

# Bioaccumulation of Chromium in Phaseolus Mungo (L.) Treated with Titanium Industry Waste

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## Abstract

Contamination of soils, ground water, sediments, surface waters and air with trace metals is one of the major environmental problems. Owing of this study focused on the investigation of the capability of the dried biomass of *Phaseolus mungo* (L.) to remove heavy metal (chromium) from titanium industry effluent. From the results it is confirmed that *P.mungo* can be effectively used for the treatment of heavy metal polluted industry effluent.

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

## Introduction

Soils may become 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 Cr 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].

This study is designed to check whether commercially important pulse could remediate metal contamination of soils by titanium industry wastes.

## Materials and Methods

### Collection of solid waste

Solid wastes were collected from Titanium factory and stored in plastic bags. The solid wastes are deposited along the passage way of effluents discharged from the titanium factory. The solid wastes comprise of materials precipitated from the liquid effluent.

### Neutralizing solid waste

About 200 g of lime (calcium oxide) were added to 1 kg of titanium solid waste and mixed well. The pH was checked using a pH meter (ELICO LI 120) and adjusted to 7 (neutral). If the pH is less than 7.0, lime is added and if high, little quantity of solid waste was added.

### Experimental design

#### i. Preparation of solid waste-amendments

a. Neutralized solid waste and amendments were mixed in 1:1 proportion. The ratio 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.

b. 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.

c. 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 studies

d. Phytoremediation of Titanium industry effluent was carried out with *Phaseolus mungo* (L.) plant. This commercially important crop was grown extensively in India. The use of a commercially important plant in bioremediation carries dual benefits of grain production as well as toxicity alleviation.

#### Culture of *P. mungo*

e. 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 sown in the soil mixture in each pot. Twenty

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seeds were sown in each pot. Six replicates for each proportion of soil-amendments mixture and control (only soil) were maintained. Necessary watering was done daily and care was taken to protect the plants from pests.

f. 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.

g. *P. mungo* plant was grown till it started flowering. Flowering occurred in about 25-60 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.

### Preparation of sample (Acid digestion)

One gram of powdered sample was placed in a 250 ml digestion tube and 10 ml of concentrated HNO<sub>3</sub> was added. The sample was heated for 45 min at 90°C, and then the temperature was increased to 150°C at which the sample was boiled for atleast 8 h until clear solution was obtained. Exactly 5 ml of concentrated HNO<sub>3</sub> was added to the sample atleast 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 HNO<sub>3</sub> was added to the sample. The solution was filtered with whatman No. 42 filter paper and 20.45 m millipore filter paper. The contents were transferred to a 25 ml volumetric flask and made up by adding distilled water.

### Estimation of heavy metals (Cr)

Samples were estimated for chromium using atomic absorption spectrophotometer. A shimadzu type Atomic Absorption Spectrophotometer (AAS) 6300 model with Air-C<sub>2</sub>H<sub>2</sub> flame type of an average fuel flow rate of between 0.8-4.0 L min<sup>-1</sup> and the support gas flow rate between 13.5-17.5 L min<sup>-1</sup> was used for sample analysis and operated as per the equipment manual. The single element hollow cathode lamps for respective metals were of Hamamatsu photonics co. Ltd-L24 33 series. The atomic absorption analysis standards for the given elements were purchased from Inorganic ventures Inc. and Sisco Research Laboratories Ltd. Calibration curves for various elements obtained from these standards were of first order reaction. The samples for Cr analyses was 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 purchased 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 94.8 to 102.3%. The samples were finally injected into the flame AAS and the readings were directly measured in a computer.

### Results

Chromium concentration was maximum in the phytal parts of *P. mungo* grown on titanium industry solid waste mixed with organic amendmets (1:1, 2:1 and 3:1 ratio). The highest value (Cr) was observed on the 60<sup>th</sup> day of exposure (1:1 ratio) and the values were 0.523 ± 0.001 in roots, 0.209 ± 0.032 in stems and 0.196 ± 0.036 µg/g in leaves and the minimum value was observed on the 0 day and 0.412 ± 0.047 in roots, 0.210 ± 0.030 in stems and 0.140 ± 0.026 µg/g in leaves. On the 30<sup>th</sup> day, bioaccumulation was 0.517 ± 0.055, 0.262 ± 0.026 and 0.181 ± 0.036 µg/g in roots, stems and leaves (Table 1).

In *P. mungo* (2:1 ratio) maximum bioaccumulation of chromium was on the 60<sup>th</sup> day of exposure and the concentration were recorded as 0.623 ± 0.002, 0.301 ± 0.028 and 0.224 ± 0.021 µg/g in roots, stems and leaves. On the 30<sup>th</sup> day of exposure, bioaccumulation was recorded as 0.602 ± 0.031, 0.278 ± 0.029 and 0.214 ± 0.027 µg/g of chromium in roots, stems and leaves. Minimum concentration was recorded on 0 day and the values were 0.420 ± 0.032, 0.207 ± 0.022 and 0.144 ± 0.012 µg/g in roots, stems and leaves (Table 1).

In *P. mungo*, the highest bioaccumulation of chromium was observed on 60<sup>th</sup> day of exposure (3:1 ratio) and was 0.697 ± 0.001, 0.429 ± 0.002 and 0.253 ± 0.001 µg/g in roots, stems and leaves. On the 30<sup>th</sup> day, bioaccumulation was 0.678 ± 0.066, 0.321 ± 0.024 and 0.281 ± 0.028 in roots, stems and leaves. The lowest bioaccumulation was on 0 day of exposure and the concentration was 0.471 ± 0.045, 0.279 ± 0.017 and 0.160 ± 0.028 µg/g in roots, stems and leaves (Table 1).

### Discussion

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. The use of *P.mungo*, crop plant widely cultivated in different parts of our country. This plant an excellent phytoremediation agents and using this for removal of soil contaminants yielded a good harvest of grains as well as served the purpose of environmental clean-

S.no	Plants	Solid Waste	No. of days grown											
			0				30				60			
			soil mixture	Roots	Stems	leaves	phytal plants				soil mixture	Roots	Stems	leaves
							soil mixture	Roots	Stems	leaves				
Concentration of Chromium (µg/g)														
1	<i>P.mungo</i> (1:1)	0.90 ± 0.07	0.112 ± 0.050	0.412 ± 0.047	0.210 ± 0.030	0.140 ± 0.028	0.101 ± 0.030	0.517 ± 0.066	0.262 ± 0.026	0.181 ± 0.036	0.075 ± 0.047	0.523 ± 0.001(26.94)	0.299 ± 0.032(42.38)	0.196 ± 0.036(39.99)
2	<i>P.mungo</i> (2:1)		0.213 ± 0.025	0.420 ± 0.032	0.207 ± 0.022	0.144 ± 0.012	0.14 ± 0.031	0.602 ± 0.031	0.278 ± 0.029	0.214 ± 0.027	0.082 ± 0.006	0.623 ± 0.002(48.81)	0.301 ± 0.028(45.41)	0.224 ± 0.021(55.55)
3	<i>P.mungo</i> (3:1)		0.383 ± 0.033	0.471 ± 0.045	0.279 ± 0.017	0.160 ± 0.028	0.678 ± 0.066	0.678 ± 0.066	0.321 ± 0.024	0.281 ± 0.028	0.126 ± 0.014	0.697 ± 0.001(48.19)	0.429 ± 0.002(53.76)	0.253 ± 0.001(58.12)

Note: percent chromium bioaccumulation (60 days) within parantheses  
Deviations significant at p ≤ 0.05 (t ± test)

Table 1: chromium (in µg/g) in the phytal parts of plants grown on titanium industry solid mixed with 1:1, 2:1 and 3:1 proportions of organic amendmets (n=6; X ± SD).

up. Eventhough the different phytal parts accumulated metals , the grains or the edible parts rarely accumulated them, thus remaining non-toxic to human consumers. Torresdey et al [11] found that Cr being concentrated in the roots and not translocated to the aerial parts of the plant by determining the uptake and accumulation of Cr by *Convolvulus arvensis* (L.). 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 60 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 60th 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. Zaranyika and Nadapwadza and Yang et al. [12,13] reported that roots have higher concentration of heavy metals than shoot while in some plant species like *Talimum traingulare* there was higher concentration in the shoots than the roots. Sharma and Dubey [14] 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 [15] 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 [16,17] the highest Cu concentration of 16.85 µg/g in the roots of *Eichhornia crassipes* and the lowest (1.01 µg/g) in *Hydrilla verticillata*. In shoots the highest Cu content (8.67 µg/g) and the lowest (1.01 µg/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.).

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