

Heavy Metals Toxicity and Associated Health Risks in Vegetables Grown under Soil Irrigated with Sewage Water

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Abstract Use of waste water for vegetables irrigation has become a frequent practice approximately in all the big cities. Waste water enrich with inorganic and organic material that are crucial for the growth of plant but also comprises heavy metals which might be detrimental for animals and humans if their concentration increases than tolerable limit. To monitor this state, a study was planned to determine the accumulation of heavy metals through the irrigation of waste water into the agricultural fields and their translocation into the vegetables. The analysis showed that most of the water and soil samples contained the heavy metals concentration was above the safe limit set by WHO and FAO. Although the samples having heavy metals within the safe limit were also couldn't be as safer when regularly used. They may cause on the other hand, in vegetable, almost all the edible part and leaf of vegetables sample were contaminated and heavy metals accumulation was higher than the recommended limits of WHO/FAO. Such a high concentration when consumed by human beings may cause loss of arthritis, headache, appetite, hypertension, exhaustion, renal dysfunction, intestinal pain, hallucinations, restlessness and vertigo. Noticeable ingestion of heavy metals by children and adults were also found in the results through the consumption of vegetables irrigated with sewage water of the area.

Keywords Heavy Metals, Lead (Pb), Copper (Cu), Iron (Fe), Wastewater, Health Risks

1. Introduction

Heavy metals are considered as one among the most important environmental concerns because of their toxicity and accumulation in body (5, 12, 23, 27, 51, and 53). Heavy metals are those metals which have a specific density of more than 5 g/cm³ and harmfully affect the living organisms as well as environment and living

organisms (25). These metals are essential to maintain the normal body physiology and functioning when present in very low concentrations. However, they become lethal when certain threshold levels exceed. Many studies have been conducted throughout the world in relation to plants and soil pollution with heavy metals through irrigation by urban and industrial effluents (27, 32). Vegetables are important components of a healthy and perfect diet of human beings (8, 49). Evidence from various studies in recent years have indicated that the consumption of various types of vegetables can significantly prevent chronic heart diseases and some types of cancers, especially cancers of the gastrointestinal tract such as colon cancer (13, 27, 32, 43). Vegetables were contaminated with heavy metal due to the sewage water irrigation, addition of metal-based pesticides, fertilizers, transportation, the harvesting process, industrial emissions, and storage or at the point of sale (43). Water unavailability in arid and semi-arid regions promotes the reuse of water resources, and the use of sewage for agricultural irrigation is the primary form of wastewater reuse (43). According to the Environmental Protection Agency (EPA), lead is considered as a very harmful pollutant. Distribution of lead in the body depends on the blood supply to different tissues. 95% of lead is deposited in bones in the form of insoluble phosphate (25, 45). Increased concentration of lead in blood significantly affects a person's IQ (13, 43, 46, 53, 55, 60). Other metals, such as copper (Cu) and Iron (Fe) are very important for maintaining physiological and biochemical functions in the body (43). Copper (Cu) naturally presented in vegetables can be contaminated after contact with and therefore, it accumulates in the tissues of plants. The most important sources of Cu for contamination of the vegetables are mining activities, agriculture, waste and sludge from wastewater treatment. The small amount of Cu is essential for humans, but if its value increases, it is dangerous to human health (13). Iron (Fe) is the other metal that is widely distributed in the environment so that it is available in most foods, water, and air. Due to its effect on the

activity of enzymes and protein production, Fe is an important element for human life (44). However, iron toxicity causes free radicals generation (25). Although iron is essential for body growth but when the concentration of iron exceeds from permissible limits, it may cause DNA strand breaks, sometimes results in cell death as well as injures the gastrointestinal tract in the body (25). The present study has been designed to compare metal contents (Cu, Fe, Pb) of waste irrigation water, soils and vegetables in Faisalabad around different sites of Paharang Drain, with the maximum acceptable levels set by World Health Organization (WHO) and its impact on human health.

2. Material and Methods

2.1. Vegetable Sample Collection, Preparation and Analysis

The samples were collected around the vicinity of Paharang Drain in Faisalabad, where waste water (sewage & industrial) was used by farmers for raising of vegetables for many years. In the field, Vegetables (spinach, *Amaranthus caudatus*; Coriander, *Coriandrum sativum*; cauliflower, *Brassica oleracea*; and tomato, *Lycopersicon sculentum*) sample were collected randomly in triplicate from the same field. In laboratory, vegetable samples were washed through distilled water to get rid of soil particles then sun dried, crushed and ground for heavy metals determination (1, 11, 16, 30, 40, 42). 0.5 gram of each of the fine powdered samples was weighed into test tubes and tri-acid mixture ($\text{HNO}_3 + \text{HClO}_4 + \text{H}_2\text{SO}_4$; 5: 2: 1) was used to digest the samples (30) at 100°C on the hot plate for two hours in a fume cupboard (11). The digested samples were cooled and filtered through the use of Whatman No.42 filter paper and volume was made up to 100ml through distilled water (3) for heavy metal analysis (Pb, Fe, Cu) through AAS (Atomic Absorption Spectrophotometer).

2.2. Soil Sample Collection, Preparation and Analysis

Soil samples were collected into polyethylene bags from two different depths (0-15 and 15-30 cm) (11, 22) by the use of spiral auger of 2.5 cm diameter from the same field (14, 30), labeled, properly tied and transported to the laboratory (42). Soil samples were spread on glass plates and then air dried followed by oven drying at 105°C for six hours (11).

Samples after air-drying were crushed and passed through a mesh sieve of 2 mm and stored in plastic jars (8, 11, 16, 19, 30, 52). To estimate heavy metals concentration, 25 g from each of the ground soil samples was weighed into a 125 ml beaker and mixed with 50 mL of DTPA (diethylene triamin penta acetic acid) extracting solution having pH 7.3 and set aside on a reciprocal shaker at 120 rpm for 2 hours (19, 22). The aliquot was centrifuged for 5 minutes at 5000 rpm (27) then the supernatants were collected for heavy metal (Pb, Fe and

Cu) determination (33) using atomic absorption spectrophotometer (11).

2.3. Water Sample Collection, Preparation and Analysis

The sampling of waste water was done from water courses of same field from the same sampling site as selected for soil and vegetable samples were collected so that the value of irrigation water may be monitored which was being applied to the field rather than at drains/outlets of pumping stations. Water samples were preserved in plastic bottles containing 2 mL concentrated nitric acid to avoid from any of the microbial activity during storage (8, 30). Water samples were carefully filtered through filter paper (Whatman # 42) and stored at 4°C until analysis (Ehsan et al., 2013; Al-Jabbobi et al., 2014).

Heavy metal (Pb, Fe, Cu) concentrations in waste water was calculated on Atomic Absorption Spectrophotometer (11, 16) using respective hollow cathode lamp (4).

2.4. Calculations

2.4.1. Daily Intake of Metals

Following equation is used to calculate the daily intake of metals (DIM):

$$\text{DIM} = \text{M (metal concentration)} \times \text{K (factor)} \times \text{I (food intake)} / \text{W (average weight)}$$

Where,

M = Concentration of heavy metal in plants (mg/kg)

K = Conversion factor

I = Daily vegetables intake

W = Average body weight

The conversion factor is 0.085 which is used to convert the weight of fresh green vegetable to dry weight (47). The standard child and adult body weights were considered to be 32.7 and 55.9 kg, respectively, whereas average daily intakes of vegetable for children and adults were considered to be 0.232 and 0.345 kg/ person/day, respectively (21, 39, 58).

2.4.2. Health risk Index (HRI)

The estimated exposure of test crops and oral reference dose ratio of each metal was calculated to determine the health risk index (12, 27, 53). Oral reference doses were 0.04 for Cu and 0.004 mg/kg/day for Pb (57). Projected exposure is obtained by dividing daily intake of heavy metals by their safe limits. The $\text{HRI} < 1$ means the exposed population is assumed to be secure while an index more than 1 is considered as not safe for human health (56).

Following equation is used to determine health risk index:

$$\text{HRI} = \text{DIM} / \text{RfD} \quad (56)$$

3. Results and Discussion

The maximum permissible limits of heavy metals in

vegetables and soils have been established by standard regulatory bodies for instance Food and Agricultural Organization (FAO), World Health Organization (WHO) and Ewers U, Standard Guidelines in Europe as shown in Table 1 below:

Table 1. Maximum Allowable Limits of Heavy Metal in Irrigation Water, Soils and Vegetables ($\mu\text{g/g}$)

Chemical Element	Maximum permissible level in irrigation water		Maximum permissible level in soils		Maximum permissible level in vegetables	
	($\mu\text{g/ml}$)	(mg/L)	($\mu\text{g/g}$)	(mg/kg)	($\mu\text{g/g}$)	(mg/kg)
Cu	0.017	0.2	100	140	73.00	30
Fe	0.500	-	50000	-	425.00	-
Pb	0.065	5.0	100	300	0.30	2.5

3.1. Waste Water Analysis

The average concentrations of Fe, Cu and Pb in the irrigation water used for Spinach, Coriander, Cauliflower and Tomato fields in the vicinity of Paharang drain, Faisalabad have been illustrated in Figs. 1 to 3.

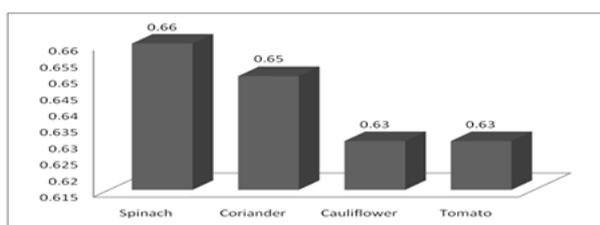


Figure 1. Mean concentration (mg/L) of Fe in urban drainage used for irrigating vegetables

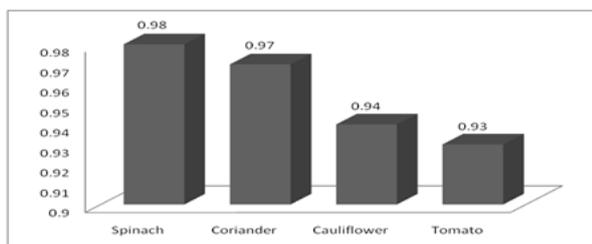


Figure 2. Mean concentration (mg/kg) of Cu in urban drainage used for irrigating vegetables

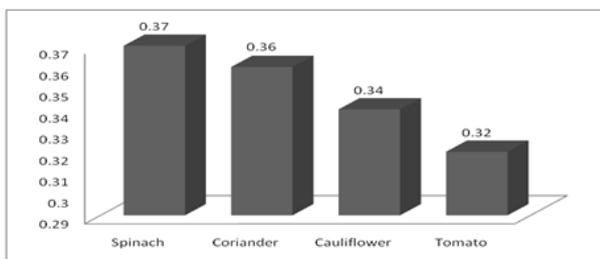


Figure 3. Mean concentration (mg/kg) of Pb in urban drainage used for irrigating vegetables

The heavy metals concentrations in all the irrigation waters analyzed during the experiment are higher than the Recommended Permissible Levels. The results regarding heavy metal content in sewage/industrial water are presented in fig 1 to fig 3. The obtained results showed that in sewage/industrial water samples, the concentration of Cu, Fe and Pb is illustrated as 0.96, 0.64 and 0.35 mg/L, respectively. These results clearly exhibited that waste water samples had concentration of Cu above the safe limits and pollution level in the water that was being used for irrigation ranged between very severely to excessive polluted (11) whereas Fe and Pb contents were below the safe limits.

3.2. Soil Analysis

The average heavy metals concentrations in surface soils that were irrigated with sewage water have been illustrated in Figs 4 to 6.

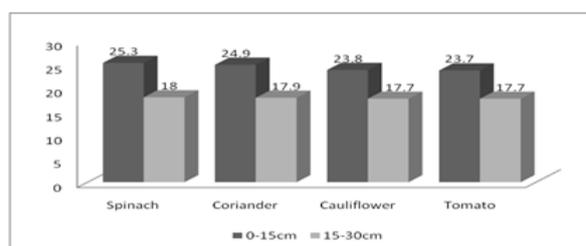


Figure 4. Mean Concentrations (mg/kg) of Fe in soils of different vegetables farms

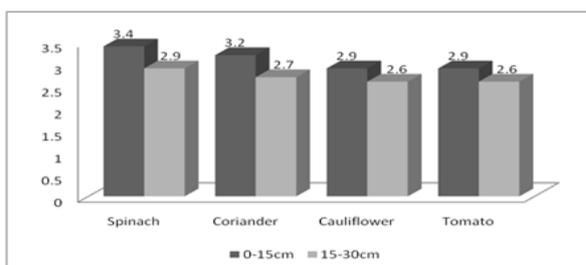


Figure 5. Mean Concentrations (mg/kg) of Cu in soils of different vegetables farms

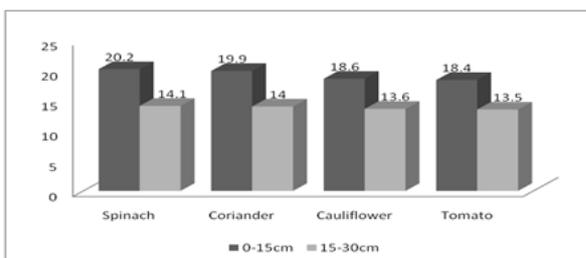


Figure 6. Mean Concentrations (mg/kg) of Pb in soils of different vegetables farms

DTPA extractable heavy metals concentration of soil samples, collected from the same point where the water samples were collected is presented in fig 4 to 6. The

results showed that Fe, Cu and Pb content on average in upper and lower layer of soil is observed as 24.4 & 17.8mg, 3.1 & 2.7 mg and 19.3 & 13.8 mg, respectively. The concentrations of Fe, Cu and Pb in all soil samples were below their respective maximum acceptable limit set by World Health Organization (11, 30)).

3.3. Plant Analysis

Average heavy metals concentrations in farm produce irrigated with sewage water is given below

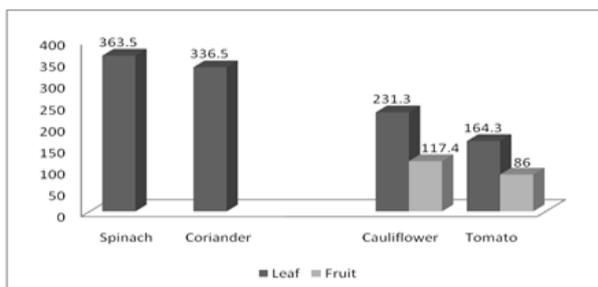


Figure 7. Mean Concentrations (mg/kg) of Fe in vegetables irrigated with waste water

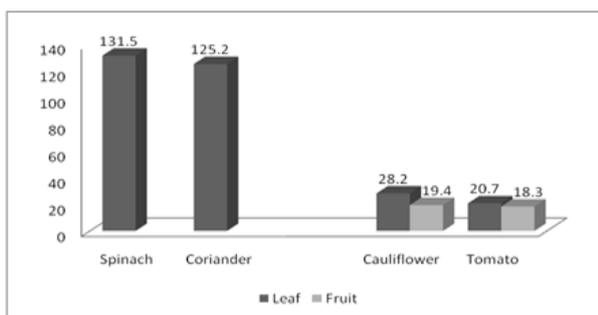


Figure 8. Mean Concentrations (mg/kg) of Cu in vegetables irrigated with waste water

Daily Intake of Metal and Health Risk Index

Table 2. Daily intake of metal (DIM) for children and adults (mg/kg/person/day)

Metal	Spinach		Coriander		Cauliflower		Tomato	
	Child	Adult	Child	Adult	Child	Adult	Child	Adult
Cu	0.079	0.068	0.075	0.065	0.017	0.015	0.012	0.011
Fe	0.219	0.190	0.202	0.176	0.139	0.121	0.099	0.086
Pb	0.037	0.032	0.033	0.029	0.029	0.025	0.025	0.022

Table 3. Health risk index for heavy metals in vegetables grown with sewage water

Metal	Spinach		Coriander		Cauliflower		Tomato	
	Child	Adult	Child	Adult	Child	Adult	Child	Adult
Cu	1.98	1.70	1.88	1.63	0.43	0.38	0.30	0.28
Pb	9.25	8.0	8.25	7.25	7.25	6.25	6.25	5.5

It is very essential to estimate the exposure level to observe the health risk of any pollutant. Food chain is the most important pathway among several possible pathways of exposure to humans. The highest intakes (Table 2) of Cu, Fe and Pb were from the consumption of Spinach while lowest intake of these metals was from the consumption of Tomato for both children and adults. It was indicated in the table 3 that leafy vegetables were more susceptible to heavy metals and posing more health risk than other vegetables.

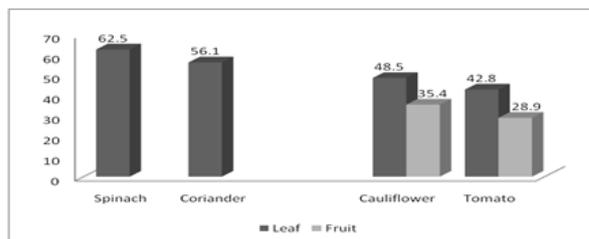


Figure 9. Mean Concentrations (mg/kg) of Pb in vegetables irrigated with waste water

The results presented in Fig 7 to 9 showed that Fe, Cu and Pb contents of vegetable leaves ranged on the average 164.3-363.5 mg/kg, 20.7-131.5 mg/kg and 42.8-62.5 mg/kg respectively. It was revealed from the results that all vegetable leaf samples have heavy metal concentration higher than the permissible limits set by the WHO, 1996 (11, 16, 30). It was studied that a plant with elevated concentration of lead fastens the ROS (reactive oxygen species) production, causing damage in lipid membrane that eventually leads to the chlorophyll and photosynthetic processes damage and suppresses the overall plant growth (25, 41). A huge instability ion uptake by plants was experienced by Pb treatment even at low concentrations which ultimately leads to considerable metabolic changes in photosynthetic capacity and eventually in a strong plant growth inhibition (25). Calcium can be substituted by lead even in picomolar concentration affecting protein kinase that regulates neural excitation and memory storage (20, 25).

Vegetable fruit samples were also analyzed to assess their heavy metal concentration and results are given in the fig 7-9. The heavy metal contents in vegetable fruit samples ranged were Cu, 18.3-19.4; Fe, 86- 117.4, Pb, 28.9-35.4 mg/kg. The content of heavy metals in all vegetable fruit samples was above the critical limits set by WHO (6, 60).

4. Discussion

In Pakistan, the farmers blindly use untreated sewage/industrial water for vegetable production especially in peri-urban areas. In present survey sewage/industrial waste water samples from ten different localities around paharnng drain of Faisalabad district were analyzed for heavy metal concentration. The results clearly exhibited that waste water samples had concentration of Cu above the safe limits and pollution level in the irrigation waters ranged between severely to excessive polluted (11) whereas Fe and Pb contents were below the safe limits. Constant use of such sewage/industrial water for irrigation over longer period may cause the heavy metals accumulation up to toxic levels for animal and plant health (29, 30). This implies that the sewage water is not safe to use for the irrigation of vegetables. Elevated heavy metals level in sewage water was also investigated by other scientists in Pakistan and they found excessive concentration of Cu, Cd, Mn and Pb in sewage water samples from Peshawar (15). Jagtap et al., (2010) reported higher contents of Zn, Cu, Pb, Ni, Cd and Cr in waste water samples from Rawalpindi Area. (16, 17, 52) found same results in industrial water of Faisalabad and (35) in sewage water of Attok area. Parallel studies were conducted in Vinayakiya Nallah region of Jodhpur district in India according to which it was concluded that metals in irrigation water have a harsh impact on vegetation and such vegetables should not be consumed.(11). There was a surprising trend observed regarding the heavy metal concentration in soil samples which were collected from the same point where the water samples were collected. The concentrations of Fe, Cu and Pb in all farm soils in the sampling are beneath their respective acceptable levels in soil set by World Health Organization (11, 30).

It is interesting that heavy metal content of wastewater were high whereas their concentration in soil was low. This might be due to the insolubility of metals because of high soil pH. Factors like soil pH, quantity of organic matter, redox potential of soil and rate of addition of metals mainly affect their adsorption and retention in soil. Related results were documented (29) in Faisalabad city then in Sheikhpura (26) and Muridke area while Mian and Ahmad (1997) reported the same trend in Rawalpindi area. Butt et al., (2005) investigated more or less similar results in Faisalabad city. Hamid et al., 2016 reported more and less similar results in the district of Lahore.

The main concern of the study was to evaluate the commonly consumed vegetables for the heavy metal concentration due to the irrigation of waste water as vegetables are used for common human nutrition and the edible portions of different vegetables varied as some vegetables are consumed as leaf like spinach, coriander and the others are used as fruit or root like tomato and cauliflower. Therefore the concentration of heavy metals was investigated in both leaf and fruit of vegetables. Leafy

vegetables like spinach and coriander and leaf portion of other two vegetables (cauliflower and tomato) showed higher concentration than the edible portion of the vegetables. It was revealed from the results that all vegetable leaf and edible portion samples have heavy metal concentration higher than the permissible limits set by the WHO (16, 30, 60). It was also experienced that the uptake of heavy metal by vegetables is not only affected by plant species and physicochemical characteristics of soil but temperature and rain fall also exert substantial effect. Farid et al., (2003) collected spinach, bitter gourd, okra, pumpkin and eggplant samples and observed that they were contaminated with heavy metals. Similarly Ronaq et al., (2005) collected spinach and turnip samples from market and found that these vegetables were unsafe for eating due to higher heavy metal concentration.

Similar results were reported by many scientists. Barman et al., (2000) and Liu et al., (2006) reported excessive heavy metals concentration in edible portion of vegetables. Arora et al., (2008) and Maleki and Zarasvand, 2008 have reported the buildup of heavy metals in vegetables. Ahsan et al., (2011) investigated heavy metal contents in edible portion of vegetables and found that Zn, Cu, Pb, Ni, Cd and Cr contents were higher than the safe limits. DIM for adults and children through consumption of contaminated vegetables may cause harsh health risks by heavy metals ingestion grown with waste water. Human risk assessment quantification from wastewater irrigated vegetables consumption is of major importance in countries like Pakistan, where wastewater irrigation practice is still unchecked. There are numerous exposure pathways which generally depend on contaminated sources of air, water, soil, food and consuming population (10, 39) but the routes of exposure via food chain is one of the key pathways of heavy metals exposure to human (38). The vegetables contamination with detrimental metals could have a direct impact on the nearby inhabitant's health, because vegetables grown by peri-urban inhabitants, because vegetables produced from peri-urban areas are locally consumed most of the time. Therefore, the contamination of vegetables could be a matter of great concern for local residents. In present study, daily intake of metals was highest in case of tomato. Similar results were reported (5) who found highest Zn, Cu, Fe and Mn intakes from the consumption of waste water irrigated carrot, spinach, mint and methi and (39) who found highest daily intake in spinach sampled from Multan district irrigated with sewage water.

5. Conclusions

The results of current study revealed that heavy metals concentration in the soil that was irrigated with waste water was above the levels of toxicity. The highest concentration of copper, iron and lead was observed in upper layer of soil

(0-15 cm) as compared to the lower soil layer (15-30 cm). The reason was the permanent sewage water irrigation of upper soil while the sewage water penetration below 15 cm was less. Similarly, the leaves of the vegetables accumulate more heavy metal concentration as compared to the edible portion (fruit). 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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