

Studies on Bioaccumulation of Cr(VI) in Phaseolus Mungo with Titanium Industrial Waste

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ABSTRACT

Rutile is an interesting, varied and important mineral. Rutile is a major ore of titanium, a metal used for high tech alloys because of its light weight, high strength and resistance to corrosion. Rutile is also unwittingly of major importance to the gemstone markets. It also forms its own interesting and beautiful mineral specimens. Contamination of soils, ground water, sediments, surface waters and air with trace metals is one of the major environmental problems. This study focused on the investigation of the capability of the dried biomass of Phaseolus mungo (L.) to remove heavy metal (hexavalent chromium) from titanium industry effluent. From the results it was confirmed that P.mungo can be effectively used for the treatment of heavy metal polluted industry effluent.

Keywords : Titanium dioxide rutile, Seeds of Phaseolus mungo; Titanium industry waste; Red soil; Sand and vermi compost.

I. INTRODUCTION

Innovative processes for treating industrial wastewater containing heavy metals often involve technologies for reduction of toxicity in order to meet technology-based treatment standards. Titanium (Ti) occurs naturally in soils and as highly purified titanium dioxide (TiO₂) in many commercial products that have been used for decades. Titanium dioxide (TiO₂) is a chemically inert white pigment used in a wide range of consumer products from paints, paper and toothpaste to plastics and cement. The key raw materials used in the production of TiO₂ are ilmenite and rutile. India has reserves of ilmenite and rutile, accounting for 35% and 18% of the total world reserves respectively, largely found in coastal regions of Andhra Pradesh, Tamil Nadu, Kerala and Odisha. Anatase and rutile are the two major types of TiO₂ which are manufactured by the sulphate and chloride process, respectively. Soils are polluted with high concentration of toxic metals and their remediation requires excavation and removal of soils to secured landfills, an expensive technology that requires sites restoration involving secondary environmental and legal problems. But phytoremediation of heavy metal contaminated soil basically involves the extraction or

inactivation of metals in soils [1]. Plants known as hyper-accumulators have been shown to accumulate hundred or thousand times more metals than normal plants [2]. Plants uptake of pollutants from water is one of the pathways considered in models aimed at assessing the hazard of chemical contaminants in water [3]. Sunflower is reported to have high metal accumulating ability, yet low Chromium [Cr(VI)] tolerance compared to other agronomic crops. It is known that Cr predominantly exists in two forms in soil; as a trivalent cation and divalent dichromate anion. Cr(III) readily precipitated in soil, whereas greater environmental pollution problems occurred with the more mobile and toxic Cr(VI) [4,5]. Roots uptake metals through the main root with subsequent translocation to above ground tissues [6, 7]. Aquatic plants play an important role as a transportation link for metals from the sediments up to shoots. Only a fraction of the metals absorbed is transferred from the roots to the above ground parts [8, 9]. The chemical modification and spectroscopic studies have showed that the cellular components included carboxyl, hydroxyl, sulfate, phosphate, amino, amide, imine and imidazole moieties which have metal binding properties and are therefore, the functional groups in these plants

[10]. The biosorption capacity of immobilized biosorbents for Cr (VI) was found to depend on pH, contact time, biosorbent dose and initial concentration of Cr (VI). In this study, the maximum uptake of Cr (VI) was 93.4, 96.2 and 98.6 mg respectively at a pH 1.5 and with an increase in pH up to 11 the metal uptake decreased gradually upto 39.95, 52.35 and 66.48 mg respectively for acid treated, untreated and base treated fungal biosorbents. Increase in biosorbent dose up to 1g of biomass and contact time up to 60 min resulted in an increase in biosorption from 20.2, 16.8 and 28.3 mg at a biosorbent dose of 0.1g 100 ml⁻¹ to 93.4, 96.2 and 98.6 mg at a biosorbent dose of 1.0 g 100 ml⁻¹ and then further increase in adsorbent dose and contact time did not result in more Cr (VI) adsorption by per unit weight of biosorbent [11]. The hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red-violet color was produced. The reaction was very sensitive, the absorptivity based on chromium being about 40000 lg⁻¹ cm⁻¹ at 540 nm wavelength. *Aspergillus niger* was previously isolated from wastewater treatment plant of NIT Warangal and routinely maintained by streaking on a rose bengal agar medium and incubating at 25°C [12].

II. METHODS AND MATERIAL

Solid wastes were collected from one of the Titanium factories from Srikakulam District, Andhra Pradesh and stored in plastic bags. The solid wastes were deposited along the passage way of effluents discharged from the titanium factory. The solid wastes contain materials precipitated from the liquid effluent. About 200 g of calcium oxide (lime) lime were added to 1 kg of titanium solid waste and mixed well. The pH was checked using a pH meter and adjusted to neutral pH of 7.0. In order to get neutral pH, lime and solid waste were added as required. Neutralized solid waste and amendments were mixed in 1:1 proportion. The composition of the mixture was 1 Kg solid waste, 0.5 Kg red soil, 0.25 Kg sand and 0.25 Kg vermin compost. The ingredients were mixed well and the pH was checked. Solid waste-amendments mixture was prepared in 2:1 ratio by mixing 4 Kg neutralized solid waste with amendments such as 1 Kg soil, 0.5 Kg sand and 0.5 Kg vermin compost. 3:1 mixture of solid waste-amendments mixture was obtained by adding 6 Kg neutralized titanium solid waste to amendments such as 2 Kg soil, 1 Kg sand and 1 Kg vermin compost. Phytoremediation of Titanium industry effluent was

carried out with *Phaseolus mungo* (L.) plant. This commercially important crop was grown extensively in Andhra Pradesh and Telangana, India. The use of a commercially important plant in bioremediation carries dual benefits of grain production as well as toxicity alleviation. Earthen pots were used as the culture vessels. The pots were filled with solid waste-amendment mixtures (1:1, 2:1 and 3:1 ratios). Then *P. mungo* seeds were mixed with the soil mixture in each pot. Thirty seeds were used in each pot. Six replicates for each proportion of soil-amendments mixture and only soil as control were maintained. Necessary watering was done daily and care was taken to protect the plants from pests. Only plant based pesticides were used. Neem oil and neem seed kernel extract were sprayed on the leaves on weekly basis and this prevented the attack of both chewing and sucking pests. Root application of neem seed oil cake was done to manage root nematodes. *P. mungo* plant was grown till it started flowering. Flowering occurred in about 30-50 days. The plants were plucked out soon after flowering and shade-dried. Partially dried plants were cut into smaller twigs and dried in a hot air oven till the weights stabilized. The fully dried plants were powdered in a blender and once again dried in a hot air oven at 90°C. The partially powdered plant parts were pulverized using a mortar and pestle [13].

Preparation of sample (Acid digestion)

One gram of powdered sample was placed in a 250 ml digestion tube and 10 ml of concentrated HNO₃ was added. The sample was heated at 90°C for 45 min and then the temperature was increased to 150°C at which the sample was boiled for at least 8 h until clear solution was obtained. Exactly 5 ml of concentrated HNO₃ was added to the sample 3 times and digestion was allowed until the volume was reduced to about 1 ml. The interior walls of the tube were washed down with a little distilled water and the tube was swirled throughout the digestion process to keep the wall clear and prevent the loss of the sample. After cooling, 5 ml of 1 percent HNO₃ was added to the sample. The solution was filtered with whatman filter paper and millipore filter paper. The contents were transferred to a 25 ml volumetric flask and made up by adding distilled water [14].

Estimation of Cr (VI)

Samples were estimated for Cr (VI) using atomic absorption spectrophotometer. An Atomic Absorption Spectrophotometer (AAS) with Air-C₂H₂ flame type of an average fuel flow rate of between 0.6-4.0 L min⁻¹ and the support gas flow rate between 10.0-18.0 L min⁻¹ was used for sample analysis and operated as per the procedure mentioned in the equipment manual. Calibration curves for various elements obtained from these standards were of first order reaction. The samples for Cr (VI) analyses were aspirated with the help of an Automatic sampler for Atomic Absorption Spectrophotometer measurements. Series of reference standards-1, 2 and 3 ppm for the metal was prepared from the prepared stock solution. The standards were prepared by pipetting 0.1, 0.2 and 0.3 mL respectively of the metal reference standards and made up to 100 mL and mounted on the automatic sampler for standard calibration curve measurement. Percentage recovery rates of metal ranged from 92.2 to 98.6%. The samples were finally injected into the flame AAS and the readings were directly measured in a computer.

III. RESULTS AND DISCUSSION

P. mungo plant was grown till it started flowering. Flowering occurred in about 30-50 days as shown in Figure 1. Chromium (VI) concentration was maximum in the phytal parts of *P. mungo* grown on titanium industry solid waste mixed with organic amendments (1:1, 2:1 and 3:1 ratio). The highest value Cr (VI) was observed on the 50th day of exposure (1:1 ratio) and the values were 0.631 ± 0.002 in roots, 0.312 ± 0.023 in stems and 0.246 ± 0.028 $\mu\text{g/g}$ in leaves and the minimum values were observed on the 0 day and 0.524 ± 0.036 in roots, 0.322 ± 0.027 in stems and 0.192 ± 0.023 $\mu\text{g/g}$ in leaves. On the 30th day, bioaccumulation was 0.620 ± 0.045 , 0.342 ± 0.019 and 0.264 ± 0.028 $\mu\text{g/g}$ in roots, stems and leaves respectively as shown in Table 1. In *P. mungo* (2:1 ratio) maximum bioaccumulation of Cr (VI) was on the 50th day of exposure and the concentration were recorded as 0.810 ± 0.018 , 0.411 ± 0.024 and 0.340 ± 0.021 $\mu\text{g/g}$ in roots, stems and leaves respectively. On the 30th day of exposure, bioaccumulation was recorded as 0.800 ± 0.026 , 0.365 ± 0.026 and 0.298 ± 0.019 $\mu\text{g/g}$ of chromium in roots, stems and leaves. Minimum concentration was recorded on 0th day and the values were 0.610 ± 0.027 , 0.288 ± 0.017 and 0.213 ± 0.015 $\mu\text{g/g}$ in roots, stems and leaves respectively (Table 1 and 2). In *P. mungo*, the highest bioaccumulation of

chromium was observed on 50th day of exposure (3:1 ratio) and was 0.820 ± 0.001 , 0.610 ± 0.018 and 0.354 ± 0.001 $\mu\text{g/g}$ in roots, stems and leaves respectively. On the 30th day, bioaccumulation was 0.824 ± 0.058 , 0.410 ± 0.020 and 0.343 ± 0.023 in roots, stems and leaves respectively. The lowest bioaccumulation was on 0 day of exposure and the concentration was 0.660 ± 0.023 , 0.464 ± 0.012 and 0.257 ± 0.021 $\mu\text{g/g}$ in roots, stems and leaves respectively (Table 1 and 2).



Figure 1: Flowers of cultivated *P. mungo* (30 days)

Phytoremediation of industrial wastes is done by employing submerged and floating aquatic plants, most of which are weeds. Phytoremediation involves absorption of the effluent components into the different phytal parts of the plant system, alleviating the habitat pollution load. Torresdey et al [15] found that Cr(VI) being concentrated in the roots and not translocated to the aerial parts of the plant by determining the uptake and accumulation of Cr(VI) by *Convolvulus arvensis* (L.). Zaranyika and Nadapwadza and Yang et al. [16, 17] reported that roots have higher concentration of heavy metals than shoot while in some plant species like *Talium traingulare* there was higher concentration in the shoots than the roots. Sharma and Dubey [18] found that lead is easily absorbed and accumulated in different plant parts, and the roots were the primary sites for absorption of water and minerals including heavy metals and root had more heavy metal load than the shoot and acted as a storage organ. Mishra and Tripathi [19] reported that Cr is a non essential element and its compounds are highly toxic and detrimental to the growth and development of the plants. Cr is easily absorbed by the roots and then transported through the vascular system [20, 21] the highest Cu concentration of 16.85 $\mu\text{g/g}$ in the roots of *Eichhornia crassipes* and the lowest (1.01 $\mu\text{g/g}$) in *Hydrilla verticillata*. In shoots the highest Cu content (8.67 $\mu\text{g/g}$) and the lowest (1.01 $\mu\text{g/g}$) were recorded in *Ipomoea aquatic* (Forsk) and the

highest Pb concentration was measured in the roots of *H. verticillata* (4.24 µg/g), whereas the lowest (1.02 µg/g) was found in shoots of *Marsilea minuta* (L.).

Table 1: Cr(VI) (µg/g) in the plants grown on titanium industry solid mixture (n=6, X±SD), 0 and 30 days

S. No	Plant Ratio	Solid Waste	No. of days grown							
			0				30			
			Soil mixture	Roots	Stems	Leaves	Soil mixture	Roots	Stems	Leaves
1	<i>P.mungo</i> (1:1)	0.90±0.070	0.112±0.050	0.524 ± 0.036	0.322 ± 0.027	0.192 ± 0.023	0.101±0.030	0.620 ± 0.045	0.342 ± 0.019	0.264 ± 0.028
2	<i>P.mungo</i> (2:1)		0.213±0.025	0.610 ± 0.027	0.288 ± 0.017	0.213 ± 0.015	0.14±0.031	0.800 ± 0.026	0.365 ± 0.026	0.298 ± 0.019
3	<i>P.mungo</i> (3:1)		0.383±0.033	0.660 ± 0.023	0.464 ± 0.012	0.257 ± 0.021	0.678±0.066	0.824 ± 0.058	0.410 ± 0.020	0.343 ± 0.023

Table 2: Cr(VI) (µg/g) in the plants grown on titanium industry solid mixture (n=6, X±SD), 50 days and % Cr(VI) in parenthesis.

S.No.	Plant Ratio	Solid Waste	No. of days grown			
			50			
			Soil mixture	Roots [%Cr(VI)]	Stems [%Cr(VI)]	Leaves [%Cr(VI)]
1	<i>P.mungo</i> (1:1)	0.90±0.070	0.075±0.047	0.631 ± 0.002 (28.92)	0.312 ± 0.023 (46.86)	0.246 ± 0.028 (42.76)
2	<i>P.mungo</i> (2:1)		0.082±0.006	0.810 ± 0.018 (50.18)	0.411 ± 0.024 (48.11)	0.340 ± 0.021 (57.53)
3	<i>P.mungo</i> (3:1)		0.126±0.014	0.820 ± 0.001 (52.41)	0.610 ± 0.018 (56.65)	0.354 ± 0.001 (61.66)

IV. CONCLUSION

The use of *P.mungo*, crop plant widely cultivated in different parts of our country. This plant as an excellent phytoremediation agent and using this for removal of soil contaminants yielded a good harvest of grains as well as served the purpose of environmental clean-up. Even though the different phytal parts accumulated metals, the grains or the edible parts rarely accumulated them, thus remaining non-toxic to human consumers. The roots, stems and leaves of *P.mungo* accumulated metal found in the titanium industry effluent. The mobilization of these metals was recorded after 30 and 50 days of growth in the waste-amendments mixture. Chromium very effectively bioaccumulated in the phytal parts of plants. Roots accumulated more chromium than stems and leaves and the accumulation

was maximum on the 50th day. The individual metal concentrations in living tissues are generally low and must be maintained within narrow limits to secure optimum biological performances. Chromium as well as other metals are absorbed by root and shoot systems and may be stored and mobilized according to demand. From the results it was confirmed that *P.mungo* can be effectively used for the treatment of heavy metal polluted industry effluent.

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VI. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

VII. REFERENCES

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