

Phytoremediation Potential of Plants for Heavy Metal Contaminated Soil

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Abstract

This research looks at how some plant species' development and metabolic characteristics are affected by heavy metal pollution in mining soils. Two mining sites provided soil samples, which were compared to control soil from a university campus. The growth, protein, carbohydrate, and proline content of the chosen species—*Sorghum bicolor*, *Vigna mungo*, *Eleusine coracana*, *Brassica juncea*, and *Telfairia occidentalis*—were assessed in a greenhouse environment. The findings showed that plants growing in polluted soils had lower amounts of protein and carbohydrates, and that *Vigna mungo* and *Sorghum bicolor* were more resistant to metal stress. In polluted soils, elevated proline buildup indicated a stress reaction. The research emphasizes the detrimental effects of heavy metal pollution on plant health and raises the possibility that tolerant species might be useful in phytoremediation.

Keywords: Heavy metals, Phytoremediation, *Sorghum bicolor*, *Vigna mungo*, Biochemical analysis, Soil contamination, Protein content, Proline accumulation.

INTRODUCTION

Rapid industrial expansion and urbanization have made the disposal of industrial effluents a critical issue. An additional method of treatment and disposal that has gained popularity recently is the application of municipal and industrial effluents to land. Depending on the business they come from, these effluents might include significant levels of different organic and inorganic compounds, heavy metals, and even helpful plant nutrients. Heavy metal buildup in land and water bodies is a consequence of the careless dumping of untreated or inadequately treated effluents. Peri-urban agriculturally farmed regions are most severely impacted by this issue. Because heavy metals do not break down in the environment, they may linger for a very long time in soil and water. Increased levels of metal buildup in contaminated soils may lead to a decline in soil fertility, microbial activity, and general soil quality, as well as a loss in yield and the introduction of harmful substances into the food chain. While cleaning up polluted places is essential, using environmental remediation techniques may be costly and invasive. Therefore, it's critical to create inexpensive and ecologically friendly techniques. In recent years, there has been a strong push to use plants that accumulate metals for environmental cleanup. This has given rise to the idea of "phytoextraction," which is a subset of the more general term "phytoremediation".

A collection of hazardous metals and metalloids linked to pollution and toxicity is referred to as heavy metals. These elements have atomic weights more than iron's and densities greater than 6 Mg m⁻³. Traditionally, all of the micronutrient cations—iron, manganese, copper, zinc, and nickel—are categorized as heavy metals. Depending on their concentration in plants or other organisms, these metals may cause toxicity or deficiencies. Moreover, lead, cadmium, chromium, mercury, selenium, and arsenic are categorized as heavy metals; yet, only plants and animals—including humans—can be poisonous to these elements. Cadmium, chromium, copper, lead,

mercury, and zinc are the heavy metal pollutants that are most often found.

Phytoremediation uses plants' innate capacity to absorb, collect, and detoxify contaminants to manage heavy metal-contaminated soil in a novel and environmentally friendly way. Significant threats to the environment and human health arise from heavy metal pollution, which is often caused by mining, industrial processes, and agricultural methods that release harmful substances into the soil. These metals, which include copper, zinc, chromium, lead, and cadmium, may have a negative impact on soil health and plant development. If they get into the food chain, they might endanger ecosystems and human health.

When compared to traditional remediation techniques like excavation and chemical treatments, phytoremediation provides a more economical and sustainable option. Some plant species have developed special physiological and biochemical responses that allow them to absorb large amounts of metal stress, store metals in their tissues, and even change them into less harmful forms. This procedure contributes to the restoration of ecological balance in addition to lowering metal concentrations in the soil.

This research examines the phytoremediation capacity of a few chosen plant species in soil polluted with heavy metals, with an emphasis on the biochemical parameters, biomass accumulation, and growth responses of the plants. This study seeks to contribute to the development of practical techniques for the rehabilitation of polluted areas by finding and classifying tolerant species, therefore enhancing agricultural production and environmental sustainability.

1. PLANT SPECIES ASSESSMENT FOR PHYTOREMEDIATION IN HEAVY METAL-CONTAMINATED SOILS

An Appraisal of Plant Species for Phytoremediation in Heavy Metal-Exposed Soils

Phytoremediation is sustainable and environmentally-friendly rehabilitation through the application of natural endogenous capacities of plants to absorb and collect contaminants while detoxifying the contaminates. Eleusine coracana, millet, Brassica juncea, sometimes mustard; Sorghum bicolor, sometimes called sorghum, Vigna mungo, also black gram, and Telfairia occidentalis, pumpkin as well have been selected for this study because of their ability to combat heavy metal pollution.

The variation in the germination of these species was remarkable due to the type of soil applied. In fact, sorghum and black gram continued quite actively growing during forty-five days. It showed that the plants survived fairly well even in the metal-contaminated soils. Only fifteen days of survival was observed in the survival of millet and mustard plants, showing that their tolerance to heavy metal stress is indeed very limited. One of the standout features is that pumpkin seeds did not germinate in mine soils, which suggests a sensitivity to contamination.

In order to assess the phytoremediation potential, parameters of growth that included measurements of root length, shoot length, leaf numbers, and biomass were taken after 15, 30, and 45 days. From the above results, it was inferred that in control soils, the plants grown were of better performance as compared to those grown in contaminated soils. Among the species, it has been identified that the most productive species is sorghum, which showed greater accumulations of biomass as compared with other species cultivated on mine soils.

The investigation has shown that the concentration of heavy metals affects the growth and development of plants. The results indicate that increased concentration of cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) lowered biomass. Several factors, which include reduced uptake of water and osmotic stress, led to poor development under polluted settings.

Generally, this evaluation identifies the requirement for appropriate plant selection that would ensure proper phytoremediation processes are undertaken while focusing on the survival capacity of sorghum in contaminated soils with heavy metals and at the same time allow remediation of such soils.

2. MATERIALS AND METHODS

2.1. Gathering, Preparing, and Characterizing Soil

Two Indian mining sites provided the soil that was gathered. Samples of control dirt were collected from a neighboring university campus. Using a sterile spatula to scrape the top surface, samples were taken at a depth of 20 cm. The soils were collected, allowed to air dry at ambient temperature, and then sieved through a 2 mm screen to get rid of any remaining stones and dust. For additional assessment, the examples were kept aseptically in clean plastic packs.

Physiochemical boundaries like pH, electrical conductivity, KMnO₄-N, Olsen-P, NH₄-Ac-K, and natural carbon were estimated in both test and control soil tests. These investigations were carried out at a soil testing laboratory utilizing conventional operating techniques. A combination of concentrated HCl and HNO₃ (4:1, v/v) was used

to digest 500 mg of soil in order to measure the total metal concentrations in the soil. Prior to analysis, the samples underwent filtering and acidification using HNO₃. The amounts of heavy metals in the acid digests were examined at SAIF, IIT Madras, using flame atomic absorption spectrophotometry (AAS).

2.2. Greenhouse Experiment

Experiments conducted in greenhouses were used to assess the phytoremediation effectiveness of certain plants. Three kilograms of test and control soil were put inside 18 cm breadth by 13 cm long polythene packs in a completely randomized factorial plan with three reiterations. We got the seeds of the accompanying yields from Tamil Nadu Agrarian College (TNAU), Coimbatore: dark gram (*V. mungo*), pumpkin (*T. occidentalis*), mustard (*B. juncea*), sorghum (*S. bicolor*), and millet (*E. coracana*). In the polythene packs with mine and agrarian soil as controls, the seedlings were planted. Every plant in the greenhouse received regular waterings and was exposed to natural light.

2.3. Biochemical Analyses: Photosynthetic Pigment Assay

Utilizing updated techniques, the photosynthetic pigment test was carried out. To get rid of dirt particles, fresh leaves were taken out of the greenhouse and given a tap water wash. 100 mg of leaf samples were crushed in an ice-cold mortar and pestle and combined with 1 milliliter of 80% acetone (v/v). Following homogenization, the materials were centrifuged for 10 minutes at 5000 rpm. Following centrifugation, the supernatant's pigment hue decreased, and its carotenoid and chlorophyll a/b levels were determined. 470, 645, and 663 nm were the absorbance measurements made using a UV-visible spectrophotometer. The amounts of carotenoid, chlorophyll a, and chlorophyll b were calculated using accepted formulae.

2.4. Protein Assay

Utilizing a spotless mortar and pestle and phosphate cradle, 100 mg of leaf tests were gauged and squashed as per the Lowry et al. convention for the protein measure. Bovine serum albumin (BSA) was created as a stock standard arrangement at a grouping of 1 mg/ml. Clean test tubes were loaded up with a few dosages of BSA (0.2, 0.4, 0.6, 0.8, and 1.0 ml), and the last volume was brought to 1 ml utilizing sterile refined water. To test the examples, 0.2 ml was pipetted into sterile test cylinders and afterward weakened with refined water to a last volume of 1 ml. After completely blending five milliliters of scientific reagent into each cylinder, the combination was permitted to sit at room temperature for ten minutes. Each test tube was then loaded up with 0.5 ml of Folin-Ciocalteu reagent, and the blend was hatched for a further 30 minutes. Using a UV spectrophotometer set to detect absorbance at 660 nm, the protein content of the test samples was computed using the relevant formula.

$$\text{Amount of protein in sample} = \text{Concentration of sample} \\ \times \text{Dilution factor} / 1000.$$

2.5. Proline Assay

Utilizing a mortar and pestle that had been recently cleaned, 0.5 g of leaf tissue tests were homogenized in 5 ml of 3% sulfosalicylic corrosive to evaluate the proline focus. To get the unmistakable filtrate required for proline evaluation, the homogenate was gone through Whatman No. 1 channel paper. Two milliliters of the plant remove, frigid acidic corrosive, and ninhydrin reagent were added to clean test tubes. The response blend became block red in the wake of being cooked for an hour at 100°C in a bubbling water shower. Following cooling, the fluid was moved to an isolating pipe and 4 ml of toluene was added. After complete blending, the toluene stage was isolated, and involving toluene as the clear, the absorbance of the isolated stage was estimated at 520 nm utilizing an UV spectrophotometer. A standard curve generated from known proline concentrations was used to assess the free proline content of the samples.

$$\mu \text{ Moles per g tissue} = \mu \text{g proline} / \text{ml of toluene} \times 5 / \\ 115.5 \times \text{sample}.$$

2.6. Statistical Analysis

The information is shown as the average of three duplicates ($\pm$ SD) for every characteristic examined, such as growth, pigments produced by photosynthetic processes, and biochemical tests.

3. RESULT AND DISCUSSION

3.1. Examination of the Soil's Physical Properties and Heavy Metal Content

Table 1 provides an overview of the experimental soils' physical characterisation findings. The test soils were found to be sandy clay loams with low to medium levels of potassium and phosphorus, as well as low to medium

amounts of nitrogen (204 kg/ha and 162 kg/ha in mine soils). Mine soils were found to have an alkaline pH ranging from 8.21 to 8.48, while control soil had a neutral pH of 7.86. Different from typical agricultural soils, higher pH values may make heavy metals more soluble. Control soil had an electrical conductivity (EC) of 0.15, while mine soils had EC values of 0.19 and 0.15, both of which are regarded as safe. The organic carbon level of the Burn & Co. soil was medium, while the Dalmia soil had a low amount. This indicates that metals are more readily absorbed by plants because they are less likely to bond with organic matter.

Table 1: Physiochemical analysis of soil samples from mining waste dumps in Tamil Nadu's Salem district

Sample Details	Parameter	Unit	Value	Interpretation
Control Soil	pH	–	7.86	Normal
	EC	dSm ⁻¹	0.15	Harmless
	KMnO4-N	Kg ha ⁻¹	196	Low
	Olsen-P	Kg ha ⁻¹	20	Medium
	NNNH4-Ac-K	Kg ha ⁻¹	124	Medium
	Organic Carbon	%	0.70	Medium
Dalmia Soil (Test Sample)	pH	–	8.21	Alkaline
	EC	dSm ⁻¹	0.19	Harmless
	KMnO4-N	Kg ha ⁻¹	162	Low
	Olsen-P	Kg ha ⁻¹	20	Medium
	NNNH4-Ac-K	Kg ha ⁻¹	37	Low
	Organic Carbon	%	0.10	Low
Burn & Co Soil (Test Sample)	pH	–	8.48	Alkaline
	EC	dSm ⁻¹	0.15	Harmless
	KMnO4-N	Kg ha ⁻¹	204	Low
	Olsen-P	Kg ha ⁻¹	10	Low
	NNNH4-Ac-K	Kg ha ⁻¹	169	Medium
	Organic Carbon	%	0.65	Medium

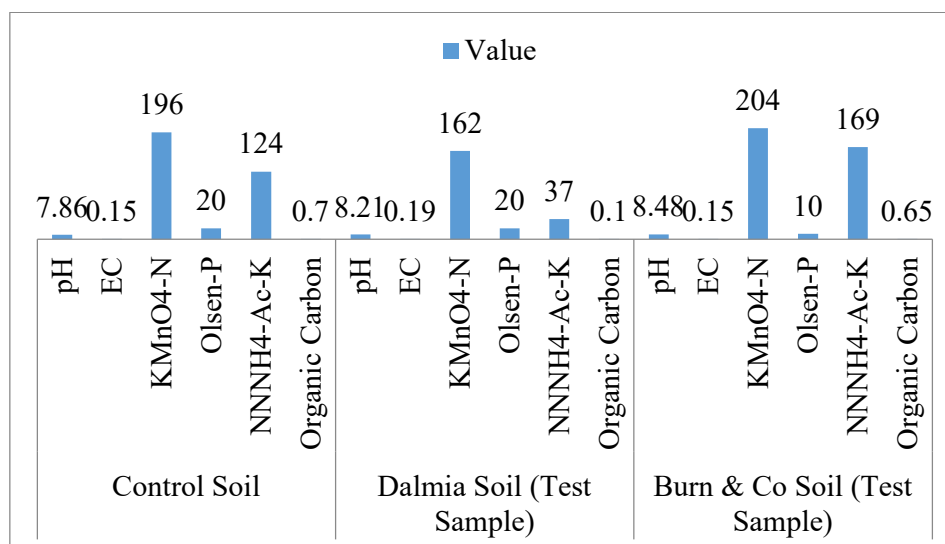


Figure 1: Physiochemical analysis of soil samples from mining waste dumps

After examination, the centralizations of weighty metals (Cd, Cr, Cu, Pb, and Zn) are displayed in Table 2. High amounts of Pb, Zn, and Album have been tracked down in mine tailings and trash, as per prior research. The discoveries showed that the measures of weighty metals (Zn, Cu, and Cr) in mine dirt were more prominent than those in control soil. Coming up next are as far as possible for weighty metals: Zn 5.0 ppm, Pb 0.05 ppm, Cu 0.05 ppm, Cr 0.02 ppm, and Disc 0.005 ppm.

Table 2: Control and mining soils (Dalmia and Burn & Co. soils) were subjected to heavy metal analysis.

Sample Details	Metals	Concentration (mg/L)
Control Soil	Cd	BDL
	Cr	0.012
	Cu	0.018
	Pb	BDL
	Zn	0.022
Dalmia Soil (Test Sample)	Cd	BDL
	Cr	0.176
	Cu	0.043
	Pb	BDL
	Zn	0.149
Burn & Co Soil (Test Sample)	Cd	BDL
	Cr	0.049
	Cu	0.018
	Pb	BDL
	Zn	0.038

3.2. Growth Parameters and Biomass

Eleusine coracana, Brassica juncea, Sorghum bicolor, Vigna mungo, and Telfairia occidentalis were the plant species picked for the examination. The percentage of these plants that germinate in various soil types. After 45 days, sorghum and black gram showed improved growth and showed resistance to soil polluted with metals. On the other hand, pumpkin seeds did not grow in mine soils after 45 days, while millet and mustard seeds barely lived for 15 days. The most direct reactions to heavy metal stress are often alterations in plant development.

In this study, growth metrics and biomass—such as root and shoot length, leaf count, and wet and dry biomass—are measured and the findings are presented at intervals of 15, 30, and 45 days. The biomass and growth characteristics of plants in the control and mining soils differed significantly. In order to assess how effective plants are in phytoremediation, biomass is essential. Sorghum was the most productive plant in mine soil, with a lower total biomass of all five plants than in control soil.

As Cd, Cu, Pb, and Ni concentrations increased to 40 ppm and higher, plant biomass dropped. Plants that are under stress from metals may have a decreased ability to absorb water and an osmotic pressure that results in poor development and biomass. Lead has an effect on biomass accumulation and growth, which probably has an effect on plant metabolism.

3.3. Protein Assay

Proteins are essential to the growth and development of plants. Plants cultivated in control soil showed higher protein contents at 15, 30, and 45-day intervals in this investigation. On the other hand, across the same time periods, plants growing in mine soils showed a drop in protein levels. At 15 days, the protein content of black gram plants grown in Dalmia soil was 2.013 mg g⁻¹, whereas at Burn & Co soil it was 2.024 mg g⁻¹. These values were substantially lower than the protein content of black gram plants cultivated in control soil. The plants grown in mine dirt had less protein on day forty-five.

The decrease in protein concentrations is linked to the movement of heavy metals inside the plants, which interact with sulfhydryl groups in proteins and negatively impact their normal function. Plant growth and development are hampered overall by this disturbance. Similar results were seen by Unnikrishnan et al. (2013), who found that when soil chromium concentrations increased, tree species' protein content declined.

3.4. Carbohydrate Assay

The plants that are cultivated in the control and mining soils provide the carbohydrates. In general, plants grown in mine soils exhibited lower amounts of total carbohydrates than plants grown in control soils. Carbohydrate content in Sorghum in mine soils dropped to 0.47 mg g⁻¹ in Burn & Co. soil and 0.391 mg g⁻¹ in Dalmia soil by the 45th day. At this period, black gram also showed a decrease in its carbohydrate content.

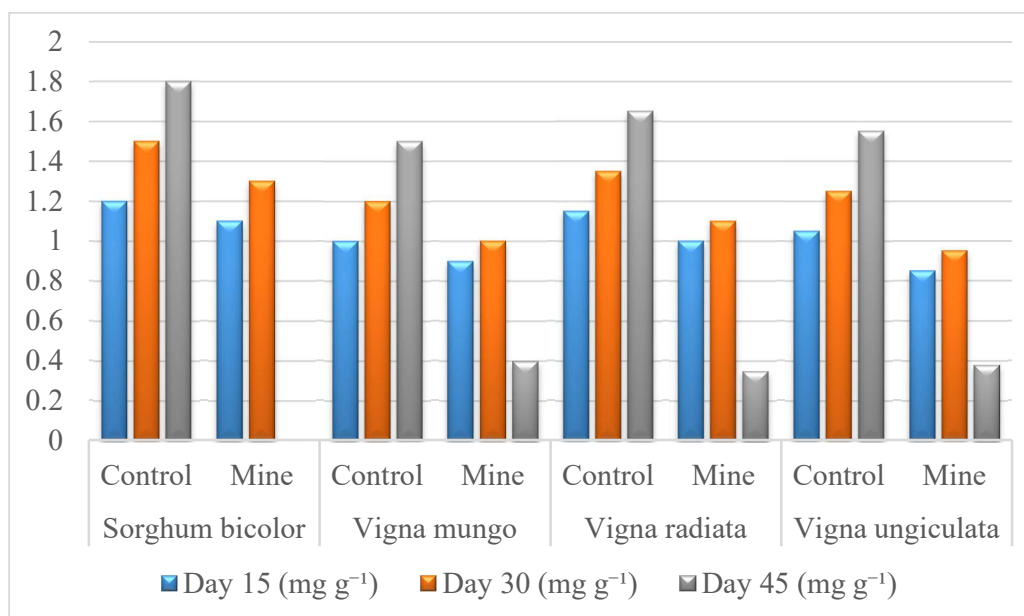


Figure 2: Effect of Mine Soil on the Accumulation of Heavy Metals in Different Plant Species

Plants cultivated in mine soils, such as *Vigna radiata* (100–30 mg g⁻¹), *Vigna mungo* (50–40 mg g⁻¹), and *Vigna unguiculata* (60–35 mg g⁻¹), exhibited decreased carbohydrate content compared to those grown in agricultural settings, according to similar results published by Mathiyazhagan and Natarajan. Plants with greater levels of heavy metals, such cadmium (Cd), have less nutritional value. Significantly less carbohydrate synthesis in Cd-contaminated soils than in control (up to 66.66% after 12 days of exposure).

3.5. Proline Assay

In response to environmental stress, proline accumulates and serves as a suitable solute for osmotic correction. In this research, plants growing in mine soils had the greatest proline level when compared to control plants. Proline levels in sorghum were 0.176 mg g⁻¹ and 0.076 mg g⁻¹, while those in black gram were 0.124 mg g⁻¹ and 0.117 mg g⁻¹, respectively, higher than in the control group.

Sorghum in mine soils increased significantly in proline at the end of the 45th day, reaching 0.858 mg g⁻¹; during the 30th and 45th days, control plants exhibited comparable proline content. reduced proline content (0.4 mg g⁻³ FW) in *P. glabra* treated as a control. Furthermore, proline content in copper-treated sunflower leaves, confirming the idea that heavy metal stress might promote proline accumulation.

4. CONCLUSION

Sorghum bicolor and *Vigna mungo* showed the highest tolerance to metal stress, demonstrating better growth and biomass accumulation compared to other species. This study assessed the effects of heavy metal-contaminated soils on the growth and biochemical characteristics of selected plant species. The substantial decline in protein and carbohydrate levels in mining soils was linked to elevated heavy metal concentrations, which were thought to interfere with protein function and reduce nutritional quality. Plants from polluted soils had higher proline contents, which suggested a stress response mechanism; nevertheless, this did not improve overall growth or metabolic activities. The results demonstrate how harmful heavy metal pollution is to plant health and raise the possibility that certain species might be good fits for phytoremediation techniques meant to lessen soil contamination. To achieve successful phytoremediation, future studies should focus on improving growing conditions and choosing plants with the best qualities.

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