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Phytoremediation of Chromium by Chickpea (*Ciecer arietinum L.*) and Mung bean (*Vigna radiata L.*) A comparative study

Saibalini Behera[1*], Suryakanta Bahira[1], Gurudutta Pattnaik[2] and Puspashree Puhan[3]

[1]Research Scholar, School of Pharmacy and Life Science, C.U.T.M, Bhubaneswar, Odisha, India.

[2]Principal, School of Pharmacy and Life Science, C.U.T.M, Bhubaneswar, Odisha, India.

[3]Assistant Professor, Department of Botany, B.J.B. Autonomous College, Bhubaneswar, Odisha, India

*Corresponding Author E-mail: beherasaibalini1000@gmail.com

Abstract

The present study have objective to test the toxic effect of chromium on species of chickpea (*Ciecer arietinum* L.) and Mung bean (*Vigna radiata* L.) comparatively. The plants are grown on the soil containing chromium as anhydrous Potassium dichromate ($K_2Cr_2O_7$) under normal environmental conditions. The study includes effect of different concentration at 10, 30, 50 ppm of chromium on plant growth and its accumulation in the species. The plants were harvested separately after specified time interval of 10, 20, 30 days and the accumulation of chromium in the plant species were observed.

Keywords: Chromium, Potassium dichromate, Chickpea, Mung bean, Phytoremediation.

Introduction

Chromium present in nature in the form of chromites ($FeCr_2O_4$) in ultramafic and serpentine rocks or combined with other metals in the forms like crocoite ($PbCrO_4$), bentorite Ca6 (Cr, Al)^2 $(SO_4)^3$ and tarapacaite (K_2CrO_4), vauquelinite ($CuPb_2CrO_4P\ O_4OH$), among others. Cr is the 17th most abundant element in the Earth crust. Chromium are used in leather processing and finishing, in the production of refractory steel, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals. Several forms of chromium are Cr(0), Cr(III) and Cr(VI). Cr(0) exist in solid and metallic form usually released from steel and alloys industry. The trivalent chromium Cr(III) exist in oxides, hydroxide and sulphate forms. It is present in the soil and aquatic environment by combining with the organic matter. It is insoluble in water hence less toxic. The hexavalent chromium Cr(VI) exist in chromate (CrO_4^{2}), dichromate ($Cr_2O_7^{2}$) and CrO_3 form. Among all the three forms Cr(VI) is the most toxic form as it have high oxidizing potential, high solubility and mobility properties.

Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and wood preservation. Leather industry is the major cause for the high influx of chromium to the biosphere accounting for 40% of the total industrial use (J. Barnhart, 1997). Chromium (VI) is considered the most toxic form which usually occurs, associated with oxygen as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$) oxyanions. Chromium (III) is less toxic, less mobile and is mainly found bound to organic matter in soil and aquatic environments. It is also one of the important pollutants among other pollutants like cadmium, lead, mercury and aluminum.

Presently there are no suitable techniques available for its removal from the environment. The various methods such as excavation, landfill, leaching and electroclamation are not useful for its removal due to high cost, less efficiency and loss of soil fertility. Therefore the development of Phytoremediation Technology for removal of heavy metal contamination is necessary for the environment.

Phytoremediation

Phytoremediation (from ancient Greek phyto, meaning “plant”, and Latin remedial meaning “restoring balances”) Refers to the technologies that use living plants to clean up soil, air, and water contaminated with hazardous chemicals. Phytoremediation is a cost–effective plant–based approach that use for removal of heavy metal contamination from the environment. Certain plant have natural ability to remove or degrade the toxic metal in the soil, water and air, these plants are referred as hyper accumulators.

About 30 plant species were tested by Ebbs *et al*., (1997) in hydroponic levels of Cu, Cd and Zn which have ability to accumulate heavy metals. Among these species many *Brassica sp*. Such as *B.juncea L. , B.juncea L. czern, B.napus L. , B. rapa L.* have ability to accumulate Zn and Cd moderately. These species are also highly effective in removing Zn from the contaminated soils. Presently more than 400 plant species have been identified as hyper accumulator plants representing less than 0.2% of all angiosperms. The high biomass plant such as willow or low biomass plant but having high hyper accumulating characteristics such as *Thalaspi* and *Arabidopsis* species are use for removal of toxic metal from soil (Landberg and Greger, 1996). The hyper accumulators that have been most extensively studied by scientific community include

Thalaspi sp., Arabidopsis sp., *Sedum alfredii* sp. (both genera belong to the family of Brassicaceae and Alyssum). *Thalaspi sp*. Are known to hyperaccumulator more than one metal, i.e., *T. caerulescens* for Cd, Ni, Pb and Zn (Prasad freitas, 2003). Among the genus *Thalapsi*, the hyperaccumulator plant *Thalaspi caerulescens* received much attention and has been extensively studied as potential candidates for Cd and Zn contaminated soils.

Chickpea (*Cicer arietinum* L.)

Chickpea [*Cicer arietinum* (L.)] belongs to genus Cicer, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. It is known as gram or Bengal gram or chana. Chickpea is an herbaceous annual plant which branches from the base. It is almost a small bush with diffused, spreading branches. The plants grows 20-50 cm high and has small, feathery leaves on either side of the stem. Chickpeas are a type of pulse, with one seedpod containing two or three peas. It has white flowers with blue, violet, or pink veins. Chickpeas are a nutrient dense food, providing rich content of protein, dietary fiber, and certain dietary minerals such as irons and phosphorus.

Mung bean (*Vigna radiate* L.)

The Mung bean (*Vigna radiata*) alternatively known as the green gram, which is a plant species in the legume family. The Mung bean is mainly cultivated in India, china, and south East Asia. It is used as an ingredient in both savory and Sweet dishes. Mung bean is commonly used in various cuisines across India. Mung bean is germinated by leaving them in water for four hours of daytime light and spending the rest of the day in dark. Mung bean spourts can be grown under artificial light for four hours over the period of a week. They are usually simply called “bean sprout”.

Aims and Objectives

The main objective of the study is to collect the suitable varieties of chickpea (*Cicer arietinum L.*) and Mung bean (*Vigna radiate L.*) Seeds and find out the effect of Cr(VI) on growth and development of plant species under natural conditions and also to study the root and shoot measurement assessment of different toxicity of treated plant species. The present study also has aim to evaluate the accumulation potential from different concentration of Cr(VI) ions mg/g on the plant.

Effect of chromium on plant

Various physiological activities like growth of root, stem, leaf, seed Germination, photosynthesis, Mineral Nutrition and also production yield of the plant is affected by the heavy metal pollution.

The growth of root and shoot, dry matter were Adversely affected by heavy metals (Huma Naz *et.al*, 2015). Presence of Cr in the external environment leads to changes in the growth and development pattern of the plant. Seed germination is the first physiological process of plant so the level of tolerance to Cr is measured when the seed are germinates in a medium containing Cr (Peralta *et al*., 2001). The Seed of the weed *Echinochola colona* when germinate in a medium containing 200µM Cr, it is observed that the germination reduced by 25% (Rout *et al.*, 2000). Peralta *et al.*,(2001) observed that 40ppm of Cr(VI) reduced by 23% .Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Breckle, 1991; Goldbold and Kettner, 1991; Tang *et al*., 2001). Prasad *et al*., (2001) reported that the order of metal toxicity to new root primordial in *Salix viminalis* is CD>Cr>Pb, whereas root length was more affected by Cr than other heavy metals studied. Root length and dry weight of the important arid tree C*aesalpinia pulcherrima* was inhibited by 100ppm Cr (Iqbal *et al*., 2001). There was a significant reduction in plant height in sinapsis alba when Cr was given at the rates of 200 or 400mg kg^{-1} soil along with N<P<K and S fertilizers (Hanus and Thomas, 1993). Leaf growth, area development and total leaf number decisively determine the yield of crops. Leaf number per plant reduced by 50% in wheat when 0.5mM Cr was added in nutrient solution (Sharma and Sharma, 1993). Tripathi *et al.*,(1999) found that leaf area and biomass of Albizialebbek seedlings was severely affected by a high concentration (200ppm) of Cr(VI) Chromium stress is one of the important factors that affect photosynthesis in term of CO_2 fixation, electrontransport, photophosphorylation and enzymes activities (Clijsters and Van Assche, 1985). Cr due to its structural similarity with some essential elements can affect mineral nutrition of plants in a complex way. Interaction of Cr with uptake and accumulation of other inorganic nutrients have received maximum attention by researches. Cr (III) and Cr (VI) are taken up by the plants by different mechanisms (Zaccheo *et al.*, 1985). Barcelo *et al.*, (1985) described the inhibition of P, K, Zn, Cu and Fe translocation within the plants parts when bean plants were exposed to Cr in nutrient solutions.

Materials and Method

Materials

One variety from each species such as mug bean (*Vigna radiate L.*) and chickpea (*Cicer arietinum L.*) which is generally available in market. Then the seeds were thoroughly washed under tap water. The damaged seeds were floated on water which was discarded from the rest seeds. The seeds were kept in individual pots for overnight. The mung beans soaked water and germinated within 12 hours. The sprouted Mung beans got after day morning, but the chickpeas soaked water then wrapped tightly in a dark place for about 24 hours. The chickpeas sprouted.

Then the sprouted chickpeas mung beans planted in the pots containing soil sample was collected from river bed of Dayanadi, near itipur, Dhauli Bhubaneswar.

Standard curve preparation

Spectrophotometer is used in the present study. Instrumentation calibration standards for transfer of 1ml, 2ml, 3ml, 4ml and 5ml. standard solution of Cr(VI) to 100ml volumetric flask add appropriate amount of matrix modifier and dilute to volume with distilled water. So the standard solution will be 5, 10, 15, and 20µg/L respectively. Details preparation procedure of Cr(VI) standard solution has been using manuals of spectrophotometer. A calibration curve of Cr(VI) standard solutions are presented in Fig.1

Table-1 Absorbance of known concentration of Cr(VI)

Known conc. of Cr.(VI)	Absorbance
05	0.033
10	0.056
15	0.081
20	0.107

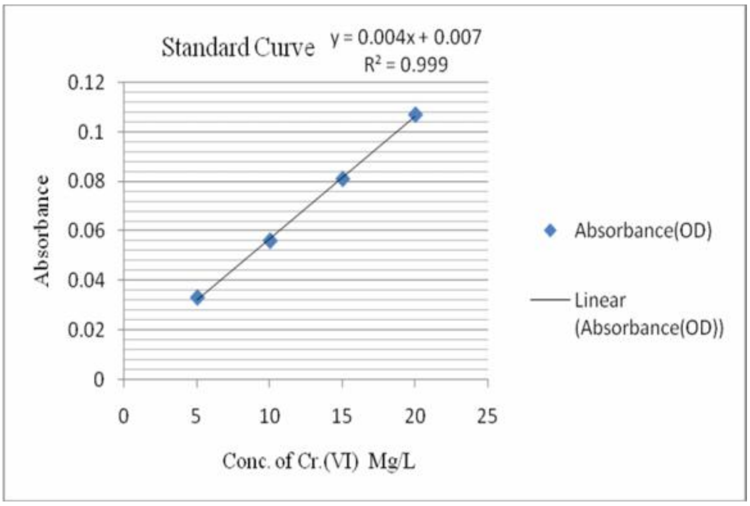

Figure 1: Calibration Curve of Cr (VI) Standard Solution

Chromium Accumulation Analysis

After preparation of standard curve, chromium accumulation in shoot and root work was done. For this work procedure is: 1st two flasks were taken, in one flask is blank and other is sample content. In blank flask, there is 100ml of distilled water was added and then 0.25ml of H_2PO_4 and

2ml of diphenyl carbazide solution was added. In sample content flask, 1st 15ml of distilled water was added then 5ml of leaf extract was added. This is collected as 100mg of leaf from different concentrations of each sample and then homogenized that leaf with acetone and volume was adjusted to 5ml, then 0.25ml of H_3PO_4 and 2ml of diphenyl carbazide solution was added. After that, the two flasks were kept in a dark for 5-10 minutes for colour formation. At last absorbance was measured in spectrophotometer at 540nm. This same procedure was done in different concentration (control, 10ppm, 30ppm, 50ppm) of each sample (Mung bean and chickpea). This same experiment was repeated in 10th, 20th and 30th days.

Results and discussion

Effects of Cr(VI) in root length of mung bean (*Vigna radiate* L.) and chickpea (*Cicer arietium L.*) at different concentrations and exposed times were presented in Table-2. In mung bean and chickpea treated with Cr(VI) solution, the root were significantly increased with respect to and temperature. After 10days, the highest root was obtained 2cm and 6.8cm in mung beam and chick pea respectively which were treated with 10ppm Cr(VI) solution and presented in Table 2. After 20days the highest root length was obtained 3.8cm and 7.9cm respectively which were treated with 10ppm Cr(VI) solution and presented in Table-2 and after 30days the highest root length was obtained 4.7cm and 9cm respectively which were treated with 10ppm Cr(VI) solution and presented in Table-2. Likewise in the plant which were treated with 30ppm and 50ppm shows significant growth.

Table 2: Root length of both plant species

Conc. of Cr(VI)mg/L	Time interval in Days					
	Day-10		Day -20		Day-30	
Control	Mung	Chickpea	Mung	Chickpea	Mung	Chickpea
Control-0	3cm	8.6cm	4.5cm	12.5cm	6cm	14.8cm
10	2cm	6.8cm	3.8cm	7.9cm	4.7cm	9cm
30	1.8cm	6cm	3.2cm	6.7cm	4cm	7.3cm
50	1.5cm	4.9cm	2.5cm	5.7cm	3.2cm	6.5cm

Table 3: Shoot length of the both plant species

Conc. of Cr(VI)mg/L	Time interval in Days					
	Day-10		Day -20		Day-30	
control	Mung	Chickpea	Mung bean	chickpea	Mung	Chickpea
Control-0	11cm	19cm	13cm	21.4cm	15cm	23.1cm
10	8cm	17.6cm	8.6cm	18.4cm	9.4cm	19.1cm
30	7cm	14.7cm	7.5cm	15.6cm	8.2cm	16.4cm
50	6cm	11.8cm	6.4cm	12.5cm	6.9cm	13.2cm

Table 4: Accumulation of Cr(VI) by Mung bean and chickpea plants treated with different Concentration of Cr.

Conc.of Cr(VI)mg/L	Time interval in days					
	10days		20days		30days	
	Mung	Chickpea	Mung	Chickpea	Mung	Chickpea
10	0.261	1.210	0.432	1.890	0.851	2.121
30	0.301	1.670	0.450	2.320	0.872	3.110
50	0.467	2.010	0.592	3.120	0.981	4.010

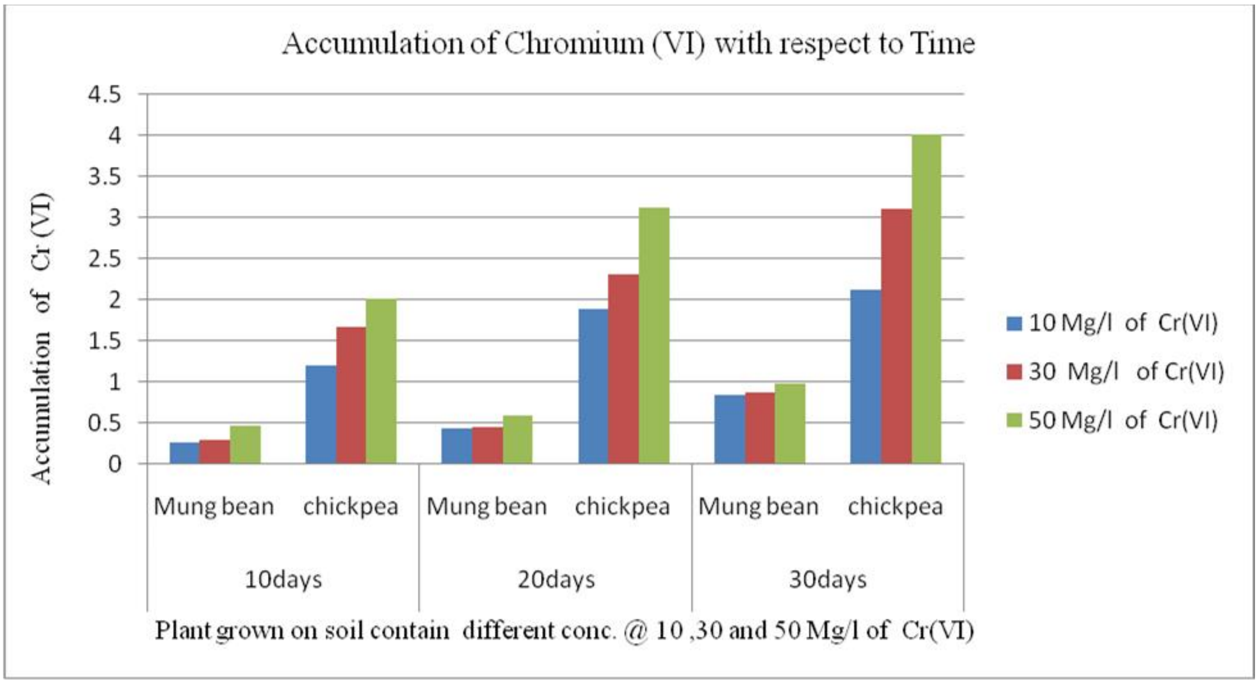

Figure 2: Accumulation of chromium (VI)

Effects of Cr(VI) in shoot length of Mung bean (*Vigna radiate* L.) and chickpea (*Cicer arietium L.*) at different concentrations and exposed times were presented in Table-3. In Mung bean and chickpea treated with Cr(VI) solution, the shoot were significantly increased with respect to and temperature. After 10days, the highest shoot was obtained 8cm and 17.6cm in Mung beam and chick pea respectively which were treated with 10ppm Cr(VI) solution and presented in Table 3. After 20days the highest shoot length was obtained 8.6 cm and 18.4 cm respectively which were treated with 10ppm Cr(VI) solution and presented in Table-3 and after 30days the highest shoot length was obtained 9.4 cm and 19.1cm respectively which were treated with 10ppm Cr(VI) solution and presented in Table-3. Likewise in the plant which were treated with 30ppm and 50ppm shows significant growth.

Accumulation of Cr(VI)

In the present study the accumulation of hexavalent chromium ions by mung bean (V*igna radiate* L.) and chickpea (*Cicer arietinum* L.) at ambient temperature with different concentrations and exposures of times was analyzed and were presented in table-4 and figure 2. Mung bean species treated with 50mg/l of Cr(VI) after 30days accumulated the highest level of metal ions 0.981mg/g dry weight. Figure-2 Similarly chickpea species treated with 50mg/l of Cr(VI) after 30 days accumulated the highest level of metal ions4.01mg/g dry weight Figure-2.

Conclusion

The increasing Cr (VI) concentration and days, the shoot and root length of Mung bean (V*igna radiate* L.) and chickpea (*Cicer arietinum* L.) plants gradually increases in length. It may be due to the presence of suitable cations and anions that have been influencing due to effect of the nutrient uptake, which helps in plants physiological metabolic activity can grow easily. The phytoremediation techniques are relatively new methods for abetment of hazardous ions from water, soil and air. From the above figures, it is clear that the species of Cr are toxicant different degrees at different stages of plant growth and development and also that the toxicity is concentration and medium dependent. The toxic properties of Cr(VI) originate when the Cr(VI) reduce to Cr(III) in the cell and also from its action as it is an oxidizing agent. Cr(III) occurring inside the cell .Cr(III), on the other hand, apart from generating reactive oxygen species, if present in high concentrations, can cause toxic effects due to its ability to coordinate various organic compounds resulting in inhibition of some metallo-enzyme systems. In mung bean, the maximum value of accumulation of Cr(VI) ions is 0.981mg/g at 50ppm concentration whereas the minimum value is 0.261mg/g at 10ppm concentration; but in chickpea, the maximum value of accumulation of Cr(VI) Is 4.01mg/g at 50ppm and the minimum is 1.21mg/g at 10ppm. Therefore, chickpea *Cicer arietinum* L.) has more accumulating power than the Mung bean *(Vigna radiate* L.), hence it can be used to remove the toxic element like Cr(VI) from the soil.

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