OPEN ACCESS Plant Health 01 (01) 2022. 09-16 DOI: 10.33687/planthealth.01.01.4534

Check for updates

Available Online at EScience Press
Plant Health
ISSN: 2305-6835
https://esciencepress.net/journals/planthealth

Comparatively Study and Analysis of Heavy Metal in the Cultivated Wetland of Baien Stream, Shakargarh, Pakistan

[a]Sundus Akhtar, [a]Ayesha Shafqat, [a]Najma Rani, [a]Iqra Younas, [a]Anza Afzal
[a] Department of Botany, Minhaj University Lahore, Pakistan.

ARTICLE INFO

Article History
Received: July 25, 2022
Revised: Septemner 22, 2022
Accepted: November 16, 2022

Keywords
Vegetables
Contaminated soil
Irrigation water
Heavy metals
Atomic absorption spectrophotometer
Shakargarh

ABSTRACT

Heavy metal pollution of soil has increased globally in recent years. In many dry and semi-arid locations throughout the world, wastewater is seen as a novel and unusual supply of water for agricultural development. As a result, soil and plant monitoring for a variety of factors, including heavy metals and hazardous contaminants, is necessary. The farmers in Shakargarh use wastewater and tube wells for crop production, however workers and customers are concerned about the possible public health hazards linked with the use of wastewater. This investigation aimed to determine the health threats of using wastewater for crop production by estimating heavy metal concentrations in vegetable crops. Vegetables samples (radish and turnip), soil and irrigation water were collected from critical points and examined for the detection of heavy metals (copper, chromium, cadmium, cobalt, and arsenic) by atomic absorption spectrophotometer. Vegetable parts were collected at the growing stage and at the time of harvesting from farmers' fields. Three soil sampling campaigns were carried out from the radish and turnip plots. Irrigation water samples were collected from different water bodies including wastewater and the tube well water. The following metals were detected: copper, chromium, cadmium, cobalt, and arsenic in the tested samples. It was observed that the high concentration of these metals in the roots of radish at fruiting stage: Cu (39 mg kg^{-1}), Cr (201 mg kg^{-1}), Cd (1.3 mg kg^{-1}), Co (54 mg kg^{-1}), and As (17.3 mg kg^{-1}). It was found that mean concentration of these metals in the roots of turnip at fruiting stage: Cu (34 mg kg^{-1}), Cr (178 mg kg^{-1}), Cd (2.1 mg kg^{-1}), Co (49 mg kg^{-1}), and As (14.1 mg kg^{-1}). The general pattern for the distribution of heavy metals in different parts of the plant was roots > stem > leaves > fruits. The incidence of heavy metals in soil samples was highest for chromium followed by copper, arsenic, cobalt and cadmium. The incidence of heavy metals in irrigation water samples was highest for chromium followed by copper, cadmium, arsenic, and cobalt. These concentrations were significantly greater than the permissible level recommended by FAO / WHO. It can be inferred that the health threats associated with the consumption of vegetables are currently very significant. Therefore, it is recommended that irrigation water, soil, and vegetables be monitored on a regular basis to safeguard the health of workers and consumers.

Corresponding Author: Sundus Akhtar
Email: dr.sundas@mul.edu.pk

INTRODUCTION

Metals and metalloids linked to contamination and probable toxicity have been referred to as "heavy metals in environmental pollution literature. A heavy metal has a high specific gravity (greater than 5) (Csuros and Csuros, 2016). Heavy metal pollution has spread across the globe, instigating environmental disruption and posing serious health threats to humans (Khan *et al.*, 2007). Pb, Cd, Hg and As are often considered as priority pollutants as they are very toxic, are released in a large concentrations and are persistent in the environment (Wang and Chen, 2014). Zn and Cu are essential elements for organisms. For instance, immune system functioning, cognitive abilities are dependent on zinc in living entities. However, these elements may be toxic if present in higher concentrations (Parveen *et al.*, 2017).

Soil and water pollution by heavy metals is the main topics of discussion of all environmental problems. The amount of nutrients in soil becomes very poor due to contamination of soil with heavy metals. Heavy metals deposition in the plants and the soil causes environmental problems (Wuana and Okieimen, 2011). Polluted water is used to cultivate crops, contaminating food grains and vegetables; it also reduces plant productivity, which has a negative impact on human health (Thompson and Darwish, 2019). Pakistan is primarily an agricultural country comprising semi-arid and arid regions used for agriculture (Butt *et al.*, 2005). Around 32,500 hectares of land in Pakistan is being irrigated with wastewater (Ensink *et al.*, 2002). The fruits and stems of plants and vegetables that were grown in contaminated soil absorbed and accumulated these contaminants (Shehata and Galal, 2020).

The use of wastewater to irrigate crops and vegetables is a common practice in Shakargarh, Pakistan. The present study has undertaken to investigate the contamination of heavy metals in selected plants like turnip and radish from agriculture land and irrigation water of Shakargarh, District Narowal.

MATERIALS AND METHODS

The entire experiment was conducted at Department of Baotany, Minhaj University Lahore, Pakistan. For Heavy metal analysis, atomic absorption spectrophotometery were carried out from institute of soil survey of Pakistan. The samples of radish and turnip were taken from random sites at Shakargarh's fields.

Types of crops

Vegetable crops radish and turnip were used as sample materials.

Sample preparation

The radish and turnip samples were thoroughly cleansed with distilled water. The stem, root, and fruit were separated from the rest of the samples. The samples were first dried for 24 hours in an oven set to 70 to 80 °C, and then they were dried in dry air. The dry samples were then sieved after being ground in a mortar.

Digestion of the vegetable samples

Each sample was precisely weighed at 0.5 g and added in 4 mL of Nitric acid (HNO_3) and 1 mL of perchloric acid ($HClO_4$) for digestion. The contents of each sample were filtered after they were cooled. After that, add 25 mL of distilled water to the filter to dilute it. Prepare the Blank solution in the same way as the Sample solution, but without adding the sample. (Rashid *et al.*, 2016)

Digestion of the water samples

Add 50 mL distilled water and 10 mL concentrated HNO_3 to a beaker. Heat the mixture until it has a volume of 40 mL. The combination was then filtered, and the filtrate was diluted with 50 mL distilled water. All of the subsequent samples of turnip and raddish were made in the same way. Prepare the Blank solution in the same way as the Sample solution, but without adding the sample.

Digestion of the soil samples

Nitric acid and perchloric acid digestion methods were utilized to prepare soil samples (Rashid *et al.*, 2016). Each sample weighed 1 gramme and was put in a digestive tube, followed by 10 ml of concentrated HNO_3. To oxidize all oxidizable materials, the entire mixture was boiled for 30-40 minutes. After cooling the sample mixture, 5 mL of 70% $HClO_4$ was added. It was cooked once more till the dense white vapours appeared. After the contents had cooled, added 20 mL distilled water and heated again to halt the departure of thick white vapours. The solution was then cooled again, filtered, and placed in a volumetric flask. The volume was then increased to 25 ml with filtered water up to the mark. Prepare the Blank solution in the same way as the Sample solution, but without the sample.

Determination of heavy metals

Using the dilution procedure, working standards of various concentrations based on the heavy metals were created from Perkin Elmer standard solutions (1000 ppm). The assessment of heavy metals was carried out by atomic absorption spectrophotometer. All of the samples were evaluated once the appropriate setup for the standardization procedure was completed. The metal content of the blank was deducted from the calculated value to correct the result of each heavy metal. All of the readings were noted three times. (Woldetsadik *et al*., 2017)

Statistical analysis

The well-organized data were used in a suitable statistical

 DOI: 10.33687/planthealth.01.01.4534

analysis. This study employed standard deviation, analysis of variance, mean, and range using the SPSS 15.0 programme.

RESULTS AND DISCUSSIONS

The results revealed tremendous variations in metal uptake by different parts at vegetative (root, shoot, leaf) and fruiting (bulb, shoot, leaf and flower) stages of *R. sativus* and *B. rapa.* It was also found that the copper (Cu), chromium (Cr), cobalt (Co), cadmium (Cd) and arsenic (As) uptake concentration in fruiting stage of both plants *R. sativus* and *B. rapa* was higher than the vegetative stage. Moreover, each tested metal uptake trend in the said plant parts during vegetative stage was root > shoot > leaves. Furthermore, the trend of metal (Cu, Cr, Co, Cd and As) uptake during fruiting stage was bulb > shoot > leaves > flower.

Table 1. lists the suggested allowed limits for the presence of heavy metals in vegetables and agricultural plants. In recent decades, unanticipated pollutants' negative influences on crop quality have posed a danger to food safety and human health in recent decades; therefore, more studies are essential that describe heavy metal contamination in soil and food crops in terms of the dangers to human health in sewage water irrigated areas.

Table 1. Permissible limits of various heavy metals in plants.

Heavy metals	Allowed limits (mg Kg^{-1})	Reference
Copper	10	(WHO, 1996)
Chromium	1.3	(WHO, 1996)
Cadmium	0.02	(Husain *et al*, 195; WHO, 1996)
Cobalt	50	(WHO, 1996)

At the vegetative stage, measurements of copper (Cu) in radish (*Raphanus sativus*) and turnip (*Brassica rapa*) plant sections revealed that the roots of both plants had greater concentrations of copper 36 and 31 mg kg^{-1} respectively. At the fruiting stage, 39 mg kg^{-1} and 34 mg kg^{-1}, respectively, of copper were found in greater concentrations in the roots of turnip and radish plants. The findings indicate that the mean Cu contents in radish and turnip plants are higher than the maximum level advised by the WHO/FAO. The order of build-up for Cu in various parts of the plants was found to be root > bulb > stem > leaves > flower (Figure 1 a and b).

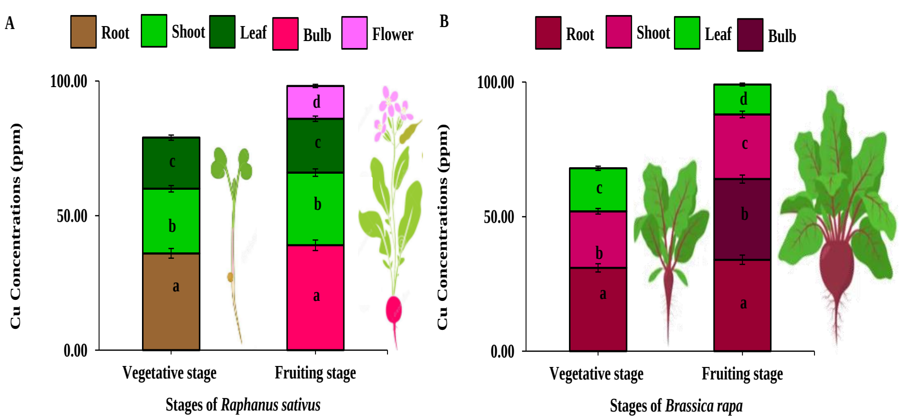

Figure 1. Copper (Cu) uptake by *Raphanus sativus* and *Brassica rapa* at different stages of plant. (Alphabets: Least significant difference of three replicates, vertical bars: Standard error of three replicates).

It was established that heavy metals accumulated more in the roots of vegetables than other parts since roots were considered as entrance routes of heavy metals from the soil water. Our findings are supported by preceding studies that described contents of heavy metals above the acceptable levels for human consumption in all the vegetables (Singh *et al.*, 2010). Our findings contradict a prior research that described the levels of have heavy metals below the acceptable levels except lettuce in which concentration of cadmium was beyond the safe limit (Azeem and Rashid, 2019). Several studies reported the accumulated concentration of Cu in all vegetables was below the permissible limits (Woldetsadik *et al.*, 2017).

According to our findings, the roots of turnip and radish plants had greater concentrations of chromium at the vegetative stage (198 mg kg^{-1}) and (160 mg kg^{-1}), respectively. Chromium levels greater than the WHO-recommended acceptable limits were found in turnip and radish plant's roots 201 and 178 mg kg^{-1}, respectively during the fruiting stage. The order of build-up for Cr in various parts of the plants was found to be root > bulb > stem > leaves > flower (Figure 2 a and b).

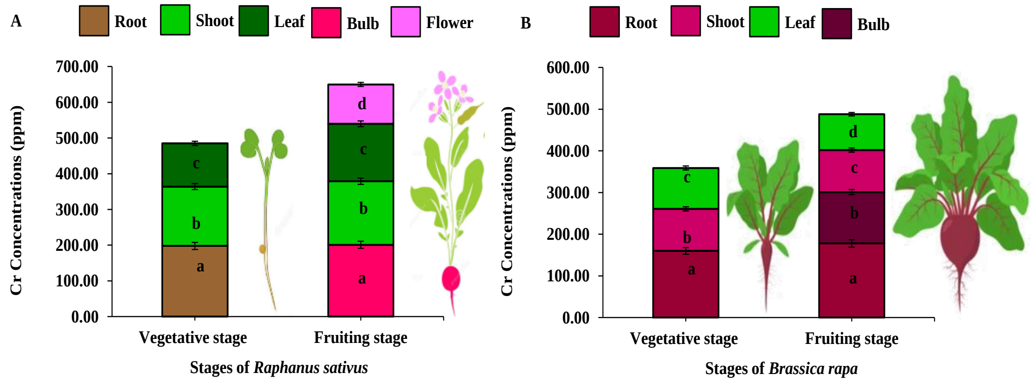

Figure 2. Chromium (Cr) uptake by *Raphanus sativus* and *Brassica rapa* at different stages of plant. (Alphabets: Least significant difference of three replicates, vertical bars: Standard error of three replicates).

The increased amount of Cr in our study poses a risk to the customers' health. Our results were in agreement with that obtained by preceding findings that described contents of heavy metals beyond the safe levels for human consumption in all the vegetables (Singh *et al.*, 2010). Our findings conflict with earlier research that described the levels of Cr in vegetable plants within permissible limits (Galal *et al.*, 2021; Woldetsadik *et al.*, 2017). The quantification of cadmium at vegetative stage was higher in the roots of radish and turnip (0.9 mg kg^{-1}) and (1.8 mg kg^{-1}) respectively. At the fruiting stage, radish and turnip plants' roots had the highest concentrations of cadmium, measuring 1.3 and 2.1 mg kg^{-1}, respectively. These levels are above the WHO/FAO recommended maximum limit, posing a health risk to consumers. The order of accumulation for Cd in various parts of the plants was found to be roots > bulb > stem > leaves > fruits (Figure 3 a and b).

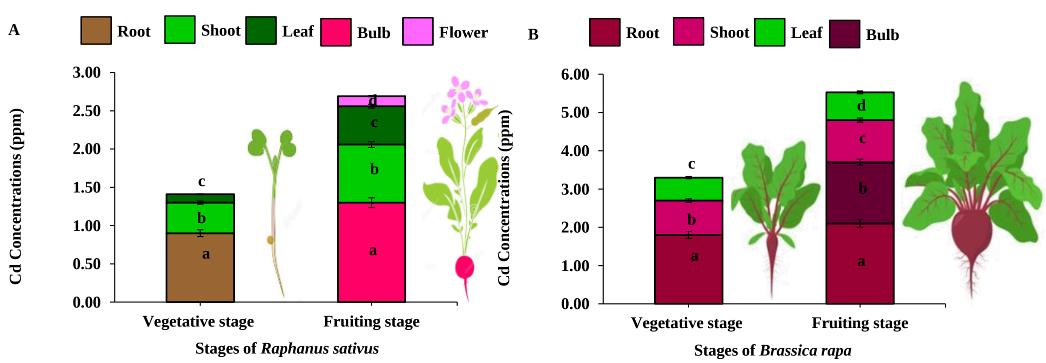

Figure 3. Cadmium (Cd) uptake by *Raphanus sativus* and *Brassica rapa* at different stages of plant. (Alphabets: Least significant difference of three replicates, vertical bars: Standard error of three replicates).

The results of our study are supported by previous findings that reported accumulation of Cd in radish plants was higher than the permissible limits (Woldetsadik *et al.*, 2017) . Our findings contradict earlier research that indicated the levels of Cd in vegetable plants within permissible limits (Woldetsadik *et al.*, 2017).

Cobalt (Co) concentration measurements in vegetative stage plant sections showed greater concentrations in the roots of turnip (51 mg kg^{-1}) and radish (43 mg kg^{-1}). The higher levels of cobalt, 54 mg kg^{-1} and 49 mg kg^{-1} were found in turnip and radish plant roots respectively, during the fruiting stage, above the safe limits recommended by the WHO. The order of accumulation for Co in various parts of the plants was found to be bulb > roots > stem > leaves > Flower (Figure 4 A & B). Our findings conflict with earlier research that indicated the amounts of cobalt in vegetable plants within permissible limits (Woldetsadik *et al.*, 2017).

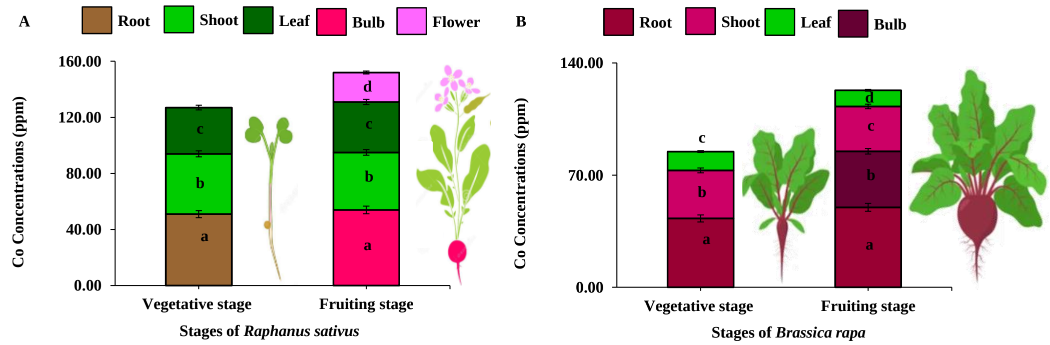

Figure 4. Cobalt (Co) uptake by *Raphanus sativus* and *Brassica rapa* at different stages of plant. (Alphabets: Least significant difference of three replicates, vertical bars: Standard error of three replicates).

According to estimates of arsenic (As) in plant parts during the vegetative stage, the roots of turnip and radish plants had greater concentrations of the toxic substance 15 and 14 mg kg^{-1}, respectively. At the fruiting stage, arsenic levels higher than the safe WHO limits were found in the roots of turnip and radish plants, at 17.3 and 14.1 mg kg^{-1}, respectively. The order of accumulation for As in various parts of the plants was found to be bulb > roots > stem > leaves > flowers (Figure 5 a and b). Previous studies that showed greater concentrations of arsenic in vegetable plants confirm our findings (Galal *et al.*, 2021). The heavy metals accumulation in the plant components throughout plant life is shown by the greater levels of heavy metals during the fruiting stage. It has been documented that the low levels of heavy metals in vegetables can harm people's health if they consume them frequently (Rana *et al.*, 2014).

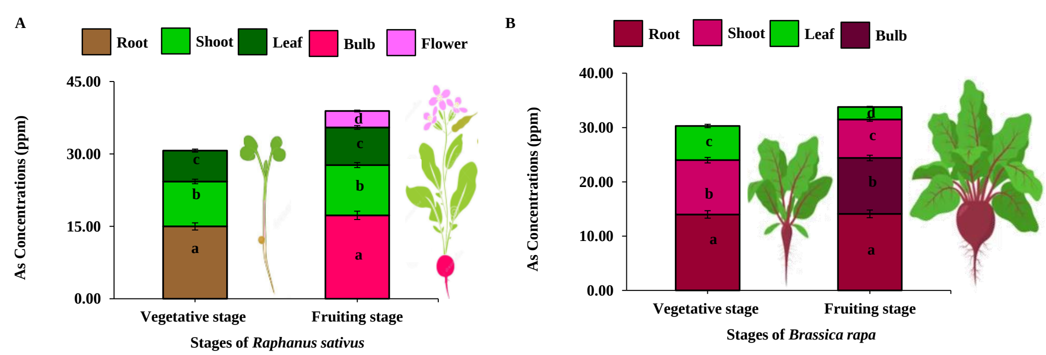

Figure 5. Arsenic (As) uptake by *Raphanus sativus* and *Brassica rapa* at different stages of plant. (Alphabets: Least significant difference of three replicates, vertical bars: Standard error of three replicates).

Heavy metal levels in soils are high in many parts of the world, particularly in developing countries and regions. Heavy metals have contaminated soil ecosystems around the world as a result of numerous human activities, and metal mobility in the food chain has become a human health danger. Determining the concentrations of contaminants (heavy metals) in soils is crucial since they can contaminate food and water. The suggested allowable levels for heavy metals incidence in soil samples are provided in table 2.

Table 2. Permissible limits of various heavy metals in soil.

Heavy metals	Allowed limits (mg Kg^{-1})	Reference
Copper	20	(WHO, 1996)
Chromium	100	(WHO, 1996)
Cadmium	0.03	(Husain *et al*, 195; WHO, 1996)
Cobalt	8	(WHO, 1996)
Arsenic	14	(WHO, 1996)

Raphanus sativus and *Brassica rapa* revealed greater concentration during fruiting stage, with corresponding values of 42 ppm and 45 ppm, which were over the acceptable limits recommended by the WHO. Copper was quantified in soil samples taken from several chosen areas. When *Raphanus sativus* and *Brassica rapa* were in the fruiting stage, soil samples from several chosen fields had somewhat higher concentrations of chromium, measuring 146 ppm and 234 ppm, respectively. These values were over the safe limits recommended by the WHO. Cadmium concentrations in soil samples quantified from *Raphanus sativus* and *Brassica rapa* during the fruiting stage were higher, at 1.4 ppm and 2.4 ppm, respectively, than the safe limits recommended by the WHO. Our findings showed that the levels of cobalt in soil samples taken from several chosen fields, including those where *Raphanus sativus* and *Brassica rapa* were in the fruiting stage, were higher than the WHO-recommended safe limits at 9.1 ppm and 10.1 ppm, respectively. *Raphanus sativus* and *Brassica rapa* soil samples obtained from several chosen fields had higher concentrations of arsenic, measuring 22 ppm and 19 ppm, respectively, during the fruiting stage. These levels were over the safe limits recommended by the WHO. Chromium was the heavy metal with the highest prevalence in soil samples, followed by copper, arsenic, cobalt, and cadmium (Table 3). Previous studies that showed greater contents of heavy metals in soil samples confirm these results (Galal *et al.*, 2021). A recent research reported that the heavy metal concentrations in irrigation soil samples did not surpass the recommended maximum limits (RMLs) (Woldetsadik *et al.*, 2017) which is against our findings.

Table 3. Concentrations of heavy metals in soil of *Raphanus sativus* and *Brassica rapa* collected from Shakargarh, during vegetative and fruiting stage.

Heavy metal	Concentrations of heavy metals in soil (ppm)			
	Raphanus sativus		*Brassica rapa*	
	Vegetative stage	Fruiting stage	Vegetative stage	Fruiting stage
Copper	30[c]	42[b]	43[ab]	45[a]
Chromium	134	230	146	234
Cadmium	1.1	1.4	21	2.4
Cobalt	8.3	9.1	9.9	10.1
Arsenic	15.3	16	17	17

In order to maintain the health and safety of the crops in sewage water irrigated areas, it is necessary to check for the presence of harmful heavy metals in irrigation water samples. The recommended permissible limits for heavy metals incidence in irrigation water samples are presented in table 4. *Raphanus sativus* and *Brassica rapa* revealed greater concentrations of copper throughout the fruiting stage with quantification results of 76.5 ppm and 73 ppm, respectively. These values were over the acceptable limits recommended by the WHO. Chromium content was found to be greater during the fruiting stage in irrigation water samples from *Raphanus sativus* and *Brassica rapa*, respectively, and was found to be 102 and 110 ppm, respectively that were beyond the safe levels recommended by the WHO. Our findings showed that irrigation water samples from several chosen crops, including *Raphanus sativus* and *Brassica rapa* during fruiting stage, had higher cadmium concentrations above the safe WHO standards of 43 ppm and 45 ppm, respectively.

Table 4. Permissible limits of different heavy metals in irrigation water samples.

Heavy metals	Allowed limits (mg Kg^{-1})	Reference
Copper	0.55	(WHO, 1996)
Chromium	0.017	(WHO, 1996)
Cadmium	0.02	(Husain *et al*, 195; WHO, 1996)
Cobalt	005	(WHO, 1996)
Arsenic	0.1	(WHO, 1996)

Table 5. Concentrations of heavy metals in soil of *Raphanus sativus* and *Brassica rapa* collected from Shakargarh, during vegetative and fruiting stage.

Heavy metal	Concentrations of heavy metals in irrigation water (ppm)			
	Raphanus sativus		*Brassica rapa*	
	Vegetative stage	Fruiting stage	Vegetative stage	Fruiting stage
Copper	75	76.5	64	73
Chromium	86	102	91	110
Cadmium	37	43	41	45
Cobalt	12	15	19	23
Arsenic	25	30	35	40

In our investigation, we found that irrigation water samples from *Raphanus sativus* and *Brassica rapa* during the fruiting stage had higher cobalt concentrations of 15 ppm and 23 ppm, respectively, which were beyond the safe limits recommended by the WHO. *Raphanus sativus* and *Brassica rapa* had higher concentrations of arsenic during the fruiting stage, estimating values of 30.1 ppm and 40 ppm for arsenic in irrigation water samples obtained from several chosen fields. These values were beyond the safe levels recommended by the WHO.

Chromium had the greatest content in irrigation water samples, followed by copper, cadmium, arsenic, and cobalt. The concentration of heavy metals in wastewater used for vegetable irrigation shows that the water used for irrigation in Shakargarh was highly contaminated. Previous studies that showed greater contents of heavy metals in soil samples confirm these findings (Galal *et al.*, 2021).

A recent investigation reported that the heavy metal concentrations in irrigation soil samples did not surpass the recommended maximum limits (RMLs) (Woldetsadik *et al.*, 2017) which is against our findings. A recent study reported that Zn was found in the greatest quantity in irrigation wastewater, followed by Pb, Cr, Ni, Cu, and Cd. Contents of heavy metals in pure irrigation water were below detectable levels (Singh *et al.*, 2010). Similar results were reported in an investigation on heavy metal distribution in vegetables and soil samples in Shakargarh (Azeem and Rashid, 2019).

REFERENCES

Azeem, M. and A. Rashid. 2019. Determination of Heavy Metals in Vegetables Grown in Sewage Irrigated Fields of Shakargarh, Pakistan. *International Journal of Alternative Fuels and Energy*, 3(2:, 36-40.

Butt, T. A., B. A. McCarl, J. Angerer, P. T. Dyke and J. W. Stuth. 2005. The economic and food security implications of climate change in Mali. *Climatic change*, 68(3): 355-378.

Csuros, M. and C. Csuros. 2016. Environmental sampling and analysis for metals. CRC Press.

Ensink, J. H., W. Van Der Hoek, Y. Matsuno, S. Munir and M.

R. Aslam. 2002. Use of untreated wastewater in peri-urban agriculture in Pakistan: *Risks and opportunities*, (Vol. 64). IWMI.

Galal, T. M., L. M. Hassan, D. A. Ahmed, S. A. M. Alamri, S. A. Alrumman and E. M. Eid. 2021. Heavy metals uptake by the global economic crop (*Pisum sativum* L.) grown in contaminated soils and its associated health risks. *PLoS ONE*, 16(6): e0252229. https://doi.org/10.1371/journal. pone.0252229.

Khan, M. A., I. Ahmad and I. U. Rahman. 2007. Effect of environmental pollution on heavy metals content of Withania somnifera. *Journal of the Chinese Chemical Society*, 54(2): 339-343.

Parveen, N. Zaidi, N and Danish, M. 2017. Development of SVR-based model and comparative analysis with MLR and ANN models for predicting the sorption .. capacity of Cr(VI). *Process Safety and Environmental Protection, 107:* 428-437.

Rana, S. V. S. 2014. Perspectives in Endocrine Toxicity of Heavy Metals—A Review. *Biological Trace Element Research*.160; 1–14.

Rashid, M. H., Z. Fardous, M. A. Chowdhury, M. K. Alam, M. L. Bari, M. Moniruzzaman and S. H. Gan. 2016. Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: an evaluation of six digestion methods. *Chemistry Central Journal,* 10: 7. https://doi.org/10.1186/s13065-016-0154-3.

Shehata, H. S. and T. M. Galal. 2020. Trace metal concentration in planted cucumber (*Cucumis sativus* L.) from contaminated soils and its associated health risks. *Journal of Consumer Protection and Food Safety,* 15: 205-217.

Singh, R., Gautam, N., Mishra, A., and Gupta, R. (2010). Heavy metals and living systems: An overview. Indian journal of pharmacology, 43(3), 246-253. https://doi.org/10.4103/0253-7613.81505.

Thompson, L. A. and W. S. Darwish. 2019. Environmental Chemical Contaminants in Food: Review of a Global Problem. *Journal of Toxicology*, 2019: 2345283. https://doi.org/10.1155/2019/2345283.

Wang, J. and C. Chen. 2014. Chitosan-based biosorbents: modification and application for biosorption of heavy metals and radionuclides. *Bioresource technology,* 160: 129-141.

Wani, P. A., Khan, M. S., and Zaidi, A. (2008). Effects of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. Bulletin of environmental contamination and toxicology, 81(2), 152-158.

Woldetsadik, D., P. Drechsel, B. Keraita, F. Itanna and H. Gebrekidan. 2017. Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa, Ethiopia. *International Journal of Food Contamination*, 4(1): 1-13.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.