

Heavy Metal Contamination in Vegetables and Soil Irrigated with Sewage Water and Associated Health Risks Assessment

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Abstract: The current study was carried out to investigate the impact of waste water on the quality of vegetables and soil according to the heavy metals in Faisalabad Pakistan. However, contamination of vegetables due to heavy metals causes potential health risk. For this purpose, samples each of water, soil and vegetables (edible portion & leaves) were collected from diverse locations around Faisalabad city. Waste water from Paharang Drain was being used as a source of irrigation in study areas. Therefore, the potential problem of contamination arise due to the effect of some heavy metals like manganese (Mn), nickel (Ni) and zinc (Zn) contents of various vegetables [spinach (*Amaranthus caudatus*), Coriander (*Coriandrum sativum*), cauliflower (*Brassica oleracea*) and tomato (*Lycopersicon sculenetum*)] produced Paharang Drain adjacent area, Faisalabad, Pakistan was checked by using atomic absorption spectrophotometer (AAS). All the plant samples were used in the study are the basis of human nutrition in those areas. The farmers of the concerned areas were consecutively using sewage water to grow their vegetables that showed the maximum heavy metals contamination. The contamination exceeds from the Maximum Acceptable Limits, set out by WHO. The results of the study were detrimental according to human health. The tested vegetables were not safe for the use of human food, particularly those directly consumed by humans.

Keywords: Heavy metals, sewage water, WHO, Mn, Ni, Zn, DIM, HRI.

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1. Introduction

Pakistan is a country with limited sources of fresh water (Nawaz et al., 2016). So, wastewater becomes an attractive source to irrigate crops most probably in urban areas because of easy accessibility, cheap and reduced fresh water availability (Hamid et al., 2016; Najam et al., 2015; Saini et al., 2014). In developing countries, municipal or industrial wastewater are commonly used for the purpose of irrigation (Ahmed et al., 2016; Saini et al., 2014). Irrigation with wastewater for long term might lead towards the heavy metal's accumulation in farming soils and plants (Hamid et al., 2016; Saini et al., 2014). Heavy metals are reserved under the category of environmental pollutant because of their poisonous

effects to animals, plants and human population (Hamid et al., 2016; Saini et al., 2014). Vegetables comprise a significant part of the human diet since they restrain proteins, carbohydrates, minerals, vitamins and trace elements (Bigdeli and Seilspour, 2008). Various studies showed heavy metals as main vegetables contaminants (Sharma et al., 2008).

Crops and vegetables that grown in soil irrigated with sewage water contaminated with heavy metals (Bao et al., 2014; Elahi et al., 2018; Farid et al., 2018; Zhao et al., 2012) poses a potential health risk to human beings and wildlife (Al-Jaboobi et al., 2014; Saini et al., 2014; Khan et al., 2009). A few of micronutrients like Mn, and Zn, are fundamentally essential for diverse physiological functions of animals and plants. Even though the most of plants

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needed these elements in the smallest quantities, farming soils are often deficient with one or more of these micronutrients. These elements could also contribute in other activities such as: (ii) redox processes (i) forming the protein and pigment structure (iii) osmotic pressure regulation (iv) the ionic balance maintenance and (v) acting as enzyme component of the cells (Oves et al., 2016).

Similarly, Zn plays an important function in protein synthesis, cellular division and amplification and contributes in lipid, carbohydrate and metabolism of nucleic acid (Oves et al., 2016). Various in-vivo and in-vitro studies showed that Zinc has valuable effects in both type-1 and type-2 diabetes. Likewise, many nickel damaging effects are due to the obstruction with the necessary metals metabolism like Ca(II), Cu(II), Fe(II), Mg(II), Mn(II) or Zn(II) which can modify or suppress modify the carcinogenic and toxic nickel effects. Nickel showed to be immunotoxic, changing the activity of all specific types concerned in the immunological response would result in asthma or contact dermatitis (Cempel and Nikel, 2006).

In view of the ever increasing evidence and significance of heavy metal contamination, the current work reported daily metal intake by human body on average basis, health risk index related with heavy metal ingestion and heavy metal concentration in water, soil and selected vegetables irrigated with the wastewater of Faisalabad, as the Paharng Drain receives large volumes of effluent from households and industries at Faisalabad and Faisalabad is considered as one of the most polluted cities in the world owing to being a hub of industries.

2. Materials and Methods

2.1. Plant Sampling and Analysis

Freshly matured leaves and fruits of (spinach, *Amaranthus caudatus*; Coriander, *Coriandrum sativum*; cauliflower, *Brassica oleracea*; and tomato, *Lycopersicon sculenetum*) were sampled at harvesting stage. The samples were transferred to the laboratory in plastic bags and cleaned with distilled water frequently air dried and then finely chopped (Hamid et al., 2016; Bigdeli and Seilsepour, 2008). Samples were dried in oven at 65°C -70°C to a constant weight followed by fine grinding (Al-Jaboobi et al., 2014; Maleki and Zarasvand, 2008) and stored in dry, clean, and high-density polyethylene bottles (100 ml capacity) with caps. After then, half gram of sample dry matter was weighed into 50-ml beaker, with the

addition of 10 ml analytical grade acids mixture HNO₃: HClO₄ in the ratio 5:1. Samples were digested at a temperature of about 190°C for 1.5 hour. After cooling, digested samples were filtered with Whatman filter paper 42 and solution was made up to final volume in a volumetric flask with distilled water (Al-Jaboobi et al., 2014; Khan et al., 2015). The metal (Mn, Ni and Zn) concentrations was checked by atomic absorption spectrometry (AAS) (Derakhahan et al., 2016; Hamid et al., 2016; Al-Jaboobi et al., 2014). Guidelines for heavy metals acceptable limits in vegetables were adopted from FAO- WHO (2007).

2.2. Water Sampling, Handling and Analysis

The sewage water samples were collected arbitrarily from the similar sites from where vegetables and soil samples were collected around paharng drain of Faisalabad. Thirty samples were taken in polyethylene bottles (250ml) which were sterilized with HNO₃ (Siddique et al., 2014). The HNO₃ acid was added for the purpose to avoid any microbial activity and preservation of the water samples (Bigdeli and Seilsepour, 2008; Khan et al., 2013). Then samples were filtered through Whatman 42 filter paper and stored in storage flasks (Rehman et al., 2014; Siddique et al., 2014; Hassan et al., 2013). Concentration of metals has been evaluated by the Atomic Absorption spectrophotometer (AAS) (Vanselow and Liebig, 1948). Standards were prepared with distilled water as matrix for every metal (Saini et al., 2014).

2.3. Soil Sampling and Analysis

Surface soil (0-15cm and 15-30cm) samples of the vegetable's farms were collected separately by using spiral auger of 2.5 cm diameter (Khan et al., 2013; Perveen et al., 2012; Dikineya and Aerola, 2010) and labeled properly. The soil samples were then air-dried and crushed to the particle size that would pass by a 2-mm mesh sieve and then stored in plastic jars (Hamid et al., 2016; Hassan et al., 2013; Nazemi, 2012; Siddique et al., 2014). Twenty-five (25) g of soil was taken and added 50 ml of DTPA solution into soil samples and constantly shaken for 2 hours on plane shaker and after 2 hours of shaking the mixture was filtered (Al-Jaboobi et al., 2014).

A sample having all substances excluding soil was run with samples as blank. Read every metal concentration by Atomic Absorption Spectrophotometer (Saini et al., 2014). Concentration of Mn, Ni and Zn present in vegetables, wastewater and soil samples were calculated on Atomic

Absorption Spectrophotometer (Shimadzu-7000, Japan) by using respective hollow cathode lamp.

2.4. Daily intake of metals

Following equation was used to determine the daily intake of metals (DIM):

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

where $C_{(\text{metal})}$ represents the concentration of heavy metals in plants (mg kg^{-1}), $C_{(\text{factor})}$ denominated as conversion factor (0.085) that is used to convert the fresh weight of green vegetables into dry weight as described by Rattan et al., (2005), $D_{(\text{food intake})}$ represents daily intake of vegetables for children and adults on average and considered to be 0.232 kg/person/day for children and 0.345 kg/person/day for adults and $B_{(\text{average})}$ is the average body weight of children and adults were considered to be 32.7 kg and 55.9 kg, respectively, as used in preceding studies (Muhmood et al., 2015; Wang et al., 2005; Ge, 1992).

2.5. Health risk index

The estimated exposure of test crops and oral reference dose ratio of each metal was calculated to determine the health risk index (Khan et al., 2008; Cui et al., 2004). Oral reference doses were 0.033 mg/kg/day for Mn, 0.02 mg/kg/day for Ni and 0.30 mg/kg/day for Zn (US-EPA IRIS, 2006). Projected exposure was obtained by dividing daily intake of heavy metals to their safe limits. The HRI <1 means the exposed population is expected to be safe. (USEPA, 2002).

Following equation is used to determine health risk index:

$$\text{HRI} = \text{DIM} / \text{RfD} \text{ (US-EPA, 2002)}$$

Where DIM was the daily intake of metals and Rfd mentioned reference oral dose.

3. Results

The Pakistan is categorized as a developing country which is facing various severe issues that badly damage human health. Shortage of fresh water availability is one of the biggest problems which we have faced in the current scenario, that is ultimately affect the agriculture sector of the country in terms of failure to fulfill the basic needs agriculturally produced to feed the growing population. To overcome this issue, the farmers are blindly using untreated sewage/industrial water for vegetable production especially in peri-urban areas.

3.1. Sewage Water Analysis

In current study sewage/industrial wastewater samples from ten different localities around paharang drain of Faisalabad district were analyzed for heavy metal concentration. The results regarding heavy metal content in sewage/industrial water are described in Table 1. The results obtained by the study showed that in wastewater samples, the concentration of Mn, Ni and Zn ranged from 0.21-1.69, 0.02-0.27 and 0.24-1.09 ml/L with average values of 0.87 ± 0.50 , 0.15 ± 0.07 and 0.63 ± 0.28 mL/L.

These results clearly exhibited that 100 and 72 % wastewater samples had concentrations of Mn and Ni above the safe limits whereas Zn contents were below the safe limits. Constant irrigation by the use of sewage/industrial water for over a longer time may cause buildup of heavy metals up to toxic levels for animal and plant health (Kirkham, 1983).

Table 1. Heavy metal concentration of wastewater used for irrigation (ml L^{-1})

Element	Range	Mean	SD	WHO safe limits (ml L^{-1})
Mn	0.21-1.69	0.87	0.50	0.2
Ni	0.02-0.27	0.15	0.07	0.2
Zn	0.24-1.09	0.63	0.28	2.0

SD, Standard deviation; WHO, World Health Organization.

Table 2. Heavy metal status of fields receiving wastewater (mg kg^{-1})

Soil Depth (0-15 cm)				
Element	Range	Average	Standard Deviation	WHO safe limits (mg kg^{-1})
Mn	9.37-36.23	23.0	9.2	-
Ni	0.65-1.78	1.2	0.4	75
Zn	2.01-8.17	5.5	2.1	300
Soil Depth (15-30 cm)				
Mn	7.06-29.95	19.2	7.9	-
Ni	0.39-1.58	1.1	0.4	75
Zn	1.77-7.15	4.9	1.9	300

Table 3. Heavy metal accumulation in leaves of vegetables (mg kg⁻¹)

Element	Range	Average	Standard Deviation	WHO safe limits (mg kg ⁻¹) *
Spinach				
Mn	60.1-158.4	111.6	33.5	61.05
Ni	25.9-35.9	31.3	3.3	0.00
Zn	71.5-154.7	115.5	28.5	297.40
Coriander				
Mn	58.3-151.4	107.6	32.1	43.95
Ni	25.1-33.6	29.7	2.9	0.00
Zn	69.7-150.6	112.5	27.8	61.36
Cauliflower				
Mn	38.1-76.4	56.8	13.1	-
Ni	11.9-35.7	24.1	8.5	-
Zn	38.2-123.9	81.7	28.2	-
Tomato				
Mn	27.5-87.8	56.9	20.8	14.93
Ni	9.7-29.8	19.3	6.7	0.03
Zn	38.1-120.1	79.7	27.2	46.20

Elevated heavy metals level in sewage water were also investigated by other scientists in Pakistan and they found excessive concentrations of Cu, Mn, Pb and Cd in sewage water samples from Peshawar (Ehsan et al., 2011) and (Jagtap et al., 2010) reported higher contents of Cd, Cr, Ni, Pb and Zn in wastewater samples from Rawalpindi Area. Similar results were reported by Siddique et al., (2014) using industrial water (Faisalabad, Pakistan) and Lone et al., (2003) using sewage water (Attok, Pakistan). (Ehsan et al., 2013 and Khan et al., 2013) investigated comparable results in wastewater of Lahore District.

3.2. Soil Analysis

Soil samples were collected from the equivalent point where the water samples were collected is presented in Table 2. The results showed that Mn, Ni and Zn content in the upper layer of soil (0-15 cm) ranged from 9.37-36.23, 0.65-1.78 and 2.01-8.17 mg/kg, respectively, with an average value of 23.0 ± 9.2 , 1.2 ± 0.4 and 5.5 ± 2.1 mg/kg. The results from soil depth (15-30 cm) showed in Table 2, according to which, the concentration of Mn, Ni and Zn content in deep layer of soil (15-30 cm) ranged from 7.06-29.95, 0.39-1.58 and 1.77-7.15 mg/kg, respectively, with an average value of 19.2 ± 7.9 , 1.1 ± 0.4 and 4.9 ± 1.9 mg/kg. According to the WHO (1996) and FAO (1985) limits, all soil samples were in safe range.

It is interesting that heavy metal content of wastewater was 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, amount of organic matter, redox potential of soil and

rate of addition of metals mainly affect their adsorption and retention in soil (McBride, 1994). Similar results were obtained by Siddique et al., 2013 in Faisalabad city and Hamid et al., (2016) Lahore.

3.3. Plant Analysis

Vegetables are used for common human nutrition and the edible portions of different vegetables varied. Some vegetables are consumed as leaf like spinach, coriander whereas 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 selected vegetables. The results presented in Table 3 showed that Mn, Ni and Zn contents of vegetable leaves of spinach, coriander, cauliflower and tomato ranged from (60.1-158.4, 58.3-151.4, 38.1-76.4 and 27.5-87.8), (25.9-35.9, 25.1-33.6, 11.9-35.7 and 9.7-29.8) and (71.5-154.7, 69.7-150.6, 38.2-123.9 and 38.1-120.1) mg/kg, respectively, with average values of (111.6 ± 33.5 , 107.6 ± 32.1 , 56.8 ± 13.1 and 56.9 ± 20.8), (31.3 ± 3.3 , 29.7 ± 2.9 , 24.1 ± 8.5 and 19.3 ± 6.7) and (115.5 ± 28.5 , 112.5 ± 27.8 , 81.7 ± 28.2 and 79.7 ± 27.2) mg/kg. It was revealed from the results that all vegetable leaf samples have heavy metal concentration higher than the acceptable limits set by the WHO, 1996.

The uptake of heavy metals by vegetables is not only affected by plant species and physicochemical characteristics of soil but temperature and rain fall also exert substantial effect. Zaidi et al., (1997) collected vegetables and pulses samples from Rawalpindi/Islamabd markets and reported the similar results as were found by Randhawa (2014).

Table 4. Heavy metal accumulation in edible portion of vegetables (mg kg⁻¹)

Element	Range	Average	Standard Deviation	WHO safe limits (mg kg ⁻¹) *
Cauliflower				
Mn	34.5-64.3	51.6	9.9	-
Ni	9.63-35.2	22.5	8.9	-
Zn	35.1-56.8	45.6	7.3	-
Tomato				
Mn	29.2-59.9	45.9	10.4	14.93
Ni	8.7-22.9	15.4	4.5	0.03
Zn	31.3-50.9	41.5	7.2	46.20

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. Samples collected from Malaysia and reported similar results as reported by Aweng (2011). Samples of vegetables were analyzed by Ahmed et al., (2012) grown in surroundings of Rawalpindi revealed the same result about contamination of vegetables with heavy metals by sewage water. Siddique et al., 2014 collected spinach samples from different locations in Faisalabad and reported the concentration of heavy metals higher than the safe limit.

Vegetable fruit samples were also analyzed to assess their heavy metal concentration and results are given in Table 4. The heavy metals content (Mn, Ni and Zn) in cauliflower and tomato fruit samples ranged from (34.5-64.3 and 29.2-59.9), (9.63-35.2 and 8.7-22.9) and (35.1-56.8 and 31.3-50.9) mg/kg, respectively with an average values of (51.6 ± 9.9 and 45.9 ± 10.4), (22.5 ± 8.9 and 15.4 ± 4.5) and (45.6 ± 7.3 and 41.5 ± 7.2) mg/kg. The heavy metals contents in all vegetable fruit samples were above the critical limits (WHO, 1996; Asaolu, 1995). Similar results were reported by many scientists. Ahsan et al., (2011) investigated heavy metal contents in edible portion of vegetables and found that Cd, Cr, Cu, Ni, Pb and Zn contents were higher than the safe limits. Arora et al., (2008) have reported the buildup of heavy metals in edible portion of vegetables. Liu et al., (2006) and Barman et al., (2000) also reported excessive heavy

metals concentration in edible portion of vegetables. Although, once the heavy metals ingested by the human body through any exposure, it would stimulate the immune system and may cause anorexia, nausea, vomiting, dermatitis and gastrointestinal abnormalities (Chui et al., 2013; Tchounwou et al., 2012). The heavy metals toxicity can damage or disrupt the mental as well as central nervous systems (Gybina and Prohaska, 2008), damage lungs (Kampa and Castanas, 2008), livers (Sadik, 2008), kidneys (Reglero et al., 2009), and other important organs (Lindemann et al., 2008), change the composition of blood, (Cope et al., 2009).

The exposure of human beings to heavy metals for long term has also shown muscular, physical and neurological impairments. The degenerative processes are similar to Alzheimer's disease (Kampa and Castanas, 2008), Parkinson's disease (Guilarte, 2011). So, this is a matter of consideration to reduce the use of wastewater especially, in developing countries which are already suffering from various serious issues including health issues with limited resources.

3.4. Daily Metals Intake and Health Risk Index

It is essential to measure the exposure level in order to investigate the risks associated with the health of any pollutant. Among the various human exposure pathways to heavy metals, food chain is the significant pathway (Khan et al., 2008). The highest intakes (Table 5) of Mn, Ni and Zn were from the spinach consumption. While the lowest intake of metals through tomato consumption for both children and adults.

Table 5. Average Daily intake of heavy metals

Metal	Spinach		Coriander		Cauliflower		Tomato	
	Child	Adult	Child	Adult	Child	Adult	Child	Adult
Mn	0.067	0.058	0.064	0.056	0.034	0.029	0.034	0.029
Ni	0.018	0.016	0.017	0.015	0.014	0.012	0.011	0.01
Zn	0.069	0.06	0.067	0.059	0.049	0.042	0.048	0.041

Table 6. 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
Mn	2.03	1.757	1.939	1.696	1.03	0.878	1.03	0.878
Ni	0.9	0.8	0.85	0.75	0.7	0.6	0.55	0.5
Zn	0.23	0.2	0.22	0.19	0.16	0.14	0.16	0.136

DIM for adults and children through the use of contaminated vegetables might cause rigorous health risks by heavy metals ingestion grown with wastewater. Daily metals intake was highest in case of green vegetables (spinach, *Amaranthus caudatus*; Coriander, *Coriandrum sativum*) in the present study. Similar results were found by Arora et al., (2008) who obtained highest Cu, Fe, Mn and Zn intakes from the consumption of wastewater irrigated carrot, methi, mint and spinach, respectively. Khan et al., (2008) also obtained highest intakes of Cd, Cr, Cu, Ni, Pb and Zn from of *Brassica napus* L, *Raphanus sativus*, *Spinacia oleracea*, and *Lactuca sativa* consumption for children and adults.

Human risk assessment quantification from wastewater irrigated vegetables consumption is of primary importance in developing countries like Pakistan, where wastewater irrigation practice is still unchecked (Muhmood et al., 2015). There are numerous exposure pathways that mainly depend on polluted sources of food, air, soil, water, and consuming population (Muhmood et al., 2015; Caussy et al., 2003) but the routes of exposure via food chain is one of the main pathways of heavy metals exposure to human (Muchuweti et al., 2006). For identifying unfavorable heavy metals effects on human health, the vegetables contamination with harmful metals could have a direct health impact in close proximity inhabitants, because most of the vegetables produced from peri-urban areas are consumed locally. Therefore, the contamination of vegetables could be a matter of great anxiety for local inhabitants (Table 6).

4. Conclusion

Accumulation of heavy metals (Mn, Ni and Zn) in vegetables is due to the presence of high concentration in soil and irrigation water. Different vegetables have varied amount of heavy metal, which show difference in their uptake capacity. Heavy metals concentration in irrigation water, soil and vegetables was above the MRL. This trend revealed that Irrigation water was a determining factor in determination of heavy metals in vegetables. On the

basis of results, it can be concluded that irrigation with sewage water is the main factor that contribute in accumulation and make vegetables unfit for human consumption. By avoiding irrigation with sewage water, heavy metal concentration can be reduced. This research will be helpful for the country to build database for policy making to control this alarming situation in the country.

List of Abbreviations: AAS: Atomic absorption spectrophotometer; Cd: Cadmium; Cr: Chromium; Cu: Copper; DIM: Daily intake of metals; g: Gram; HRI: Health Risk index; mg kg⁻¹: milligram per kilogram; ml L⁻¹: milliliter per Liter; Mn: Manganese; Ni: Nickel; Pb: Lead; Rfd: Reference oral dose; WHO: World health organization; Zn: Zinc

Competing Interest Statement: The authors declare that they have no conflict of interest.

Author's Contribution: F.J. designed the study. F.J., A.A. and M.S. conducted the experiment. F.J. A.A. and M.S. performed statistical analysis while F.J. wrote the manuscript. All the authors read and approved the final manuscript.

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