

Heavy metals (Cd, Pb & Zn) accumulation in Cauliflower (*Brassica oleracea*. Var. *Botrytis*) and associated health risks assessment in three Districts of Punjab, Pakistan

ZEESHAN REHMAN¹, RANA ABRAR HUSSAIN¹, SHAISTA JABEEN², SAKHAWAT ALI², ZAHAR NOREEN¹ & IRUM MUKHTAR^{1*}

¹Division of Science and Technology, University of Education, Township Campus Lahore.

²PCSIR (Pakistan Council of Scientific and Industrial Research), Lahore Pakistan.

ABSTRACT

Continuous discharge of untreated urban and industrial waste water is a source of heavy metals in soil is a major threat to ecological system and human health. Present study was carried out to evaluate human health risks in association with food chain contamination of heavy metals from urban and industrial wastewater irrigation. During present investigation, Pb²⁺, Cd²⁺ and Zn²⁺ concentrations were analyzed in mostly cultivated/consumed *Brassica oleracea*. Var. *Botrytis* species in Lahore (LHR), Narowal and Kasur districts of Punjab, Pakistan. The average concentration of elemental Zn in sewage-irrigated samples was the highest (153.4233mg kg⁻¹), followed by Cd²⁺ (70.47333 mg kg⁻¹) and Pb (65.79667mg kg⁻¹). Results showed higher Pb²⁺ and Cd²⁺ level in *B. oleracea* than daily intake of metals (DIM) standard limits, cultivated on wastewater. Whereas health risk index (HRI) was found maximum (23.22 mg kg⁻¹) for this vegetable cultivated on waste water in Kasur region. From the health point of view, the long-term consumption of contaminated vegetables is one of the causes of carcinogenic and non-carcinogenic health risks.

Keywords: Heavy metals, cauliflower, urban and industrial wastewater, health risk, intake of metals

INTRODUCTION

Urban and industrial development has caused an alteration to the environment by increasing the waste materials and pollutants worldwide. Due to industrial and municipal activity, land and water contamination with toxic heavy metals is a common problem. Heavy metals are significant due to their toxicity for ecological, evolutionary, nutritional and environmental effects. Soil and water contaminants are primary cause of heavy metals exposure to humans. Consumption of contaminated food crops, water or inhalation of dust can cause toxic metals to enter in human body (Jaishankar *et al.*, 2014; Balkhair & Ashraf, 2016). In many developing countries, sewage water is an irrigation source for food crop cultivation to a certain extent (Allende & James, 2015; Balkhair & Ashraf, 2016). Vegetables easily take up heavy metals and accumulate them in their edible parts (Sipter *et al.*, 2008) and contribute to an increasing risk to heavy metals exposure to human due to consumption of such contaminated food. Long term usage of

contaminated food may lead to toxic metals buildup in different human body organs resulting in several clinical and physiological problems (Mahmood & Malik, 2014). Therefore, heavy metals in vegetables are alarming if soils and irrigation waters are contaminated.

Wastewater irrigation is an alternate to low fresh water supply and to increase the yield of vegetable crops in urban and pre-urban agricultural lands. However, these effluents are one of the major causes of toxic metals loading in amended soils and crops (Mahmood & Malik, 2014). Wastewater irrigation is a common practice in peri-urban areas of Lahore, Narowal and Kasur districts in Punjab province. These cities and their surroundings are sites for many industries like leather tanneries, textile, electroplating mills, iron foundries pigment factories. These industries are discharging unchecked heavy metals and hazardous chemicals every day in main city drainages. Various vegetables cultivated on industrial and sewage effluents are being sold and

Author's Contribution: R.A.H., Conceived the idea and supervised the study; Z.R., Performed experiment and compiled data; S.J., S.A., & Z.N., Gave technical support during experimental work; I.M., Analyzed data and wrote manuscript.

*Corresponding author: erumm21@yahoo.com

supplied to these main cities. Serious health problems can develop as a result of accumulation of heavy metals because of dietary intake of contaminated food crops (Perveen *et al.*, 2011; Nawaz UI Hassan *et al.*, 2013). Health risk assessment of heavy metals has been done in many developed countries especially for daily consumed vegetables (Milacic & Kralj, 2003). However, a very few studies have been carried on heavy metal contamination in vegetables in Pakistan (Mahmood & Malik, 2014). There is an urgency of systematic assessments to make timely decisions to avoid severe health problems due heavy metals entering the food chain with invisible toxicity. Cauliflower (*Brassica oleracea*) is a most planted and consumed vegetable crop in Punjab province. It is a good source of variety of vitamins, minerals and dietary fibers. However, this vegetable is widely irrigated with waste waters in some areas. The experiential data on cauliflower plants growth due to heavy metal accumulation is rarely available for multi-metal-contaminated soil. Therefore, the aim of this research is to evaluate heavy metal accumulation in cauliflower and associated health hazards due to its consumption by human being.

MATERIALS AND METHODS

Sample Sites Plant Sampling

Agricultural areas in surrounding of Lahore, Narowal and Kasur cities were selected as the study area. Cauliflower cultivation areas were selected on basis of wastewater from urban drains and fresh water irrigation. Cauliflower (flower portion) samples were collected from different sites in region of Narowal, Lahore and Kasur and labeled as fresh (FICS) and waste water irrigated (WICS). From each site, a total of 6 samples was collected, consisting of 3 fresh and 3 waste-water irrigated fields respectively followed by sample washing with tap water to remove soil debris. Edible parts from each sample were separated from non-edible portion. Edible parts of cauliflower were oven dried for 12-15 days at 50°C, ground into fine powder and stored, passed through a 2-mm-mesh sieve and stored in labeled polythene bags at room temperature. Dried samples were carried to Pakistan Council of Scientific and Industrial Research (PCSIR) laboratory, Lahore, Pakistan for further analysis.

Digestion of Samples

Containers for the analysis were cleaned carefully by a detergent, rinsed with tap water followed by soaking in acid (2+1 HCl) and rinsed

with metal-free water. From each dried cauliflower sample, 1 gm grounded fine powder was added to a flask containing concentrated HNO₃ (4 ml) and HCl (12 ml). Sample acid mixture was allowed to stand for at least 12 h. Later mixture was boiled for 2 h, cooled, rinsed with 15 mL of deionized water. Digested mixture was filtered through pre-washed Whatman No. 540. Volume of each filtrate was made up to 100 ml by adding ultra 2M HNO₃. The prepared samples were stored at 4°C in acid-washed polyethylene bottles (Mapanda *et al.*, 2005).

Heavy Metal Analysis

Concentrations of heavy metals were determined by a flame atomic absorption spectrophotometer (AAS, Model-A, Shimadzu Analyst-800, Japan) by using respective hollow cathode lamp using standard solutions of metals. The target heavy metals included Cadmium (Cd), Lead (Pb), and Zinc (Zn) (Perkin Elmer; 2000). Calculations were made by using the following formula;

$$\frac{\text{Concentrations (mg/L)} \times \text{Dilution Factor (mL)}}{\text{Weight of Sample (g)}}$$

Daily Intake of Metals (DIM)

Daily intake of different heavy metals in adult on consumed cauliflower was calculated by survey based data. DIM was calculated according to Chary *et al.* (2008);

$\text{DIM} = C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{food intake}} / B_{\text{average weight}}$
Where C_{metal} , C_{factor} , $D_{\text{food intake}}$ and $B_{\text{average weight}}$ 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 cauliflower intake was calculated on survey data of 200 consumers with an average body weight of 58 kg.

Health Risk Index (HRI)

To evaluate human health risk of heavy metals, cauliflower samples grown on the both fresh and wastewater were collected from the study regions to calculate metal concentration and health risk index (HRI). Value of HRI depends upon the daily intake of metals (DIM) and oral reference dose (RfD). The health risk index for Cd, Pb and Zn by consumption of cauliflower samples were calculated as reported in literature (Jan *et al.*, 2010)

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

RfD is a standard per day exposure of metal to the human body that has no hazardous effect during life time (US-EPA IRIS, 2006). Where DIM represents the daily intake of metals and Rfd represents reference oral dose. Rfd value for Pb, Cd and Zn is 0.004, 0.001 and 0.30 (mg/kg bw/day), respectively (US-EPA IRIS, 2006). The health risk index of current investigation was also compared with previous studies (Khan *et al.*, 2010; Jan *et al.*, 2010).

RESULTS

Concentration of Heavy Metals in Vegetables

Concentration of heavy metals in cauliflower grown in WW and FW was compared along with the permissible limits set (European Union, 2002; Awashthi, 2000), showed in Table III. Results revealed that Zn²⁺, Pb²⁺ and Cd²⁺ concentration was significantly higher in cauliflower in WW than those grown in FW. However, concentration of Pb²⁺ and Cd²⁺ from cauliflower grown on WW, exceeded the permissible limits (European Union, 2002), while was in the range of Indian safe limits (Awashthi, 2000). Concentration of Cd²⁺ in all cauliflower samples, exceeded to the EU safe limits (European Union, 2002), irrigated with FW. Cauliflower samples showed maximum Cd²⁺ concentration (70.47333 mg kg⁻¹), obtained from Lahore region (Table III) followed by Narowal (56.55 mg kg⁻¹). Alternatively, the maximum Pb²⁺ concentration (65.79 mg kg⁻¹) was found in Kasur samples grown on WW. However, Pb²⁺ concentration (0.0001 mg/kg) was not considerable in FW samples from Narowal. Among heavy metal concentration in cauliflower grown on WW the trend appeared as Zn > Cd > Pb while in samples grown at FW the trend appeared as Zn > Pb > Cd.

DIM and HRI of Heavy Metals

Values of DIM calculated at average age 47 years, is presented in Table I. These data revealed that the values of DIM were high in case of cauliflower grown on WICS. Furthermore, cauliflower cultivated in LHR was found to be the highest for DIM in relation to Pb²⁺ followed by Zn²⁺ and Cd²⁺. However, DIM tendency in cauliflower cultivated in Narowal was in the order of Zn²⁺ > Cd²⁺ > Pb²⁺.

Maximum value of HIR (23.50) was found for cauliflower, grown in Kasur (Table II). However, HRI of Zn²⁺, Cd²⁺ and Pb²⁺, ranging from 0.07–0.09, 0.0–5.45 and 11.60–23.50 respectively. Results of HRI were found to be lower than those of Khan *et al.* (2010) and Jan *et al.* (2010). However,

highest HRI value was found for Cd²⁺ followed by Pb²⁺ and Zn²⁺ in cauliflower samples collected from Kasur.

DISCUSSION

Agricultural practices on industrial and municipal sewage is the main route of heavy metal accumulation in food crops (Balkhair & Ashraf, 2016). Long term WW irrigation can change physicochemical properties of soil and leads to heavy metal uptake by crops, especially leafy vegetables. In the present investigation, Cd²⁺, Pb²⁺ and Zn²⁺ concentration in FW was found in the range of Indian permissible limits (Awashthi, 2000), which is being used for cauliflower cultivation in Lahore, Narowal and Kasur areas. Present study has also found the average levels of Cd (90.23 mg/kg-1), Pb (59.26 mg/kg-1) and Zn (101.01 mg/kg-1) that contradicts with previously reported study (Mahmood & Malik, 2014). Previous studies have reported that vegetables crops can show high levels of heavy metals due to gradual accumulation if irrigated with sewage and industrial effluent (Gupta *et al.*, 2010; Balkhair & Ashraf, 2016). However, low metal accumulation in some plants species i.e. wheat (*Triticum aestivum*) Garlic (*Allium sativum*) and Eggplant (*Solanum melongena*) and food crops depend on physical and chemical nature of the soil, temperature, pH and available organic matter in soil. In different vegetable parts, the concentrations of heavy metals are reported in the order of leaf > stem > root > tuber > bulb > fruit > seed (Santamaria *et al.*, 1999; Zurera-Cosano *et al.*, 1989).

Current data showed very high concentration as compared to previously reported average levels of Cd, Pb and Zn in different vegetables (Cao *et al.*, 2014; Singh *et al.*, 2010; Zhuang *et al.*, 2009). Results also demonstrated a high bio-available concentration of heavy metals in all cauliflower samples, irrigated with WW as compared to FW irrigated. Continuous adding of metals by irrigation with WW and low leaching metals into the lower layers of soil, may be a reason of high concentration of Cd²⁺, Pb²⁺ and Zn²⁺ in cauliflower. Previously, Cd²⁺ and Pb²⁺ concentrations were also reported higher but within standard limits (European Union, 2002) in and around the LHR (Younas & Shahzad, 1998). Similarly, higher concentration of Cd²⁺, Zn²⁺, Ni²⁺, Cu²⁺, Cr²⁺ and Pb²⁺ in WW irrigated soil have been reported from other areas in Pakistan (Jan *et al.*, 2010).

A variation in Cd²⁺, Zn²⁺ and Pb²⁺ metal concentration may be due to heavy metal concentration in WW and plant uptake capability of

heavy metals (Pandey *et al.*, 2012). However, concentration of Cd²⁺ was exceeding the safe limits in Lahore and Kasur areas where cauliflower is irrigated with WW. Edible parts of the vegetables contaminated with heavy metal may route the uptake of these contaminants in humans. Cd²⁺ has been reported to cause problem and disorder in different body organs and systems like kidney, liver, testis, ovaries, gastric, nervous and cardiovascular system (Amna *et al.*, 2015; Tauqeer *et al.*, 2016 ; Wu, 2015).

According to DIM analysis, consumption of contaminated cauliflower may cause health complications due to high Cd²⁺ and Pb²⁺ grown in Lahore, Narowal and Kasur districts.

This study suggests that DIM and HRI related to cauliflower is not safe for consumption. This vegetable pose severe health risk with regard to the Cd²⁺, However FW cauliflower was found totally safe for local consumption.

Conclusions and Recommendations

Wastewater irrigation has caused sufficient toxic metals load in cauliflower in Lahore, Narowal

and Kasur districts as compared with the ground water worldwide. Current study revealed that WW irrigated cauliflower crops grown at Lahore, Narowal and Kasur were enriched with Pb²⁺, Cd²⁺ and Zn²⁺. The extent of heavy metal recorded are in order of Zn²⁺ > Cd²⁺ > Pb²⁺ in crop samples grown in WW. Results also indicated that cauliflower have a capability to accumulate the heavy metals if irrigated with WW. HRI values indicated that cauliflower containing high pose a serious health risk, particularly with Cd²⁺. Long-term use of WW irrigation can cause severe risk to consumer's health. To avoid the entrance of metals into the food chain, it is needed to take urgent measures to implement environment protection laws to monitor and standardized the industrial and municipal effluents in different districts. Furthermore, continuous monitoring of the soil, plant and water quality to prevent heavy metals contamination in vegetable crops are prerequisites to minimize health risks to consumers.

Table 1. The range and mean concentrations (mg kg⁻¹) of heavy metals in Cauliflower Samples (CS) grown in Waste Water and Fresh Irrigated Water in different districts in Punjab

Region	Sample	Cd	Pb	Zn
NRL	FICS	3.50667	---	63.24333
	WICS	56.55	27.19333	77.58333
KSR	FICS	5.363333	18.85	84.30667
	WICS	40.57667	65.79667	153.4233
LHR	FICS	6.316667	17.4	52.27333
	WICS	70.47333	25.74333	72.05333
Eu*	NA	3	100	300
Ind.St*	NA	3-6	250-500	300-600

EU*= European Union Standard European Union (2006)

Ind. St*= Indian Standard, Awasthi (2000)

FICS=Freshwater irrigated cauliflower samples

WICS=waste water irrigated cauliflower samples

Table 2. Daily Intake of Metal (DIM) in cauliflower at three respective sites; Narowal, Lahore and Kasur

Samples	Zinc	Lead	Cadmium
NRL _F	0.021	----	0.016
NRL _W	0.0258	0.0090	0.01885
LHR _F	0.0281	0.0063	0.018
LHR _W	0.0051	0.0219	0.013
KSR _F	0.0174	0.0058	0.021
KSR _W	0.0257	0.00858	0.0235

NRL_F= Narowal (fresh), NRL_W= Narowal (waste)
 LHR_F= Lahore (fresh), LHR_W= Lahore (waste)
 KSR_F= Kasur (fresh), KSR_W= Kasur (waste)

Table 3. Health Risk Index (HRI) in cauliflower at three respective sites; Narowal, Lahore and Kasur

Samples	Zinc	Lead	Cadmium
NRL _F	0.07	---	11.6
NRL _W	0.086	2.25	18.85
LHR _F	0.093	1.575	18.00
LHR _W	0.017	5.475	13.00
KSR _F	0.058	1.45	21.00
KSR _W	0.085	2.145	23.50

NRL_F= Narowal (fresh), NRL_W= Narowal (waste)
 LHR_F= Lahore (fresh), LHR_W= Lahore (waste)
 KSR_F= Kasur (fresh), KSR_W= Kasur (waste)

REFERENCES

- Allende, A., & Monaghan, J., 2015. Irrigation Water Quality for Leafy Crops: A Perspective of Risks and Potential Solutions. *Int. J. Environ. Res. Public Health.*, **12(7)**: 7457–7477
- Amna, A., Masood, S., Mukhtar, T., Kamran, M. A., Rafique, M., *et al.*, 2015. Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of *Linum usitatissimum* in association with *Glomus intraradices*. *Environ. Monit. Assess.* **187**:311. doi: 10.1007/s10661-015-4557-8
- Awashthi, S. K., 2000. Prevention of Food Adulteration Act no 37 of 1954. Central and State Rules as Amended for 1999, Ashoka Law House, New Delhi.
- Balkhair, K. S & Ashraf, M. A., 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi J. Biol. Sci.*, **23(1)**: 32–44
- Cao, S. X., Zhao, X., Ma, J., Dong, T., & Wei, F., 2014. Health risks from the exposure of children to As, Se, Pb and other heavy metals near the largest coking plant in China *Sci. Total Environ.*, **472**: 1001–9
- Chary N.S., Kamala C.T. & Raj, D.S.S., 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicol. Environ. Saf.*, **69(3)**:513–524.
- European, U., 2002. Heavy Metals in Wastes, European Commission on Environment ><http://ec.europa.eu/environment/waste/studies>

- Gupta, S., Satpati S., Nayek, S. & Garai, D., 2010. Effect of wastewater irrigation on vegetables in relation to bio-accumulation of heavy metals and biochemical changes. *Environ. Monit. Assess.*, **165 (1-4)**:169-177
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. & Beeregowda. K. N., 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.*, **7(2)**: 60-72
- Jan, F. A. Ishaq, M., Khan, S., Ihsanullah, I., Ahmad, I., & Shakirullah, M., 2010. A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (lower Dir). *J. Hazard. Mater.*, **179**:612-621
- Jan, F. A., Ishaq, M., Khan, S., Ihsanullah, I., Ahmad, I. & Shakirullah, M., 2010. A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (lower Dir) *J. Hazard. Mater.*, **179**:612-621
- Mahmood, A. & Malik. R. N., 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian J. Chem.*, **7**: 91-99
- Mapanda, F., Mangwayana, E., Nyamangara, J., & KE, G. 2005 The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agric. Ecosyst. Environ.*, **107(2-3)**:151-65.
- Milacic, R. & Kralj, B., 2003. Determination of Zn, Cu, Cd, Pb, Ni and Cr in some Slovenian foodstuffs. *E r. Food Res. Technol.*, **217**:211-214
- Nawaz, H., Mahmood, Q., Waseem, A., Irshad, M. F & Pervez, A., 2013. Assessment of Heavy Metals in Wheat Plants Irrigated with Contaminated Wastewater. *Pol. J. Environ. Stud.*, **22(1)**: 115-123
- Pandey, R., Shubhashish, K. & Pandey J., 2012. Dietary intake of pollutant aerosols via vegetables influenced by atmospheric deposition and wastewater irrigation. *Ecotoxicol. Environ. Saf.*, **6** :200-208
- Perveen, S., Ihsanullah, I., Shah, Z., Shah, W. S. S. & Shah, H. H., 2011. Study on accumulation of heavy metals in vegetables receiving sewage water. *J. Chem. Soci. Pak.*, **33**, 220, 2011.
- Qadir, M. A. Ghafoor, G. & Murtaza, 2000. Cadmium concentration in vegetables grown on urban soils irrigated with untreated municipal sewage. *Environ. Dev. Sustain.*, **2**:13-21
- Santamaria, P., Elia, A., Serio, F. & Todaro E., 1999. A survey of nitrate and oxalate content in fresh vegetables. *J. Sci. Food and Agric.*, **79 (13)**: 1882-1888
- Singh A., Sharma R. K., Agrawal M. & Marshall, F. M., 2010. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food Chem.Toxicol.*, **48**: 611-619
- Sipter E., E. Rozsa, K. Gruiz, E. Tatrai & V. Morvai, 2008. Site-specific risk assessment in contaminated vegetable gardens. *Chemosphere.*, **71**: 1301-7
- Tauqeer, H. M., Ali, S., Rizwan, M., Ali, Q., Saeed, R., Iftikhar, U., et al., 2016. Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. *Ecotoxicol Environ. Saf.*, **126**, 138-146.
- Wu, Z. C., 2015. *Screening of High/Low Cadmium Accumulation Brassica Napus Cultivars And Research on the Biochemical Mechanisms*. Doctoral thesis, Huazhong Agricultural University, Wuhan
- Younas, M. & Shahzad, F., 1998. Assessment of Cd, Ni, Cu and Pd pollution in Lahore, Pakistan. *Environ. Internat.*, **24**: 761-766
- Zhuang P., McBride M. B., Xia H., Li N. & Li. Z., 2009. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Sci. Total Environ.*, **407**: 1551-61
- Zurera-Cosano, G., Moreno-Rojas, R., Salmeron-Egea, J. & Lora, R. P., 1989. Heavy metal uptake from greenhouse border soils for edible vegetables. *J. Sci. Food and Agric.*, **49 (3)**: 307-314.