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Effect of chelators on morpho- physiological parameters of paragrass (*Bracharia mutica*) grown in over burden soil of Sukinda chromite mine

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ABSTRACT

Presence of heavy metals like chromium in agricultural soils, surface and ground water is of foremost concern to environment. Several physical, chemical and biological practices were used to alleviate Cr from over burden soil (OBS) of chromite mine of Sukinda. Amongst different techniques, phytoremediation is a green technology to attenuate the Cr from contaminated soil. The phytoremediation ability of a paragrass plant can be improved by using different types of chelators like Ethylene diamine tetra acetic acid (EDTA), Diethylene triamine penta acetic acid (DTPA), citric acid (CA) and salicylic acid (SA). The pot culture experiments were carried out in the Department of Botany, Utkal University, Bhubaneswar (Odisha) to study the morpho-physiological parameters, antioxidant enzyme (guaiacol peroxidase and catalase) activities as well as accumulation and translocation of various heavy metals from the chromite mine soil. The results showed that growth, chlorophyll, protein contents and uptake of chromium were decreased with the treatments of chromium (46.25 mg kg^{-1}) but application of chelators enhanced the length, biomass, chlorophyll, protein content and uptake of chromium in contaminated soil. Paragrass treated with Cr-CA (46.25 mgkg^{-1}) exhibited an increase in all the physico-chemical parameters of plants. Application of chelators in over burden soil also improved the activity of enzymes (guaiacol peroxidase and catalase), bio concentration factor (BCF), total accumulation rate (TAR), tolerance index (TI) and transportation index (Ti) of paragrass plants. The activies of guaiacol peroxidase was enhanced in plant by the application of citric acid, EDTA and Mg by 0.494, 0.462 and 0.431(mg/protein/min), respectively but the catalase activities was increased in citric acid, salicylic acid, DTPA by 0.439, 0.431 and 0.431(mg/protein/min) respectively. Salicylic acid, citric acid and Mg effectively increased the BCF, TAR, TI and Ti of paragrass plant. Thus chelators and metal ion play a significant role in inducing the growth, combating against oxidative stress as well as in increasing the bioaccumulation of chromium in both roots and shoots.

Keywords: Bio concentration factor, paragrass, phytoaccumulation, transportation index

INTRODUCTION

In our country huge resources of chromium are mostly deposited in Odisha. The state Odisha is gifted with enormous amount of mineral deposit of chromite ore. Odisha alone contributes about 98% of total Cr deposits. Chromium is stable in soil in both trivalent and hexavalent form. Both differs in terms of mobility, bioavailability and toxicity. Hexavalent form of chromium (Cr^{+6}) is more toxic than trivalent form (Cr^{+3}). It was observed that lower concentration of Cr induces the growth of plants but if the concentration of Cr is high in soil it inhibits the length and biomass of the plants (Patra *et al.*, 2018a; 2019). As a heavy metal, chromium has the ability to induce oxidative damage to lipids, proteins, nucleic acids, etc. by producing reactive oxygen species (ROS). The ROS are extremely combative it attack on polyunsaturated fatty acid of membrane and it induces lipid peroxidation of cell membrane. All ROS are highly toxic to plant while the amount of ROS exceeds the resistance mechanism of a plant is assumed to be in a condition of oxidative strain. To with stand the oxidative damage, plants have the defense system that inhibits the reactions promoted by oxygen or peroxides. The defense system consists of oxidative inhibitor enzymes like catalase, peroxidase, superoxide dismutase, etc. and the non-enzymatic compounds like proline, carotenoid, ascorbate, tocopherol, etc (Patra *et al.*, 2018a). The morpho-physiological alterations occurred in plants by exposure to Cr either by changing the activity of antioxidant enzymes or by producing the ROS. The toxic effect of Cr includes reduction of length of root and shoot, loss of chlorophyll pigment, low yielding, inhibition of germination of seeds, immature abscission of plant parts, inhibition of enzyme action, and decreased the biosynthesis of various bio molecules. Other negative impacts

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of Cr on plants were reduction of length and biomass, destruction of cell membrane and induction of oxidative stress (Patra *et al.*, 2019). Instantaneous drooping of leaves and decrease of plant growth has also been observed when the plants were exposed to varied concentration of chromium.

In order to view the toxicity of chromium, different efforts have been taken for mitigation of chromium through phytoremediation technology (Patra *et al.*, 2018b; 2018c). This technique can be used for reduction of toxic metals from contaminated soil and observed that it is economically practicable and effective method as compared to other traditional techniques (Ali *et al.*, 2013). The plantation of green plants on contaminated soil shows more effective in many ways (i) rhizofiltration (ii) phytostabilisation (iii) phytoextraction of toxic metals (iv) sustainable management of contaminated soil. The phytoextraction ability of plants can be improved by the supplementation of of chelators named as induced phytoextraction (Patra *et al.*, 2018c). The objective of the experiment was to attenuate the toxicity of chromium in soil using paragrass by the application of chelators and metal ion. Thus, the current experiment intended to study the morpho-physiological, toxicological and phytoextraction potentiality of non-edible plant species. Almost no experiment has been conducted for alleviation of chromium from over burden dumps of Sukinda chromite mine by using paragrass through chelate based phytoremediation technology.

MATERIALS AND METHODS

The experiment was carried out at Department of Botany, Utkal University, Bhubaneswar, Odisha in 2018. Three uniform plantlets of paragrass were planted in a poly bag containing 50% of OBS and different types of chelaters in equi molar ratio like Cr (46.25 mg kg^{-1}), Cr–EDTA (46.25 $mgkg^{-1}$), Cr–DTPA (46.25 $mgkg^{-1}$), Cr–Citric acid (46.25 mg kg^{-1}), Cr–Salicylic acid (46.25 $mgkg^{-1}$), and Cr–Mg (46.25 $mgkg^{-1}$). The plantlets were harvested for morpho-physiological analysis and bioavailability of Cr in roots and shoots of plants after 60 d of plantation. The length, fresh and dry weights of roots and shoots were recorded after 60 d. For measurement of dry weight the plant materials were oven dried at 80^0C for about 3 d. Total chlorophyll, protein and proline contents in plants were analyzed by adopting the method of Porra (2002), Lowry *et al.* (1951) and Bates *et al.* (1973), respectively. The activities of antioxidant enzymes i.e., Catalase and guaiacol peroxidase were determined by Chance and Maehly (1955) and Bergmeyer et al (1974), respectively. The treated and control soils were dried in air and crushed with the mortar and pestle then digested by nitric acid and hydrochloric acid in the ratio of 1:3. The roots and shoots of 60 d grown paragrass plants were studied for total Cr content. The total Cr content in soil, roots and shoots was determined by analyzing those extracted liquid samples in Atomic Absorption Spectrophotometer (A Aanalyst 200 Perkin Elmer). Metal accumulation in plants was analyzed for calculation of Bio-concentration Factor (BCF), Transportation index (Ti) and Total Accumulation Rate (TAR) as per formulae. (Ghosh and Singh, 2005; Zurayk *et al.* 2002).

$$\text{BCF} = \frac{\text{Average chromium concentration of the plant tissue } \left(\text{mgkg}^{-1}\right)}{\text{Chromium added in soil } \left(\text{mgkg}^{-1}\right)}$$

$$\text{Ti} = \frac{\text{Cr concentration of shoot } \left(\text{mgkg}^{-1}\right)}{\text{Cr concentration of root } \left(\text{mgkg}^{-1}\right)} \times 100$$

$$\text{TAR} = \frac{\left(\text{shoot concentration} \times \text{shoot biomass} + \text{root concentration} \times \text{root biomass}\right)}{\left[\left(\text{Shoot biomass} + \text{root biomass}\right) \times \text{Days of growth}\right]}$$

Where TAR is in $mgkg^{-1}day^{-1}$, biomass is in g dry mass, and concentration is in $mgkg^{-1}$ dry mass.

Stress tolerance indices for various growth parameters were determined using the formulae of Wilkins (1957).

$$\text{Tolerance Index} = \frac{\text{Dry weight of treated plants}}{\text{Dry weight of control plants}} \times 100$$

The data presented in figure and tables are mean ± SE (Standard error of the mean of three replicates). One way analyses of variance (ANOVA) were carried out for inter comparison of various treatments where F values were detected at 5% level.

RESULTS AND DISCUSSION

Effect of Cr stress on growth

There was an improvement of length and biomass of root and shoot of paragrass plant in over burden soil as compared to control. The growth of plant was enhanced further by the application of chelators and metal ion to over burden soil. However, the application of EDTA, citric acid and Mg were more effective in improving the length, fresh and dry weight of roots and shoots as compared to salicylic acid and DTPA (Fig. 1 and Fig. 2). Chromium affects plant growth and metabolism by decreasing nutrient uptake and photosynthetic abilities. It was observed that, chelating agents augmented the availability of micronutrients in rhizospheric regions (Patra *et al.*, 2018c). For example, iron is easily accessible to the plant in Fe- EDTA form and increased the growth and biomass of plants by enhancing the chlorophyll synthesis. Stimulation of plant growth is therefore, due to the supply of nutrients by chelate mobilization of cations in the soil and the enhancement of uptake by their roots. However, application of chelators was effective for counteracting the toxic effects of chromium. High concentration of chromium affects the plant growth by reducing the availability of nutrients in rhizospheric regions and photosynthetic abilities (Mohanty and Patra 2011).

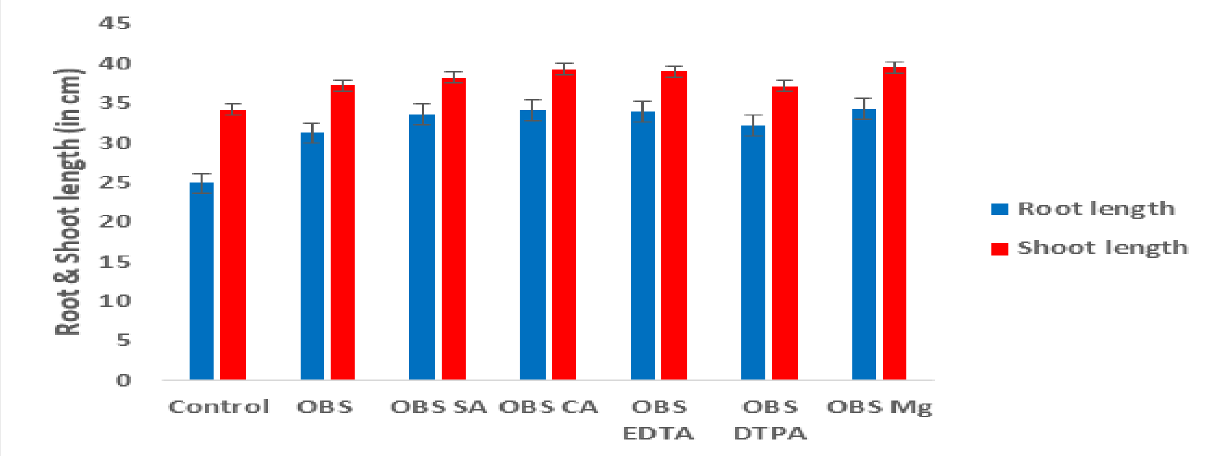

Fig 1: Effect of chelators and ion on length of root and shoot of paragrass plants

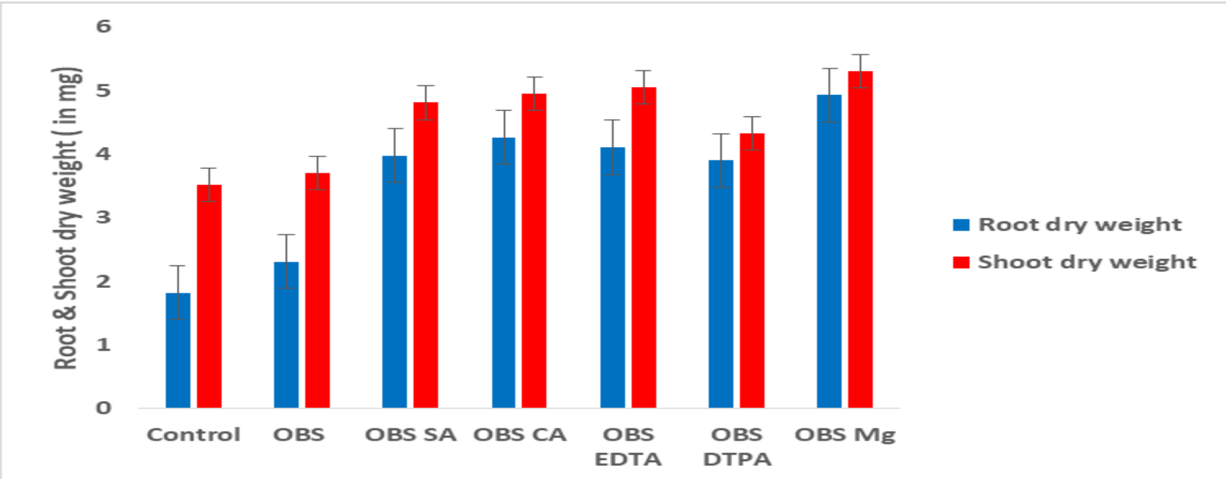

Fig 2: Effect of chelators and ion on fresh and dry weight of root and shoot of paragrass plants

Effect of Cr on biochemical parameters

Application of chromium rich over burden soil (46.25 $mgkg^{-1}$) to paragrass increased all the biochemical parameters like total chlorophyll, protein, carbohydrate and proline content in plants. These biochemical parameters increased further by the addition of chelators and metal ion (Table 1). In chromium treated soil, the magnesium competes with chromium for binding in a heterotrimeric enzyme complex. The over burden soil treated with Mg ion had maximum chlorophyll, protein and carbohydrate content. A noticeable increase in proline amount was observed in OBS treatment as compared to control. Accumulation of proline in paragrass plant in over burden soil is a significant feature for recognizing the effect of stress on plant (Mohanty *et al.*, 2011). An enhancing the proline level is considered to support the cells in osmoprotection as well as regulating the redox potential, scavenging hydroxyl radicals in the defense against denaturation of several molecules (Khan *et al.*, 2002). The total chromium in root and shoot was positively correlated with total chlorophyll and protein.

Table 1: Effect of chelators and ion on on total chlorophyll, protein, carbohydrate and proline in paragrass plants

Treatments	Total chlorophyll (mg /g fresh wt.)	Protein (mg /g fresh wt.)	Carbohydrate (mg /g fresh wt.)	Proline (mg/g fresh wt.)
Control	0.1645±0.022	0.1484±0.002	0.0316±0.006	0.0151±0.0004
OBS	0.1783±0.032	0.1511±0.007	0.0368±0.011	0.0217±0.002
OBS SA	0.1987±0.024	0.2115±0.023	0.0792±0.0004	0.0259±0.003
OBS CA	0.2149±0.017	0.2847±0.012	0.0678±0.017	0.0271±0.0009
OBS EDTA	0.1989±0.021	0.2154±0.016	0.0731±0.005	0.0321±0.007
OBS DTPA	0.1924±0.014	0.2198±0.07	0.0483±0.001	0.0211±0.005
OBS Mg	0.2198±0.012	0.2989±0.021	0.1683±0.023	0.0125±0.017

Effect of Cr on activity of antioxidant enzymes

The activities of antioxidant enzymes (catalase and guaiacol peroxidase) were increased with increase in chromium concentration but decreased at very high concentration. Application of Cr-Mg, Cr-CA and Cr-SA showed a significant increase in root guaiacol peroxidase activity in paragrass seedlings whereas enhanced catalase activities were noted through the application of Cr-Mg, Cr-CA and Cr-DTPA (Fig. 3). This study indicated that the application of chelators increased the activity of antioxidant enzymes which helps in detoxifying the free radicals which were created during stress condition. This demonstrated that oxidative inhibitor enzyme synthesis was enhanced with the application of a chelator, which helps scavenging more free radicals and defending the plant from Cr stresses. The antioxidant enzymes may scavenge the ROS formed by the presence of Cr. These antioxidants mitigate oxidative damage and reduce stress phenomena. The activity of peroxidase and catalase has been studied in response to chromium toxicity in several plant species (rice, wheat, green gram) and lower plants like mosses (Panda 2003). All the enzymes activities were highly positively correlated with the total chromium at roots.

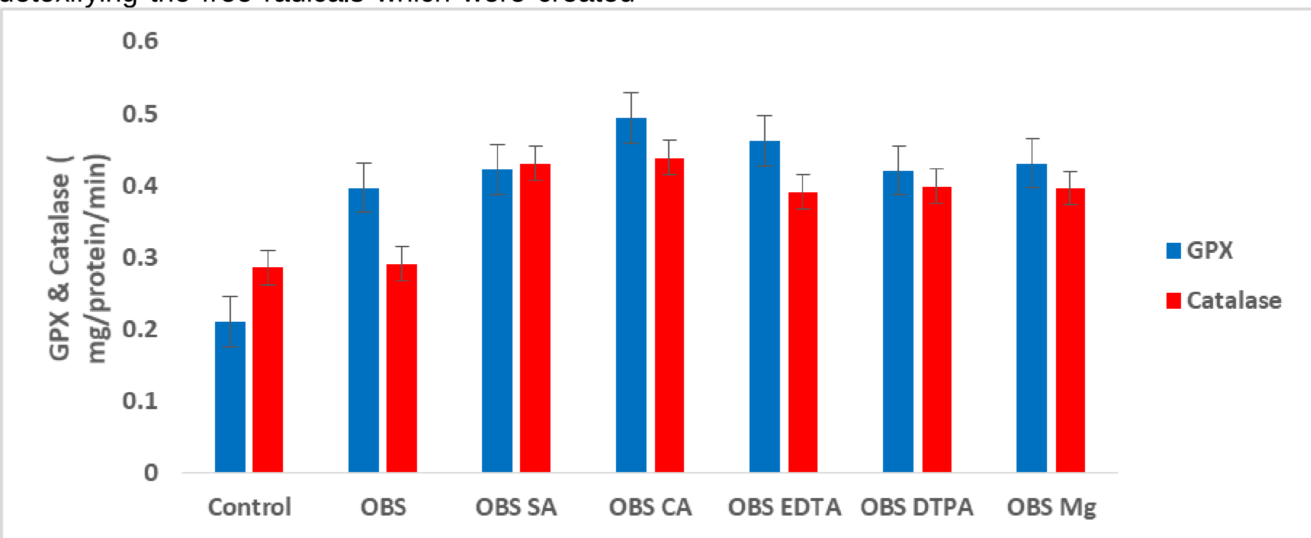

Fig. 3: Effect of chelators and ion on GPX and catalase activity of paragrass plants

Bioavailability of chromium in root and shoot of paragrass

The bioavailability of chromium in root and shoot of plants enhanced with the use of chelators and metal ions. The accumulation of chromium was more in roots than shoot of paragrass plants (Fig. 4). The results indicated that chelators enhanced the availability of chromium in the rhizospheric region in contaminated soil. The transport and accumulation of chromium depends on the formation of complexes that act to enhance Cr uptake and availability in plants (Torresdey *et al.*, 2005; Zhuang *et al.*, 2007). The complexes are made with different organic compounds, i.e., citric acids, salicyclic acid, EDTA, DTPA etc. The Cr- EDTA resulted in more uptake of uptake chromium from mining soil than other chelator.The translocation of metal from soil to plant increased with the application of chelating agents. The phytoextraction capability of a plants can be evaluated by determining the bio concentration factor (BCF) and transportation index (Ti). An increase in the BCF and Ti values in the presence of chelator's and metals ions enhanced the mobilization of chromium from soil to roots and subsequently to shoots (Table 2). Tolerance index of paragrass plant also increased with the application of chelators and metal ion at high concentration of chromium. Salicylic acid was more effective to enhance the tolerance index of plant in stress condition.

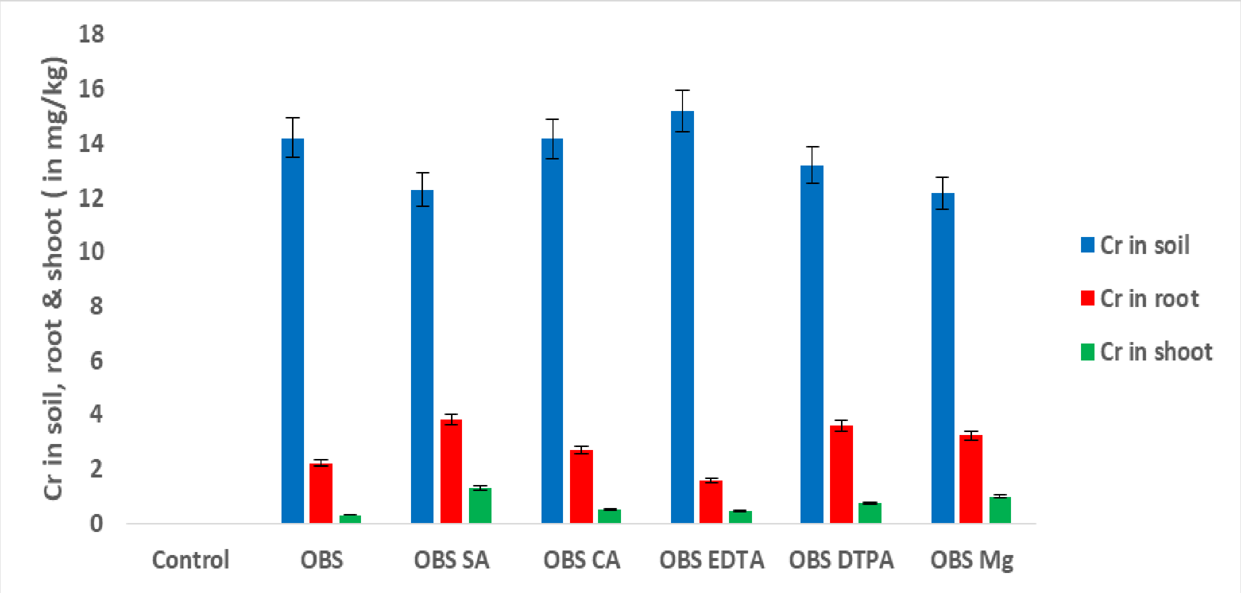

Fig. 4: Effect of chelators and ion on bioavailability of Cr in soil, root and shoot

Table 2: Effect of chelators and ion on bio-concentration factor, total accumulation rate, tolerance index and transportation index

Treatments	Bio concentration factor (BCF)	Total accumulation rate (TAR)	Tolerance index (TI)	Transportation index (Ti)
OBS	0.1356	0.0172	6.01	14.09
OBS SA	0.2756	0.0405	10	34.21
OBS CA	0.1724	0.0252	7.12	18.14
OBS EDTA	0.1102	0.0159	8.81	27.5
OBS DTPA	0.2351	0.0349	6.1	20.83
OBS Mg	0.2286	0.0345	7.63	30.15

It may be concluded from the results that chromium induced severe morpho-physiological changes in paragrass plants. The results will help to suggest the evolved phytoremediation technology for reduction of chromium level and its practical application in mining sites. The plant can be considered as green tools for combating chromium stress in chromite mining site at Sukinda areas.

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