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Uptake of Five Heavy Metals by Spinach, Lettuce and Coriander Irrigated with Industrial Waste Waters

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Abstract. Heavy metals are non-essential harmful substances that are transferred from environment to human body *via* food chain. The present study, therefore, investigated the uptake concentration of heavy metals including Cd, Cr, Cu, Fe and Mn by spinach, lettuce and coriander irrigated with Steel Mill and Khwar (stream) effluents. The concentration of heavy metals was within the safe limits in Tap water but it was high in Khwar effluent followed by Steel Mill effluents. The results indicated that Cd and Cu contents were within permissible limit in the tested vegetables.The concentration of Cr, Fe and Mn in the three vegetables irrigated with Steel Mill and Khwar effluents were above the FAO/WHO permissible limits. The order of concentration of heavy metals in vegetables and effluents was Khwar effluent > Steel Mill effluent > Tap water. The accrual order of heavy metals was spinach > lettuce > coriander. The relative order of heavy metals concentration in 3 vegetables irrigated with Khwar effluents was Mn> Fe > Cr > Cu > Cd. It was concluded that neither the untreated industrial waste water be used for irrigating crops nor vegetables and crops raised on such polluted water be consumed by humans and their domestic animals.

Keywords: spinach, lettuce, coriander, industrial waste, heavy metals contents, Pakistan.

Introduction

The use of industrial and domestic waste water for irrigating crops has rendered soil and irrigation water unsuitable for growing crops and consumption by humans and animals (Abbas *et al*., 2021; Ashraf *et al*., 2021; Gupta *et al*., 2021; Haroon *et al*., 2021; Sharma *et al*., 2020; Waseem *et al*., 2014). Heavy metals once added to soil move on to water, plants and animals including humans through food chain; where by they cause serious health problems (Fongea *et al*., 2021; Haque *et al*., 2021; Huang *et al*., 2021; Kasozi *et al*., 2021; Oloruntoba *et al*., 2021; Iqbal *et al*., 2020; Jabeen *et al*., 2020; Ahmad *et al*., 2019; Zwolak *et al*., 2019 Hassan *et al*., 2021, 2015). Vegetables irrigated with industrial or municipal waste water accrue excessive quantities of various heavy metals that cause physiological and biological disorders and diseases in living organisms. Thus, thorough screening of vegetables irrigated with waste water for heavy metal contents is required before human consumption (Ashraf *et al*., 2021; Hassan *et al*., 2021; Li *et al*., 2021; Sakiyo *et al*., 2020; Lv *et al*., 2019). Some studies conducted in Pakistan (Ehsan *et al*., 2021; Khalid *et al*., 2021; Iqbal *et al*., 2020; Ishaq *et al*., 2020; Jabeen *et al*., 2020) suggest that heavy metal concentration in vegetables and crops irrigated with industrial effluents or city waste water contain heavy metals concentration exceeding the permissible limits. Leafy vegetables efficiently accumulate heavy metals (Abbas *et al*., 2021; Celik and Kunene, 2021; Jabeen *et al*., 2020; Ahmad *et al*., 2019; Uddin *et al*., 2019; Begum *et al*., 2017; Ahmad *et al*., 2014; Waseem *et al*., 2014). The uptake of heavy metals depend on plant species, variety, parts and habitat (Javani and Asadi-Gharneh, 2020; Nworie *et al*., 2019; Zwolak *et al*., 2019; Aurangzeb *et al*., 2014, 2011).

Our earlier investigations (Aurangzeb *et al*., 2014, 2011) concluded that wild plants growing along Khwar (polluted water channel) and plants irrigated with effluents accumulated heavy metals exceeding the permissible level. Therefore, the present study was carried out to assess the uptake efficiency of heavy metal concentrations in three most common leafy vegetables namely; spinach, lettuce and coriander following irrigation with tap water (control), Steel Mill and Khwar effluents. The findings were expected to will add to our existing knowledge about the ecology of heavy metal pollution in our daily food intake.

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Materials and Methods

Effluents from a waste water channel (hence forth referred as Khwar effluent) were collected in clean plastic containers near the Agricultural University, Peshawar, Pakistan. Effluents from Steel Mill were collected at source from Hayatabad Industrial Estate, Peshawar. These effluents were immediately shifted to lab. The Khwar effluent also received wastes from other industries and domestic refuses during its 3-4 km passage. Therefore, it was a mixture of various pollutants at the collection point near Agriculture University.

Healthy seeds of spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*) and coriander (*Coriandrumsativum*) were grown in pots (15x23 cm tapering down to 15 cm) containing equal amounts of loamysoil during October in net-house in Peshawar University Campus. Pots were arranged in a completely randomized block design. One week after emergence of seedlings, pots were thinned to 4 uniform healthy plants. The pots were initially flooded with tap water (control), Khwar or Steel Mill effluents. Soil moisture was maintained at field capacity by gravimetric method. Any loss of water was compensated by providing tap water or effluents to the respective treatments. After 7 weeks above ground parts were harvested, oven dried at 65 °C for 72 hand milled.

For heavy metal determination, 1 g dried plant sample was digested in 15 mL of HNO_3, $H_2SO_4$and $HClO_4$ mixture (5:1:1) at 80 ºC until atransparent solution was obtained. Water/effluent samples (50 mL) were digested with10 mL of concentrated HNO_3 at 80 ºC until they became transparent. These transparent solutions were then filtered through Whatman # 42 filter paper and diluted to 50mL with distilled water. The concentrations of Cd,Cr, Cu, Feand Mn in the filtrate were determined by using Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, Inc., USA) following (Estefan *et al*., 2013 Singh *et al*., 2010).

Results and Discussion

Original concentration of effluents. The primary sources of heavy metals are soil, water and air that enter the living organisms through food chain (Sharma *et al*., 2020; Ali *et al*., 2019; Waseem *et al*., 2014). Therefore, compared the three types of irrigation sources by growing the test vegetables. It was seen that the original heavy metal concentration of tap water, Khwar and Steel Mill effluents (Table 1) initially varied. The concentration was least in tap water with a range from 0.003 to 0.83 mg/Kg, which were within the FAO/WHO (2011) safe limits. The concentration of heavy metals in Khwar effluents, ranging in between 0.04-22.01 mg/Kg, was higher than the Steel Mills effluents that ranged from 0.03 to 20.37 mg/Kg. Thus, the trend in heavy metals concentration in deceasing order was: Khwar effluents > Steel Mill effluents > Tape water. (Hassan *et al*., 2021; Naz *et al*., 2019) reported low amount of heavy metals in irrigation water than in the sewage water. The concentrations among heavy metals in decreasing order were: Fe (0.83 mg/Kg) >Mn (0.08 mg/Kg) > Cr (0.03 mg/Kg) > Cd (0.01 mg/Kg) > Cu (0.03 mg/Kg) in tap water (control treatment). The declining concentration of heavy metals in Steel Mill effluent was: Mn (20.37 mg/Kg) > Fe (14.7 mg/Kg) > Cr (2.84 mg/Kg) > Cu (0.16 mg/Kg) > Cd (0.03 mg/Kg). The Khwar effluent had order of: Mn (22.01 mg/Kg) > Fe (15.74 mg/Kg) > Cr (3.91mg/Kg) > Cu (0.60mg/Kg) > Cd (0.04 mg/Kg). Copper and Cadmium had the lowest concentration among the three irrigation sources. The concentrations of Cr, Fe and Mn in Steel Mill and Khwar effluents were above the permissible limits for their use as irrigation water. Similar findings (Ashraf *et al*., 2021; Hassan *et al*., 2021; Haroon *et al*., 2021; Oloruntoba *et al*., 2021) reported high concentration of heavy metals beyond the permissible level for irrigation of crops and vegetables supporting the present results. Khan *et al*. (2016) stated that the uptake and accumulation of heavy metals by vegetables irrigated with waste water were more than tube well irrigated vegetables, which exceeded permissible limits of FAO/WHO (2011). Heavy metals present in water or soilsare ultimately transferred to plants, animals and human body through food chain.

Comparative uptake of heavy metals by three vegetables irrigated with effluents. The heavy metal concentrationsin three vegetables irrigated with tape water (control), Khwar and Steel Mill effluents was higher (Table 2) than the original contents in the irrigation waters (Table 1). The overall concentration of heavy metal in spinach varied from 0.003 to 0.08 mg/Kg in tap water. It was in between 0.002 to 46.74 mg/Kg in Steel Mill effluents and 0.03 to 63.96 mg/Kg in Khwar effluents. In lettuce, the uptake of heavy metals ranged from 0.001 to 0.05 mg/Kg in tapwater; while lettuce grown with Steel Mill effluents contained 0.02 to 7.98 mg/Kg and 0.02 to 8.46 mg/Kg heavy metals in Khwar effluents (Tables 1 and 2). Similarly, coriander had taken in heavy metals within the range of 0.001 to 0.007

Table 1. Original concentrations of heavy metals (mg/L) in tap water, Steel Mill and Khwar effluents.

Heavy metals	Tap water	Steel mill effluent	Khwar effluent	Average for individual heavy metal	Range for individual heavy metal	NEQSa standards
Cd	0.01	0.03	0.04	0.02	0.01-0.03	0.1
Cr	0.03	2.84*	3.91*	2.26*	0.03-3.91	1.0
Cu	0.003	0.16	0.60	0.25	0.003-0.60	1.0
Fe	0.83	14.70*	15.74*	10.42*	0.83-15.74	2.0
Mn	0.08	20.37*	22.01*	14.50*	0.08-22.01	1.5

a.National Environmental Quality Standards (NEQS) for the municipal and liquid industrial effluents. (Environmental Protection Agency, Pakistan, 2007)
*Exceed NEQS standards

Table 2. Uptake of 5 heavy metals (mg/L) by three vegetables irrigated with tap water (control), Steel Mill and Khwar effluents.

Treatments	Cd	Cr	Cu	Fe	Mn	Range within each effluent
	A.	**Spinach**				
Control (Tap water)	0.01	0.01	0.003	0.08	0.05	0.003-0.08
Steel mill effluent	0.02	1.33*	0.03	46.74*	6.48*	0.02-46.74
Khwar effluent	0.03	1.54*	0.22	63.96*	6.78*	0.03-63.96
Average value for three effluents	0.02	0.96*	0.08	36.92*	4.43*	-
	B.	**Lettuce**				
Control (Tap water)	0.001	0.01	0.001	0.05	0.05	0.001-0.05
Steel mill effluent	0.02	1.01*	0.02	7.98*	5.89*	0.02-7.98
Khwar effluent	0.02	1.03*	0.13	8.46*	6.04*	0.02-8.46
Average value for three effluents	0.01	0.68*	0.05	5.49*	3.99*	-
	C.	**Coriander**				
Control(Tap water)	0.001	0.01	0.01	0.07	0.04	0.001-0.07
Steel mill effluent	0.014	0.97*	0.02	2.64	3.39*	0.014-3.39
Khwar effluent	0.03	1.15*	0.03	3.64	3.66*	0.03-3.66
Average value for three effluents	0.015	0.71*	0.02	2.12	2.36*	-
Overall range across the 3 vegetables	0.001-0.03	0.01-1.54	0.001-0.22	0.05-63.96	0.04-6.78	-
FAO/WHO (2011) permissible limit	0.2	0.1	0.2	5.0	0.2	-

*Values exceed FAO/WHO(2011) permissible limits

mg/Kg, 0.014 to 3.39 mg/Kg and 0.03 to 3.66 mg/Kg respectively in tap water, Steel Mill effluent and Khwar effluents. Vegetables irrigated with Khwar effluents generally had high amount of heavy metals than Steel Mill effluent and tap water. Spinch was more efficient in accumulating heavy metal than lettuce and coriander. This agrees with Abbas *et al*. (2021), who also reported that spinach accumulated higher amounts of heavy metals followed by lettuce and fenugreek. Heavy metal concentrations generally declined in order of spinach >lettuce > coriander. The relative order of heavy metals concentration in 3 vegetables irrigated with Khwar effluents was: Mn> Fe > Cr > Cu > Cd. The present findings agree with Iqbal *et al*. (2020), who reported that vegetables irrigated with waste water gathered high concentration of various heavy metals.

The uptake of individual heavy metal by three vegetable is discussed below:

Cadmium (Cd). Cadmium is non-essential highly toxic element for living organisms (Haider *et al*., 2021; Huang *et al*., 2021). Vegetables irrigated with tap (control) water had 0.001 to 0.03 mg/Kg Cd contents with an overall average of 0.02 mg/Kg. Spinach had 0.02 mg/Kg and 0.03 mg/Kg with Steel Mill and Khwar effluents irrigation, respectively (Table 2). All these values are within safe limits (0.2 mg/Kg) of FAO/WHO (2011). High amountof Cd in spinach and some other crops was also reported by (Patel *et al*. 2019; Eze *et al*., 2018).

The Cd accrual in lettuce grown with Steel Mill and Khwar effluents was similar (0.02 mg/Kg) and within permissible limits (0.2 mg/Kg). Coriander irrigated with Steel Mill and Khwar effluents, respectively build up 0.014 mg/Kg and 0.03 mg/Kg Cd contents both values were below permissible limits. Contrary to our findings, other workers (Abbas *et al*., 2021; Ashraf *et al*., 2021; Hassan *et al*., 2021; Haque *et al*., 2021; Haroon *et al*., 2021; Huang *et al*., 2021; Kasozi *et al*., 2021; Ishaq *et al*., 2020) reported high concentration of Cd in various vegetables grown in heavy metal contaminated water. No visible trend was traced for Cd uptake in the present study as the range of concentration varied in between 0.02 to 0.03 mg/Kg in the three tested vegetables.

Chromium (Cr). FAO/WHO (2011) permissible limit for Cr concentration in vegetables is 0.1 mg/Kg. The three test vegetables grown with control water had Cr contents of 0.01 mg/Kg, which was within the safe limits.The Cr concentration found in spinach (range: 1.33-1.54 mg/Kg), lettuce (range: 1.01-1.03 mg/Kg) and coriander (range: 0.97-1.5 mg/Kg) grown with Steel Mill and Khwar effluents was above the FAO/WHO (2011) permissible limits (Table 2). The findings are in line with contemporary findings (Patel *et al*., 2019; Souri *et al*., 2018; Eze *et al*., 2018), they reported high Cr amounts in spinach, lettuce and coriander that exceeded the permissible point. Similarly, Gupta *et al.* (2021) reported high heavy metal contents including Cr in vegetables in spinach that crossed the acceptable limits. The present findings are also strengthened by others workers (Ashraf *et al.*, 2021; Haque *et al*., 2021; Haroon *et al*., 2021; Sharma *et al*., 2020) in this regard.

Copper (Cu). Copper as a bio-catalystis required for body pigmentation but most plants are deficient in Cu amount for growth.The FAO/WHO (2011) safe limit for Cu in plant is 0.2 mg/Kg. The acquirement of Cu differed among the vegetables and irrigation sources (Table 2). The copper accumulation varied between 0.001 to 0.01 mg/Kg in three tested vegetables. It was 0.03 mg/Kg in spinach, 0.02 mg/Kg in lettuce and 0.02 mg/Kg in coriander with Steel Mill effluent irrigation. When grown with Khwar effluents, Cu values were respectively, 0.22 mg/Kg, 0.13 mg/Kg and 0.03 mg/Kg in spinach, lettuce and coriander. All these values were within permissible limits. Some other studies (Patel *et al*., 2019; Eze *et al*., 2018; Latif *et al*., 2018; Souri *et al*., 2018; Adedokun *et al*., 2017; Nisar *et al*., 2016; Salawu *et al*., 2015) however, witnessed high levels of Cu in spinach, brinjal and other crops. Najam *et al*. (2015) and Agarwal *et al*. (2007) recorded FAO/WHO (2011) exceeded limits of 50 mg/Kg and 59 mg/Kg Cu contents in spinach and lettuce. Gupta *et al*. (2008) also reported Cu contents that not only exceeded our recorded values but also FAO/WHO (2011) the permissible limit in spinach (22 to 48 mg/Kg), lettuce (17.6 to 37.8 mg/Kg) and coriander (20.8-28.6 mg/Kg).

Iron (Fe). Iron governs cell homeostasis, synthesis of chlorophyll and activation of respiratory enzymes in plants. However, high concentration causes many respiratory problems (Latif *et al*., 2018). The permissible level of Fe in plants is 5 mg/Kg (FAO/WHO, 2011). In the present case (Table 2), the overall range of Fe contents varied from 0.05 to 63.96 mg/Kg among the investigated three vegetables with 3 irrigation sources. Except coriander (2.64-3.64 mg/Kg), Fe amount in spinach (46.74-63.96 mg/Kg) and lettuce (7.98-8.46 mg/Kg) grown with Steel Mill effluents crossed the FAO/WHO (2011) safe limits. Like this study, Salawu *et al*. (2015) recorded 25.66 mg/Kg Fe contents in spinach. Uddin *et al*. (2019) also stated very high level of Fe (356.71 mg/kg) in leafy vegetables that exceed our values and permissible limits. The present findings are also in line with (Latif *et al*., 2018; Nisar *et al*., 2016) also reported that heavy metals contents in soil and vegetables including spinach and coriander that were beyond the allowable limits of WHO/FAO (2011). Salama *et al*. (2015) based on the 13 years observation concluded that Fe contents decreased in 9 vegetables; increased in 14 crops and remained unchanged in the other cases. All These findings support our results. It is obvious that the concentrations of heavy metals including Fe are never permanent in plants.

Manganese (Mn). The FAO/WHO (2011) safe limit for Mn contents in plants is 0.2 mg/Kg. The Mn contents were in between 0.04 to 0.05 mg/Kg in 3 vegetables grown in tap water remained within the safe limits. But, vegetables irrigated with Steel Mill effluents differed in the order of spinach (6.48 mg/Kg) > lettuce (5.89 mg/Kg) > coriander (3.39 mg/Kg) these all crossed the safe limits.The Mn uptake by the tested vegetables irrigated with Khwar effluents was above the safe limits. It was in order of: spinach (6.78 mg/Kg) > lettuce (6.04 mg/Kg) > coriander (3.66 mg/Kg). High amount of Mn (32.10 to 33.91 mg/Kg) in spinach and other leafy vegetables was also observed by Uddin *et al*. (2019) that supports the present findings. Jabeen *et al*. (2020 and Nisar *et al*. (2016) also reported FAO/WHO (2011) The level of heavy metals in soil and vegetables including spinach and coriander reported by (Jabeen *et al*., 2020; Nisar *et al*., 2016) and exceediy by FAO/WHO (2011), that ase present findings. Like the present findings, Latif *et al*. (2018) also noted 137.3 mg/Kg Mn contents in spinach, which is far higher than recoded in the present case. Many contemporary studies (Hassan *et al*.,2021; Nworie *et al*., 2019; Eze *et al*., 2018; Najam *et al.*, 2015; Agarwal *et al*., 2008) have achieved high Mn values in plants including vegetables that support the present findings.

Conclusion

The findings suggest that uptake of 5 analyzed heavy metals in three vegetables grown with tap water were within the acceptable limits. However, vegetables irrigated with Khwar and Steel Mill effluents augmented the uptake of Cr, Fe and Mn concentration above the allowable limit. Since, Khwar effluents is a mixture of various industrial and domestic waste water, therefore, it contained elevated concentration compared to pure Steel Mill effluents. It was also observed that higher the concentration of heavy metals in effluents, higher was the uptake by the vegetables. The overall uptake of heavy metals by vegetables was in order of spinach > lettuce > coriander. Heavy metals are transferred from contaminated soil and water into biological system through food chain. Heavy metals concentrate in kidneys, lungs, liver, bones and other parts of human body and cause many health disorders and deterioration of biological activities. It is, therefore concluded that neither the untreated industrial waste water and effluents should be used for irrigation purposes, nor vegetables grown with heavy metal contaminated water should be consumed as human diet.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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