

A Study on Phytoremediation of Heavy Metal Contaminated Soil through *Abelmoschus Esculentus* L.

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Abstract-Heavy metal contamination is a serious threat to soil and water resources as well as human health. Phytoremediation is a promising technique to reclaim contaminated soil and water. A pot experiment was conducted where *Abelmoschus esculentus* L. (okra) was grown in heavy metal (Ni and Cr) contaminated soil at concentrations of 50 mg kg⁻¹ and 100 mg kg⁻¹. After 60 days of the experiment, the plants were harvested and reduction of metal in soil, metal uptake and accumulation patterns in test plants were investigated. Effects of Ni and Cr on plant growth and physiology (Chlorophyll and proline content) were also assessed. The reduction percentage of Ni and Cr in soil was in the order of Cr 50 (2.32%) > Ni 50 (1.73%) > Cr 100 (1.24%) > Ni 100 (1.13%). The uptake of Ni and Cr was more in roots as compared to the shoots. The translocation of metals from root to shoot increased with increasing concentration. Due to addition of Ni and Cr in soil, inhibition in length and biomass of shoot and root was observed in comparison to the control. The concentration of chl a, chl b and total chlorophyll decreased with increasing metal concentration in soil, The proline content was seen to increase with increasing metal dosage. The findings suggest that *A. esculentus* holds potential for phytoremediation (phytostabilization) to reduce heavy metal contamination (Ni and Cr) in soil.

Key words: Phytostabilization, Phytoextraction, Heavy metal accumulation, Bioabsorption coefficient, RCF and Translocation factor.

Introduction

Anthropogenic activities including industrialization, mining, smelting, chemical fertilizers, pesticides, burning of fossil fuels, municipal garbage, and pollution around the world have been rising

dramatically since the turn of the 20th century (Ozyigit *et al.*, 2022). In order to meet the high demands of the growing population, agricultural and industrial output has expanded manifold, resulting in addition of significant amounts of solid, liquid, and gaseous waste to the environment (Nkoh *et al.*, 2022). Soils have been contaminated with a variety of contaminants, such as heavy metals, pesticides, fertilizers (Kumari *et al.*, 2022) and microplastics (Iqbal *et al.*, 2023) which further accumulate in soil biota, crop plants and endanger human health (Li *et al.*, 2023).

Heavy metals pose a major threat to ecology, agriculture, and natural fauna (Iqbal *et al.*, 2023). Some of these are essential for plants but many are not required at all. Plants require vital heavy metals, like as copper, zinc, chromium, iron, nickel, and manganese for proper growth and biological activities (Asgari Lajayer *et al.*, 2019). These play a vital role in both plants and animals due to their redox properties, chemical coordination, and maintenance of regulatory processes like homeostasis, degradation, transport, and binding to target cells (Khan *et al.*, 2019). Heavy metals that aren't necessarily dangerous but have an impact on plants are cadmium, lead, mercury, and arsenic (Ahmad *et al.*, 2023). Such metals are well-known pollutants due to their toxicity, persistence, bio-accumulative nature, and lack of biodegradability (Usman, 2022; Jehan *et al.*, 2021). Heavy metal accumulation in the plants can affect chemical and physiological processes, which can lead to chlorosis, growth suppression, yield decrease, minimal nutrient absorption, root browning, and plant mortality (Khan *et al.*, 2023).

Physical remediation such as surface capping, landfilling, and encapsulation; chemical remediation, such as stabilization, solidification, soil flushing, soil washing, and electro kinetics; and bioremediation,

such as the use of plants and microorganisms, have all been used to remove heavy metals from contaminated sites (Li *et al.*, 2019). Metal toxicity, soil type, soil conditions, cost, and ultimate use of the land all have a considerable impact on the applications of these techniques (Priya *et al.*, 2023). Physical and chemical remediation techniques used to remove heavy metals from the soil and water are disruptive, expensive, labor-intensive, and lead to further issues (Naeem *et al.*, 2023).

Phytoremediation is the use of plants to absorb heavy metals and minimize their dangerous effects on the environment (Bhat *et al.*, 2022). It is an effective and affordable technology to clean-up contaminated soil and water by remove inactive metals and metal pollutants (Ugwuechendu *et al.*, 2024). Phytoremediation can be done in several ways, including Phytodesalinization, phytodegradation, phytoextraction, Phytofiltration, and phytovolatilization (Anum *et al.*, 2022). Several phytoremediator plants including vegetable crops have been studied in the last decades to determine their phytoremediation potential. For instance, *Hopea odorata* (Arifin *et al.*, 2012), *Lycopersicon esculentum*, *Rumex acetosa*, and *Solanum melongena* (Yashim *et al.*, 2014), *Acacia pycnantha* and *Eucalyptus camaldulensis* (Nirola *et al.*, 2015), *Ipomoea aquatica*, *Abelmoschus esculentus*, *Amaranthus dubius*, *Hibiscus sabdariffa* and *Telforia occidentalis* (Ng *et al.*, 2016; Hassan *et al.*, 2018; Akpokodje and Uguru, 2019), *Artemisia alba* (Turra (Branković *et al.*, 2019), *Tetraena qataranse* (Usman *et al.*, 2019), *Pteris vittata*, *Epipremnum aureum*, *Mucuna bracteata* and *Imperata cylindrica* (Tang and Angela, 2019), and *Pisum sativum* (Galal *et al.*, 2021) have all been assessed under different spike conditions of several concentrations of heavy metals.

Abelmoschus esculentus (L.) Monench (Okra) is an economically important vegetable crop cultivated in many countries such as India, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Myanmar, Pakistan, Thailand, Malaysia, Brazil, Ethiopia, Cyprus, Ghana and the Southern United States (FAOSTAT, 2008; Omokaro *et al.*, 2023). It is a widely grown, fast-growing vegetable. The part used are its fruits which are consumed boiled, sliced or fried either in fresh or in dried form (Fatokun and Chedda, 1983; Fatin *et al.*, 2021; Omokaro *et al.*, 2023). It has a tender texture which is highly mucilaginous and useful in soup thickening also. Okra seed is rich in proteins

up to 20% and 20% oil (Rao *et al.*, 1991; Fatin *et al.*, 2021). The okra seed flour could also be used to fortify cereal flour (Adelakun, 2009). The plant is a nutritionally important summer vegetable and an excellent source of potassium, calcium and unsaturated fatty acids. In vitro fresh watery extract of its seeds restricts growth of cancerous cells (Khare, 2007). Okra plant has potential to uptake heavy metal from crude oil-contaminated soil. Thus, Okra can clean up contaminated soil as a cost-effective and environmentally friendly method (Ugwuechendu *et al.*, 2024).

The aim of the study was to assess the phytoremediation potential of *Abelmoschus esculentus* and characterize its uptake and accumulation of nickel and chromium. The effects of the two heavy metals on growth and physiology of the plant were also assessed.

Materials and Methods

Pots were prepared with 5 kg garden soil. Seeds of *A. esculentus* (F⁻¹ Hybrid Indravati Plus) were procured from National Seed Corporation, Sikandra, Agra (U.P.). Metals were applied to the pots in aqueous solution as NiCl₂ and CrCl₃.6H₂O. Uniform seeds were planted in triplicate in the pots. Control pots were set up with test plant without any metal treatment.

The pots were kept in random block design and watered as and when required in such a way to prevent loss of contaminants by leaching. The study was conducted in green house for 60 days. At 60 DAT (days after treatment) three replicates of each treatment were taken apart for metal analysis of soil/stem/root. Soil samples were homogenized, air dried, passed through 20 mm mesh sieve and stored in clean zip lock polythene bags in a refrigerator until further analysis. Plant samples were first washed with distilled water to remove adhering mud and further washed with deionized water 3-4 times and dried at room temperature in a dust free chamber. Soil/plant samples (0.5g) were digested using wet digestion method with 10 ml aqua regia. Ni and Cr content in samples was estimated by Atomic Absorption Spectrophotometer (ANALYST 100, Perkin Elmer, USA).

Chlorophyll (Arnon, 1949) and proline (Bates *et al.*, 1973) were also estimated in test plants to assess stress due to Ni and Cr. Growth parameters i.e., shoot and root length and biomass (dry) were measured at end of study. Data is expressed as mean of three values + standard deviation.

Result and Discussion

Table 1: Reduction in Ni and Cr content in soil

Treatment	Ni content (mg kg ⁻¹)	
	Initial	Final
50 mg kg ⁻¹	46.1±0.78	45.3±0.35
	97.2±1.23	96.1±2.14
Cr content (mg kg ⁻¹)		
50 mg kg ⁻¹	47.3±4.55	46.2±3.07
	96.3±3.81	95.1±2.46

Reduction in Ni and Cr content in soil

Percent reduction of Ni and Cr content in soil of both treatments at final day of study (60 DAT) is presented in Table 1. The reduction percentage of Ni and Cr in soil was in the order Cr 50 > Ni 50 > Cr 100 > Ni 100. Metal reduction % in soil was 1.73% and 1.13% for Ni 50 and 100 treatments, and 2.32% and 1.24% for Cr 50 and 100 treatments. Thus, *A. esculentus* seems to be more effective at absorbing Ni and Cr at lower concentrations. Malik *et al.* (2017) also found greater reduction in soil metal content at lower doses of Cd (25 mg kg⁻¹) and Pb (50 mg kg⁻¹) compared to their higher doses at 50 and 100 mg kg⁻¹, respectively.

Table 2: Heavy metal uptake in *A. esculentus* at final day of experiment

Treatment	Ni	
	Shoot	Root
50 mg kg ⁻¹	23.35±2.25	30.29±1.99
100 mg kg ⁻¹	20.3±1.5	21.52±2.13
Cr		
50 mg kg ⁻¹	13.4±2.2	19.1±1.6
100 mg kg ⁻¹	22.3±3.4	28.1±2.5

Heavy metal uptake in *A. esculentus*

Metal uptake in roots and shoots of *A. esculentus* is presented in Table 2. At lower dose of Ni (50 mg kg⁻¹), uptake of Ni 30.29 mg kg⁻¹ in roots and 23.35 mg kg⁻¹ in shoots was observed at the end of the 60 day study. For Cr the corresponding readings are 19.1 and 13.4 mg kg⁻¹ in root and shoot, respectively shoots. Thus, both Ni and Cr were preferably partitioned in the roots. This trend was also true for the higher dose of metals. However, at the higher dose Ni uptake decreased while that of Cr as seen to

increase. Thus, uptake of Ni decreased with increasing metal concentration in soil, while Cr uptake increased in proportion to metal concentration.

Heavy metal accumulation characteristics in *A. esculentus*

To evaluate the phytoremedial potential of the plant, metal accumulation characteristics like BAC (Bioabsorption coefficient), RCF (Root concentration factor) and TF (Translocation factor) were used. These are simple ratios of metal concentrations viz.—BAC (shoot/soil); RCF (root/soil); and TF (shoot/root). Plants with RCF >1 and TF <1 indicate the capability of roots to accumulate greater metal content from substrate and such plants are considered suitable for phytostabilization (Briggs *et al.*, 1983; Polder *et al.*, 1995). On the other hand plants exhibiting both TF and BAC values >1 are generally considered promising metal Phytoextractors (Fitz and Wenzel, 2002). Higher TF values indicate more allocation of absorbed metal to aboveground biomass. Ni and Cr accumulation characteristics for the test plants at 60 DAT are presented in Table 3.

Table 3: Heavy metal accumulation characteristics of *A. esculentus* at final day

	50 mg kg ⁻¹	100 mg kg ⁻¹
Ni		
BAC	0.51	0.21
TF	0.77	0.94
RCF	0.66	0.22
Cr		
BAC	0.29	0.23
TF	0.70	0.79
RCF	0.41	0.29

BAC, TF and RCF values for both doses of Ni and Cr are <1. The TF values also suggest that the translocation of Ni and Cr from root to shoot increased with increasing concentration in soil. The ratios reiterate that the test plant did in fact sequester greater amounts of the metals in roots. This does not indicate a textbook fit in either category of phytoremediation as described above; however, a second look at the data in table 3 indicated that the test plant does hold promise for

phytostabilization of the two metals studied, more so for Ni. It must be kept in mind that phytoremedial potential is a function of both metal accumulation in the plant's tissues as well as its biomass. In the latter, our test plant is better than many hyperaccumulators.

Previously several researchers investigated metal accumulation potential of *A. esculentus*. Motesharezadeh and Savaghebi (2012) reported metal accumulation potential of okra with higher amounts of Cd compared to Pb in roots specifying the higher absorption and ability of dissolution of Cd. Subhashini (2013) revealed higher accumulation of Pb in roots compared to leaves and stems of *Catharanthus roseus*. Hassan *et al.* (2018) reported better accumulation of Zn in okra compared to rosselle and spinach.

Effect of Heavy metals on Physiology of *A. esculentus*

Chlorophyll content

At the end of study period, it was found that chlorophyll 'a', chlorophyll 'b' and total chlorophyll content decreased in all plants treated with Ni and Cr treatment when compared to the control, as seen in Figures 1 and 2. Chlorophyll 'a' content in control was 1.98 mg kg⁻¹ fresh weight. In Ni treatments, this value fell to 1.66 mg kg⁻¹ f. wt. in a dose dependent manner while in Cr treatments chlorophyll 'a' was seen to be lesser at lower dose and slightly greater (1.70 mg kg⁻¹ f. wt.) than that of control at higher dose.

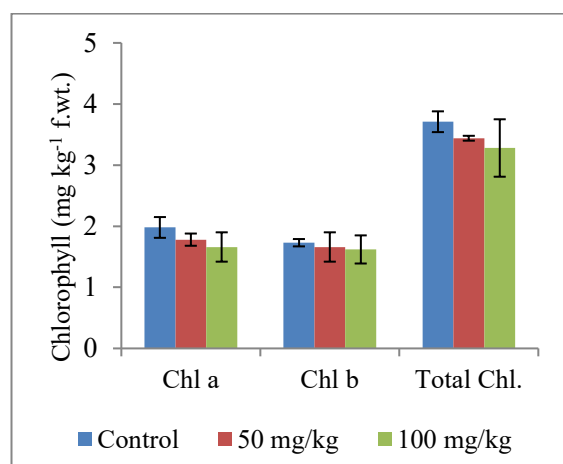


Figure 1: Effect of Ni on chlorophyll concentration of *A. esculentus* leaf

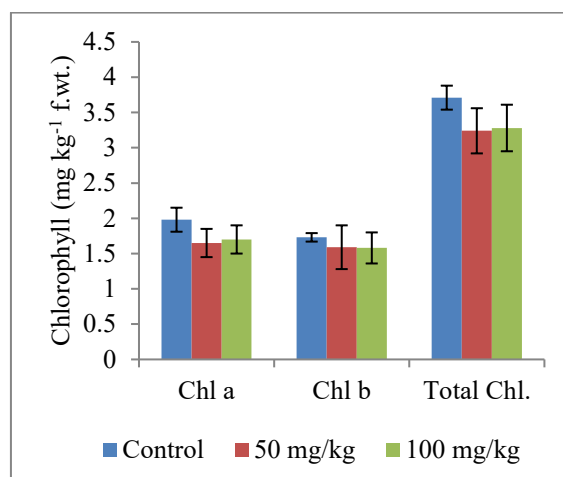


Figure 2: Effect of Cr on chlorophyll concentration of *A. esculentus* leaf

Cr was slightly more phytotoxic to chlorophyll 'b' content than Ni at both concentrations. The chlorophyll 'b' content in control was 1.73 mg kg⁻¹ f. wt. which fell to 1.62 mg kg⁻¹ f. wt. in Ni treatment at higher dose. However in both Cr treatments chl 'b' values recorded were 1.59 mg kg⁻¹ f. wt.

Total chlorophyll content in *A. esculentus* control was 3.71 mg kg⁻¹ f. wt. which was reduced to 3.28 mg kg⁻¹ f. wt at 100 mg Ni kg⁻¹ in soil in a dose dependent fashion. At 50 mg Cr kg⁻¹ in soil total chlorophyll content was 3.24 mg kg⁻¹ f.wt. which increased slightly to 3.28 mg kg⁻¹ f. wt. in response 100 mg Cr kg⁻¹. Overall, total chlorophyll was also reduced due to metals in soil.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990). In various investigations reduction in chlorophyll content was observed due to contamination of heavy metals in soil as shown in the present study. Batool *et al.* (2014) mentioned that heavy metals like Ni and Cd influence morphological and physiological characters and causing a reduction in plant growth parameters. Al-Janabi *et al.* (2019) reported a reduction in chlorophyll content in *Typha domingensis* in the presence of the Ni, Zn, Mn and Co based on metal concentrations in substrate. Zengin and Munzuroglu (2005) recorded progressive decrease in total chlorophyll of *Phaseolus vulgaris* with increasing concentrations of Cu, Hg, Cd, and Pb.

Proline content

As shown in Figures 3 and 4, all treatments showed higher level of proline in comparison to control. Cr was seen to be more phytotoxic than the Ni. Proline content in control plants was $0.17 \mu\text{g g}^{-1}$ f.wt. which increased to $0.25 \mu\text{g g}^{-1}$ f.wt. at $100 \text{ mg Ni kg}^{-1}$ in substrate in proportion to Ni content. A similar trend was observed in Cr treatments with $0.29 \mu\text{g g}^{-1}$ f.wt. proline at $100 \text{ mg Cr kg}^{-1}$ in soil. Even at the lower dose of Cr proline content was more (0.21) than that of Ni (0.19).

Proline is an osmoprotectant that protects the plant under water stress conditions. Malik *et al.* (2017) recorded higher level of proline content in okra plant under Cd and Pb stress. Significant increment in proline content in *Phaseolus vulgaris* was observed by Zengin and Munzuroglu (2005) after ten-day exposure of Cu, Hg, Cd, and Pb. Singh *et al.* (2012) also reported higher proline concentration in *Vigna mungo* under Pb and Ni treatments.

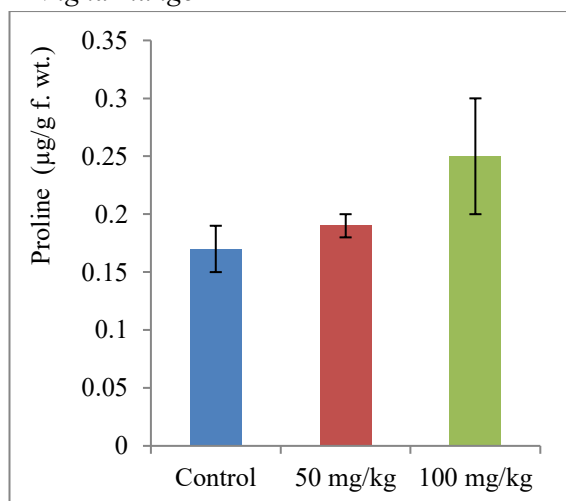


Figure 3: Effect of Ni on proline content in *A. esculentus* leaf

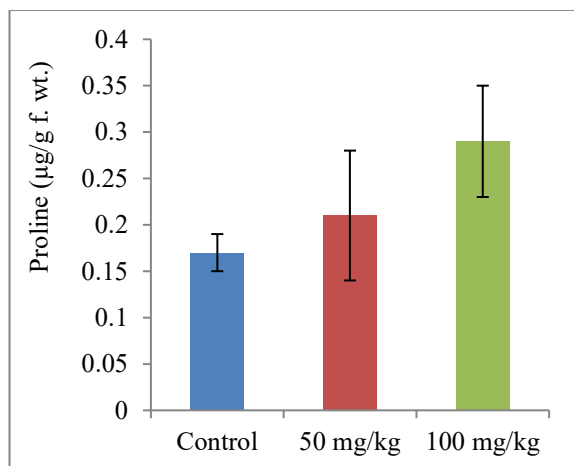


Figure 4: Effect of Cr on proline content in *A. esculentus* leaf

Effect of Heavy metals on Growth of *A. esculentus*

Effect of Heavy metals on shoot and root length

Table-4 shows the shoot and root length of Ni and Cr treated plants respectively. Shoot length of plants treated with Ni was slightly lesser than control value (19 cm). Decrease in shoot length of Cr treated plants was to a slightly greater extent and dose dependent.

The root length of control plants was 13.2 cm which was reduced to 10.5 cm and 11.3 cm in Cr and Ni treatments at 100 mg kg^{-1} . The decrease was dose dependent for both metals.

Table 4: Effect of Heavy metals on Shoot and Root length of *A. esculentus*

Treatment	S. L. (in cm)	R. L. (in cm)
	Ni	
Control	19.0±1.3	13.2±1.3
50 mg kg ⁻¹	18.7±0.9	12.9±0.3
100 mg kg ⁻¹	18.8±1.3	11.3±2.1
Cr		
50 mg kg ⁻¹	18.4±2.0	11.8±1.9
100 mg kg ⁻¹	17.8±0.7	10.5±0.7

Thus, addition of Ni and Cr to the growth matrix was seen to inhibit growth in terms of root and shoot length in all treated plants as compared to control. Cr was more inhibitive of shoot/root length than Ni. A reduction in the length was observed with both shoot and root under Pd and Cd contamination in the study of Malik *et al.* (2017). Le *et al.* (2017) reported that Pb slightly affected root length of *Brassica oleracea* and *A. esculentus* and Cd inhibited the shoot length of *A. esculentus*.

Murugalakshmikumari and Ramasubramanian (2014) reported gradually reduction of shoot length, root length, fresh weight and dry weight of *A. esculentus* with increasing concentration of Pb. Reduction in shoot length, root length, productivity (kg/acre) and number of leaves per individual were noted in okra plants irrigated with industrial wastewater (Ahmed *et al.*, 2022). Galal *et al.* (2021) recorded a reduction in growth parameters of *Cyperus alopecuroides* as a result of the high concentration of heavy metals in irrigation water.

Effect of Heavy metals on shoot and root biomass

Shoot and root dry biomass of treated plants and control are exhibited in Table 5. Deleterious effect of metals in growth substrate of test plants was more pronounced due to Cr. Shoot biomass of control plants was 0.46 g which reduced to 0.38 and 0.41 g in Cr and Ni treatments at higher dose. Root biomass of control plants was 0.19 g which reduced to 0.09g in both Cr and Ni treatments at higher dose. The reduction in both shoot and root biomass was dose dependent.

Table 5: Effect of Heavy metals on Shoot and Root Biomass of *A. esculentus*

Treatment	S. B. (in g)	R. B. (in g)
Ni		
Control	0.46±0.12	0.19±0.05
50 mg kg ⁻¹	0.43±0.13	0.14±0.04
100 mg kg ⁻¹	0.41±0.01	0.09±0.01
Cr		
50 mg kg ⁻¹	0.42±0.16	0.13±0.04
100 mg kg ⁻¹	0.38±0.14	0.09±0.01

Fresh weight of *Brassica oleracea* and *Abelmoschus esculentus* sprouts was decreased upon Pb and Cd exposure with significant reduction of *A. esculentus* fresh weight in Pb incubations (Le *et al.*, 2017). Bautista *et al.* (2013) demonstrated strong impacts of Cd exposure on seed germination, root length and fresh weight of *Lactuca sativa*, *Beta vulgaris* and *Spinacia oleracea* with lesser effects of Cr on the parameters.

Hayat *et al.* (2013) reported reduction in growth parameters like vegetative growth, fresh weight, dry mass of *Cicer arietinum* under Cd stress. Ahmed *et al.*, 2022 showed reduction in fresh and dry leaf weight, stem weight and yield in okra plant irrigated with industrial wastewater. Galal *et al.* (2021) recorded reduction in the biomass and production of *C. alopecuroides* when irrigated with heavy metal contaminated water. Batool *et al.* (2014) also found reduced productivity and biomass in *C. alopecuroides* due to Ni and Cd in irrigation water.

Conclusion

Phytoremediation is a low cost-effective technique that uses the potential of plant to remediate heavy metal pollutant present in soil. This study demonstrates *A. esculentus* L. as a moderate phytostabilizer of Ni and Cr as metal uptake was more in roots as compared to the shoots with low root to shoot transfer ratio. It works more effectively on lower concentration of the heavy metals. Compared to previous research on hyperaccumulators, this work highlights the role of an economically important vegetable crop. Due to metal accumulation, shoot/root length, biomass as well as chlorophyll content of test plant were reduced. Proline concentration increased and possibly helped the plant withstand metal stress. Overall, it can be concluded that *A. esculentus* holds phytoremediation (phytostabilization) potential with scope for further studies.

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