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ORIGINAL RESEARCH ARTICLE

Cr^{+6}- induced growth, biochemical alterations and Chromium bioaccumulation in *Cassia tora* (L.) Roxb.

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Abstract: The present *in vivo* pot culture experiments showed hexavalent Chromium (Cr^{+6}) induced growth, physiological and biochemical alterations and Chromium bioaccumulation in 60 days *Cassia tora* (L.) Roxb. (Coffe Pod) plants. Root and shoot length, fresh and dry matters considerably decreased with increasing concentration of Cr^{+6}. However, in 10 ppm shoot dry weight increased. High dose Cr^{+6} application showed decreased chlorophyll, protein and catalase activity. The activity of Catalase increased with 10 ppm of Cr^{+6} supply to the soil which declined with 50 ppm of Chromium in leaves. Total sugar, reducing sugar, starch and total carbohydrate contents also decreased with increase Cr^{+6} concentration. Proline and peroxidase activities increased with increasing concentration of Cr^{+6}. Chromium bioaccumulation was more in roots than leaves and stems. Bio-Concentration Factor (BCF), Total Accumulation Rate (TAR) indicated highest values i.e. 0.051 and 0.070 respectively in 50 ppm whereas Transportation Index (TI) was highest (0.814) in 10 ppm of Cr^{+6}. The results of the present study revealed that the phytoremediation ability of Coffe pod plants growing under wild conditions can be used *to* combat Cr $^{+6}$ stress in chromium contaminated areas.

Key words: Biochemical alterations; *Cassia tora*; Cr^{+6}application; Cr-bioaccumulation plant growth

Introduction

Chromium is an important toxic environmental pollutant. The open caste recovery and extensive use of chromium in industrial activities leads to the release of large quantities of toxic hexavalent chromium to environment. Hexavalent chromium (Cr^{6+}) stress is one of the major abiotic stress problems in chromite mining sites. Plants that are exposed to environmental contamination by chromium are affected in diverse ways, including a tendency to suffer metabolic stress. The studies on attenuation of toxic effects of chromium using plants in Cr^{+6} amended in soil are limited (Chaturvedi *et al.,* 2015). One of the approaches, i.e., the moving of Cr contaminated top soil to landfills, is costly and amplify environmental threats. Sources of new native hyperaccumulator plants for use at contaminated sites are needed and constitute a key objective of current phytoremediation research activities. Therefore, more informations are warranted to know the potential phytoaccumulators to exploit the situation in an effective, affordable and eco-friendly ways.

Phytodetoxification is a process which brings detoxification of heavy metals through plant-based chelation, reduction, and oxidation mechanisms. (Salt *et al*., 1998; Zayed and Terry, 2003; Panda and Patra, 1997; Mohanty and Patra, 2011; Chaturvedi *et al.,* 2015). The use of different plant species for cleaning contaminated soils and waters by phytoremediation mechanism has gainedimportance as a low cost technology. *Cassia* is a wild legume in tropics and few reports suggest its role as hyper accumulator activity (Siringoringo, 2000; Shirbhate and Malode, 2012). In this context, pot culture study with chromium amended soils was designed to analyze the phytotoxic effects of Cr^{+6} on bioavailability in *Cassia tora* (Coffe pod). Further, efforts were made to verify the effects of varied concentrations of chromium on growth, physiological and biochemical alterations. The observations has been correlated with plant tolerance to Cr^{+6} and its bioaccumulation in roots and shoots of the test plant.

Material and Methods

Plant Materials

Cassia tora (L.) Roxb. (Coffe pod) seeds were collected from the mature pods of the *Cassia tora* palnts grown as a wild weed from Utkal University campus which is hilly area at Bhubaneswar, India. The seeds were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes followed by thoroughly washing in tap water and distilled water before use for sowing in culture pots for plant growth and analysis. Seeds were sown in poly pots containing garden soil and green manure (3:1) for germination, propagation and plant growth. Before sowing of seeds in poly pots, the soils were amended with different concentration of hexavalent Chromium (Cr^{+6} source: $K_2Cr_2O_7$)

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solutions viz. 10ppm and 50 ppm respectively. The control pot was run at the same time without Cr treatment. The same aged leave as per the method adopted by Patra and Misha (1979) were collected from 60 days grown plants of *Cassia tora* and used for biochemical and enzymatic analysis.

Growth Culture Experiment

The plant growth analyses was undertaken by measuring root and shoot length, fresh weight and dry weight of roots and shoots of 60 days plants grown in Chromium amended soils using different concentrations of hexavalent Chromium (10 ppm and 50 ppm).

Toxicological Interpretation:

The toxicological effects of ionic $Cr^{+6}$was expressed in terms of % phytotoxicity, and tolerance index (TI) which were calculated by the methods as described previously (Iqbal and Rahmati, 1992; Labra *et al.,* 2006 and; Datta *et al.,* 2011).

Biochemical Analysis

Chlorophyll and Carotenoid contents of leaves of *Cassia tora* were analyzed using 60 days grown plants. The pigments were extracted using alkaline cold acetone (80% v/v) and calculated by the Arnon's method (1949) with minor alteration (Porra, 2002). Toal sugar was analyzed by Anthrone reagent (Yoshid *et al.*, 1972) and reducing sugar by Nelson's method (1944). Protein and Proline contents were analyzed and estimated as done previously (Patra and Mishra, 1979, Bates *et al.*, 1973). Catalase (CAT) and peroxidase (POD) enzyme assay and activities were measured as per the method Chance and Maehly (1955) with modification (Patra *et al.*, 1978 and Patra and Mishra (1979).

Total Cr Bioavailability in Plant tissues.

Cassia tora seedlings grown up to 60 days in different concentrations of of Cr^{+6} (0, 10 and 50 ppm) were analyzed for total Cr content in roots, shoots and leaves (Bonnet *et al.*, 1991). Root, stem and leaves of 60 days treated *Casia tora* plants from different pots of were oven dried and grinded separately to fine powders. Nitric acid and Perchloric acid in the ratio of 10:1 as acid mixture was used for digestion of powdered plant samples for extraction of total Cr content of roots, stems and leaves using MDS-8-Microwave Digestion Unit. The acid digested solutions were filtered and the final volume was made up to 100 ml. Total Cr bioaccumulation was estimated from different plant parts using extracted liquid samples in an Atomic Absorption Spectrophotometer (Perkin Elmer, AAanalyst 200, USA). Plant biomass was used for analysis of Bio-Concentration Factor (BCF), Total Accumulation Rate (TAR) and Transportation Index (Ti) as described previously (Zurayk *et al.*, 2002; Ghosh and Singh, 2005a).

Statistical analysis

The experiments were conducted in triplicates for each treatment and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates.

Results

Study of Growth Indices Impairment in Response to Cr^{6+} Stress

The growth parameter studies on 60 days Coffe pod plants showed visible deterioration in growth with increasing supply of Cr^{+6}. Root and shoot length, fresh and dry matters of Coffe pod considerably decreased with increasing concentration of Cr^{+6} in comparison to control (Fig.1, 2 and 3). However, in 10 ppm shoot dry weight increased. Root growth inhibition is a primary toxic effect of heavy metals and this parameter is an ideal index to measure the degree of tolerance (Wong and Bradshaw, 1982). The roles of heavy metals on growth impacts and tissue ionic concentration has been described (Paiva *et al.*, 2000). The work also states that dry weight of shoot and root significantly reduced significantly with increase in heavy metal level. The reduction becomes more harmful with elevated levels of heavy metals. Therefore, plants showed greater variations ranging from morphological characters to physiochemical characters. In the present study, plant height and fresh weight were reduced at higher concentration of Cr^{+6} (50 ppm). This is probably due to the reason that toxicity of heavy metals significantly inhibited root vitality, checking plant roots from absorbing mineral ions and leading to reduction of plant growth and development (Datta *et al.*, 2011; Mohanty *et al.*, 2015).

Table 1: Toxicological interpretation of 60 days old *Cassia tora* plants grown in Cr^{+6} contaminated soil.

Treatment	% phytotoxicity to root	% phytotoxicity to shoot	Root tolerance index	Shoot tolerance index	Plant tolerance index
Control	0	0	100	100	100
Cr^{+6}-10 ppm	15.15	29.07	84.84	70.92	77.03
Cr^{+6}-50 ppm	16.91	38.90	83.08	61.09	70.74

Table 2: Bio concentration Factor (BCF), Transportation index (Ti) and Total Accumulation rate (TAR) of *Cassia tora* plant.

Treatment (ppm)	Transportation Index (Ti)	Bio-Concentration Factor	Total Accumulation Rate (TAR) (mg $kg^{-1}day^{-1}$)
Control	0.00	0.00	0.00
Cr^{+6}-10	0.814	0.050	0.064
Cr^{+6}-50	0.696	0.051	0.070

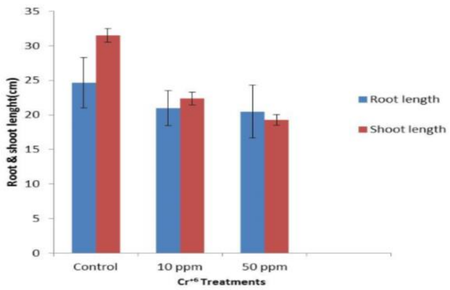

Figure 1: Effect of different concentration of Cr^{+6} on Root and shoot length of 60 days old *Cassia tora*

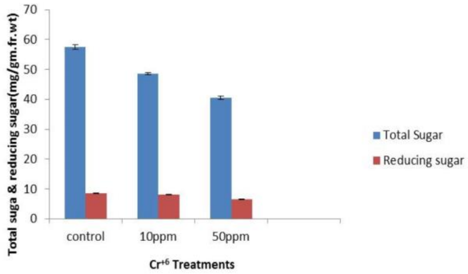

Figure 5: Effect of different concentration of Cr^{+6} on leaf total sugar and reducing sugar contents of 60 days old *Cassia tora*

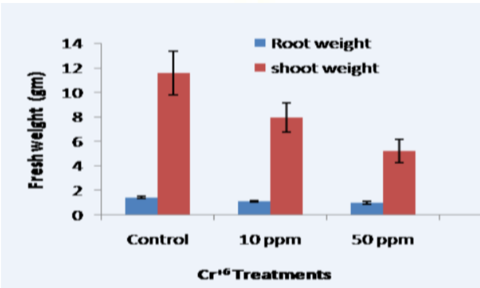

Figure 2: Effect of different concentration of Cr^{+6} on Root and shoot weight of 60 days old *Cassia tora*

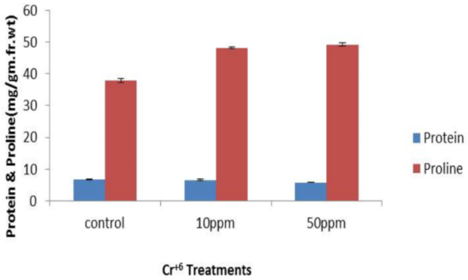

Figure 6: Effect of different concentration of Cr^{+6} on leaf protein and proline contents of 60 days old *Cassia tora*

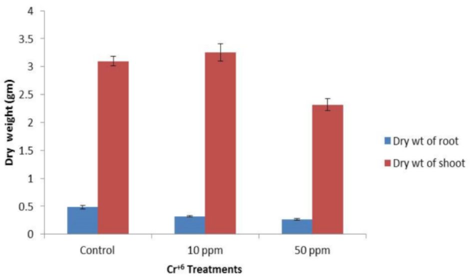

Figure 3: Effect of different concentration of Cr^{+6} on dry weight of Root and shoot of 60 days old *Cassia tora*

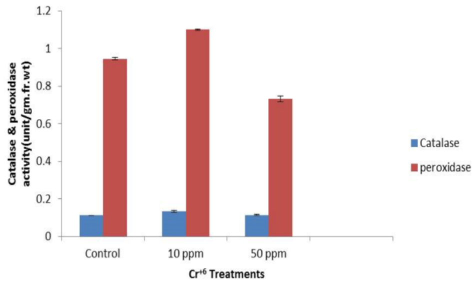

Figure 7: Effect of different concentration of Cr^{+6} on leaf catalase and peroxidase activities of 60 days old *Cassia tora*.

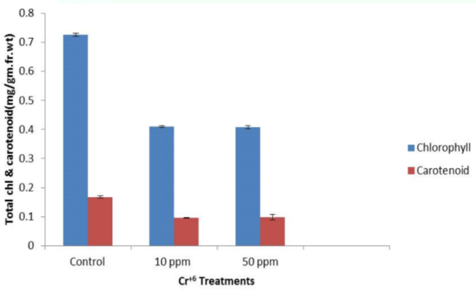

Figure 4: Effect of different concentration of Cr^{+6} on leaf chlorophyll and carotenoid content of 60 days old *Cassia tora*.

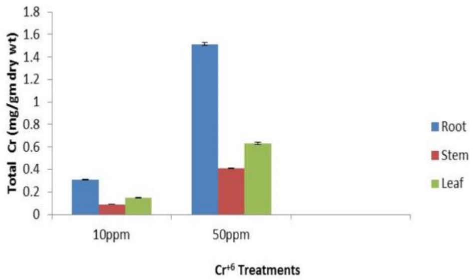

Figure 8: Effect of Cr^{6} on total Cr bioaccumulation (mg/kg dry mass of plant) in 60 days grown *Cassia tora* plants.

Toxicological Interpretation

The levels of phytotoxicity (%) in roots and shoot of 60 days grown *Cassia tora* under different concentrations of Cr^{+6} treatments showed an increase tendency with supplementary Cr^{+6} concentration (Table 1). The highest phytotoxicity values of root (16.91%) and shoot (38.90%) was found in plants supplied with 50 ppm of Cr^{+6}. Results from the tolerance studies showed that root, shoot and plant TI decreased with increasing Cr^{+6} supply. Root tolerance has been found better as compared to shoot.

Toxic Effects of Cr^{6+}on Biochemical Parameters.

A significant deterioration in chlorophyll and carotenoid contents of Coffe pod leaves were observed at higher Cr concentration (50 ppm) (Fig.4). Less reduction in chlorophyll and carotenoid content was observed with increased Cr stress which indicates that renewed biosynthesis of chlorophyll and carotenoid pigments are taking place preventing the acceleration of chlorophyll reduction. Decreased content of chlorophyll is a common symptom of heavy metal toxicity. The inhibitory role of Cr on biosynthesis of pigments may be a metal specific action which can also block the photosynthetic electron transport chain and thus degrade chlorophyll pigment (Bonnet *et al.,* 2000; Zeid, 2001). Total sugar, starch, reducing sugar and non-reducing sugar contents were highest in control grown up to 60 days (Fig 5). Different carbohydrate compounds decreased with increase in chromium concentrations from 10 to 50 ppm levels due to toxic effects which prevented carbohydrate synthesis during plant growth.

The data for protein and proline contents were given in Fig 6. The protein content decreased with increase in chromium concentration. The reduction in protein levels with increasing concentration of Cr^{+6} may be due to degradation by proteases by Cr phytotoxicity (Prassad, 1996; Romero-Puertas *et al.*, 2002). Proline level was found highest in leaves of 50 ppm chromium treated pots grown up to 60 days. Catalase and Peroxidase activities of leaves of 60 days old plants were given in Fig.7. Catalase activity increased initially using 10 ppm of Cr^{+6}. Similar trend for peroxidise was found at 10 ppm chromium treatment. However, the use of 50 ppm Cr inhibited biosynthesis of antioxidant enzymes (Catalase and Peroxidase) Metal stress inhibits enzyme activities possesing functional sulfhydryl group which also affects the normal protein form by disrupting the pathways and protein synthesis (Nagoor, 1999).

Chromium Bio-accumulation in Cassia tora

Chromium content of roots, stems and leaves showed a high level of differences (Fig. 8). Cr accumulated more in roots as compared to aerial parts during plant growth and development. The maximum Cr accumulation was observed in root (1.515) using 50 ppm Cr^{+6} treatment. Cr accumulation in stems was less (0.09) as compared to root and leaves. The aerial parts of the coffe pod plants showing less Cr as compared to roots is a familiar feature as noted by other workers (Ghosh and Singh 2005a, b; Erenoglu *et al.,* 2007; Mohanty and Patra 2011; 2012). High Cr accumulation in roots and less uptake to aerial portion of the plant is the most common metal tolerance character evidenced by (Ghosh and Singh, 2005a). Cr accumulation and Bio-concentration factor (BCF) is usually increased with increasing Cr concentration (Ghose and Singh a, b). In the present study, Cr accumulation and BCF increased with increasing Cr^{+6} concentrations (Table 2). The Total Accumulation Rate (TAR) increased with increasing concentration of Cr^{+6}. High concentrations of Cr application has been correlated with high Cr bioaccumulation and shoot translocation in *Cassia tora.* Ti values was found highest (0.814 mg kg^{-1}) using concentration of Cr^{+6} 10 ppm. The plants also showed a general trend of fall in Ti value with an increase in chromium concentration as reported by other (Ghosh and Singh, 2005b).

Discussion

The present study revealed deleterious impacts of varying concentrations of Cr^{+6} on growth parameters, photosynthetic pigments, antioxidant enzymes and chromium bioaccumulation which suggests the potentiality of *Cassia tora* plants for Cr uptake, translocation and bioaccumulation. The study explains the impact of Cr toxicity and gives the idea of attenuation of toxic chromium from the polluted soil as the plants permit to tolerate chromium stress when grown in chromium amended soil. Therefore, the findings of the present research will help to lay down ideas of chromium detoxification and attenuation toxicity by suitable phytoremediation methods.

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