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Chromium Phytotoxicity in Tree Species and its Role on Phytoremediation

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Abstract: Background: The use of tree species to assess the phytoremediation capacity and phytotoxicity effect of chromium was highly recommended for the clean environment. Many studies have considered the rates of polluted levels reductions from the soil. **Aim:** Relatively this paper describes experiments which examined the influence of tree species to reduce the chromium levels in a short span of time from the soil and the physiological changes while growing. *Pithecellobium dulce*, *Tamarindus indicus*, *Pongamia glabra*, *Cassia auriculata* and *Azadirachta indica* were cultivated in pot soils were treated with different concentrations (10, 30, 50 mg L^{-1}) of chromium, prepared from the stock solution of potassium dichromate. **Result:** The chromium accumulation potential of the tree species is higher in the order of *Pongamia glabra*>*Cassia auriculata*>*Pithecellobium dulce*>*Azadirachta indica*>*Tamarindus indicus*. The decrease in morphological parameters (root length and shoot length) and biochemical parameters (protein, chlorophyll, carotenoid, leghaemoglobin nitrogenase enzyme and proline) were observed in the experimental plant due to chromium accumulation. **Conclusion:** The study suggested that continuous deposition of chromium would denature the soil medium. The application of phytoremediation technology seems to be essential for making a good environment.

Key words: Phytotoxicity, chromium, treespecies, biochemicals, phytoremediation

INTRODUCTION

With a tremendous improvement in scientific field and industrialization, there has been a significant increase in use and release of chemicals and heavy metals into the environment (Bhattacharyya *et al*., 2005; Ganesh *et al*., 2008). Among the heavy metals, chromium (Cr(VI) plays a significant role in polluting the environment. The chromium discharging industries are alloy manufacturing, dyes and pigments, electroplating, metal finishing, petroleum refining, leather tanning and wood preservation factories (Aleves *et al*., 1993; Mishra and Doble, 2008). In soil environment, the most stable oxidation states of chromium are Cr(III) and Cr(VI). Even at low concentrations, it may produce mutagenesis and carcinogenesis (Chidambaram *et al*., 2009). Land application of tannery waste as organic fertilizer has led to extensive Cr contamination of agricultural areas (Lakshmi and Sundaramoorthy, 2001). It has also been reported that even the lower concentrations of chromium (72 ppm) showed inhibitory effect on plant growth (Sundaramoorthy *et al*., 2006). Metals like chromium with phytotoxic effect have decreased plant biomass production, biochemicals and minerals (Sankar Ganesh *et al*., 2006). In addition to this, its presence in excess amount within the plant causes stunted growth, poor root development and discoloration of leaves (Chidambaram *et al*., 2009). It caused the changes in transpiration and nutrient balance due to chromium toxicity (Sharma *et al*., 2003; Scoccianti *et al*., 2006; Yu *et al*., 2010). Because Cr(VI) is relatively mobile, easily soluble in water, and simply permeable the biological membrane and enter the cell (Kazi *et al*., 2008). The chromium seems to act as toxic to agriculture crops and reduces the agricultural yield. Phytoremediation is an eco-friendly and novel technologies in which the plants are used for the removal of inactivate contaminants in soil and water (Merkel *et al*., 2005). The contaminants are absorbed subsequently, stored and metabolized by the plants. The present study deals with in terms of the comparative study of chromium phytotoxicity growth performance, biochemical changes and phytoremediative capacity of five selected tree species on chromium contaminated soil.

MATERIALS AND METHODS

Tree species: The seeds of *Pithecellobium dulce*, *Tamarindus indicus*, *Pongamia glabra*, *Cassia auriculata* and *Azadirachta indica* were collected from Forest Research Station, Coimbatore, India. The earthen pots were filled with 10 kg of garden soil and treated with

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different concentrations (10, 30 and 50 mg L^{-1}) of chromium. Then, the seeds were sown in the pots containing chromium and irrigated with tap water. Control set was maintained without chromium treatment. The seedlings were allowed to grow in polluted soil upto 120 days. After the experiment, the soil samples were collected from the pots and they were analysed to know the variation in their physico-chemical properties. All the experimental sets were maintained in triplicate. The accumulation of chromium was analysed in both shoot and root of tree species.

Heavy metal preparation: Cr solution was prepared by dissolving 5.916 g of $K_2Cr_2O_7$ in 1000 mL of distilled water. From this stock solution, various concentrations (10, 30 and 50 mg L^{-1}) of chromium solution were prepared and used as treatment solutions.

Soil analyses: The experimental soil samples were collected manually from the depth of 5-20 cm in each pot and labeled separately. Cr(VI) content was determined after digestion with HCl and quantification was carried out using atomic absorption spectrophotometer. The soil nitrogen (Subbiah and Asija, 1956), Phosphorus and potassium (Jackson, 1958), copper, iron, zinc, manganese and chromium (Piper, 1966) of both control and treated soil were estimated. All the parameters were determined in triplicate.

Plant growth and biomass: After 120th day tree plant samples were removed from the earthen pots and various morphological parameters like shoot length, root lengths, root nodules biomass dry weight were recorded and analyzed to estimation.

Photosynthetic parameters: The 0.5 g of fresh leaf material was taken and ground with 10 mL of 80% acetone. The supernatant was saved and the residue was re-extracted with 10 mL of 80% acetone. The supernatant was utilized for chlorophyll estimation. The absorbance was read at 645, 663 and 450 nm. The values were calculated using the formula proposed by Arnon (1949) for chlorophyll and Kirk and Allen (1965) for carotenoid contents.

Estimation of protein: The protein content was estimated by following the method of Lowry *et al.* (1951). Plant sample was homogenized in 10 mL of 20% Trichloro acetic Acid (TCA). The homogenate was centrifuged at 800 rpm for 10 min. The supernatant was discarded and pellet was re-extracted with 5 mL of 0.1 N NaOH. One milliliter of the extract was taken in a test tube and 5 mL of reagent 'C' was added. This solution was mixed well and kept in dark for 10 min. Later 0.5 mL of folin phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in Spectrophotometer.

Estimation amino acid: The 0.5 g of plant sample was homogenized in 10 mL of 80% ethanol. The homogenate was centrifuged at 800 rpm for 10 min. One mL of the extract was taken in the test tube and 1 mL of 0.1 N HCl was added to neutralize the sample. To this, one ml of ninhydrin reagent was added and the test tube was heated for 20 min in a water bath. Later, 5 mL of the diluent solution was added and heated again in water bath for 10 min. the extraction and estimation was done by following the methods of Moore and Stein (1948). The test tubes were cooled and the value of absorbances was read at 570 nm in a Spectrophotometer.

Estimation of proline: The 0.5 g of plant material (root and shoot) was homogenized using 10 mL of 3% aqueous sulpho salicylic acid. The homogenate was filtered through Whatmann No. 1 filter paper. Two mL of acid ninhydrin was added with (1.25 g of Ninhydrin+30 mL of glacial acetic acid+20 mL of 6 m phosphoric acid) 2 mL of glacial acetic acid. The sample was heated for one h at 100°C in a water bath and 4 mL of Tolune was added to it. This solution was mixed well and the absorbance was read at 520 nm in a Spectrophotometer by the method followed by Bates *et al.* (1973). The results were expressed as μmol g^{-1} fresh weight.

Estimation of leghaemoglobin content: The nodules were washed and weighed. They were crushed in tris-acetic acid buffer. The extract was centrifuged at 3000 g for 20 min and 0.1 to 1.0 mL of supernatant was taken and it was made upto final volume of 4.0 mL by tris-acetic acid buffer so as to get an absorbance reading between 0.2 and 0.4. Later, 2 mL of freshly prepared benzidine reagent was added. The rate of colour formation was noted by observing the change in optical density by using UV-Spectrophotometer at 540 nm. A standard graph was prepared by plotting the absorbance at the end of 30th sec against different concentrations (0.8 -1.5 μg mL^{-1}) of ox-blood haemoglobin. The leghaemoglobin content of the test sample was calculated from the Standard graph and expressed in mg g^{-1} of nodules on fresh weight basis by the method followed by Hardy *et al.* (1968).

Nitrogenase enzyme estimation: Nitrogenase activity in root nodules of test crop was estimated at various sampling days by using acetylene reduction assay method. One gram of freshly collected root nodules were

washed thoroughly in distilled water. They were placed in 65 mL serum vials and closed with rubber stoppers. The 6.3 mL of air from the serum vial was evacuated at 28°C for one h with the sterile disposable syringe. At the time of assay, 6.3 mL of acetylene gas was injected by using the sterile disposable. These bottles were incubated at 28°C for 1 h. At the time of assay, 0.5 mL of the gas sample was withdrawn after flushing twice and injected into gas chromatography and tested for ethylene production. The factor 0.006 was arrived by injecting pure ethylene gas. The nitrogenase activity was expressed as a mole of ethylene produced per gram of nodules per hour according to the procedure by Hardy *et al.* (1968).

Chromium accumulation: After harvesting, the plants were wiped with 0.01 N HCl and washed thoroughly with tap water, finally rinsed with distilled water in order to remove surface absorbed metal ions. The plants were divided into root and shoots and oven-dried at 80°C for 48 h. The dried samples were ground and digested with HNO_3 (APHA, 1998). Then chromium content was analyzed using atomic absorption spectrophotometer.

Statistical analysis: The experimental studies were carried out with Completely Randomized Design (CRD). Each treatment was conducted in triplicate and the Standard Deviation (SD) was calculated. The data was expressed in ±SD triplicate.

RESULTS AND DISCUSSION

Plant growth and biomass: The root length and shoot length of the tree species seedling grown in different concentrations of Cr contaminated soil is shown in Fig. 1. After 120 days the maximum shoot length (37.6 cm) and root length (21.2 cm) were observed in control treatment of *Pongamia glabra*. The shoot length (25.2 cm) and root length (12.7 cm) were reducedat 50 mg kg^{-1} chromium treated plants. The lowest growth of shoot (6.3 cm) root (3.4 cm) was reported in 50 mg kg^{-1} chromium treated *Tamarindus* indicus plants. Moreover, our result showed that *Pongamia glabra* tolerates high amount of chromium accumulation than the other tested species. Some morphological symptoms of yellowing of leaves appeared in the tree species when it is exposed to 30-50 mg kg^{-1} of Cr contaminated soil. Similar views were reported by various authors (Vajpayee *et al*., 2001; Rotkittikhun *et al*., 2007; Norwood *et al*., 2007). The phytotoxicity of heavy metals and extreme infertility of the contaminated soil were the major limiting factors for the plant growth. The morphological growth parameters like biomass and nodules were showed in Fig. 2 and 3. Highest morphological parameters were observed in control pots when compared to high concentration of chromium irrigated pots. The highest dry weight content 6.2 g seedlings and number of root nodules (39.2 nodules $plant^{-1}$) were observed in *P. glabra* control seedlings and it was reduced at 4 g $seedlings^{-1}$ and 18.7 nodules $plant^{-1}$ on 50 mg kg^{-1} treated control plants. The lowest dry weight yield was reported in *Azadirachta indica* seedlings (0.6 g sec^{-1}) on highest treatment of chromium in this study. The lowest number of root nodule was reported in *Cassia auriculata* (24 nodule $plant^{-1}$) at control and it was reduced to 11.8 nodules $plant^{-1}$ at 50 mg kg^{-1} chromium treated plants. *Tamrindus indicus* and *Azadirachta indica* were omitted this study because of the absence of root nodules. The plants grown on chromium treatment soil

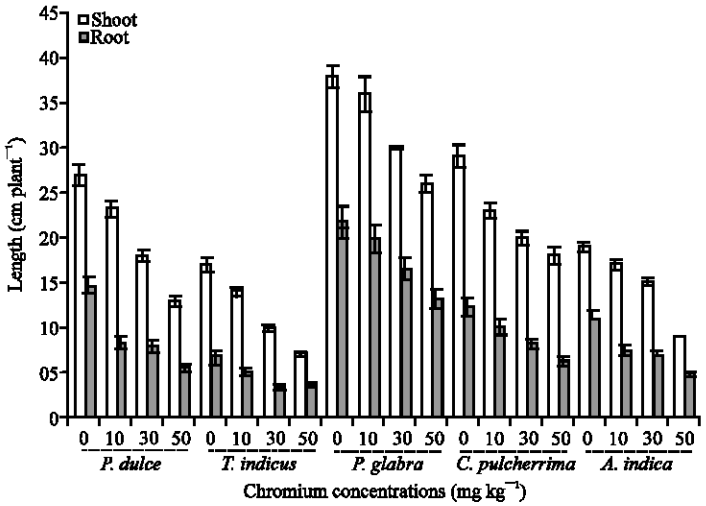

Fig. 1: Shoot and root length of tree species under Cr(VI) treatment

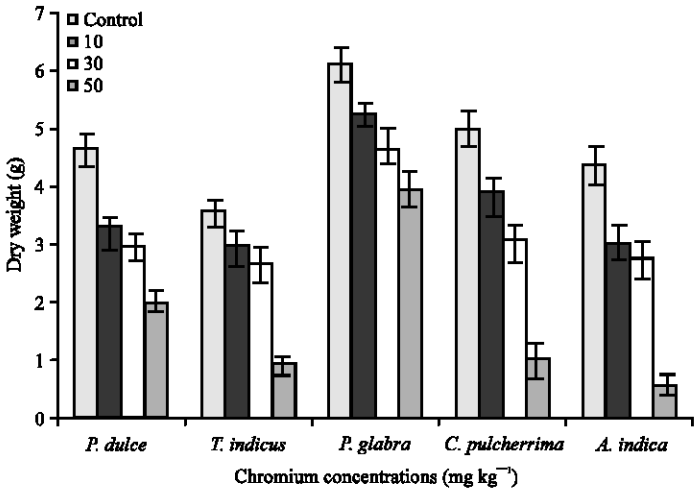

Fig. 2: Dry weight of tree species under Cr(VI) treatment

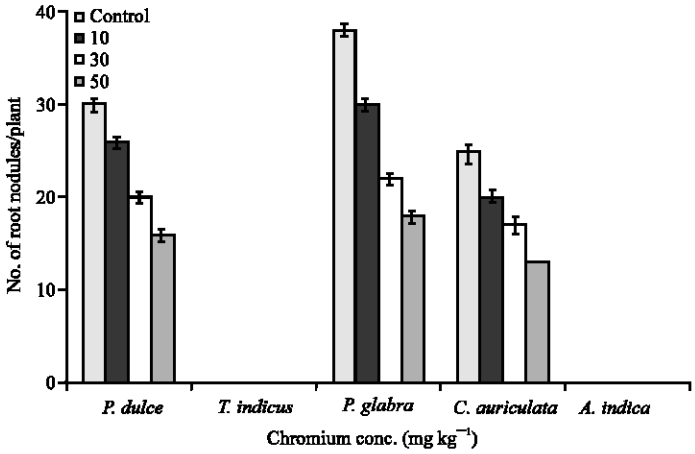

Fig. 3: Number of root nodules in tree species under Cr(VI) treatment

showed reduction in biomass growth, when compared to plants grown in control pots. The reduction of biomass and inhibition of growth may be due to the lack of water absorption capacity and osmotic pressure by the metal stress at higher concentrations. The presences of chromium create abiotic stress and damages in the external part of root system and inhibit the cell elongation and cell division (Chidambaram *et al*., 2009).

Photosynthetic pigments: Accumulation of Cr by the tree species and crops is known to produce significant physiological responses. The changes in the pigment content such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids after being exposed to chromium are shown in Fig. 4 and 5, respectively. The result shows that all the photosynthetic pigments were high in control and declined correspondingly with the increase in Cr concentration. The highest Chlorophyll 'a' (1.83 mg g^{-1} FW) content was observed in control seedlings of *Pithecellobium dulce*. *T. indicus* seedling shows highest content (0.78 mg g^{-1} FW) of chlorophyll 'b'. The highest content of carotenoid content were reported in control plants of *C. auriculata* (0.62 mg g^{-1} FW) and the lowest content was reported in (0.22 mg g^{-1} FW) at 50 mg kg^{-1} chromium treated *A. indica* seedlings. The impaired α-aminolevulinic acid dehydratase leads to reduced photosynthetic pigments by chromium treatment (Jana and Choudhuri, 1982). It may also be due to the presence of chromium, like metals have the potentials to alter the rate of photosynthesis by disturbing the structure of chloroplast, leading to the changes in the fatty acid composition inhibiting photosynthetic pigments and enzymes of Calvin cycle in chromium treated seedlings (Vazquez *et al*., 1987)

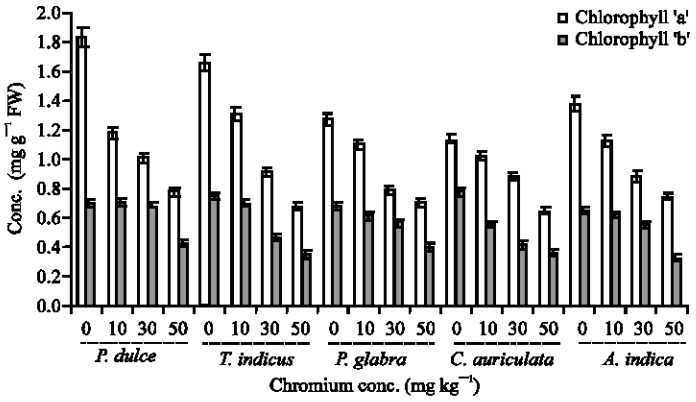

Fig. 4: Chlorophyll 'a' and 'b' content level in tree species under Cr(VI) treatment

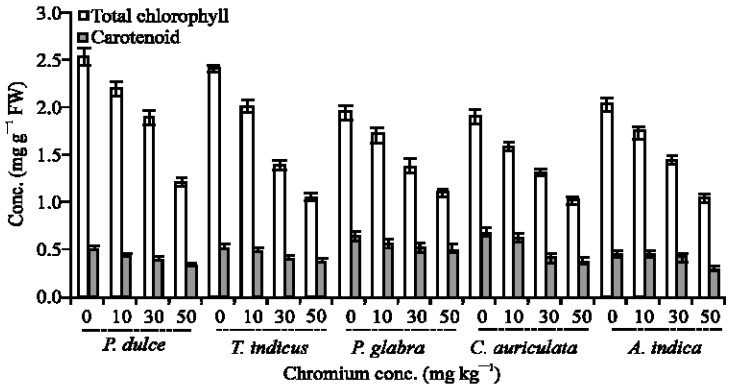

Fig. 5: Total chlorophyll and carotenoid content of tree species under Cr(VI) treatment

photophosphorylation (Vajpayee *et al*., 2001; Chandra and Kulshreshtha, 2004; Shanker *et al*., 2005). So, the treated tree species has low pigment contents and poor growth.

Amino acid and proline content: The amino acid and proline contents of five tree species after 120th day of chromium treatment are presented in Fig. 6 and 7. Amino acid and proline contents increased in chromium treatments when compared to control. The highest content (3.11 mg g^{-1} FW) of amino acid was observed in 50 mg kg^{-1} chromium treated *C. auriculata* and the highest proline content (1.5 mg g^{-1} FW) was observed in 50 mg kg^{-1} chromium treated *P. dulce*. The lowest amino acid (2.54 mg g^{-1} FW) and proline (0.4 mg g^{-1} FW) content was reported in control treated *P. glabra*. The increasing trend of proline and amino acids in chromium treated plants may be due to the stress in chromium treated soil. The capability of plants for chromium induced proline and amino acid accumulation. It could be brought of chromium metal and also by water deficiency. This water deficiency and water stress develop in the leaves and roots under the condition of chromium (VI) stress (Chidambaram *et al*., 2009).

Protein content: Protein content plays a major role in plant growth and development. The protein content of tree species is gradually decreased with increase of chromium concentration. (Table 1). Highest protein content (8.21 mg g^{-1} FW) were reported in control seedlings of *P. glabra* and the lowest protein content (1.98 mg g^{-1} FW) were observed at 50 mg kg^{-1} chromium treated *T. indicus* seedlings. Degradation in protein content might be the result of increased activity of protease or other catabolic enzymes under chromium stress (Rai *et al*., 1992). During transport of heavy metals in plants, sulphydryl group of protein may be reduced causing deleterious effect in the normal protein form and also while disturbing the protein metabolism automatically the plant mechanisms will affect so that the plant growth and development were inhibited.

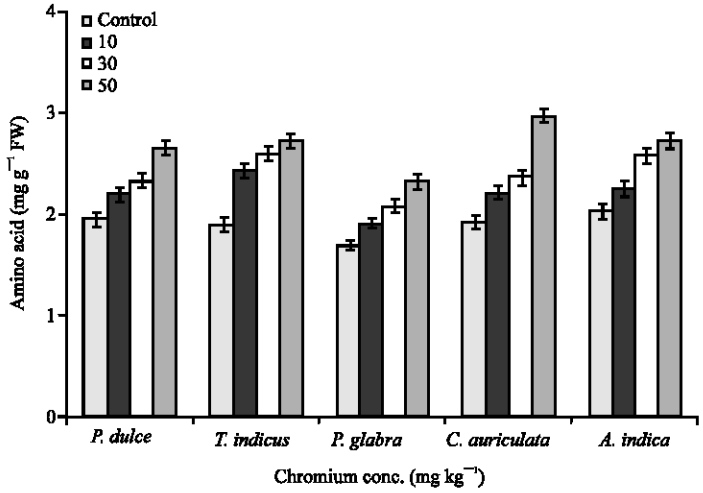

Fig. 6: Amino acid content of tree species under Cr(VI) treatment

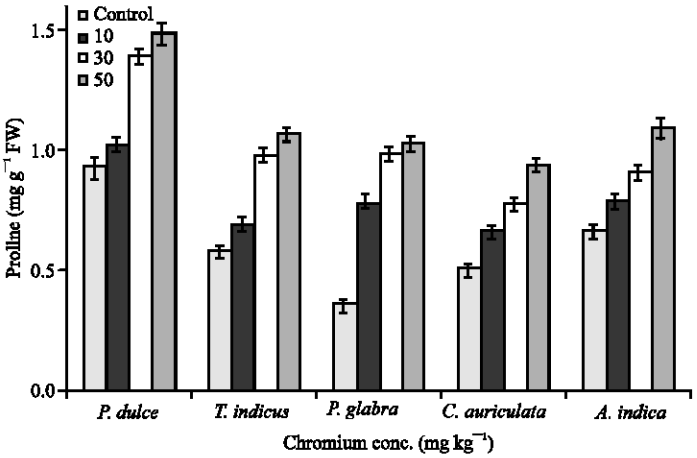

Fig. 7: Proline content of tree species under Cr(VI) treatment

Table 1: Effect of different concentrations of chromium on protein content of tree species

Species	Cr concentration (mg kg^{-1})	Protein content (mg g^{-1} FW)
Pithecellobium dulce	Control	7.2±0.36
	10	6.01±0.30
	30	3.29±0.16
	50	2.01±0.10
Tamarindus indicus	Control	4.01±0.20
	10	3.79±0.18
	30	2.54±0.12
	50	1.98±0.09
Pongamia glabra	Control	8.21±0.41
	10	6.08±0.30
	30	3.20±0.16
	50	3.11±0.15
Cassia auriculata	Control	7.28±0.36
	10	5.92±0.30
	30	3.81±0.18
	50	2.22±0.12
Azadirachta indica	Control	5.81±0.49
	10	4.27±0.20
	30	3.18±0.16
	50	2.01±0.10

Values are Means±SD

Leghaemoglobin (LHG) content: The changes of leghaemoglobin content are presented in Fig. 8. The highest level of leghaemoglobin content (7.31 μmol C_2H_2 nodule FW) was estimated in control treatment of *Pongamia glabra* and the lowest content (1.4 μmol C_2H_2 nodule FW) was observed in 50 mg kg^{-1} chromium treated Cassia auriculata. Among five plants, the leghaemoglobin (LHG) estimation was done in Leguminosae members such as *Pithecellobium dulce*, *Pongamia glabra*, *Cassia auriculata*. The LHG content gradually decreased while increasing the chromium level. At the same time, the presence of LHG induced the phytoextraction capacity of the plant by means of nitrogen fixation. The root nodules were absent in other two plants *Tamarindus indicus* and *Azadirachta indica*. The reduction of LHG and accumulation of chromium contents might be due to the conversion and translocation of metals by means LHG synthesis and nitrogenase enzyme.

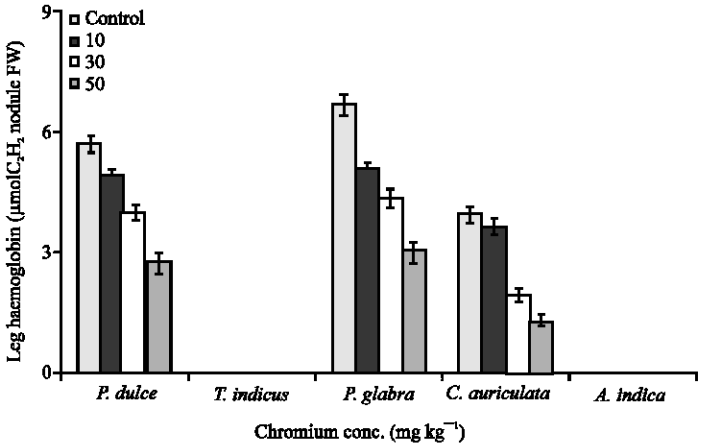

Fig. 8: Leghaemoglobin content of tree species under Cr(VI) treatment

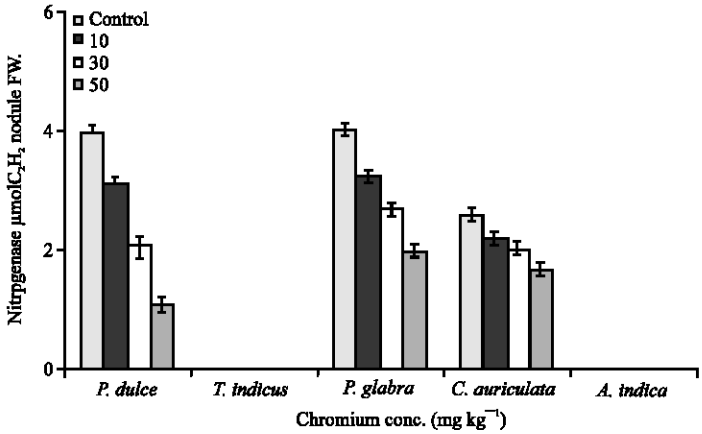

Fig. 9: Nitrogenase enzyme content of tree species under Cr(VI) treatment

Nitrogenase enzyme (NGE): The nitrogenase enzyme results showed a high level of NGE present in control treatment plants. Among the five plants NGE estimation was aslo done only in Leguminosae plants. Among the nodule having plants, *Pongamia glabra* shows (4.22 μmol C_2H_2 nodule FW) a high nitrogenase enzyme (Fig. 9) when compared with other plants. The lowest content (1.12 μmol C_2H_2 nodule FW) was reported in 50 mg kg^{-1} chromium treated *Pithecellobium dulce*. The presence of NGE promotes the extraction of chromium from soil to plant tissue. The absorption of chromium by root nodules may be due to the symbiotic relationship and also due to the transportation of chromium by root nodules.

Chromium accumulation and physicochemical analysis of soils: The phytoremediation of contaminated soils is getting more attraction in current day research and development (Banerjee *et al*., 2008). *Phytoremediation*, is a method used by plants to detoxify pollutants through physical, chemical and biological process (Wanzel *et al*., 1999). It is a practice for the detoxification of heavy metal from polluted areas. The technology is based on the ability of the plant to absorb the toxic elements and transport into the aerial parts of plants. Their distribution cannot be controlled and they can readily be processed for receiving valuable metals (Cunningham and Ow, 1996). Recent works provide evidence as in some members of the family *Brassicaceae* (Kumar *et al*., 1995*;* Zayed *et al*., 1998). Plants can accelerate bioremediation in surface soil with their ability to stimulate soil microorganism through the release of nutrient, from the soil and transport oxygen to the rhizosphere. Abundance of metabolically active microorganisms in rhizosphere transforms heavy metal from one oxidant state to another (Garbisu and Alkorta, 2001). The selected tree species accumulate high levels of Cr. The metal concentration in the plant increased with increase in concentration of chromium in

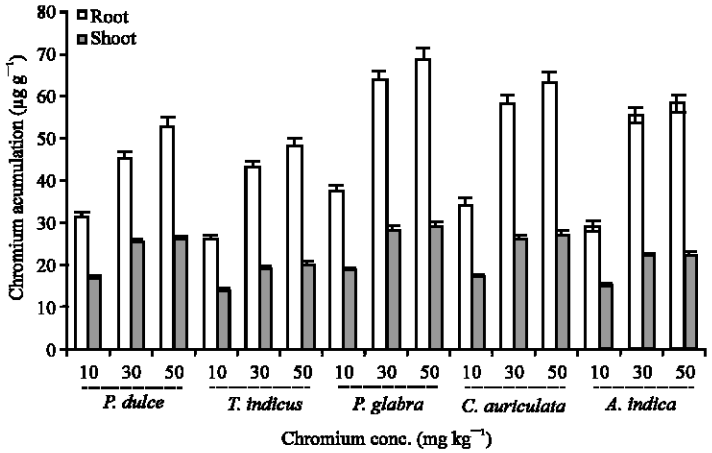

Fig. 10: Accumulation of chromium in root, shoot of tree species

Table 2: Average physicochemical composition of the soil sample and chromium polluted soil

Parameters	Garden soil	Contaminated soil (10 mg kg^{-1} chromium)	Contaminated soil (30 mg kg^{-1} chromium)	Contaminated soil (50 mg kg^{-1} chromium)
pH	6.65	6.6	6.0	5.43
EC	0.20	0.29	0.39	0.46
Nitrogen	382.00	282.0	282.0	282.0
Phosphorus	280.00	280	275	269
Potassium	75.00	100	130	150
Copper	6.10	6.10	6.10	6.10
Zinc	3.25	3.25	3.25	3.25
Iron	4.15	4.15	4.15	4.5
Manganese	3.17	3.17	3.09	3.04
Chromium	-	10	30	50

All parameters except pH and EC are expressed in mg L^{-1}

the soil. The physico-chemical characteristics of contaminated and normal soil such as pH, EC and Cu, Zn, Mn and Cr were analyzed and the results are given in Table 2. The maximum degradation of chromium was observed in *Pongamia glabra* grown soil followed by *Cassia auriculata* and the minimum degradation was observed in *Tamarindus indicus*, grown soil (Fig. 10). The uptake of Cr was highest in 50 mg kg^{-1} *Pongamia glabra* (root 68.2 and shoot 27.9 µg g^{-1}) and it was followed by *Cassia auriculata>Pithecellobium dulce >Azadirachta indica>Tamarindus indicus* (root 49.2 and shoot 22.5 µg g^{-1}). In this study, the highest chromium concentration was observed in root than shoots of the above plants after 120 days of growth. A similar trend was also reported previously (Barbosa *et al*., 2007*;* Jean *et al*., 2008), that chromium retained in the root present in soluble form in vacuoles of root cells and chromium in root protoplast. So, the maximum chromium accumulation was recorded in root when compared with other parts of plant. Poor translocation of Cr to the shoot is also expected, as plant are exposed with form of Cr and the tendency to accumulate Cr in the roots seems to be a common phenomenon in all plant species (Yadav *et al*., 2009) and also the bioavailability of metals to plants is maintained by numerous factors related with soil and environmental conditions (Sundaramoorthy *et al*., 2010). The metal accumulation by plants can be mainly active or passive process. It is probably due to binding of metals to the ligands and it reduced its mobility from roots to aerial parts (WHO, 1990; Jean *et al*., 2008). Accumulation of chromium by various plant species is well studied (Sinha *et al*., 2005). In the study, the leguminous plants show high degradation and accumulation of chromium because of plant rhizobacteria. The presence of bacteria changes the nature of chromium in solubilizing form (Abou-Shanab *et al*., 2003*;* Jing *et al*., 2007*;* Yadav *et al*., 2009). The accumulation of chromium from external medium into root cells is either due to diffusion of metal iron along the concentration gradient formed or due to mass how driven transpiration stream (Greger, 1999). The same thought reported earlier (Giller *et al*., 1998) that rhizobacteria play important role in recycling of plant nutrients, maintenance of soil structure and detoxification of chemicals, therefore bacteria enhance the remediation potential of plants.

CONCLUSION

This study is focused on the phytodegradation of chromium by growing five types of tree species. Among

them, *Pongamia glabra* accumulated high amount of chromium in both roots and shoots. Though chromium is more toxic, *Pongamia* is able to tolerate its high level presence. All plants used for the study degrade a considerable amount of chromium to clear the soil contamination level and reduce environmental risk to some extent. Finally, the article concluded that nature is made use of here, in attempting to create a good and healthy environment.

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REFERENCES

APHA, 1998. Standard Method for the Examination Of Water and Water Pollution Wastewater. 24th Edn., American Public Health Association, Washington, DC, USA.

Abou-Shanab, R.A., J.S. Angle, T.A. Delorme, R.L. Chaney and P. Van Berkum *et al*., 2003. Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. N. Phytol., 158: 219-224.

Aleves, M.M., C.G.G. Beca, R.G.D. Carvalho, J.M. Castanberia, M.C.S. Periera and L.A.T. Vasconcdos, 1993. Chromium(II) removal in tannery wastewater polishing by *Pinus sylvestris* Barti. Water Res., 27: 1333-1338.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenyloxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.

Banerjee, A., D. Nayak, D. Chakrabortty and S. Lahiri, 2008. Uptake studies of environmentally hazardous (51)Cr in *Mung beans*. Environ. Poll., 15: 423-427.

Barbosa, R.M.T., A.A.F. de Almeida, M.S. Mielke, L.L. Loguercio, P.A.O. Mangaberia and F.P. Gomes, 2007. A physiological analysis of *Genipa americana* L.: A potential phytoremediator tree for chromium polluted watersheds. Environ. Exp. Bot., 61: 264-271.

Bates, L.S., R.P. Waldren and I.D. Deare, 1973. Rapid determination of pre proline for water studies. Plant. Soil., 39: 205-207.

Bhattacharyya, P., A. Chakraborty, K. Chakrabarti, S. Tripathy and M.A. Powell, 2005. Chromium uptake by rice and accumulation in soil amended with municipal solid waste compost. Chemosphere, 60: 1481-1486.

Chandra, P. and K. Kulshreshtha, 2004. Chromium accumulation and toxicity in aquatic vascular plants. Bot. Rev., 70: 313-327.

Chidambaram, E., P. Sundaramoorthy, A. Murugan, K.S. Ganesh and L. Baskaran, 2009. Chromium induced cytotoxicity in blackgram (*Vigna mungo* L.). Iran. J. Environ. Health Sci. Eng., 6: 17-22.

Cunningham, S.D. and D.W. Ow, 1996. Promises and prospects of phytoremediation. Plant Physiol., 110: 715-719.

Ganesh, K.S., L. Baskaran, S. Rajasekaran, K. Sumathi, AL.A. Chidambaram and P. Sundaramoorthy, 2008. Chromium stress induced alterations in biochemical and enzyme metabolism in aquatic and terrestrial plants. Colliods Surf. B: Biointerfaces, 63: 159-163.

Garbisu, C. and I. Alkorta, 2001. Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol., 77: 229-236.

Giller, K.E., E. Witter and S.P. Mcgrath, 1998. Toxicity of heavymetals to microorganism and microbial process in agricultural soil. Soil. Biol. Biochem., 3: 389-414.

Greger, M., 1999. Metal Availability, Uptake Transport and Accumulation in Plants. In: Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, Prasad, M.N.V. (Ed.). Springer-Verlag, Heidelberg, Germany, pp: 51-57.

Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns, 1968. The acetylene-ethylene assay for N_2 fixation: Laboratory and field evaluation. Plant Physiol., 43: 1185-1207.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ., USA.

Jana, S. and M.A. Choudhuri, 1982. Senescence in submerged aquatic angiosperms: Effects of heavy metals. New Phytol., 90: 477-484.

Jean, L., F. Bordas, C. Gautier-Moussard, P. Vernay, A. Hitmi and J.C. Bollinger, 2008. Effect of citric acid and EDTA on chromium and nickel uptake and translocation by *Datura innoxia*. Environ. Pollut., 153: 555-563.

Jing, Y., Z. He and X. Yang, 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J. Zhejiang Univ. Sci. B, 8: 192-207.

Kazi, T.G., H.I. Afridi, N. Kazi, M.K. Jamali, M.B. Arain, N. Jalbani and G.A. Kandhro, 2008. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol. Trace Elem Res., 122: 1-18.

Kirk, J.T.O. and R.L, Allen, 1965. Dependence of chloroplast pigments synthesis on protein synthetic effects of actidione. Biochem. Biophys. Res. Cann., 27: 523-530.

Kumar, P.B.A.N., V. Dushenkov, H. Motto and I. Raskin, 1995. Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol., 29: 1232-1238.

Lakshmi, S. and P. Sundaramoorthy, 2001. Biochemical and mineral content changes of the paddy seedlings under tannery effluent treatment. J. Environ. Poll., 8: 13-17.

Lowry, O.H., N.J. Rosendrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Merkel, N., R. Schultze-Kraft and C. Infante, 2005. Phytoremediation in the tropics-influence of heavy crude oil on root morphological characteristics of graminoids. Environ. Pollut., 138: 86-91.

Mishra, S. and M. Doble, 2008. Novel chromium tolerant microorganisms: Isolation, characterization and their biosorption capacity. Ecotoxicol. Environ. Saf., 71: 874-879.

Moore, S. and W.H. Stein, 1948. Photometric method for use in the chromatography of amino acids. J. Biol. Chem., 176: 367-388.

Norwood, W.P., U. Borgmann and D.G. Dixon, 2007. Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. Environ. Pollut., 147: 262-272.

Piper, C.S., 1966. Soil and Plant Analysis. Hans Publishers, Bombay, India, Pages: 368.

Rai, U.N., R.D. Tripathi and N. Kumar, 1992. Bioaccumulation of chromium and toxicity on growth, photosynthetic pigments, photosynthesis, *in vivo* nitrate reductase activity and protein content in a chlorococcalean green algae *Glaucocystis nostochinearum*. Chemophere, 25: 1721-1732.

Rotkittikhun, P., R. Chaiyarat, M. Kruafrachue, P. Pokethitiyook and A.J.M. Baker, 2007. Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thysanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study. Chemosphere, 66: 45-53.

Sankar Ganesh, K., P. Sundaramoorthy and A.L.A. Chidambaram, 2006. Chromium toxicity effect on blackgram, soybean and paddy. Pollut. Res., 25: 257-261.

Scoccianti, Y., R. Crinelli, B. Tirillin, V. Mancinelli and A. Speranza, 2006. Uptake and toxicity of Cr(III) in clerly seedlings. Chemosphere, 64: 1695-1703.

Shanker, A.K., C. Cervantes, H.L. Tavera and S. Avudainayagam, 2005. Chromium toxicity in plants. Environ. Int., 31: 739-753.

Sharma, D.C., C.P. Sharma and R.D. Tripathi, 2003. Phytotoxicity lesions chromium in maize. Chemosphere, 51: 63-68.

Sinha, S., R. Saxena and S. Singh, 2005. Chromium induced lipid peroxidation and enzymatic and non-enzymatic antioxidants and bacoside-a content in medicinal plant *Bacopa monnieri* L. Chemosphere, 55: 227-255.

Subbiah, B.V. and G.L. Asija, 1956. A rapid procedure for the estimation of available nitrogen in soils. Curr. Sci., 25: 259-260.

Sundaramoorthy, P., A. Chidambaram, K.S. Ganesh, P. Unnikannan and L. Baskaran, 2010. Chromium stress in paddy: (I) Nutrient status of paddy under chromium stress; (ii) Phytoremediation of chromium by aquatic and terrestrial weeds. C.R. Biol., 333: 597-607.

Sundaramoorthy, P., K.S. Ganesh, L. Baskaran, K. Sumathi and S. Rajasekaran, 2006. Germination behaviour of some agricultural crops under chromium treatment-short communication. Bull. Biol. Sci., 6: 99-101.

Vajpayee, P., R.D. Tripathi, U.N. Rai M.B. Ali and S.N. Sinha, 2000. Chromimum (VI) accumulation reduced chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphaea alba* L. Chemosphere, 41: 1075-1082.

Vajpayee, P., U.N. Rai, M.B. Ali, R.D. Tripati, U. Yadav, S. Sinha and S.N. Singh, 2001. Chromium induced physiological changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. Bull. Environ. Contam. Toxicol., 67: 246-256.

Vazquez, M.D., C. Poschenriedr and J. Barcello, 1987. Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.). Ann. Bot., 59: 427-438.

WHO, 1990. Chromium, Nickel and Welding (IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans). Vol. 49, World Health Organization, Lyons, France, ISBN-13: 978-9283212492, pp: 256.

Wanzel, W.W., E. Lombi and D.C. Adriano, 1999. Biochemical of Metal Polluted Soils. In: Heavy Metal Stress in Plants: From Molecules to Ecosystem, Prasad, M.N.V. and J. Hagemeyer (Eds.). Springer, Berlin, Germany, pp: 273-303.

Yadav, S.K., A.A. Juwarkar, G.P. Kumar, P.R. Thawale, S.K. Singh and T. Chakrabarti, 2009. Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by *Jatropha curcas* L.: Impact of dairy sludge and biofertilizer. Bioresour. Technol., 100: 4616-4622.

Yu, X.Z., X.Y. Peng and L.Q. Xing, 2010. Effect of temperature on phytoextraction of hexavalent and trivalent chromium by hybrid willows. Ecotoxicology, 19: 61-68.

Zayed, A.M., C.M. Lytle and N. Terry, 1998. Accumulation and volatilization of different chemical species of selenium by plants. Planta, 206: 284-289.