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RESEARCH

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Assessment of heavy metal accumulation and health risks in okra (*Abelmoschus Esculentus* L.) and spinach (*Spinacia Oleracea* L.) fertigated with wastwater

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Abstract

In many countries like Pakistan, where crops are irrigated by wastewater, the accumulation of heavy metals is a serious problem, especially when such an irrigation is a widespread practice. The focus of this study was to know the highly toxic metals like cadmium (Cd), chromium (Cr), and lead (Pb) in water, agricultural soil, and crops, besides their probable risk to human health in the area of Vehari district. The physicochemical parameters were determined for the samples, including organic matter, organic carbon, pH, and electrical conductivity. Water used for irrigation, samples of vegetables for Cd, Cr, and Pb concentration, as well as transfer factor from soil to plants (TF) were analyzed for calculating the daily intake of metals (DIM) and their health risk index (HRI). The results show that the wastewater used for irrigation was contaminated with Cr (0.07mg/kg), Cd (0.054mg/kg), and Pb (0.38mg/kg). In the tube well, the concentrations of heavy metals were: Cd (0.053mg/kg), Pb (0.01mg/kg), and Cd (0.03mg/kg). Application of wastewater increased heavy metals concentration in soil and vegetables. Heavy metals concentrations in wastewater irrigated soil before sowing vegetables in mg/kg were: Pb (0.91), Cd (0.12), and Cr (0.48). After the application of wastewater, significant enrichment of wastewater was observed in Pb (1.93mg/kg), Cd (0.07mg/kg), and Cr (0.34mg/kg). Our study showed a high-risk index of food crops polluted with heavy metals and resultantly greater health risk to humans and animals. That is why preventive measures should be adopted to reduce heavy metals pollution to irrigation water and soils to protect both humans and animals in the Vehari district.

Keywords Heavy Metals, Wastewater, Vegetables, Health Risk, Water management

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Introduction

Wastewater has widespread uses in irrigation in diluted, treated, and untreated forms in peri-urban and urban areas of developing counties mostly (Scott et al. 2009). But in soil, accumulation of heavy metals is an outcome of applying untreated or partly treated water for the long term (Elgallal et al. 2016; Ahmed et al. 2022). In crop irrigation, the usage of wastewater has a number of advantages as well because it supplies organic matter and nutrients to the soil (that are important for plants), besides reducing water pollution and saving nutrients and water (Murtaza et al. 2010; Jayasumana et al. 2015; Hussain et al. 2022a). But, using this water for crops is associated with some disadvantages, like contamination of ground water, building of harmful microorganisms, and accumulation of heavy metals (Akram et al. 2022a; Hussain et al. 2021a). Though, the level of metals is low in the water, their high concentration has been shown in plants and soil (Alghobar and Suresha 2017; Victor et al. 2016; Hussain et al. 2020a; Dantas et al. 2021; Naz et al. 2022; Udovicki et al. 2022).

That is why using municipal and industrial waste water for irrigation purposes may accumulate heavy metals in plants and soils (Abdullahi et al. 2013; Zahoor et al. 2019). As contamination of heavy metals is harmful to humans, wildlife, and agriculture, that is why, it is a serious environmental problem (Ahmed and Slima 2018;Hussain et al. 2020b; Hussain et al. 2021b; Akram et al. 2022b). Though heavy metals are vital micronutrients and work as cofactors with many metabolic enzymes, their concentrations beyond a certain level in the soil become toxic for majority of the plants (Mustapha and Adeboye 2014;Hussain et al. 2020c; Hussain et al. 2021c). Important contaminations found in tissues and surfaces of vegetables are heavy metals (Edirisinghe & Jinadasa 2019). When these unsafe concentrations are consumed for a prolonged period, they may disrupt many biochemical and biological processes in the human body (Singh et al. 2010; Hussain et al. 2022b). According to Ahmed and Slima (2018), from heavy metals, though some trace amounts are also required by the human body including copper, zinc, cobalt, iron, and others, yet their higher levels are dangerous for humans. Few heavy metals like cadmium, arsenic, mercury, and lead do not have established benefits for humans, even though, their accumulations may cause severe illness or premature deaths (Aweng et al.2011; Ng et al. 2016; Akram et al. 2018; Ali et al. 2019; Custodio et al. 2021; Din et al. 2022; Hussain et al. 2022c;Waleed et al. 2022).

Vegetables, particularly leafy vegetables, accumulate heavy metals in higher proportions when grown in water contaminated with heavy metals in comparison when grown with uncontained water because metals are absorbed through the leaves (Khan et al. 2008; Hussain and Karuppannan 2021; Majeed et al. 2021). The growth of leafy vegetables like spinach, cauliflower, and cabbage is very good in sewage water, while some other vegetables like radish have sensitivity towards sewage water (Kapourchal & Pazira 2009; Hussain et al. 2022d). When sewage water is used for irrigation, heavy metals get accumulated in the vegetables which may result in severe hazards to the health of humans and the animals (Avci 2013). This matter is of high concern in areas where untreated sewage water is used for growing vegetables for longer periods. Bioaccumulation of heavy metals, particularly in food items, is highly dangerous to human health (Islam et al. 2021). The entry route of these metals in the human body is primarily through ingestion and inhalation, where the former is the main source of exposing humans to these metals (Chauhan and Chauhan 2014; Liang et al. 2017; Woldetsadik et al. 2017; Ametepey et al. 2018; Hussain et al. 2022e.

Belonging to the family Malvaceae, Okra (*Abelmoschus esculentus* L.) is an annual vegetable crop. It is a very good source of dietary fiber, calcium, potassium, vitamin A, magnesium, vitamin C, protein, and carbohydrates. It also contains substances like glycans that lead to the viscosity of aqueous suspension (Falade and Omojola 2010), and it has gum-like consistency (Masood et al. 2022). Iodine is used for treating goiters, and glycans are its very good source. In the treatment of backache and leucorrhoea, the powder of the okra roote is used along with sugar. Okra is also helpful to both women and men for basseting their vigor and vitality (Sabagh et al. 2020; Mubeen et al. 2021). As regards its use as a vegetable, usually its tender pods are consumed (Nkansah et al. 2021). Okra pods may be used as snacks and sun-dried in the shape of slices for use during off-seasons. An anionic polysaccharide is taken from seedpods of *Hibiscus sculentus* of okra gum, is used as a flocculent for removing solid wastes from tannery effluents (Agarwal et al. 2003; Alghobar and Suresha 2017; Bukar & Onoja 2020; Tavelli et al. 2022).

Carcinogenic effects of heavy metals like Pb and Cd have been reported (Ahmed and Slima 2018). Thought some metal elements like zinc (Zn) and copper (Cu) are very important nutrients for human health yet their higher concentrations have been reported to be toxic. For example, the level of high-density lipoproteins and immune functions may be reduced by Zn (Harmanescu et al. 2011), while surplus Cu may result in liver damage and acute intestinal and stomach aches (Liu et al. 2005; Rahman et al. 2014).

The important objectives of this research were :

1. Quantification of heavy metals (for example Cu, Pb, Cd, and Zn) in wastewater used for irrigation, soils, and crops in Vehari district (Punjab, Pakistan).

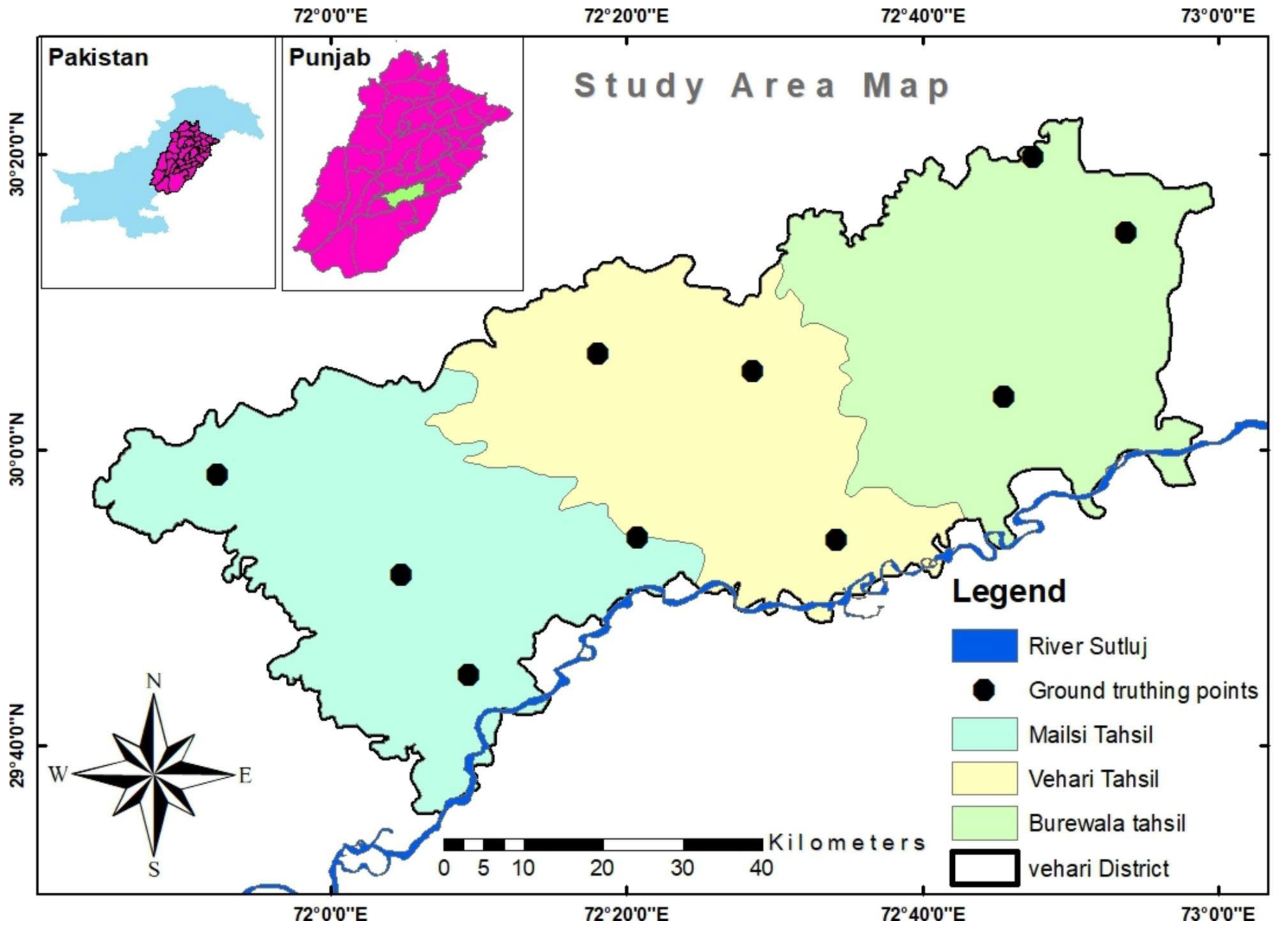

Fig. 1 Study area map

2. Estimate of risk to humans rustled by daily intake of these heavy metals in the shape of contaminated vegetables with the help of health risk index (HRI).

Materials and methods

Study area

Vehari District is situated at Latitude 30°01′59″N Longitude 72°20′59″E (Fig.1). This study was conducted in Vehari district of Punjab, Pakistan, during the spring season, of 2017. The area around this city, is ideal for agriculture being flat and alluvial plain are part of Indus basin. The study area has best cultured land which is appropriate for cotton, wheat, rice as well as many vegetables. Among commonly grown vegetables in this area are brinjal (*Solanum melongena*), radish (*Raphanus sativus*), and cauliflower (*Brassica oleracea var. acephala*), okra (*Abelmoschus esculentus*), tomato (*Lycopersicum esculentum*), carrots (*Daucus carota*) and cabbage (*Brassica oleracea var. capitata*). The purpose of growing these vegetables is for domestic use besides selling in the local market. Irrigation is through canals that supply water for agriculture purposes in the shape of a concerted network and are playing a vital role in increasing fertility of the soil in Vehari district. The climate of the Vehari is very hot in summer while mild in winter – an arid climate. However, extremely hot summers have been recorded in this city, with temperatures of about 54°C (129°F) in summer while as low as −1°C (30°F) in winter. Dust storms are very common, while average rainfall is about 127 millimeters (5 inches).

Water sampling and treatment

Samples of water were collected from canal, tube well, and three wastewater sites of Vehari. 30 number of water samples were collected in different location in Vehari district. Pre-washed with nitric acid, polyethylene bottles were used for storing samples of 1L and were stored at 4°C until analysis. Then standard methods were used for measuring the concentration of heavy metals, electrical conductivity (EC), and pH.

Soil sampling and characterization

Clean polyethylene bags were used for the collection of fifteen samples of soil to represent irrigation of soil from all the sampling plots for each plant. With the help of a soil auger, sub-samples of soil were taken at a depth of

Table 1 Heavy metals safe limits for vegetables

		Safe limits (ppm)	
Samples	**References**	Pb	Cd
Water (mg/L)	WHO/FAO(2008)	5.01	0.01
	USEPA, 2010, USEPA., 2010	0.015	0.005
Soil (mg/kg)	USEPA, 2010, USEPA., 2010	250–500 300	3.0–6.0 0.3-3.0
Plant (mg/kg)	USEPA, 2010,	5.0	0.2
	USEPA., 2010	-	-

0–20cm. Under ambient conditions, all the samples were air-dried in the laboratory and passed via a 2-mm mesh sieve. However, non-soil particles like rocks, organic debris, gravel, stones, and wooden pieces were removed. Organic carbon (OC), electrical conductivity (EC), pH, and organic matter were measured using standard methods of soil analysis.

Plant sampling and identification

The common vegetables grown in district Vehari are okra (*Abelmoscus esculentus* L.) and spinach (*Spinacia oleracea* L.). Details of safe limits of heavy metals for the vegetable have been given in Table1. With three replications, 0.500g of each plant was taken as a sample. All the vegetables were individually separated into leaves, routes, and seed sub-samples, and deionized water was used to wash them properly to remove all noticeable soil particles.

Heavy metals analysis

Digestion of vegetable and soil samples

Before grinding to powder, an oven was used to dry all the samples at 75°C for 72h. Aqua regia (nitric acid HNO_3 concerted with hydrochloric acid HCl) was used for digesting samples of 0.5g vegetable and soils overnight at room temperature. Then observing the standard method NF ISO 11,466, the mixture was boiled for two hours. Membrane (0.45μm) was used to filter digested samples after cooling; the filtrates were diluted to 50 ml with deionized water and stored at room temperature for further analysis.

Heavy metals determination

Accumulation of heavy metals in plants has severe human risk, as reported by many studies (Balkhair and Ashraf 2016). Various indices like daily intake of metals (DIM), transfer factor (TF), health quotient (HQ), and health risk index (HRI) have been used for determining health risks related with wastewater containing heavy metals (Gaetke and Chow 2003; Jaishankar et al. 2014). In the filtrate of samples, four heavy metals (Cd, Pb, Cr, and Zn) were determined with the help of atomic absorption spectrophotometer (AAS). For determining heavy metals, standard solutions were prepared by diluting a stock solution containing 100 ppm of single element AAS grade standard with distilled water. Standard solutions were used to construct calibration curves for heavy metals using AAS. Guidelines given by European Union Standard and USEPA and by WHO/FAO were used to establish maximum limits of heavy metals in water used for irrigation, soil, and vegetables.

Data analysis

Transfer factor (TF)

In plants, bioaccumulation of metals taken from the soils may be determined with the help of transfer factor (TF) (Alexander et al. 2006):

$$TF = \frac{Cplant}{Csoil} \tag{1}$$

.Where the concentration of heavy metals in soils and edible parts of vegetables is represented by Csoil and Cplant, respectively. Among types of metal under discussion, there may be considerable variation in transfer coefficient (Kachenko and Singh 2006).

Daily intake of heavy metals (DIM)

The amount of daily consumption and concentration of metals in the crops both determines DIM with the help of the following equation:

$$DIM = \frac{Cmetal \times Cfactor \times Dfoodinake}{BWaverage} \tag{2}$$

Where, Cfactor, Cmetal, Dfood intake, and BWaverage show concentrations of heavy metals in plants (mg/kg), the conversion factor.

For converting the weight of fresh green vegetables into dry weight, a conversion factor of 0.085 was used, as described by Rattan et al. (2005). In children and adults, the average daily intake of vegetables was taken as 0.232 and 0.345kg/person/day, respectively, whereas average child and adult body weights were taken as 32.7 and 55.9kg, respectively.

Health risk index (HRI)

The below-given formula was used for calculating the health risk index. Exposure of people to certain metals is said to be safe where the value of HRI is <1 (Cui et al. 2005).

$$HRI = \frac{DIM}{RFD} \tag{3}$$

.where

RFD=Oral Reference Dose.

Table 2 Average values of Physico-chemical parameters of various treatment water used for irrigation

Parameter	TW	CW	WW	W+T50%,50%	W+C50%, 50%
pH	7.61±0.04	7.33±0.02	6.91±0.2.64	7.01±0.2.83	7.11±2.0
EC (mS/cm)	0.22±0.1	0.25±0.1	0.90±0.3	0.59±0.2	0.63±0.2
HCO_3^{1} (mEq/L)	4.1±1.0	3.2±1.11	14.6±3.87	11.4±1.13	12.5±1.92
Cl^- (mEq/L)	4.1±0.13	3.2±1.8	8.7±1.1	6.2±2.15	6.0±1.17
Ca^{2+}+Mg^{2+} (mEq/L)	6.2±0.12	4.2±2.0	8.5±2.4	6.0±1.31	6.1±1.46
TSS (mg/l)	9.5±0.34	14.5±6.0	30.5±3.2	19.4±0.2.86	20.5±2.13

Table 3 Characteristics of soils collected from the study area

Parameter	TW	CW	WW	W+C50%,50%	W+T50%,50%
Before sowing					
pH	7.52±0.01	7.16±0.05	6.95±0.03	7.21±0.01	7.06±0.01
OM (%)	0.21±0.01	1.21±0.47	2.21±0.23	1.03±0.02	1.09±0.51
EC (S/m)	0.31±0.04	0.46±0.07	0.87±0.03	0.54±0.01	0.59±0.07
Okra (After Harvest)					
pH	7.78±0.02	7.31±0.7	7.19±0.04	7.42±0.02	7.22±0.03
OM (%)	0.61±0.08	1.87±0.35	3.08±0.81	2.9±0.04	2.06±0.21
EC (S/m)	0.27±0.07	0.41±0.06	1.8±0.08	0.97±0.11	0.66±0.013
Spinach (After Harvest)					
pH	7.81±0.02	7.4±0.8	7.2±0.05	7.58±0.02	7.4±0.02
OM (%)	0.73±0.6	1.91±0.34	3.2±0.91	2.7±0.03	2.08±0.05
EC (S/m)	0.29±0.8	0.37±0.06	2.24±0.09	1.07±0.04	0.99±0.08

Statistical analysis

SPSS software was used for statistical data analysis. Mean, and standard deviation have been used for measuring numerical variables. One-way ANOVA test has been applied to evaluate the significance of the variance between heavy metals concentrations in vegetables grownup in the irrigation region.

Results and discussion

Physico-chemical parameters of various treatment

The quality of waste, tube-well, and canal waters was measured for irrigation regarding their pH, EC, and chlorides. The data associated with the quality of all treatment waters is presented in Table2. Results revealed that the pH of tube well water was 7.61. Whereas, the pH value of wastewater was 6.91 and pH values for other treatments were: canal (7.33) 50% waste and 50% tube well water (7.01) and 50% waste 50% canal water (6.5) 6.74. Tube well water samples had the lowest EC (1.0 mS/cm), while the highest EC (3.1 mS/cm) was noted in the wastewater. A comparison of wastewater and tube well water ECs showed that the former had 53 times higher EC than the latter.

The chief water quality guideline measured by EC for crops production is salinity hazard (Ahmed and Slima 2018). These values have been noted within permissible limits. Total suspended solids (TSS) were higher in wastewater samples compared to tube well water (9.55mg/l). The maximum value of TSS was observed in wastewater (30. 5mg/l). Bicarbonate concentration was maximum in wastewater (14.6) and minimum in control water (4.1). The maximum concentration is 8.5 (mEq/L) in wastewater, and control has the lowest concentration of 6.2 mEq/L. A similar trend is observed in Bharose et al. (2013). Chloride (Cl^-) content in the irrigation water determines its suitability. The mean chloride content in tube well water during this study was 4.1±1.3 mEq/L (Table2). But in wastewater little variation was observed at 8.7±1.1 mEq/L. The results obtained from this study are corroborated by the finding of Bharose et al. (2013).

Soil characteristics of various treatment

In Table3, the data related to soil pH is given. The soil samples had an average pH value of 7.81. This level of pH is a vital influencing factor for bioavailability as well as transportation of heavy metals in soil. The increased pH of soil decreases the mobility of heavy metals in the soil owing to precipitation of carbonates, hydroxides, or by forming insoluble organic complexes (Nihorimbere et al. 2011). In changing the pH of the soil, irrigation through wastewater plays an important role (Sessitsch et al. 2013). The same studies also held that bacterial population related to growth of plants in metalliferous soil is also affected by pH and potentially by the accumulation of heavy metals. These bacteria are known as plant growth-promoting rhizobacteria (PGPR).

We found mean soil sample electrical conductivity of 2.21S/m; thus, according to El Fadeli et al. (2014), it may be said as moderately saline. It has been proved that the solubility of heavy metals increases with increased

Table 4 Absorption of heavy metals (mg/kg) in water samples used for irrigation purposes, collected from various peri-urban agricultural systems of Vehari district, Pakistan

Treatments	Lead Pb	Cadmium Cd	Chromium Cr
Main			
Wastewater	0.38 ± 0.1	0.054 ± 0.007	0.07 ± 0.009
Canal Water	0.063 ± 0.004	0.022 ± 0.003	0.04 ± 0.004
Tube well Water	0.053 ± 0.009	0.01 ± 0.003	0.03 ± 0.004
Sub			
W50%+C50%	0.67 ± 0.007	0.04 ± 0.004	0.06 ± 0.03
W50%+T50%	0.063 ± 0.008	0.03 ± 0.002	0.08 ± 0.004

conductivity, thereby resulting in the huge availability of metals to plants from soil.

We noticed organic carbon was 4.87% on average. OC contents were increased in the soil by use of wastewater as a source of irrigation, which resulted in increased organic complexing molecules of low molecular weight (LMW) that carry micronutrients (inclusive of heavy metals) besides increasing uptake of heavy metals (Ram et al. 2006). The studied soil had 8.40% mean concentrations of organic matter. In wheat plants, Rupa et al. (2003) reported elevated levels of organic matter for increasing heavy metals uptake. The mean concentrations in the studied samplewere Cd, Pb, Zn, and Cu at 11.22mg/kg, 57.36mg/kg, 112.71mg/kg, and 17.70mg/kg, respectively. The soil had the highest concentration of Zn, and next to it were Pb and Cu, while Cd had the lowest concentration. Our findings regarding the concentration of Pb were less compared with the findings of Singh et al. (2009) in the Marrakech region, but concentrations of Cu and Zn were noted within the respective permissible limits given by WHO/FAO (2017).

Concentration of heavy metals in various treatments of water

The data to various concentrations of heavy metals in water used as different treatments are presented in Table4. The concentration of Pb in wastewater was a extreme i.e. 0.38mg/kg. Pb concentrations were 0.053mg/kg, 0.063mg/kg, 0.067mg/kg and 0.062mg/kg respectively for control, canal, 50% waste 50% tube well and 50% waste 50% canal water. The concentration of Cr in wastewater was 0.07. Cr concentrations in other treatments water were 0.03, 0.04, 0.08 and 0.06mg/kg respectively for control, canal, 50% waste 50% tube well and 50% waste 50% canal water. The concentrations of Cd in wastewater were negligible at 0.054mg/kg. Cd concentrations in other treatment water were 0.022mg/kg, 0.01mg/kg, 0.04mg/kg, and 0.03mg/kg, respectively for the canal, tube well wastewater 50%, canal water 50%, wastewater 50%, and canal water 50%.

Table 5 Concentration of heavy metals in irrigation site

	Soil Depth (cm)		
Heavy Metals	**ST.Dev**	**Cadmium Cd**	**Chromium Cr**
Lead	0.91 ± 0.04	0.12 ± 0.02	0.48 ± 0.04
Cadmium	0.07 ± 0.004	0.07 ± 0.03	0.25 ± 0.06
Chromium	0.05 ± 0.008	0.06 ± 0.03	0.07 ± 0.05

Heavy metals in wastewater are the common limiting factors for its agronomic use, which are introduced due to the mixing of untreated industrial wastes into the municipal sewer or natural stream. Lead (Pb) content in the wastewater used in agricultural fields is more than two-fold the content found in groundwater used in irrigation. The mean Pb content was higher in the irrigated wastewater (0.38±0.1mg/kg) than in tube well water (0.053±0.009mg/kg). Higher lead content in the wastewater as compared with tube well water has been corroborated with the finding (Singh et al. 2010).

Chromium (Cr) content in the irrigated wastewater was much higher than the groundwater used in irrigation. The Cr content in the wastewater is more than ten and twenty times higher than the prescribed standard and Awashthi, 2000. But in tube well water, the value of Cr is within the safe limit as mentioned in earlier standards.

Concentration of heavy metals in soil before sowing and after harvesting

Various studies have reported higher levels of heavy metals particularly Pb, in the soil watered with untreated industrial effluent or sewage water. Contamination of soil by irrigation through sewage water was also described by Khan et al.(2020). The use of sewage water by the farmers for irrigation and nutritional purposes were reported in Faisalabad, Pakistan. Higher concentrations of Fe, Cd, and Ni were reported in soils irrigated with industrial effluents and sewage waters.

Heavy metals were detected in a wide range in samples collected from vegetable cultivation sites. The mean concentration was maximum for Pb, and then by Ni and Cd (Table4). The heavy metal concentration varied between irrigation sites. Pb concentration in soils was 0.05mg/kg, 0.07mg/kg, 0.06mg/kg and 0.07mg/kg respectively for canal, wastewater, 50% waste 50% tube well and 50% waste 50% canal. The mean information presented that the experiential value of Pb in all soil sites was higher than the permissible limit set by WHO. The Pb enrichment in the wastewater irrigated soils as related to tube well water irrigated soils is corroborated with the findings of (Naaz and Pandey 2010).

In irrigation water, level of Mn varied from 0.85 to 0.96. With the exception of Cd, concentrations of Cu, Ni, Co, Mn, and Pb in soil were initiate within their Maximum Residual Limit (MRL) as given by FAO (Table5). Cadmium had a mean concentration in the range of 1.57 to

Table 6 Concentration of heavy metals (**mg/kg**) in the edible portion of Spinach and okra samples collected from peri-urban agricultural system

Treatments	Vegetables	Lead Pb	Cadmium Cd	Chromium Cr
Main				
Wastewater	Spinach	1.84 ± 0.85	0.76 ± 0.89	0.23 ± 0.01
Canal Water	Spinach	1.23 ± 0.06	0.23 ± 0.15	0.16 ± 0.015
Tube well Water	Spinach	1.18 ± 0.02	0.19 ± 0.02	0.13 ± 0.15
Sub				
50% wastewater + 50% Canal water	Spinach	1.34 ± 0.055	0.45 ± 0.066	0.19 ± 0.016
50% wastewater + 50% Tube well water	Spinach	1.30 ± 0.020	0.42 ± 0.30	0.17 ± 0.015
Main				
Wastewater	Okra	1.08 ± 0.06	0.48 ± 0.27	0.55 ± 0.03
Canal Water	Okra	0.79 ± 0.009	0.30 ± 0.04	0.37 ± 0.02
Tube well Water	Okra	0.35 ± 0.008	0.28 ± 0.05	0.32 ± 0.026
Sub				
50% wastewater + 50% Canal water	Okra	0.68 ± 0.07	0.39 ± 0.02	0.40 ± 0.01
50% wastewater + 50% Tube well water	Okra	0.77 ± 0.06	0.37 ± 0.02	0.39 ± 0.02

2.48mg/kg. Cadmium had the highest concentration at Kot Abdul Fateh followed by Hamroot and then in Mozu Alamgir at 2.48mg/kg, 1.84mg/kg, and 1.57mg/kg, respectively. Irrigation with sewage water is the cause of this increased concentration of Cd besides the presence of various heavy metals in abundance. Irrigation of soil with untreated industrial and sewage water is a very common practice, especially when it is free of cost, and that is why farmers do not consider the health consequences of using this water.

Level of heavy metals in spinach vegetable

The information on heavy metals concentration in various vegetables are given in Table6. On a dry weight basis, the concentration of Pb in spinach was 1.84, 1.23, 1.18, 1.34, and 1.30mg/kg under waste, canal, and tube well water, 50% waste 50% canal and 50% waste 50% tube well water irrigated plots respectively. The Cd concentration in spinach was 0.76, 0.23, 0.19, 0.45, and 0.42mg/kg under the waste, canal, tube well, 50% waste 50% canal, and 50% waste 50% tubewell water watered plots, respectively in study area. The concentration of Cr in spinach were 0.23, 0.16, 0.13, 0.19, and 0.17mg/kg under waste, canal, wastewater, 50% waste 50% canal, and 50% waste 50% tube well-irrigated plots, respectively.

The values of Cd and Cr were noticed within prescribed limits as given by WHO. But, in all the samples except canal and control water, Pb had the highest concentration. Various studies have reported the use of contaminated water for irrigation purposes in vegetables in Pakistan. Higher concentrations of heavy metals were reported in soils and vegetables irrigated with sewage and tube well-watered samples, and the results are closer to the findings of our study (Kumar et al. 2018). The same outcomes were described by Gupta et al. (2010) in samples of coriander, turnip, potato, tomato, squash, and turnip. Likewise were Bukar et al. (2020) findings in leaves and fruits of spinach and okra when irrigated with sewage water in the Attock area. Samples of pulses (cuntin, colver, coriander, mung bean, black pepper, spices clover, caramay, and black been) and vegetables from markets of Rawalpindi/Islamabad and had similar findings as given by Balkhair and Ashraf (2016).

Compared with the prescribed standards, it is found that Pb and Cd content in spinach have crossed the safe limit of WHO/FAO, 2008 and EU standard, 2002 (Table6). Statistically, heavy metals content in spinach in both control and treated plots shows significant ($p < 0.0001$) variation. Wastewater-induced heavy metals enrichment in spinach was studied by Singh et al. 2010.

The results of the heavy metals concentration in okra vegetables cultivated with wastewater in the experimental plot are shown in Table7. The concentration of Pb in spinach were 1.08mg/kg, 0.79mg/kg, 0.35mg/kg, 0.68mg/kg, and 0.77mg/kg under the waste, canal, tube well, 50% waste 50% canal, and 50% waste 50% tube well water irrigated plots respectively. The concentration of Cd in spinach were 0.48mg/kg, 0.30mg/kg, 0.28mg/kg, 0.39mg/kg, and 0.37mg/kg under the waste, canal, tube well, 50% waste 50% canal, and 50% waste 50% tube well water irrigated plots, respectively. The concentration of Cr in spinach were 0.55mg/kg, 0.37mg/kg, 0.32mg/kg, 0.40mg/kg, and 0.39mg/kg under waste, canal, tube well water, 50% waste 50% canal, and 50% waste 50% tube well water irrigated plots, respectively.

All the mean values of heavy metals Pb and Cd values of both plots have crossed the prescribed safe value of WHO/FAO, 2008 (Table6). Heavy metals content in treated ladies finger shows significant level ($p < 0.0001$) accumulation except Cr ($p = 0.001$). Wastewater irrigation helps to enrich ladies' fingers with heavy metals, supported by Singh et al. 2009.

Table 7 Daily intake of metals (mg) in children and adults

Elements (Heavy Metals)	Children		Adults	
	Spinach	Okra	Spinach	Okra
Lead (Pb)	0.05600	0.05600	0.043	0.047
Cadmium (Cd)	0.00083	0.00100	0.00062	0.0012
Chromium (Cr)	0.00068	0.00080	0.00057	0.00067

Health risk

In vegetables and soils, heavy metals have been the focus of many studies in Pakistan, but quite less has been focused on reading health risks associated with these pollutants. Humans are greatly exposed to heavy metals by transfer factor (TF). Assessment of this TF factor is highly important for evaluating the health risk index related to waste-water irrigated soils (Cui et al. 2005). Calculation of this transfer factor of heavy metals from soil to vegetables has been given in Table7. For Cd, Pb, Zn, and CU, this mean TF was in the range of 0 to 0.744, 0.319 to 0.922, and 0.277 to 0.801, 0.134 to 0.905, respectively. Between these four metals, no significant difference was reported in these results.

The above differences may be associated with metal-binding capacity with the roots, the interaction between physicochemical limits, availability of metals, and species of plants grown in that particular soil. Many factors establish accumulations and absorptions of heavy metals that include organic matter, availability of nutrients, pH, moisture, and temperature, while increased uptake of Pb, Cr, Zn, and Cu in the wheat plant was associated with the presence of organic matter (Rupa et al. 2003; El fadeli et al. 2014). Estimating exposure level though quantification of exposure of routes with the pollutant is essential for evaluating the HRI of the heavy metals. Among different pathways of exposure of humans to the pollutants, the food chain keeps key value. In this area, HRI and DIM, both in children and adults, were calculated (Table7).

The daily contact of humans to pollutants or toxins that has no significant hazard to them during their life span is called the oral reference dose (RFD). The RFD value for toxic metals like CD, PB, Zn and Cu is 1.00E03 mg, 4.00E03 mg, 3.00E01 mg and 4.00E02 mg respectively. Huang et al. (2008) reported concerns for potential health risks when HRI exceeds 1.0. Except for Cu and Zc, we found HRI>1, meaning thereby is a potential risk to human health by eating such food crops. By consuming vegetables where waste water was used for irrigation, El fadeli et al. (2014) reported a similar health risk in Saudi Arabia. Moreover, side effects were reported more in children in comparison with adults. The food chain has been proved to be a vital pathway for transferring toxic metals from the environment to humans (Balkhair and Ashraf 2016).

The present research is highly significant regarding health perspectives as it aimed at estimating contamination levels and human exposure by consuming food crops grown with heavy metals.

Conclusion

The use of wastewaters for irrigation of agricultural land continuously for many years has increased the level of heavy metals in soils and resultantly in the plants. It is revealed by the results that the soils irrigated with wastewater were enriched moderately with Cd, Pb, and Cr within the permissible limit given by WHO. That is why long long-term dietary intake of vegetables grown in waste waters results in the accumulation of heavy metals in the human body and leaves its detrimental impact after many years of exposure. The potential risk of higher concentrations of Pb and Cd has been indicated by HRI>1 as a potential risk for human health because they consume plants and vegetables grown in soils that have been continuously irrigated with wastewater. In these regions, it is necessary to devise effective measures for curing contamination of toxic metals.

Authors' contributions

MAK, MI and MTJ proposed the main concept and highly involved in write-up. SH, MM, H.G.A, A.A.A, H.A.M, M. A, W.A.T, M.A.R and SF assisted in data analysis and preparation spatial map. SF, A.A.A, H.G.A,H.A.M, W.A.T and WN are involved to write-up and review. MAK, SK, SH, A.A.A, H.A.M, H.G.A, M. A, W.A.T, M.A.R and MM involved to review, editing, review and English grammar correction. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available in the article/ from the corresponding author on request.

Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

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