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

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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, Pb^{2+}, Cd^{2+} and Zn^{2+} concentrations were analyzed in mostly cultivated/consumed *Brassica oleracea*. Var. *Botyris* 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 Cd^{2+} (70.47333 mg kg^{-1}) and Pb (65.79667mg kg^{-1}). Results showed higher Pb^{2+} and Cd^{2+} 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

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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 Ul 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^{o}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 HNO_3. The prepared samples were stored at 4^{o}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, Shimadzhu Analyst-800, Japan) by using respective hallow 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;

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

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);

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

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 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)

$$\text{HRI} = {}^{\text{DIM}}/_{\text{R}_{\text{fd}}}$$

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, 0.001and 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 Zn^{2+}, $Pb^{2+}$and Cd^{2} concentration was significantly higher in cauliflower in WW than those grown in FW. However, concentration of $Pb^{2+}$and Cd^{2+} 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 Cd^{2+} in all cauliflower samples, exceeded to the EU safe limits (European Union, 2002), irrigated with FW. Cauliflower samples showed maximum Cd^{2+} concentration (70.47333 mg kg^{-1}), obtained from Lahore region (Table III) followed by Narowal (56.55mg kg^{-1}). Alternatively, the maximum Pb^{2+} concentration (65.79 mg kg-1) was found in Kasur samples grown on WW. However, Pb^{2+} 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 Pb^{2+} followed by $Zn^{2+}$and Cd^{2+}. However, DIM tendency in cauliflower cultivated in Narowal was in the order of $Zn^{2+} > Cd^{2+} > Pb^{2+}$.

Maximum value of HIR (23.50) was found for cauliflower, grown in Kasur (Table II). However, HRI of Zn^{2+}, Cd^{2+} and Pb^{2+}, 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 Cd^{2+} followed by Pb^{2+} and Zn^{2+} 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, Cd^{2+} Pb^{2+} and Zn^{2+}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 concen-tration 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 Cd^{2+} Pb^{2+} and Zn^{2+}in cauliflower. Previously, Cd^{2+} and Pb^{2+} concentrations were also reported higher but within standard limits (European Union, 2002) in and around the LHR (Younas & Shahzad, 1998). Similarly, higher concentration of Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{2+} and Pb^{2+} in WW irrigated soil have been reported from other areas in Pakistan (Jan *et al*., 2010).

A variation in Cd^{2+}, Zn^{2+} and Pb^{2} 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 $Cd^{2+}$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 contaminates in humans. Cd^{2+} 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 Cd^{2+} and Pb^{2+} grown in Lahore, Narowal and Kasur districts.

This study suggests that DIM and HRI rerated to cauliflower is not safe for consumption. This vegetable pose severe health risk with regard to the Cd^{2+}, 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 Pb^{2+}, Cd^{2+} and Zn^{2+}. The extent of heavy metal recorded are in order of $Zn^{2+} > Cd^{2+} > Pb^{2+}$ 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 Cd^{2+}. 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, Awashthi (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 $_{(waste)}$

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