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Translocation and accumulation of chromium and its phytotoxic effects on *Sesbania sesban*

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ABSTRACT

In vivo pot culture study was conducted at Utkal University, Bhubaneswar (Odisha) to study the translocation and accumulation of chromium and its phytotoxic effects on Sesbania sesban during rabi season of 2018.The results showed that hexavalent chromium (Cr^{+6}) induced phytotoxic impacts on biochemical changes and its translocation potential in 15, 30 and 45 days old sesban (Sesbania sesban L.) seedlings. Hexavalent chromium showed significant growth retardation in 45 days old sesbania seedlings. Germination of seeds at 300 mg $kg^{-1}$of Cr^{+6} exhibited 85% inhibition in germination. Results showed decreased chlorophyll, and carotenoid with increasing Cr^{+6} concentrations. The higher proline content (36.32 mg gm^{-1} fr. wt.) was observed at 300 mg kg^{-1} of Cr^{+6}. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities were significantly higher at elevated supply of Cr^{+6} than control. Chromium bioaccumulation in roots was more than shoot. Chromium accumulation in the root and shoot increased gradually with increasing chromium concentration and caused reduction in overall plant growth. Tissue specific chromium bioaccumulation was more in roots and shoots respectively at 300 mg kg^{-1}. The present study reveals that sesbania can be used for effective Cr translocation from soil to roots then shoots.

Key Words: Bioaccumulation, hexavalentchromium, antioxidantenzymes

INTRODUCTION

Ever increasing environmental stress due to pollution is a serious global issue. The pollution due to heavy metals has increased over the years due to natural and anthropogenic activities leading to degradation of rhizosphere. Most of them are toxic to plants even at lower concentrations like lead and cadmium. The toxic levels of these elements have been reported in the soil, plants and in the environment. They are translocated into different parts, accumulate and bio-magnify into the food chain. On the earth chromium is present at seventh richest component. Chromium presents in many states of oxidation form i.e Cr^{+2} to Cr^{+6}, out of them most common and stable form present in the surroundings are Cr^{+3} and Cr^{+6}. Cr^{+3} is an important element for animal and human beings. Contrasting Cr^{+3}, Cr^{+6} is most powerful, really lethal, carcinogen and might reason for fatality to aminals and human beings, if intake large quantity. Intake of plant imitative chromium containing foodstuff, mainly cereals provides a most important portion of the everyday chromium intake. For that reason, it is important to determine the form of chromium which arepresent on plant parts and its accumulation. Chromium exists in an aqueous as a hexavalent chromium Cr^{+6} and trivalent chromium Cr^{+3}. Several mutagenic, poisonous and carcinogenic effects in biological systems caused by chromium have been studied. Cr^{+6} and Cr^{+3} have different degrees of action and this because of their different ability to cross the biological membranes. Cr^{+6}enters the cell membrane possibly through the sulphate (anion) transport channel as chromate (CrO_4^{2}), whereas Cr^{+3} complexes enter the cell membrane through passive transport. Trivalent form of chromium forms stable complexes with several ligands and thereby alters the physiological functions. *Sesbania sesban* L. commonly known as sesban has proved to be particularly popular leguminous plant due to its fast growing and wide use as fuel and fodder. *Sesban* plants have high efficiency in fixing atmospheric nitrogen and producing high biomass. It is also planted as an intercrop for soil fertility enhancement because it bears nitrogen fixing root nodule. The experiment was carried out to investigate Cr toxicity, tolerance and accumulation, phytotoxicity on morphological and biochemical parameters.

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MATERIALS AND METHODS

Dry graded seeds of *Sesbania sesban* were collected from Orissa University of Agriculture and Technology (OUAT), Bhubaneswar and uniform size of seeds were selected by hand picking. Then the seeds were surface sterilized by mercuric chloride (0.1%) for 5 min, after that washed with tap water for several times followed by distilled water before they were used for germination study and pot experiment. The germination of seed was done in earthen pots having 5 kg garden soil. After seven days, seedlings grown in uncontaminated garden soil were supplemented with different Cr^{+6}concentrations (10, 50, 100, 200 and 300 mg kg $^{-1}$). Plants were grown up to 45 days and harvested for growth and biochemical analysis. Analyses of plants growth, pigment contents, biochemical alterations and antioxidants were conducted to correlate the changes associated with different types of treatments used for remediation of Cr. Analysis of pigment chlorophyll content was conducted by extracting pigments from leaves of the same age (3rd nodal position) from 15.30 and 45 days grown sesbnia plantlet (Porra, 2002). Proline estimation was done as method of Bates *et al.*, (1973). Enzyme extraction and assay were carried out at 4 °C. Catalase, peroxidase and Superoxide dismutase activity was measured as per methods of Chance and Maehly (1955) with a little modification Patra and Mishra (1979). The entire Cr bioavailability in root and shoot was determined according to Patra *et al.*(2019). The plant parts like root and shoot were collected and root parts were washed in 0.01 N HCl and then washing thoroughly with distilled water to eliminate Fe and Cr hydrous oxides which might have present in surface area of root. The digestion of sample was done by using MDS-8 (Microwave Digestion Unit) until an apparent soluble solution was obtained. The digested solutions were filtered by filter paper final volume was made up to 50 ml. By using atomic absorption spectrophotometer (Perkin Elmer, Analyst 200, USA), Cr content was estimated from different parts of plants

RESULTS AND DISCUSSION

Impact of chromium stress on Sesbania sesban on growth

Under high concentration of Cr, seeds germination was significantly more inhibited than control. On the other hand, germination fraction was significantly affected at 200 and 300 mg kg^{-1}, signifying that elevated amount of chromium formed toxic consequence in germination of seed. The consequence of dissimilar concentrations of Cr on plant height, length of root and shoot, fresh and dry weight of root and shoot of *sesbania* was recorded at 45 days after sowing (Table1). Root and shoot length of *Sesbania* seedlings were significantly affected by high concentrations of Cr^{+6} than control. The increase in chromium concentrations decreased all morphological growth parameters of Sesbania at all the sampling days.

Table 1: Growth parameters of 45 days *Sesbania* plants under different types of Cr^{+6} applications

ts Cr^{+6} (mg kg^{-1})	Root length(cm)	Shoot length(cm)	Fr.wt of root (g)	Fr. wt of shoot (g)	Dry wt.(DW) in root(g)	Dry wt. (DW) in shoot(g)	Root DW/Shoot DW(g)
Control	10.0±1.15	30.33±2.7	0.457±0.03	4.571±0.17	0.141±0.02	0.804±0.02	0.175
10	9.33±1.20	23.66±1.3	0.255±0.01	1.903±0.04	0.058±0.01	0.263±0.01	0.220
50	12.33±1.76	29.33±0.8	0.505±0.01	3.916±0.03	0.116±00	0.651±0.02	0.178
100	8.66±0.66	28.0±1.5	0.459±0.04	3.312±0.08	0.107±0.01	0.674±0.02	0.158
200	9.66±0.33	27.0±0.58	0.249±0.01	2.926±0.29	0.083±00	0.473±0.01	0.175
300	5.33±1.20	22.0±0.15	0.299±0.02	2.808±0.17	0.077±0.01	0.344±0.02	0.141

Root and shoot length of sesban seedlings were significantly affected with toxic concentrations of Cr^{+6} in comparisons to control after 45 days of growth. The effect of Cr^{+6} (300mg kg^{-1}) on root was found to be highly toxic as the length of the roots was significantly reduced as compared to control. Similar results were reported by Zayed and Terry (2003).

Effect of Cr stress on biochemical parameters

Significant deteriorations in chlorophyll and carotenoid content of sesban leaves were observed with increasing supply of Cr^{+6} (Fig. 1 and 2). Chromium stress causing reduction in chlorophyll content was attributed to ultrastructural damage (Patra *et al.*, 2018a; Patra *et al.*, 2018b; Patra *et al.*, 2018c Patra *et al.*, 2019; Patra *et al.*, 2020). Increased total chlorophyll content was observed at the lower level of Cr^{+6} (10 and 50 mg kg^{1}) obviously due to better growth of sesbania seedlings in comparison to control. Reduced chlorophyll content at elevated concentration may be due to the existence of metal ions or deprivation of chlorophyll by free radicals produced by heavy metals. Chlorophyll content ratio was found to be more or less depending upon metals used in the experiment. This indicates the positive impact of heavy metals on chlorophyll biosynthesis in the plants because at definite concentration of iron and copper which can act as catalytic and structural elements for chlorophyll biosynthesis.

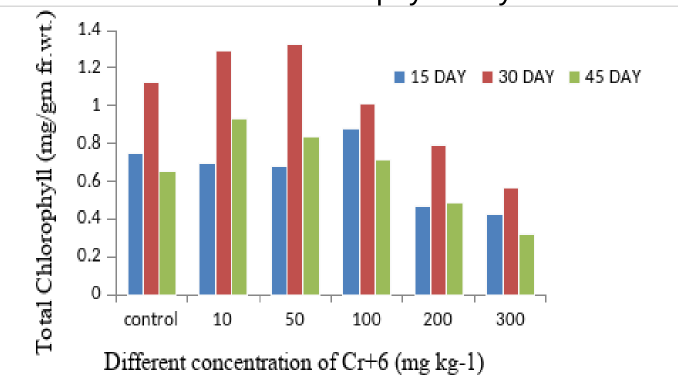

Fig.1: Effect of Cr^{+6} on total chlorophyll (mg/g fr.wt.) of *Sesbania sesban* seedlings

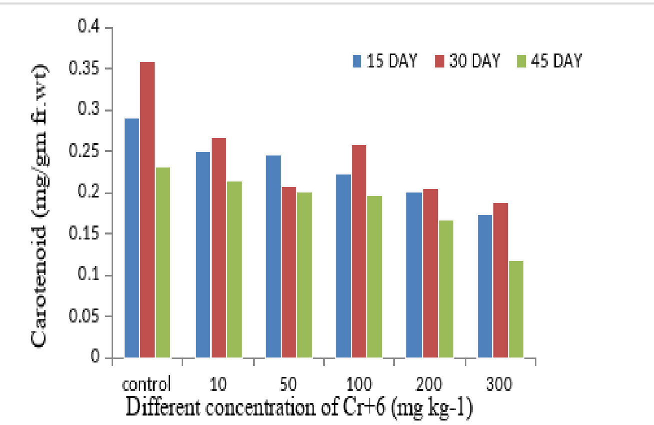

Fig.2: Effect of Cr^{+6} on carotenoid (mg/g fr.wt.) of *Sesbania sesban* seedlings

The carotenoid content of control plants was found to be highest collected at different time intervals of plant growth. The carotenoids play as accessory component for photosynthesis which protect chlorophyll from degradation during stress conditions caused by heavy metals or other stress.

Chromium showed linear increase in proline accumulation (Fig. 3) because the proline is the only amino acid that accrues to a greater extent in the leaves of many plants under stress (Mohanty and Patra, 2011).The higher proline content (36.32 mg g^{-1} fr. wt.) was observed at 300 mg kg^{-1} of Cr. Proline build up might also assist in detoxification of non-enzymatic free radical. Elevated Cr^{+6} levels showed an enhancement in proline accretion after 45 days growth in treatment soils. Elevated proline content is well thought-out to facilitate the cells both in osmoprotection and, scavenging hydroxyl free radicals and gives defence against macromolecules denaturation (Khan *et al.,* 2002).

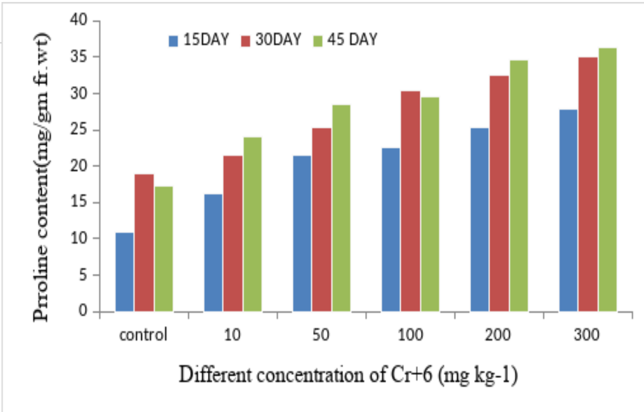

Fig.3:Effect of Cr^{+6} on proline (mg/g fr.wt.) of *Sesbania sesban* seedlings

Effect of Cr stress on activity of antioxidant enzymes

CAT activity was found highest in 45 days plants (1.42 unit/mg fr.wt.) in 200 mg kg^{-1} concentration (Fig.4). Lowest expression of CAT activity was reported from 15 days plant grown in controlled condition (0.435 unit/ mg fr.wt.). Peroxidase activity was found highest in 45 days plants (1.985unit/ mg fr.wt.) in 200 mg kg^{-1} concentration and followed by 200 mg kg^{-1}(1.78 unit/ mg fr.wt.) at 30 days (Fig 5). Lowest peroxidase activity was reported from 15 days plant grown in controlled condition (0.765 unit/

mg fr.wt.). SOD activity was found highest in 45 days plants of (1.998 unit/ mg fr.wt.) in 200 mg kg-[1] concentration and followed by 200 mg kg-[1] (1.85unit/ mg fr.wt.) at 30 days (Fig 6). (Mobin and Khan, (2007) reported similar results. The result suggested that the chromium present in soil induces oxidative stress in plant that increased the activity of antioxidents which could be a protection mechanism to mitigate the oxidative injury as the plants showed better growth despite the elevated activity of POD, CAT and SOD in the treatments than the respective control. Increase in SOD activity may be attributed to accumulation of superoxide radical, de-novo synthesis of enzymatic proteins (Verma and Dubey, 2005) and induction of SOD (Alvarez and Lamb, 1997). The higher activities of oxidative enzymes with increasing metal accumulation in the plants are probably slow shift of reductive phase to oxidative phase. The results suggested that chromium present in soil induces oxidative stress in plants.

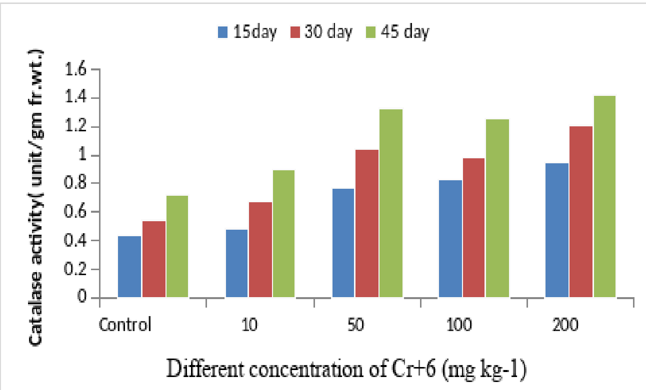

Fig. 4:Effect of Cr^{+6} on CAT activity (unit/mg fr.wt.) of *Sesbani sesban* seedlings

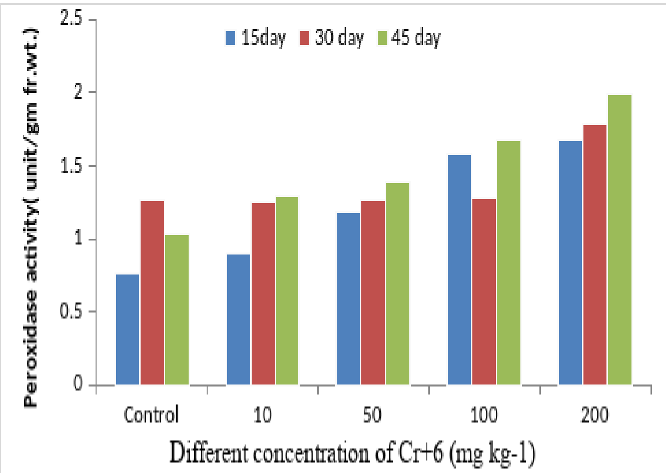

Fig.5: Effect of Cr^{+6} on POD activity (unit/mg fr.wt.) of *Sesbani sesban* seedlings

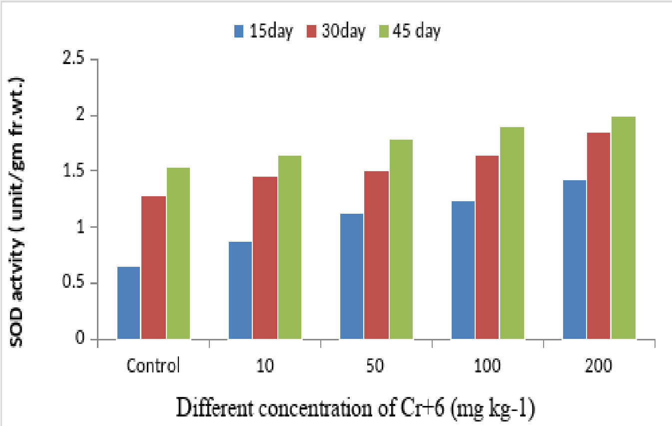

Fig.6: Effect of Cr^{+6} on SOD activity (unit/mg fr.wt.) of *Sesbani sesban* seedlings

Chromium Bio-accumulation in root and shoot

Chromium bioaccumulation in root and shoot was gradually increased with supply of elevated high Cr concentration. Heavy Cr accumulation in plant tissues and shoot translocation at high Cr concentration enable the plant to be a potential accumulator of Cr. Chromium accumulation in root stem and leaves gradually increased with supply of elevated concentrations of Cr (Zou 2009). Maximum Cr accumulation was observed in roots of sesban seedlings for all the treatments in comparison to shoot (Fig. 7). Cr The transport and build-up of chromium depends on the formation of complexes that act to enhance Cr uptake and availability in plants (Zhuang *et al.*, 2007).

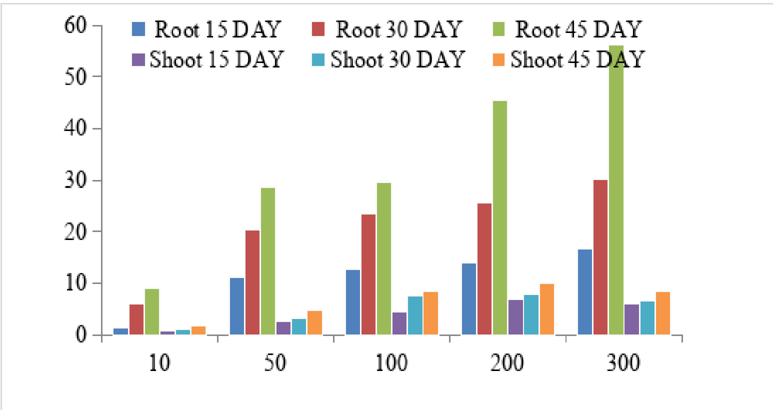

Fig: 7: Effect of different concentrations of Cr^{+6}(mg Kg^{-1}) on total Cr bioaccumulation in *Sesbania sesban*

It may be concluded that the elevated concentration of chromium triggered a substantial decline in growth, chlorophyll and carotenoid and increased activity of antioxidative enzyme in sesbania plant by elevating ROS production, accumulation of maximum amount of chromium. *Sesbania* could be a highly efficient plantswhich may be used in upcoming phytoremediation technology and grassland applications in mining wastes soil for recovery and phyostabilisation programme.

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