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# CHROMIUM TRANSLOCATION, CONCENTRATION AND ITS PHYTOTOXIC IMPACTS IN *IN VIVO* GROWN SEEDLINGS OF *SESBANIA SESBAN* L. MERRILL.

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The present *in vivo* pot culture study showed hexavalent chromium ($Cr^{+6}$) induced phytotoxic impacts and its translocation potential in 21 days old sesban (*Sesbania sesban* L. Merrill.) seedlings. $Cr^{+6}$ showed significant growth retardation in 21 days old sesban (*Sesbania sesban* L. Merrill.) seedlings. Germination of seeds at 10,000 mg $L^{-1}$ of $Cr^{+6}$ exhibit 80% inhibition in germination. Seedling survival was 67% after 7 days of seedling exposure to 300 mg $kg^{-1}$ of $Cr^{+6}$. Shoot phytotoxicity was enhanced from 6% to 31% with elevated supply of $Cr^{+6}$ from 10 mg $kg^{-1}$ to 300 mg $kg^{-1}$. Elevated supply of $Cr^{+6}$ exhibited increasing and decreasing trends in % phytotoxicity and seedling tolerance index, respectively. Elevated supply of chromium showed decreased chlorophyll and catalase activities. Peroxidase activities in roots and leaves were significantly higher at increased supply of $Cr^{+6}$. Cr bioconcentration in roots was nearly 10 times more than stems whereas leaves showed nearly double accumulation than stems. Tissue specific chromium bioaccumulation showed 53 and 12 times more in roots and shoots respectively at 300 mg $kg^{-1}$ $Cr^{+6}$ than control. The present study reveals potential of sesban for effective Cr translocation from roots to shoots as evident from their translocation factor and Total Accumulation Rate values.

*Keywords:* Antioxidative enzymes – bioaccumulation – chromium – TF-TAR – TI

## INTRODUCTION

Global industrialization, extensive mining activities and growing demands of increased human population in this twenty-first century leads to release of toxic heavy metal ions to the environment. Heavy metal phytotoxicity limits plant growth and crop cultivation in acid soils and tremendously affects the crop mortality. Fe, Mo and Mn are important heavy metals as micronutrients whereas Zn, Ni, Cu, V, Co, W and Cr are toxic elements with high or low importance as trace elements. Chromium (Cr) is the seventh most abundant element on earth [19]. Out of the different oxidation states of chromium (Cr), hexavalent ($Cr^{+6}$) and trivalent ($Cr^{+3}$) chromium are stable in nature. They differ in terms of mobility, bioavailability and toxicity. $Cr^{+3}$ are essential for animal and human health whereas $Cr^{+6}$ is a potent, extremely toxic, carcino-

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gen and may cause death to animals and humans, if ingested in large doses. Various mutagenic, toxic and carcinogenic effects have been imposed by chromium compounds in biological systems [10, 18, 20, 28]. $Cr^{+6}$ usually occurs in association with oxygen as chromate ($CrO_4^{2-}$) or dichromate ($Cr_2O^{2-}_7$) oxyanions that have a long residence time and high solubility in the water [12]. Seed germination is the first physiological process affected by $Cr^{+6}$ [22]. The metal ions exert their toxic effect on cell metabolism when it reaches a threshold level in soil. Sensitive species serve as an indicator and tolerant species which retain large amount of metals in their cell wall without causing any damage to cellular system, were detected as accumulators.

Widespread use of chromium in several industrial and mining activities leads to the release of toxic hexavalent chromium to environment. Hexavalent chromium ($Cr^{+6}$) stress is one of the major problems in chromite mining area of Orissa (India). The state of Orissa accounts for 98% chromite reserve of the country [18]. *Sesbania sesban* L. Merrill. commonly known as sesban has proved to be extremely popular leguminous agroforestry species due to its fast growth and wide use as fuel and fodder. It has also proved to be extremely tolerant of a wide range of sites including those which can be regarded as difficult such as saline, waterlogged. Sesban plants have high efficiency in fixing atmospheric nitrogen and producing high biomass. These plants are used as a fodder and forage plant in India and Taiwan [1]. It is also planted as an intercrop for soil improvement because it bears nitrogen fixing root nodules. Considering the above positive traits, the plant could be effectively employed for reclamation of mine sites which overcome the two major problems for plant establishment on mine tailings i.e. toxicity of heavy metals and deficiency of major nutrients. This warrants exhaustive investigations on phytotoxic impacts of varying doses of $Cr^{+6}$ along with its concentration in different plant tissues. There is a huge dearth of information on the toxicological responses in sesban plant under Cr stress. The present study aimed to assess the phytotoxic impacts of $Cr^{+6}$ which include growth impairment studies, physiological, biochemical and toxicological changes in 21 days old sesban seedlings exposed to varying concentrations of hexavalent chromium.

## MATERIALS AND METHODS

### *Experimental design and plant material*

Pot culture experiments were conducted in completely randomised design in the nursery site of Post Graduate Department of Botany, Utkal University, Odisha, India during the month of January to April. Dry graded seeds of sesban (*Sesbania sesban* L. Merrill.) were procured from Central Rice Research Institute, Cuttack and were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes.

### *Germination study*

The pretreated uniform healthy sesban seeds were germinated in Petri dishes over saturated cotton pads supplemented with different concentrations of $Cr^{+6}$ (source: $K_2Cr_2O_7$) viz. 5 mg $L^{-1}$, 10 mg $L^{-1}$, 100 mg $L^{-1}$, 200 mg $L^{-1}$, 500 mg $L^{-1}$, 1000 mg $L^{-1}$, 1500 mg $L^{-1}$, 2000 mg $L^{-1}$, 2500 mg $L^{-1}$, 3000 mg $L^{-1}$, 4000 mg $L^{-1}$, 5000 mg $L^{-1}$, 8000 mg $L^{-1}$, 10000 mg $L^{-1}$ along with a control for two days inside BOD incubator at 25 ± 2 °C. After 48 hours (two days) the number of germinated seeds under each treatment of $Cr^{+6}$ was recorded. The germination percentage and germination index (IG %) of seeds were calculated [20].

### *Growth of sesban seedlings*

The seeds were germinated in earthen pots (size: height 30 cm and diameter 15 cm) containing 5 kg garden soil (control: $Cr^{+6}$ –0 mg $kg^{-1}$) and after 7 days of plant growth in uncontaminated control garden soil, the pots were supplemented with selected concentrations of $Cr^{+6}$ (10 mg $kg^{-1}$, 100 mg $kg^{-1}$, 200 mg $kg^{-1}$ and 300 mg $kg^{-1}$) ($Cr^{+6}$ treatments were in values of mg per kg dry weight of soil) and were grown at the nursery site of Department of Botany, Utkal University for 21 days. So, 21 days old seedlings meant for the seedlings treated for 21 days with different concentrations of $Cr^{+6}$. Pots supplemented with half strength Hoagland nutrient solution were taken as control treatment. One mg $kg^{-1}$ Cr treatment is considered as 1 ppm.

### *Toxicological analyses*

The toxicological effects of $Cr^{+6}$ were expressed in terms of % phytotoxicity, germination index and Tolerance Index (TI), Translocation Factor (TF) and Total Accumulation Rate (TAR) which were calculated by the methods prescribed by Mohanty and Patra [19], Labra et al. [14] and Datta et al. [9].

### *Analysis of biochemical parameters*

Analysis of seedling growth, pigment content and proline accumulation was conducted using 21 days old sesban seedlings. The extraction of chlorophyll was made using cold alkaline acetone (80% v/v) and calculated as per the methods of Arnon [3] with a little modification [23]. Proline was estimated as per method of Bates et al. [5]. Enzyme extraction and assay were carried out at 4 °C. Catalase and peroxidase enzyme assay and activity were measured as per the method of Chance and Maehly [8] with a little modification [21].

### *Total Cr bioavailability in plant tissues*

Sesban seedlings treated with different concentrations of $Cr^{+6}$ for twenty one days were analyzed for total Cr content in roots and shoots [19]. Before analysis of total Cr content, the roots were rinsed with 0.01 N HCl followed by washing with distilled water for removing mixed Fe and Cr hydroxides, which may have precipitated on the root surfaces. Root, stem and leaves of 21 days old sesban seedlings from different treatment pots of $Cr^{+6}$ were oven dried and grinded separately to fine powders. Nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) in the ratio of 10:1 were added to the weighed and grinded plant powder samples (roots, stems and leaves) separately and kept for 24 hours overnight [19]. Then the acid mixed plant samples were digested and extracted for metal content using MDS-8 (Microwave Digestion Unit). The acid digested solutions were filtered by Whatman No.1 filter paper and the final volume was made up to 100 ml with deionized water. Total Cr bioaccumulation in different parts of plants were estimated by analysing those extracted liquid samples in an Atomic Absorption Spectrophotometer (Perkin Elmer, AAanalyst 200, USA).

### *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. LSD values were calculated and DMRT test was conducted showing significant variation in treatment means.

## RESULTS AND DISCUSSION

### *Seed germination affected by Cr stress*

The seed germination test under increasing concentrations of $Cr^{+6}$ ranging from 10 to 10,000 mg $L^{-1}$ showed gradual inhibition. Seeds germinated under different concentration of $Cr^{+6}$ showed significant reductions in germination percentage as compared to control (Fig. 1). The germination % ranged from 20–98% with decreasing concentrations of $Cr^{+6}$ treatments (Fig. 1). The reduced germination of sesban seeds under $Cr^{+6}$ stresses was due to the depressive effect of Cr on the subsequent transport of sugars to the embryo axis [29]. The percentage germination index (IG%) computed from combined data was reduced to 59% when the seeds are exposed to 300 mg $L^{-1}$ of $Cr^{+6}$ (Table 1). The % survival of seedlings after treatment with increasing concentrations of $Cr^{+6}$ was also reduced from 87% (control – 0 mg $kg^{-1}$ of $Cr^{+6}$) to 67% (300 mg $kg^{-1}$ of $Cr^{+6}$). The reduction in germination of sesban seeds was attributed to increased protease activity under chromium stress [9, 29].

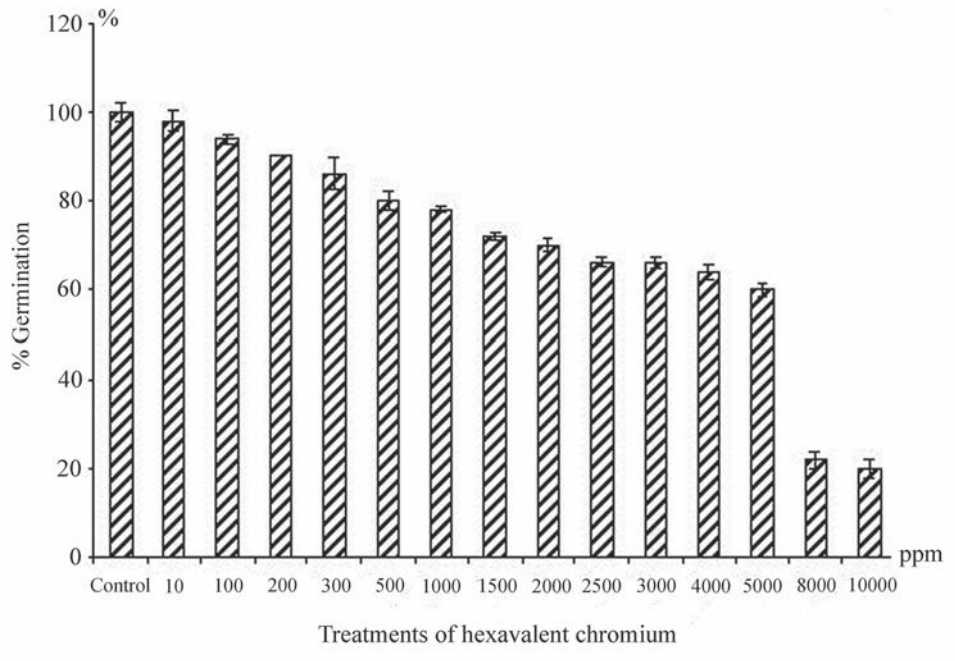


*Fig. 1.* Effect of different concentrations of $Cr^{+6}$ on % germination of *Sesban* seeds

## *Analysis of growth indices impairment in response to* $Cr^{+6}$ *stresses*

The growth parameter studies of 21 days old sesban seedlings under different treatments of $Cr^{+6}$ showed significant deterioration in seedling growth with increasing supply of $Cr^{+6}$ (Table 1). Root and shoot length of sesban seedlings were significantly (at $P \leq 0.05$) affected with toxic concentrations of $Cr^{+6}$ among the different treatments in comparison to control after 21 days of growth. The effect of $Cr^{+6}$ (300 mg $kg^{-1}$) on root was found to be highly toxic as the length of the roots have been significantly reduced as compared to control. Similar results have been reported by several other workers in other plants [7, 28]. The deleterious effect was more pronounced in root and shoot biomass of 21 days old sesban seedlings supplemented with $Cr^{+6}$ (300 mg $kg^{-1}$). Root growth inhibition is a primary toxic effect of heavy metals [26] and this parameter is an ideal index to measure the degree of tolerance. The difference in the tolerance index of roots and shoots towards different concentrations of $Cr^{+6}$ showed a declining trend with increasing concentration of $Cr^{+6}$ (Table 1). Hexavalent chromium affects plant growth and metabolism by decreasing nutrient uptake and photosynthetic abilities [4, 19].

Reduction of growth parameters was scored for all concentrations of $Cr^{+6}$ tested; at low concentration of $Cr^{+6}$ (10 mg $kg^{-1}$) the damage was not fatal. A consistent

growth inhibition was observed starting from 10 mg $kg^{-1}$ of $Cr^{+6}$ (Table 1) with the highest inhibition occurring at 300 mg $kg^{-1}$. A significant decrease in root length was observed when the sesban plants were supplemented with $Cr^{+6}$ beyond 100 mg $kg^{-1}$ concentrations. Shoot length of sesban seedlings treated with different concentrations of $Cr^{+6}$ showed significant difference from each other at $P \leq 0.05$ (Table 1). Root fresh weight values of seedlings treated with $Cr^{+6}$ (300 mg $kg^{-1}$) significantly decreased in comparison to control and other treatments.

The % phytotoxicity to roots and shoots of 21 days old sesban seedlings under different $Cr^{+6}$ treatments showed an increasing trend with increasing $Cr^{+6}$ concentration. The highest % phytotoxicity value of shoot (31.3) was found in plants supplied with 300 mg $kg^{-1}$ of $Cr^{+6}$. In case of % phytotoxicity of root, similar observation was found (Table 1). The different concentrations of $Cr^{+6}$ significantly ($P \leq 0.05$) contributed towards % phytotoxicity of root and shoot.

Tolerance Index (TI) represents the relative growth rate of the plants and is equal to the growth of seedlings under $Cr^{+6}$ treatment divided by the growth in control, the quantity multiplied by 100. TI of roots length and fresh weight are commonly used to quantify plant metal tolerance as described by Turner [24a]. The higher the TI, the better is the tolerance. Results from the seedling tolerance studies showed that fresh weight TI was better than root tolerance index (Table 1). Root lengths are less substantially impaired by Cr stress than fresh weights (Table 1). The data for IG%, % phytotoxicity and Tolerance Index indicates that sesban is less sensitive to chromium and is a tolerant species.

The results of the experiments highlight the complex nature of plants' tolerance to $Cr^{+6}$. The most likely explanation for the increased root tolerance is that there are increased activities of antioxidative enzymes like catalase (1:11:1:6) and peroxidase (1:11:1:7) in roots as compared to shoots (Table 2). These enzymes are mostly associated with the scavenging of toxic free radicals produced as a result of Cr stress. These enzymes play important role to detoxify and sequester Cr, and therefore, the roots appear to be the major site of enzyme synthesis. This finding is consistent with the results obtained from previous studies with cadmium (Cd) tolerance conducted by Zhu et al. [30]. The researches found that the activities of catalase and peroxidase in the roots of sesban plants were about 2-fold higher than in shoots (Table 2). The translocation of less toxic and reduced amount of Cr from the root to the shoot through the xylem was probably driven by transpiration as reported for other metals by Salt et al. [24]. Another possible explanation for the observed difference between fresh weight and root length tolerance is that gluthathione (GSH) and phytochelatin (PC) are involved in heavy metal tolerance rather than uptake as reported by Lee [15].

*Table 1*
Toxicological interpretations in 21 days old sesban seedlings under $Cr^{+6}$ stress

| Treatments of $Cr^{+6}$ (mg $kg^{-1}$) | Germination index (IG%) | % survival after 7 days growth of germinated seedlings | % phytotoxicity to roots | % phytotoxicity to shoots | Root tolerance index | Shoot tolerance index | Seedling tolerance index |
|---|---|---|---|---|---|---|---|
| Control (0) | 100 | 87 | 0 | 0 | 1 | 1 | 1 |
| 10 | 83.83 | 80 | 14.5 | 6.4 | 86 | 94 | 90.28 |
| 100 | 66.61 | 74 | 29.1 | 22.5 | 71 | 77 | 77.54 |
| 200 | 65.08 | 70 | 27.7 | 25.8 | 72 | 74 | 69.45 |
| 300 | 59.69 | 67 | 30.6 | 31.3 | 69 | 69 | 62.31 |

*Table 2*
Antioxidative enzyme activity of 21 days old sesban seedlings under $Cr^{+6}$ stress

| Treatments | Concentration of $Cr^{+6}$ (mg $kg^{-1}$) | Root catalase activity | Leaf catalase activity | Root peroxidase activity | Leaf peroxidase activity |
|---|---|---|---|---|---|
| Control | 0 | $780^{d} \pm 1.7$ | $394^{d} \pm 5.0$ | $1763^{d} \pm 3.8$ | $132^{e} \pm 1.9$ |
| Hexavalent Chromium ($Cr^{+6}$) | 10 | $1934^{a} \pm 6.9$ | $1135^{a} \pm 5.7$ | $1765^{d} \pm 6.4$ | $553^{d} \pm 4.4$ |
| | 100 | $1446^{b} \pm 5.6$ | $765^{b} \pm 7.8$ | $1866^{c} \pm 1.5$ | $766^{c} \pm 0.9$ |
| | 200 | $864^{c} \pm 4.8$ | $470^{c} \pm 2.2$ | $2045^{b} \pm 1.6$ | $859^{b} \pm 1.3$ |
| | 300 | $513^{e} \pm 3.8$ | $231^{e} \pm 1.4$ | $2287^{a} \pm 2.3$ | $1612^{a} \pm 1.9$ |
| | LSD* $P<0.05$ | 9.8 | 17 | 9.6 | 7.7 |

NB: Mean in a column superscribed with different letters are significantly different at LSD* $P<0.05$ by DMRT; Catalase activity as expressed in Catalase unit per gm fresh weight; Peroxidase activity as expressed in Peroxidase unit per gm fresh weight.

## *Toxic effects of $Cr^{+6}$ on biochemical parameters*

### *Effect of $Cr^{+6}$ on chlorophyll and proline content*

A significant deterioration in chlorophyll content of sesban leaves were observed with increasing supply of $Cr^{+6}$ when grown for 21 days (Fig. 2). The increased Cr stress causing reduction in chlorophyll content was attributed to ultrastructural damage [19]. Similar reports on the impacts of metal toxicity are available in different plants [13, 16]. An increased total chlorophyll content was observed at the lower level of $Cr^{+6}$ (10 mg $kg^{-1}$) treatment obviously due to better growth of sesban seedlings in comparison to control. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis. An excessive supply of chromium seems to prevent the incorporation of iron into the protoporphyrin molecule, resulting in the reduction of chlorophyll pigment [6, 9]. Similar events of reduced pigment biosynthesis have been reported under salt stress. Chromium degrades δ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting δ-aminolevulinic acid (ALA) utilization; this results in the buildup of ALA and reduction of the level of chlorophyll [25]. Chromium, mostly in its hexavalent form, can replace Mg ions from the active sites of many enzymes. $Cr^{+6}$ also cause Fe deficiency

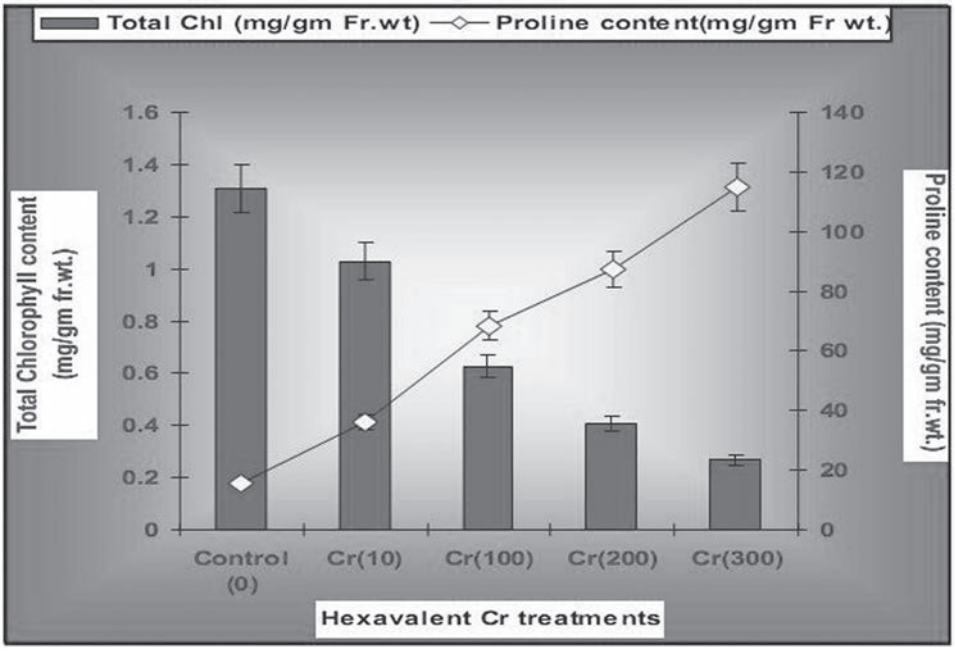


*Fig. 2.* Total chlorophyll level and proline biosynthesis in *Sesbania sesban* seedlings after 21 days exposure to $Cr^{+6}$ stresses

in stressed plants, disrupting chlorophyll biosynthesis [16, 31]. The total chlorophyll content decreases linearly with increasing concentration of $Cr^{+6}$ (Fig. 2).

Increasing concentrations of $Cr^{+6}$ shows linear increase in proline accumulation (Fig. 2). Proline content increases with the enhancing concentration of $Cr^{+6}$ (Fig. 2) because the proline is the only amino acid that accumulates to a greater extent in the leaves of many plants under stress [19]. In the present investigation, higher proline content (114.7 mg $gm^{-1}$ Fr. Wt.) was observed at 300 mg $kg^{-1}$ of Cr and the decrease may be related with the reduced growth of plant. Proline accumulation is an important parameter to recognize the stress impact on plants [19, 27].

Proline accumulation may also help in nonenzymic free radical detoxifications [17, 19]. Increasing proline level is considered to help the cells in osmoprotection as well as in regulating the redox potential, scavenging hydroxyl radicals and gives the protection against denaturation of various macromolecules [11]. Correlation coefficient ($R^2$) values exhibited good linear correlation between different increasing concentrations of $Cr^{+6}$ and proline accumulation in 21 days old sesban seedlings. In the present context, a uniform increase in the proline level was noticed when the sesban seedlings were subjected to increased concentrations of $Cr^{+6}$.

## *Effect of $Cr^{+6}$ on catalase and peroxidase activity*

A significant increase in root and leaf catalase activities were observed in sesban seedlings treated with 10 mg $kg^{-1}$ and 100 mg $kg^{-1}$ $Cr^{+6}$, respectively, but the activity showed declining trend with increasing supply of Cr (Table 2). Root and leaf peroxidase activities were significantly increased with increasing concentration of $Cr^{+6}$ (Table 2). Plants under different treatments of $Cr^{+6}$ showed significant variation in their catalase and peroxidase activities. Peroxidase (POD) and catalase (CAT) are two potent scavengers of $H_2O_2$, which minimize its accumulation and diffusion across cell membranes, preventing peroxidative damage to cell constituents. Enhancement in the activities of both roots and leaf CAT activity were recorded at low $Cr^{+6}$ stress (up to 200 mg $kg^{-1}$) and this possibly contributed to better scavenging of $H_2O_2$ in these plants. After the treatment with 300 mg $kg^{-1}$ $Cr^{+6}$ for 21 days, decreased CAT activities in roots and leaves were observed. This can be interpreted as a sign of cytotoxicity due to overproduction of reactive oxygen species (ROS) [31]. Peroxidase activity showed significant increase with elevated treatments of Cr concentration.

## *Chromium accumulation in sesban*

Chromium accumulation in root stem and leaves gradually increases with supply of elevated concentrations of Cr which corroborate the findings of other researchers in different plants [32]. Maximum Cr accumulation was observed in roots of sesban seedlings for all the treatments in comparison to stem and leaves (Fig. 3). Cr accumulation in leaves was double than that of the stems. Sesban seedlings after 21 days of

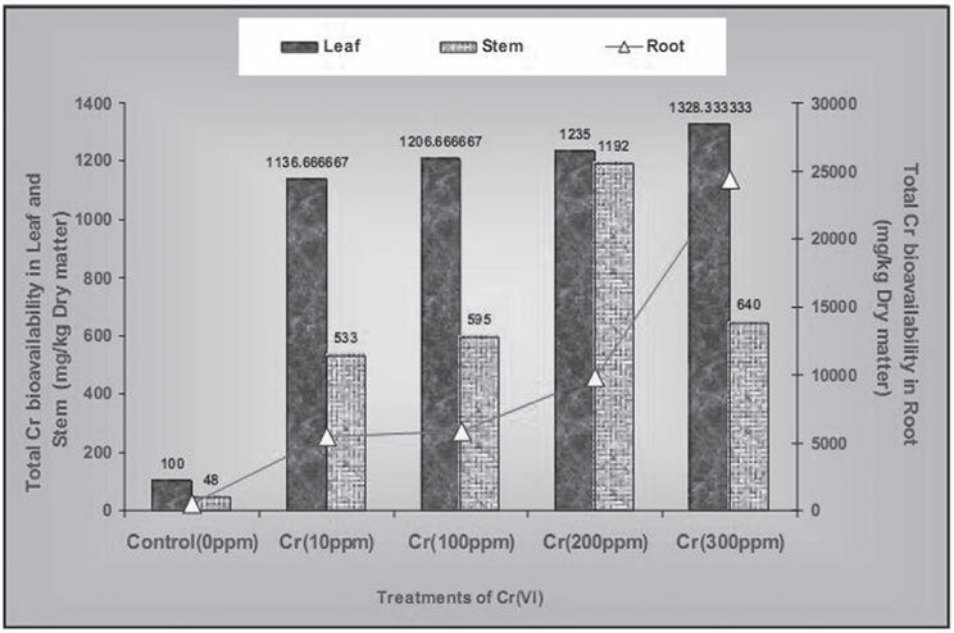


*Fig. 3.* Total Cr bioavailability in root stem and leaf tissues of 21 days old *Sesbania sesban* seedlings under $Cr^{+6}$ stress

growth in different concentrations of $Cr^{+6}$ showed significant variations in their root, stem and leaf bioaccumulation pattern. Maximum accumulation of Cr was observed for seedlings treated with 300 mg $kg^{-1}$ of $Cr^{+6}$. Shoot translocation of Cr was increased up to 200 mg $kg^{-1}$ of $Cr^{+6}$ supply as evident from their translocation factor values (Table 3). But at 300 mg $kg^{-1}$ of $Cr^{+6}$ supply the sesban seedlings showed stiff decline in Cr translocation which was due to high growth retardation. Rate of Total Accumulation of Cr in 21 days old sesban seedlings increased 25 times in treatments of 300 mg $kg^{-1}$ of $Cr^{+6}$ than control as evaluated from TAR (Total Accumulation Rate) values (Table 3).

*Table 3*
Chromium translocation and rate of accumulation in 21 days old sesban seedlings

| Treatments | Concentration of $Cr^{+6}$ (mg $kg^{-1}$) | Translocation factor | Total Accumulation Rate (mg $kg^{-1}$ $day^{-1}$) |
|---|---|---|---|
| Control | 0 | $0.022^{a}$ | $9.281^{a}$ |
| Hexavalent Chromium ($Cr^{+6}$) | 10 | $0.308^{b}$ | $107.644^{b}$ |
| | 100 | $0.309^{b}$ | $114.079^{c}$ |
| | 200 | $0.248^{c}$ | $167.909^{d}$ |
| | 300 | $0.081^{d}$ | $252.007^{e}$ |

NB: Mean in a column superscribed with different letters are significantly different at LSD* $P<0.05$ by DMRT.

## CONCLUSIONS

Recently more emphasis and priority have been given to suitable plant based remediation techniques for sustainable ecosystem and stabilizing mining environment. The present study revealed deleterious impacts of varying concentrations of $Cr^{+6}$ on germination, growth parameters indicating its phytotoxicity and tolerance ability, photosynthetic pigments, antioxidant enzymes. The study suggests the potential of sesban plants for Cr translocation which enable the plant to tolerate high chromium stress and also protect itself from Cr phytotoxicity through altering various metabolic activities. The results of the study revealed the phytoremediation devices adopted by the sesban seedlings to combat chromium stresses under field condition. A field study is recommended to see the effect of natural variables (temperature, pH, light, soil quality, etc.) on the above laboratory based results. Further research on mechanism of Cr tolerance in sesban plants needs to be thoroughly conducted. The effective translocation of Cr from roots to shoots of sesban seedlings suggests the plant as a potential green tool for phytoextraction mechanism. The findings of the present research investigation will help prescribe the evolved chromium phytoremediation technology for practical application under field condition using these tolerant species of sesban and developing its tolerance through various amendments.

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