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RESEARCH ARTICLE

Assessment of heavy metal contamination in *Trifolium alexandrium* and *Spinacia oleracea* using ICP-MS: A comparative analysis across different districts in eastern Uttar Pradesh

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Abstract

In areas near sugar mills, many plants have been found to accumulate unsafe heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), and zinc (Zn), which can pose severe threats to human and environmental health. The objective of this study is to assess the level of heavy metal concentrations in different tissues of *Trifolium alexandrinum* and *Spinacia oleracea* samples collected from Ayodhya, Bahraich, and Gonda districts in Uttar Pradesh, India. Heavy metal concentrations in plant samples were estimated using inductively coupled plasma mass spectrometry (ICP-MS). The present study revealed the variations in heavy metal distributions among plant species of different areas, with notably high levels of heavy metals in samples like *T. alexandrinum*, and *S. oleracea* collected near sugar mills. The differences observed within specific regions suggest that regional factors, such as soil and land use, influence the accumulation of heavy metals. These findings underscore the urgent need for continuous monitoring and control of heavy metal pollution to mitigate potential health and environmental risks associated with proximity to sugar mills.

Keywords: Trace elements, *Trifolium alexandrinum, Spinacia oleracea,* Bahraich, Gonda, Ayodhya, ICP-MS, Sugar mills.

Introduction

The sources of heavy metals mainly arise from industrial activities like mining, smelting, fossil fuel burning, and agricultural practices such as the use of fertilizers, amendments of soil, and disposal of land by municipal solid wastes (Kidd *et al*., 2009; Rajkumar *et al*., 2013). Various pollutants present in the environment generally harm the environmental components like air, soil, and water. Out of them, heavy metals are well known due to their persistence, toxicity in nature, and bio-accumulative potential present in the environment (Yang *et al*., 2021). Consequently, the increasing environmental contamination by heavy metals poses a severe threat to plants, animals, and human beings (Uddin *et al*., 2021). These persistent heavy metals can also build up in the food chain, leading to higher exposure levels for certain plants and animals in the environment. Long-term exposure to heavy metals can harm vital organs and neurological processes, potentially causing life-threatening diseases (Jan AT *et al*., 2016). Plants mainly absorb toxic elements due to the buildup of heavy metals in the soil. When present in excessive amounts, heavy metals can significantly affect plant growth and crop productivity (Ziwei Ding *et al*., 2018). With the global economy and industrialization accelerating, the risk of polluted farmland increases, posing a threat to human health (Khan A. *et al*., 2015). Trace elements such as copper, zinc, lead, cadmium, and arsenic can accumulate in crops, potentially leading to higher metal content in agricultural products and posing health risks to humans. Contaminated food can result in

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chronic and acute health effects, including bone fractures, kidney dysfunction, hypertension, and cancer (Houston *et al*., 2007; Rodríguez *et al*., 2018). The harmful effects of these metals depend on various factors, such as the amount, their chemical makeup, age, gender, genetics, and the nutritional health of the affected people (Traudt *et al*., 2017).

Population growth and food demands lead to increased environmental pollutants that ultimately contaminate the food chain (Duruibe *et al*., 2007). The plants are a major source of dietary heavy metals, and their contamination can have catastrophic health consequences. While some heavy metals are beneficial in trace amounts, others, like lead and mercury, are highly toxic even at low levels, especially for pregnant women and young children (Liang G *et al*., 2019; Kim Y *et al*., 2013; Reuben A *et al*., 2017). Other reports suggest that some metals, such as copper, zinc, and nickel, can be beneficial for humans, but at toxic levels, they can be harmful to human health, particularly for pregnant women and young children (Stern *et al*., 2010; Vijayalakshmi *et al*., 2018). As industrialization and agriculture intensify, the risk of heavy metal contamination in farmland grows. Soil quality directly impacts human health. Contaminated soil can lead to dangerous levels of heavy metals in crops. These elements, including zinc, lead, cadmium, and arsenic, can accumulate in the body over time and cause serious health problems. For example, cadmium exposure can lead to kidney dysfunction and cancer, while lead can cause nervous system damage (Rinaldi *et al*., 2017). Their presence in contaminated soil leads to bioaccumulation in the food chain, causing a range of health problems (Rahimi *et al*., 2019; Peralta *et al*.,2009). Therefore, the removal of heavy metals from contaminated environments is imperative.

Sugar mills are may be the sources of heavy metal pollution, which can significantly impact the environment and agriculture (Saranraj *et al*., 2014). The present study examines the presence of high levels of heavy metals accumulation in plants grown near sugar mills in the Bahraich, Gonda, and Ayodhya districts of Uttar Pradesh, India, with particular attention to *Trifolium alexandrinum* and *Spinacia oleracea*, due to their propensity to accumulate heavy metals (Yadav *et al*., 2015). Heavy metal pollution from sugar mills significantly affects agriculture in nearby areas (Kumar *et al*., 2010). Plants uptake heavy metals from the soil, concentrating them to unsafe levels, resulting in soil contamination and potentially harming their growth and productivity (Alengebawy *et al*., 2021).

Uttar Pradesh is well known for its fertile soil; hence, it is the leading producer of agricultural products with farming and animal breeding as the main activities practiced by the people (Bhatti *et al*., 2016). Berseem (*T. alexandrinum)* is commonly grown as a fodder crop, particularly during the Rabi season in Uttar Pradesh and is scientifically classified under Leguminosae or the pea family (Ali *et al*., 2012; Bhat, 2013). Berseem clover is grown on over 2 million hectares of land in India's northern, central and eastern production regions. It can produce as much as 8 tons of green fodder per crop. It has high nutritional value and is used in feeding animals, especially large herds and flocks. Berseem is recommended as a forage plant since it possesses several positive characteristics. It can transform nitrogen in the ground, enhance the soil structure and its humus composition, stop soil erosion, and minimize the growth of weeds. Because of this feature, it is used for mulching; thus, it is regarded as a green manure plant and can be incorporated into the soil to enhance fertility (Daneshnia *et al*., 2016; Khanduri *et al*., 2021). It also has herbal properties and can also be used to reclaim the land from contaminants through phytoremediation. Due to its palatability and feed value, it is used in fodder production as hay, silage and pasture. Green leafy vegetables possess a variety of chemical compounds, which are essential nutrients and non-essential compounds that can enhance an individual's health. Spinach is a green leafy vegetable containing vitamins and minerals and is well known for its phytochemicals and bioactive that go additional to functional nutrition. It has been described that phytochemicals present in spinach display the activity of free radical scavenging, gene expression and activity regulation, appetite suppression through the release of satiety factors, and anticancer, antiobesity, antidiabetic, and hypolipidemic effects. Spinach, or *S. oleracea* belongs to the family of Amaranthaceae. It is an annual plant that may even grow in winter in temperate climate zones (Ahada Chetan *et al*., 2015). Spinach can be described as a micronutrient-dense vegetable with low-calorie content, rich in niacin, zinc, dietary fiber, protein, vitamins- calcium, iron, magnesium, phosphorus, potassium, copper, manganese, etc. Spinach (*S. oleracea)* is a useful functional food containing lots of nutrients that are good for health but require more marketing to enhance its utility in meals. Hence, the objective of the present study was to understand the metal uptake potential of two different plants available at the adjacent area of sugar mills. This finding will deliver valuable insight into the phytoremediation of contaminated sites using *T. alexandrinum* and *S. oleracea*.

Materials and methods

Description of the Study Sites

Ayodhya, Gonda and Bahraich are districts of eastern Uttar Pradesh, which are located between latitudes 81'E to 82'E and longitudes 27'N to 26.5'N. In these districts, different sugar mills and industries are situated. Ayodhya is near the capital of Uttar Pradesh, which is known all over the world as lord Ram Temple. Gonda is the district next to Ayodhya, and Bahraich district is adjacent to Nepal country. Various pilgrimage sites and the famous Katarniaghat Wildlife Sanctuary are situated in the Bahraich district. All three cities are well known for their agricultural and industrial activities.

Sample Preparation

The plant was harvested by pulling out the whole plant before washing it with water to remove the debris. After that, they were rinsed with ethanol to remove surface contaminants, then rinsed repeatedly with distilled water, and then the plants were put in the oven at 40°C until they became crispy in a laboratory for a day. Further, the plant samples were ground in a blender for further processing (Balakrishna *et al*.,2021). Details of the plant samples are shown in Table 1.

Digestion of Samples

Dried plant samples weighing 1 g were placed in a test tube. After that, 5 mL of perchloric acid ($HClO_4$) was added, followed by the addition of 15 mL nitric acid (HNO_3). Details of sample codings are provided in Table 2. Any residual mixtures that had not formed a homogenous solution with the solvent were removed from the reaction vessel and allowed to stand in a pyridine-saturated fume cupboard for 24 hours. After that, the mixture was heated on a stove at 90°C, and the temperature was increased by 10°C each following 2 minutes, providing the final temperature of 170°C. Further, a few drops of hydrogen peroxide (H_2O_2) solution were added at 170°C and observed for the formation of white fumes. The solution in the flask was allowed to cool down at room temperature, and then after, the flask was maintained up to 50 mL with deionized water and subjected to filtration using Whatman filter paper number 42. At the end, the solutions were subjected to ICP-MS to determine the concentration of heavy metals present in plants (Mawari *et al*., 2022).

Operating Procedure of ICP-MS

ICP-MS is a high-throughput technique with several advancements that are applied in different branches of sciences and technologies such as earth sciences, environmental sciences, forensic sciences and technology in the fields of food, material, chemical and semiconductor industries, and nuclear technologies, etc. (Yang *et al*., 2021). However, this technique involves high ion density and high temperature useful for disturbing the atoms and elements present in a plasma and is thus the most effective for atomizing and ionizing a sample or matrix. ICP-MS (NexION 1000, PerkinElmer) is a type of mass spectrometry, an efficient analytical technique for the detection of trace elements which can determine elements at picogram per liter (pg/L). It also stands out for providing a fairly high tolerance to saline matrices and distinct element responses that are somehow independent of the compound matrix (Pröfrock *et al*.,2012). Most importantly, it is quite accurate in determining trace elements. The standard operating procedure of instrument is presented in Table 3.

Table 1: Description of the plants used

Name of the plant	*Scientific name*	*Parts used*	*Amount used*
Berseem	*T. alexandrinum*	Whole plant	1 gm
Spinach	*S. oleracea*	Whole plant	1 gm

Table 2: Sample coding

Sample coding
1. MIG24031894-B-TA
2.MIG24031895-G-TA
3.MIG24031896-A-TA
4.MIG24031897-G-SO
5.MIG24031898-B-SO
6.MIG24031899-A-SO

B: Bahraich, G: Gonda, A: Ayodhya, TA: *T. alexandrinum*, SO: *S. oleracea*

The instrument was firstly set at a power of 1100 W that ensured the formation of high-temperature plasma which atomized and ionized the sample. Then after, an alteration was done in the sample uptake rate to 2 mL/min in order to limit the manner in which the sample was introduced to the nebulizer. Further, adjustment of the nebulizer gas flow was maintained to 0. 90 L/min to spray the liquid sample in the form of an aerosol to be inhaled by the patient. In the case of plasma conditions, plasma gas flow was fixed at the rate of 15 L/min to sustain the plasma and the auxiliary gas flow at 1.20 L/min. Thus, a flow rate of 20 L/min was maintained to keep the plasma stable. By default, the pulse pattern was specified as one point per mass at the maximum peak for the best mass identification. Later lens voltage was lowered down from 12 to 6 V to enable the appropriate functioning of ions and put the pulse stage voltage on 950 V in order to enhance the ion recognition capacity. The peak hop acquisition mode was opted to scan multiple masses, but the

Table 3: Instrument operating conditions

S. No.	*Components*	*Details*
1.	ICP RF power	1100 W
2.	Sweeps/Reading	8
3.	Sample uptake rate mL/ min	2
4.	Pulse pattern	One point per mass at maximum peak
5.	Lens voltage	6.00 V
6.	Auxillary gas flow	1.20 L/min
7.	Dwell time	50 ms
8.	Argon plasma gas flow	15 L/min
9.	Acquisition mode	Peak hop
10.	Reading/replicates	1
11.	Rinse solution (v/v)	3% HNO_3
12.	Nebulizer gas flow	0.90 L/min
13.	Rinse time	180s
14.	Number of runs	6
15.	Pulse stage voltage	950 V

Table 4: Number of heavy metals detected in samples (µg/kg)

Elements	*B-TA*	*G-TA*	*A-TA*	*B-SO*	*G-SO*	*A-SO*
Arsenic	10.13 ± 1.8	9.749 ± 1.6	11.829 ± 2.1	18.342 ± 4.1	10.64 ± 1.7	19.458 ± 4.2
Zinc	3.086 ± 0.67	2.937 ± 0.52	7.442 ± 1.6	8.467 ± 1.5	8.793 ± 1.4	5.02 ± 1.3
Lead	1.572 ± 0.31	1.622 ± 0.3	3.276 ± 0.54	5.488 ± 1.3	4.455 ± 1.1	3.255 ± 0.9
Nickel	1.148 ± 0.22	0.948 ± 0.24	0.571 ± 0.18	1.061 ± 0.5	0.526 ± 0.6	1.252 ± 0.4
Cadmium	0.643 ± 0.12	0.582 ± 0.11	0.236 ± 0.1	0.927 ± 0.34	0.348 ± 0.12	0.776 ± 0.3

identification was done for the required ones. In the reading/replicates field, input 1 understands to perform the analysis a single time for each sample before proceeding to the next one. In between analyses, 3% HNO_3 (v/v) was used to rinse the solution for cleaning the system and the time lapse was 180 seconds. The experiments were repeated six times so that consistent results could be obtained (Moldovan *et al.*, 2004).

Results

All the samples analyzed from both plant species were found to contain detectable levels of five different heavy metals. These heavy metals include cadmium (Cd), arsenic (As), zinc (Zn), lead (Pb), and nickel (Ni). This investigation provides insights for the accumulation of heavy metal in the plants grown in the environment near sugar mills. Details of a detectable number of heavy metals found in plants are presented in Table 4.

From Table 4, it is seen that the concentration of arsenic is found to be high, followed by zinc (Zn), lead (Pb), nickel (Ni) and cadmium (Cd) in the plants. The accumulation of As is found to be high in the *S. oleracea* plant (19.45 ug/kg) as compared to *T. alexandrinum,* i.e., 11.8 µg/kg. The *S. oleracea* species collected from Bahraich accumulates high levels of trace elements, which can be attributed to the selective absorption of heavy metals from the soil and their subsequent movement within the plant. *S. oleracea* generally exhibited higher metal concentrations compared to *T. alexandrinum* (Figure 1).

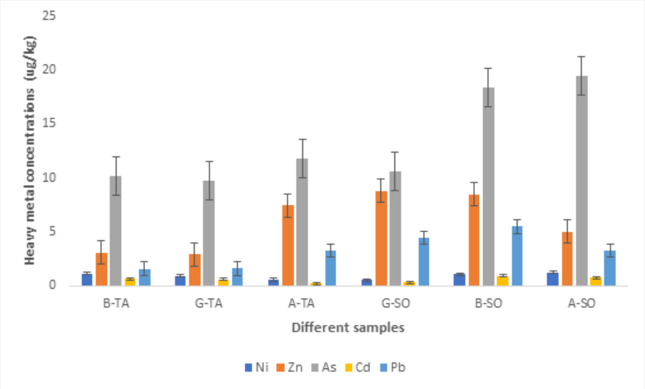

Figure 1: Comparative concentrations of arsenic (As), cadmium (Cd), zinc (Zn), lead (Pb) and nickel (Ni) in two different plant species sampled from 3 different locations

The *S. oleracea* sample has shown the highest degree of contamination of As, with a potential concentration of up to 19. 45 µg/kg. The concentration of Zn in analyzed samples was found to be 2 and 9 µg/kg. Zinc, as a micronutrient, is vital in the plant's development, the formation of different structures in the plant and the advection of various enzymes. The concentration of nickel (Ni) was also detected in very small concentrations, ranging from 0. 9 to 2 µg/kg (Figure 1). The levels of cadmium (Cd) ranged from 0. 2 to 1. 0 µg/kg, and lead (Pb) content ranged from 1. 0 and 6. 0 µg/kg. Thus, the differences in the identified heavy metal concentrations add to the concerns regarding heavy metal pollution of the environment.

Discussion

Research on the presence and buildup of heavy metal contaminants in different aspects of the environment have emerged as important issues of worldwide interest (Zwolak *et al.*, 2019; K Mandal *et al.*, 2023). Taken together, it could be concluded that the present study has brought ample evidence of the fact that the levels of metals are disparate in two different plants, namely, *S. oleracea* and *T. alexandrinum*. Consequently, the study leads to the knowledge of the fact that there are relatively higher levels of trace elements in the *S. oleracea* plant than in the *Trifolium* plant. Besides this, there could be many reasons that the *S. oleracea* plant contains a higher concentration of trace elements than those present in *T. alexandrium*. It could be one of the plausible reasons that leafy plants and all green vegetables, especially the leafy ones compared to fruits, vegetables exhibit higher growth rates and transpiration rates (Chetan *et al.*, 2012). It is also reported that increase in the rate of accumulation and translocation of metals from the roots to the shoot levels by using improved root-to-shoot mobility (S. Singh *et al.*, 2012). Most vegetables have larger leaves to carter for this area thus more likely to get contaminated by either rainwater or dust from the soil.

Three of the areas under consideration are Ayodhya, Gonda and Bahraich of Uttar Pradesh in India. Though physio-graphically and climatically all these areas differ scanty variations in the trace elements in plants. It may be detected because of differences in the microclimate, which exist due to some dominant influential factors like industrialization, farming, mining, construction, and transportation (Dong H *et al*., 2023; Hongbo Liu *et al*., 2021). Lack of zinc in the human body has several consequences for the organism. It can negatively affect the immune function, slow down the body's ability to heal, and inhibit DNA synthesis (Prasad *et al*., 2000). However, consuming high levels of zinc can also lead to side effects such as nausea, stomach upsets, and vomiting and can interfere with copper absorption. Healthy amounts of zinc intake, whether from food or supplements, are important. Despite the daily need for nickel being relatively low, it is still necessary for humans. Increased levels of nickel can have adverse effects on health, including causing skin disorders, allergies, and respiratory problems (Plum *et al*., 2010).

In animals, cadmium toxicity symptoms include kidney and bone abnormalities and cardiovascular diseases after prolonged exposure to large amounts of cadmium (Genchi *et al*., 2020). Cadmium also negatively affects plant growth and the quality of crops, highlighting its impact on agriculture. Excessive lead (Pb) reduces organ growth, mainly inhibiting root growth by decreasing cell division and elongation. Small animals used in laboratories are particularly affected by high lead consumption, leading to disorders of the hematopoietic system, nervous system, or sexual organs, causing acute and chronic poisoning (Sharma *et al*., 2005). In general, the harmful impacts of these heavy metals emphasize the significance of regulating and controlling these metals in the environment to ensure the safety of people's health and ecosystems. It is important to consider these aspects, as they contribute to soil and water pollution, hindering plant growth and reducing food safety.

Conclusion

These findings led us to identify a variation in the number of heavy metals in clover and spinach from the three sampled regions of Bahraich, Gonda and Ayodhya. Therefore, out of all the heavy metals detected in the tissues of the studied plant, it was possible to identify arsenic, cadmium, lead, nickel, and zinc. Altogether, the current study revealed that plants sampled close to the sugar mills had generally higher heavy metal concentrations to the extent that they may have been affected by the emissions or associated sources of sugar mills. Further, it was also noted that heavy metal concentrations also varied at the study area level, which might indicate local factors such as typology of the soil, type of land use and point or diffuse sources of contamination. In light of the study's previous findings, it is evident that additional attention should be focused on monitoring and managing the levels of heavy metals in nearby agricultural areas impacted by sugar mills, especially the threatening levels of contaminants affecting both the ecosystem and the safety of food. Possible risks of direct human intake of arsenic, cadmium, and lead through contaminated food, in particular edible plants, are associated with the presence of heavy metals in *S. oleracea*. Appropriate measures are warranted to control the heavy metal contamination in these areas like emission controls from sugar mills, better ways of handling wastes, and favorable management of farming activities. Thus, more concerted work should be required to reveal the mechanisms of heavy metal accumulation in plant tissues and the effects of long-term intake of food products with polluted soil as a source. Altogether, this analysis enhances the knowledge regarding the level of heavy metal contamination in the agricultural areas close to sugar mills in Bahraich, Gonda, and Ayodhya and underlines the necessity for more vigorous work to control this ecological problem and protect the population's health.

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References

Ahmadi, F., Samadi, A., & Rahimi, A. (2020). Improving growth properties and phytochemical compounds of Echinacea purpurea (L.) medicinal plant using novel nitrogen slow release fertilizer under greenhouse conditions. Scientific Reports, 10(1), 13842. https://doi.org/10.1038/s41598-020-70949-4

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics, 9(3), 42. https://doi.org/10.3390/toxics9030042

Ali, H., Naseer, M., & Sajad, M. A. (2012). Phytoremediation of heavy metals by *Trifolium alexandrinum*. International Journal of Environmental Sciences, 2(3), 1459–1469. https://doi.org/doi:10.6088/ijes.00202030031

Balakrishna, M., & Seetharm, P. (2021). Qualitative and Quantitative Evaluation of Trace Elements in Amaranthaceae Family Medicinal Plant Using ICP-MS. International Journal of Current Research and Reviews, 13(5), 58–65. http://dx.doi.org/10.31782/IJCRR.2021.13521

Bhatti, S. S., Sambyal, V., & Nagpal, A. K. (2016). Heavy metals bioaccumulation in Berseem (*Trifolium alexandrinum*) cultivated in areas under intensive agriculture, Punjab, India. SpringerPlus, 5(1), 173. https://doi.org/10.1186/s40064-016-1777-5

Chetan, A., & Ami, P. (2015). Effects of heavy metals (Cu and Cd) on growth of leafy vegetables–*Spinacia oleracea* and Amaranthus caudatus. International Research Journal of Environment Sciences, 4(6), 63.

Daneshnia, F., Amini, A., & Chaichi, M. R. (2016). Berseem clover quality and basil essential oil yield in intercropping system under limited irrigation treatments with surfactant. Agricultural Water Management, 164, 331–339. https://doi.org/10.1016/j.agwat.2015.10.036

Das, S. (2016). Amaranths: The Crop of Great Prospect. In S. Das, Amaranthus: A Promising Crop of Future (pp. 13–48). Springer Singapore. https://doi.org/10.1007/978-981-10-1469-7_3

Ding, Z., Li, Y., Sun, Q., & Zhang, H. (2018). Trace elements in soils and selected agricultural plants in the Tongling mining area of China. International Journal of Environmental Research and Public Health, 15(2), 202. https://doi.org/; https://doi.

org/10.3390/ijerph15020202

Dong, H., Gao, Z., Liu, J., & Jiang, B. (2023). Study on the Accumulation of Heavy Metals in Different Soil-Crop Systems and Ecological Risk Assessment: A Case Study of Jiao River Basin. Agronomy, 13(9), 2238. https://doi.org/10.3390/agronomy13092238

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences, 2(5), 112–118.

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. International Journal of Environmental Research and Public Health, 17(11), 3782. https://doi.org/10.3390/ijerph17113782

Houston, M. C. (2007). The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. Altern Ther Health Med, 13(2), S128–S133.

Jan, A. T., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, Q. M. R. (2015). Heavy metals and human health: Mechanistic insight into toxicity and counter defense system of antioxidants. *International Journal of Molecular Sciences, 16*(12), 29592–29630. https://doi.org/10.3390/ijms161226183

Khan, A., Khan, S., Khan, M. A., Qamar, Z., & Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: A review. *Environmental Science and Pollution Research, 22*, 13772–13799. https://doi.org/10.1007/s11356-015-4881-0

Khanduri, J., Sinha, V. B., Rautela, I., Rayal, R., & Dev, M. (2021). Fluctuation in Germination on Exposure to Salt Stress in Trifolium Alexandrium L. in Cultivars. J. Mountain Res, 16(3), 49–56. https://doi.org/: https://doi.org/10.51220/jmr.v16i3.6

Kidd, P., Barcel´o, J., Pilar Bernal, M., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R., Monterroso, C. (2009). Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ. Exp. Bot. 67, 243–259. https://doi.org/10.1016/j.envexpbot.2009.06.013.

Kim, Y., Ha, E.-H., Park, H., Ha, M., Kim, Y., Hong, Y.-C., Kim, E.-J., & Kim, B.-N. (2013). Prenatal lead and cadmium co-exposure and infant neurodevelopment at 6 months of age: The Mothers and Children's Environmental Health (MOCEH) study. Neurotoxicology, 35, 15–22. https://doi.org/10.1016/j.neuro.2012.11.006

Kumar, V., & Chopra, A. K. (2010). Influence of sugar mill effluent on physico-chemical characteristics of soil at Haridwar (Uttarakhand), India. Journal of Applied and Natural Science, 2(2), 269–279. https://doi.org/10.31018/jans.v2i2.134

Liang, G., Gong, W., Li, B., Zuo, J., Pan, L., & Liu, X. (2019). Analysis of heavy metals in foodstuffs and an assessment of the health risks to the general public via consumption in Beijing, China. International Journal of Environmental Research and Public Health, 16(6), 909. https://doi.org/10.3390/ijerph16060909

Mandal, K., & Dhal, N. K. (2023). Bioaccumulation of industrial heavy metals and interactive biochemical effects on two tropical medicinal plant species. Environmental Science and Pollution Research, 30(15), 43860–43871. https://doi.org/10.1007/s11356-023-25396-9

Mawari, G., Kumar, N., Sarkar, S., Daga, M. K., Singh, M. M., Joshi, T. K., & Khan, N. A. (2022). Heavy Metal Accumulation in Fruits and Vegetables and Human Health Risk Assessment: Findings From Maharashtra, India. Environmental Health Insights, 16, 117863022211191. https://doi.org/10.1177/11786302221119151

Moldovan, M., Krupp, E. M., Holliday, A. E., & Donard, O. F. (2004). High resolution sector field ICP-MS and multicollector ICP-MS as tools for trace metal speciation in environmental studies: A review. Journal of Analytical Atomic Spectrometry, 19(7), 815–822. https://doi.org/10.1039/B403128H

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. The International Journal of Biochemistry & Cell Biology, 41(8–9), 1665–1677.

Plum, L. M., Rink, L., & Haase, H. (2010). The essential toxin: Impact of zinc on human health. International Journal of Environmental Research and Public Health, 7(4), 1342–1365. https://doi.org/10.3390/ijerph7041342

Prasad, A. S. (2000). Effects of zinc deficiency on immune functions. The Journal of Trace Elements in Experimental Medicine, 13(1), 1–20. https://doi.org/10.1002/(SICI)1520-670X(2000)13:1<1::AID-JTRA3>3.0.CO;2-2

Pröfrock, D., & Prange, A. (2012). Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) for Quantitative Analysis in Environmental and Life Sciences: A Review of Challenges, Solutions, and Trends. Applied Spectroscopy, 66(8), 843–868. https://doi.org/10.1366/12-06681

Rajkumar, M., Prasad, M.N.V., Swaminathan, S., Freitas, H., 2013. Climate change driven plant–metal–microbe interactions. Environ. Int. 53, 74–86. https://doi.org/ 10.1016/j.envint.2012.12.009.

Reuben, A., Caspi, A., Belsky, D. W., Broadbent, J., Harrington, H., Sugden, K., Houts, R. M., Ramrakha, S., Poulton, R., & Moffitt, T. E. (2017). Association of childhood blood lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. Jama, 317(12), 1244–1251. https://doi:10.1001/jama.2017.1712

Rinaldi, M., Micali, A., Marini, H., Adamo, E. B., Puzzolo, D., Pisani, A., Trichilo, V., Altavilla, D., Squadrito, F., & Minutoli, L. (2017). Cadmium, organ toxicity and therapeutic approaches: A review on brain, kidney and testis damage. Current Medicinal Chemistry, 24(35), 3879–3893. https://doi.org/10.2174/0929867324666170801101448

Rodríguez, J., & Mandalunis, P. M. (2018). A Review of Metal Exposure and Its Effects on Bone Health. Journal of Toxicology, 2018, 1–11. https://doi.org/10.1155/2018/4854152

Saranraj, P., & Stella, D. (2014). Impact of sugar mill effluent to environment and bioremediation: A review. World Applied Sciences Journal, 30(3), 299–316. https://doi.org/DOI: 10.5829/idosi.wasj.2014.30.03.1656

Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. Brazilian Journal of Plant Physiology, 17, 35–52. https://doi.org/10.1590/S1677-04202005000100004

Singh, S., Zacharias, M., Kalpana, S., & Mishra, S. (2012). Heavy metals accumulation and distribution pattern in different vegetable crops. Journal of Environmental Chemistry and Ecotoxicology, 4(10), 170–177. https://doi.org/DOI: 10.5897/JECE11.076

Stern, B. R. (2010). Essentiality and Toxicity in Copper Health Risk Assessment: Overview, Update and Regulatory Considerations. Journal of Toxicology and Environmental Health, Part A, 73(2–3), 114–127. https://doi.

org/10.1080/15287390903337100

Traudt, E. M., Ranville, J. F., & Meyer, J. S. (2017). Effect of age on acute toxicity of cadmium, copper, nickel, and zinc in individual-metal exposures to Daphnia magna neonates. Environmental Toxicology and Chemistry, 36(1), 113–119. https://doi.org/10.1002/etc.3507

Uddin, M.M.; Zakeel, M.C.M.; Zavahir, J.S.; Marikar, F.M.M.T.; Jahan, I. (2021) Heavy metal accumulation in rice and aquatic plants used as human food: a general review. Toxics 9, 360. https://doi.org/10.3390/toxics9120360

Vijayalakshmi, S., & Kripa, K. G. (2018). Heavy metal analysis of blepharis maderaspatensis (L.) heyne ex roth. Asian J Pharm Clin Res, 11(10), 251–253. http://dx.doi.org/10.22159/ajpcr.2018.v11i10.26418

Weidong Yang, Di Liu, Yuyan Wang, Bilal Hussain, Fengliang Zhao, Zheli Ding, Xiaoe Yang, Zhiqiang Zhu, Muhammad Dawood (2021). Variations in phytoremediation potential and phytoavailability of heavy metals in different *Salix* genotypes subjected to seasonal flooding. Journal of Environmental Management 299, 113632.

Yadav, R. K., Minhas, P. S., Lal, K., Chaturvedi, R. K., Yadav, G., & Verma, T. P. (2015). Accumulation of metals in soils, groundwater and edible parts of crops grown under long-term irrigation with sewage mixed industrial effluents. Bulletin of Environmental Contamination and Toxicology, 95, 200–206. https://doi.org/10.1007/s00128-015-1547-z

Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: A Review. Water, Air, & Soil Pollution, 230(7), 164. https://doi.org/10.1007/s11270-019-4221-y