.17–35.25	1.28–3.87
Spinacia oleracea L.	M ± S.D.	2.42 ± 1.6	0.91 ± 0.86	50.4 ± 8.9	8.31 ± 5.26	3.65 ± 4	3.52 ± 2.44	5.77 ± 4.6	0.44 ± 0.26	2.9 ± 1.17	0.38 ± 0.61	1.90 ± 1.02	0.14 ± 0.14	16.58 ± 5	13.25 ± 5.7	25.86 ± 6.8	9.68 ± 6.23
	Range	1.37–4.27	0.34–3.01	41.75–65.27	3.13–13.65	0.18–11.1	1.27–6.13	2.14–10.94	0.26–0.83	1.88–4.19	0.01–0.93	1.20–3.08	0.02–0.3	11.83–21.73	9.43–19.88	18.18–31.26	6.04–16.87
Beta vulgaris L.	M ± S.D.	1.31 ± 1.6	1.03 ± 0.53	54.4 ± 18.6	4.55 ± 2.6	6.23 ± 11.01	3.96 ± 2.12	4.1 ± 2.3	1.85 ± 1.39	0.64 ± 03	0.27 ± 0.24	0.21 ± 0.02	0.42 ± 0.23	76.26 ± 10.18	7.2 ± 3.27	45.65 ± 13	3.39 ± 2.8
	Range	0.29–2.61	0.55–2.17	29.32–80.93	1.57–6.23	1.1–31.03	1.78–6.03	1.9–6.49	0.55–3.97	0.21–0.93	0.11–0.64	019–0.23	0.27–0.69	67.39–87.37	3.91–10.45	34.73–60.26	0.2–5.83
Allium sativum L.	M ± S.D.	4.1 ± 0.65	0.98 ± 0.91	51.7 ± 20.3	6.70 ± 3.16	9.85 ± 7.8	1.23 ± 1.03	4.70 ± 3.6	1.32 ± 0.82	0.38 ± 0.29	0.26 ± 0.15	0.19 ± 0.9	0.21 ± 01	21 ± 5.6	3.16 ± 2.7	24.64 ± 4.7	6.98 ± 3.6
	Range	3.18–5.01	0.38–3.34	23.87–75.09	3.21–9.39	0.87–20.67	0.28–2.33	1.17–8.35	0.11–1.98	0.71–0.31	0.12–0.49	0.09–0.27	0.11–0.31	16.84–27.095	1.19–6.34	19.71–29.05	3.11–10.34
Daucus carota L.	M ± S.D.	1.92 ± 1.02	1.04 ± 0.71	51.2 ± 16.6	5.19 ± 3.9	5.33 ± 3.46	3.67 ± 2.98	5.34 ± 3.84	0.87 ± 0.75	1.97 ± 0.96	0.26 ± 0.8	0.29 ± 0.24	0.09 ± 0.34	17.45 ± 5.8	2.87 ± 1.88	19.39 ± 6	7.56 ± 4.15
	Range	0.98–3.01	0.51–2.97	31–68.56	1.31–9.19	1.13–9.97	1.94–7.12	1.9–9.51	0.36–1.09	1.01–1.97	0.17–0.79	0.11–0.57	0.07–0.13	11.34–22.85	1.61–5.03	14.04–25.95	3.13–11.38
Coriandrum sativum L.	M ± S.D.	2.32 ± 1.7	1.40 ± 1	57.6 ± 14.3	7.39 ± 4.12	6.2 ± 5.85	4.27 ± 3.9	4.95 ± 3.4	1.65 ± 1.1	2.04 ± 0.45	0.38 ± 0.36	0.31 ± 0.25	0.37 ± 1.5	28 ± 8.6	9.17 ± 3.9	39.69 ± 7.6	8.28 ± 3.4
	Range	1.23–4.37	0.76–3.98	43.01–79.45	3.21–11.45	1.09–15.57	0.21–8.01	1.57–4.94	0.57–1.76	1.64–2.53	0.13–0.91	0.13–0.61	0.21–0.57	21–37.65	5.14–13.04	33.83–48.26	4.93–11.74
Permissible limits	EU[b]		1	50			NA[d]	20			0.43	0.2			500	50	
	Ind. St.[c]		20	NA[d]			67	30			2.5	1.5			NA[d]	50	
P			* **	* **			* **	* **			* **	NS			NS	* **	

NS = No significant difference.

[a] Heavy metal concentration in vegetables from WWZ.

[b] European Union Standards European Union (2006).

[c] Indian standards Awashthi (2000).

[d] Not available.

* Significance at $P \leqslant 0.05$.

** Significance at $P \leqslant 0.01$.

Table 4 Transfer factor (TF) of heavy metals in vegetables grown at WWZ and GWZ.

Plant species	Cr	Cr[a]	Co	Co[a]	Ni	Ni[a]	Cu	Cu[a]	Pb	Pb[a]	Cd	Cd[a]	Mn	Mn[a]	Zn	Zn[a]
Solanum tuberosum L.	0.09	0.07	1.51	6.23	0.24	0.21	0.08	0.14	0.036	0.032	0.128	0.066	0.171	0.351	0.249	0.544
Brassica oleracea capitata	0.09	0.14	1.77	5.53	0.22	0.18	0.06	0.17	0.037	0.187	0.204	0.085	0.32	0.341	0.498	0.944
Brassica oleracea	0.07	0.10	1.10	5.61	0.21	0.10	0.05	0.15	0.044	0.108	0.143	0.072	0.162	0.179	0.206	0.886
Brassica campestris L.	0.07	0.13	1.81	5.67	0.20	0.23	0.12	0.19	0.028	0.180	0.28	0.340	0.267	1.38	0.394	1.178
Brassica rapa L.	0.06	0.07	1.08	6.23	0.21	0.20	0.04	0.17	0.057	0.050	0.204	0.113	0.142	0.385	0.117	0.713
Daucus carota L.	0.07	0.12	1.30	5.90	0.26	0.13	0.06	0.20	0.036	0.189	0.068	0.599	0.082	0.327	0.35	0.647
Spinacia oleracea L.	0.06	0.06	2.08	6.37	0.25	0.22	0.03	0.14	0.055	0.042	0.1	0.066	0.38	1.505	0.42	1.142
Beta vulgaris L.	0.07	0.19	1.14	6.05	0.28	0.35	0.12	0.16	0.038	0.025	0.318	0.059	0.206	0.41	0.159	0.616
Allium sativum L.	0.07	0.09	1.68	5.99	0.09	0.19	0.08	0.18	0.036	0.128	–	0.091	0.09	0.344	0.328	0.485
Coriandrum sativum L.	0.1	0.11	1.86	6.74	0.30	0.22	0.11	0.17	0.053	0.133	0.28	0.097	0.263	0.553	0.38	0.993

[a] Heavy metal concentration in vegetables from WWZ.

0.10–0.76, 0.13–1.99, 0.29–2.42 and 0.010–0.165 respectively and the highest HRI value was found for Mn^{2+} followed by the Ni^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+} and Cr^{2+} in case of vegetables collected from WWZ. HRI was found to be the highest for Mn^{2+} and its rank appeared as $Mn^{2+} > Cd^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Pb^{2+} > Cr^{2+}$ in case of vegetables obtained from GWZ.

4. Discussion

Continuous wastewater irrigation has changed the soil physicochemical properties and has led to heavy metal uptake by food crops, predominantly vegetables. Oxidation state, heavy metals form and phase strongly influence their bioavailability (Bi et al., 2006; Lei et al., 2008). In the present study heavy metal concentration in ground water was in the range of Indian permissible limits (Awashthi, 2000) while there was higher concentration of Cu^{2+}, Ni^{2+}, Cr^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+} and Co^{2+} (Table 2) in wastewater compared with the Indian permissible limits (Awashthi, 2000), which is being used for irrigation in the cultivation of food crops particularly vegetables in the WWZ of study area. In the Lahore city 1350 million litters wastewater is generated per day (Saleemi, 1990) that drains into the Ravi River through the city drainages, managed by WASA, Lahore. Industrial and municipal sewage of city are discharged in these drainages, which is the main route of heavy metal accumulation in wastewater (Wozniak and Huang, 1982). Irrigation of agricultural land with wastewater leads to the accumulation of heavy metals in soil (Jan et al., 2010). Table 2 presents the heavy metal accumulation in soil irrigated with wastewater and ground water. Results showed that the bio-available concentration of heavy metals was higher in the soil irrigated with wastewater as compared to the soil irrigated with the ground water. Except Cd^{2+}, the metal concentration was below the permissible limits of the EU standard (European Union, 2002) and Indian standards (Awashthi, 2000) in the soil of WWZ and GWZ. Continuous removal of metals by food crops (vegetables and cereals) grown at the wastewater irrigated soil and heavy metals leaching into the deeper layers of soil may be a reason of low concentration of heavy metals than the permissible limits (Singh et al., 2010). Cd^{2+} concentration was found to be the highest in the wastewater irrigated soil followed by the concentration of Ni^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Pb^{2+} and Cr^{2+}. Concentration of Cd^{2+}, Ni^{2+}, Pb^{2+} and Cu^{2+} was also reported to be higher but within the safe limits of the EU standard (European Union, 2002) in and around the city Lahore (Younas and Shahzad, 1998). Jan et al. (2010) also reported the higher concentration of Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{2+} and Pb^{2+} in the soil irrigated with the wastewater from Peshawar, Pakistan.

In the present study metal concentration was greater in the vegetables grown in wastewater, than those grown in ground water. A variation in the metal concentration may be due to the variable factors like heavy metal concentration in soil wastewater used for irrigation and atmospheric deposition along with the plant's capability to uptake and accumulate the heavy metals (Pandey et al., 2012). One way ANOVA was used to compare the metal concentration in the vegetables grown in wastewater and those grown in ground water. Results showed significantly ($P \geqslant 0.05$) higher concentration of Cr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+} and Zn^{2+} in vegetables grown in wastewater as compared to the vegetables grown in ground water. P value in case of Cd^{2+} was non-significant because the Cd^{2+} concentration was also higher in some vegetables grown at ground water (Table 3). Cd^{2+} concentration (Table 2) was high in tube well water compared to the permissible limits set by the EU standard (European Union, 2002) but was in the safe limits of Indian standards (Awashthi, 2000). In the city of Lahore a number of steel and iron foundries and re-rolling mills that are using thousands of tons of broken ships steel coated with Cd^{2+} and Pb^{2+} and steel scrap daily are discharging Cd^{2+} and Pb^{2+} in wastewater that is being used for irrigation purposes.

During the present study it was found that the Cd^{2+}, Pb^{2+}, Cr^{2+} and Co^{2+} concentration in vegetables grown in wastewater irrigated soil was higher than the safe limits while the concentration of Ni^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} was in the range of safe limits set by the EU standard (European Union, 2002). However, metal concentration in wastewater irrigated vegetables was in the range of Indian safe limits (Awashthi, 2000). Concentration of Cd^{2+} was exceeding the safe limits in wastewater and soil of WWZ; Co^{2+}, Pb^{2+} and Cr^{2+} concentration were also higher in the wastewater (Table 2). Wastewater used for the irrigation purposes may route the uptake of heavy metals from roots to the edible parts of the vegetables. Industrial and traffic emission, burning of fossil fuel, discharge of Pb^{2+} storage batteries, sewage water, and paints/pigments may be the main sources of Pb^{2+} while Cr^{2+} is discharged from electroplating and pigment/pains industries, textile mills and tanneries in the city Lahore. In the human body Cd^{2+} induces the gastrointestinal problems and severe toxic effects on different body parts like kidney, liver, testis, ovaries, nervous system

Table 5 Daily intake of metals (mg/person/day) in vegetables grown at WWZ and GWZ.

Plant species	Cr	Cr[a]	Co	Co[a]	Ni	Ni[a]	Cu	Cu[a]	Pb	Pb[a]	Cd	Cd[a]	Mn	Mn[a]	Zn	Zn[a]
Solanum tuberosum L.	0.00135	0.0017	0.0063	0.056	0.0035	0.0064	0.0014	0.0044	0.00027	0.0005	0.00018	0.00022	0.006	0.019	0.005	0.023
Brassica oleracea capitata	0.0014	0.0032	0.0074	0.05	0.0032	0.0053	0.0009	0.0052	0.00028	0.003	0.00028	0.00028	0.011	0.018	0.01	0.04
Brassica oleracea	0.00104	0.0023	0.0046	0.05	0.0031	0.0031	0.0008	0.0047	0.00033	0.0017	0.0002	0.00024	0.006	0.01	0.005	0.037
Brassica campestris L.	0.00103	0.0028	0.0075	0.05	0.003	0.0067	0.0019	0.0057	0.00021	0.0029	0.00039	0.00113	0.001	0.07	0.008	0.05
Brassica rapa L.	0.00095	0.0015	0.0045	0.056	0.0031	0.006	0.0007	0.0051	0.00043	0.0008	0.00028	0.00038	0.005	0.02	0.003	0.03
Daucus carota L.	0.00109	0.0025	0.0054	0.053	0.0038	0.0038	0.0009	0.006	0.00027	0.003	9.40E-05	0.00199	0.003	0.017	0.008	0.027
Spinacia oleracea L.	0.00095	0.0014	0.0087	0.057	0.0037	0.0065	0.0005	0.0043	0.0004	0.0007	0.00015	0.00022	0.014	0.08	0.009	0.048
Beta vulgaris L.	0.00108	0.0043	0.0047	0.054	0.0042	0.0103	0.0019	0.0049	0.00028	–	0.00044	0.0002	0.007	0.022	0.004	0.026
Allium sativum L.	0.00103	0.002	0.007	0.054	0.0013	0.0056	0.0014	0.0056	0.00027	0.002	–	0.0003	0.003	0.018	0.007	0.02
Coriandrum sativum L.	0.00147	0.0024	0.0077	0.061	0.0045	0.0065	0.0017	0.0052	0.0004	0.0021	0.00039	0.00033	0.001	0.03	0.009	0.041

[a] DIM of vegetables grown at WWZ.

Table 6 Health risk index for heavy metals in vegetables grown at WWZ and GWZ.

Plant species	Cr	Cr[a]	Ni	Ni[a]	Cu	Cu[a]	Pb	Pb[a]	Cd	Cd[a]	Mn	Mn[a]	Zn	Zn[a]
Solanum tuberosum L.	0.0009	0.0012	0.18	0.32	0.03	0.11	0.068	0.13	0.18	0.22	0.19	0.57	0.018	0.076
Brassica oleracea capitata	0.0009	0.0021	0.16	0.26	0.02	0.13	0.069	0.75	0.28	0.28	0.35	0.55	0.037	0.132
Brassica oleracea	0.0007	0.0015	0.16	0.15	0.02	0.12	0.083	0.43	0.20	0.24	0.19	0.29	0.015	0.124
Brassica campestris L.	0.0007	0.0018	0.15	0.34	0.05	0.14	0.052	0.73	0.39	1.13	0.29	2.22	0.029	0.165
Brassica rapa L.	0.0006	0.001	0.16	0.30	0.02	0.13	0.107	0.20	0.28	0.38	0.16	0.62	0.009	0.010
Daucus carota L.	0.0007	0.0017	0.2	0.19	0.02	0.15	0.068	0.76	0.09	1.99	0.09	0.53	0.03	0.090
Spinacia oleracea L.	0.0006	0.0009	0.18	0.33	0.01	0.11	0.010	0.17	0.15	0.22	0.42	2.42	0.03	0.160
Beta vulgaris L.	0.0007	0.0029	0.21	0.52	0.05	0.12	0.071	0.10	0.45	0.20	0.23	0.67	0.012	0.086
Allium sativum L.	0.0007	0.0014	0.06	0.28	0.03	0.14	0.068	0.52	0	0.30	0.10	0.56	0.02	0.068
Coriandrum sativum L.	0.0001	0.0016	0.23	0.33	0.04	0.13	0.099	0.54	0.39	0.32	0.30	0.89	0.03	0.14

[a] HRI of vegetables grown at WWZ.

and cardiovascular system (Cooke and Johnson, 1996). Pb^{2+} causes hematological effects, neurological effects, renal failure, gastrointestinal effects, physiological disorders and carcinogenic effects (ATSDR, 2007). Cr^{2+} has epidemiological effects on the urogenital system, cardiovascular problems and carcinogenic effects (Costa and Klein, 2006).

It was found that the leafy vegetables like *C. sativum*, *S. oleracea*, *B. campestris*, *B. oleracea capitata* have a higher concentration of heavy metals than those of bulbs and tuber type vegetables. Further, in vicinity to the study area a number of industries and automobiles emit their smoke in the open air; the atmosphere of that area remains smoky and this smoke contains various toxic metals that may cause atmospheric deposition of heavy metals on the leaves of vegetables, which may be a reason of higher concentration of heavy metals in leafy vegetables. Previous reports from Pakistan have indicated that the vegetables grown in wastewater accumulate higher concentration of heavy metals than those vegetables grown at the ground water (Khan et al., 2010; Jan et al., 2010; Akbar et al., 2009).

Metal transfer factor from soil to plants is a key module of human exposure to heavy metals via food chain. Transfer factor of metals is essential to investigate the human health risk index (Cui et al., 2004). TF of metals varied significantly in different vegetables and was found to be maximum for Co^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+}. Among vegetables *C. sativum*, *S. oleracea*, *B. rapa* and *S. tuberosum* showed a higher metal transfer factor from soil to plants than other vegetables. Leafy vegetables uptake metals in higher concentration compared to the other vegetables. Leafy vegetable has a higher transpiration rate to sustain the growth and moisture content of plant that may be the reason of high uptake of metals in them (Tani and Barrington, 2005; Lato et al., 2012). Results revealed that the values of TF were found to be lower for Zn^{2+}, Mn^{2+}, Ni^{2+} and Cu^{2+} and higher for Cd^{2+}, Pb^{2+} and Cr^{2+} than those reported by Jan et al. (2010). In case of TF reported by Khan et al. (2010) TF values for Cd^{2+} and Pd^{2+} were higher and Cu^{2+}, Ni^{2+} and Zn^{2+} were lower in the present research work.

DIM for adults via consumption of contaminated vegetables may cause severe health risks by ingestion of Cd^{2+} and Mn^{2+} through *D. carota* and *B. campestris* grown at WWZ while the estimated DIM of Cr^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} were in the range of safe limits set by US-EPA IRIS (2006). DIM and HRI of the study area suggest that *S. tuberosum*, *B. oleracea capitata*, *B. oleracea*, *B. rapa*, *S. oleracea*, *B. vulgaris*, *A. sativum*, *C. sativum* grown at the WWZ were almost safe for consumption but *S. oleracea*, *D. carota* and *B. campestris* pose severe health risk with regard to the Cd^{2+} and Mn^{2+}. In case of GWZ all the vegetables were totally free from any risk.

5. Conclusion

Continuous wastewater irrigation to the agricultural land has caused an ample build up of toxic metals in wastewater irrigated soil as compared with the ground water irrigated soil. The present study revealed that wastewater irrigated soil wastewater and food crops grown at WWZ were enriched with Cr^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, and Zn^{2+}. In food crops grown in wastewater, the extent of heavy metal enrichment was in the order of $Cr^{2+} > Pb^{2+} > Cd^{2+} > Co^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$. Research revealed that the leafy vegetables have a higher capability to accumulate the heavy metals from soil compared with the others (tuber/bulbs, etc.). HRI indicated that the vegetables grown in wastewater and ground water are free from any risk; however, *S. oleracea* and *B. campestris* pose a serious health risk, particularly with Cd2 + and Mn2 +. Long-term use of wastewater as irrigation purpose may lead to the severe risk to consumer's health as, this study has already shown a severe risk to human health by two vegetables. It is suggested that an urgent attention is required for the implementation of proper means to monitor and regulate the industrial and municipal effluents.

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