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CHELATOR APPLICATION IMPROVES THE ANTIOXIDANT DEFENSE ACTIVITY, CHROMIUM UPTAKE AND OIL CONTENT IN LEMON GRASS GROWN IN CHROMIUM CONTAMINATED OVER BURDEN DUMPS OF MINING AREA

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Abstract

Phytoremediation potential of lemongrass was evaluated by pot culture experiment using chromium contaminated over burden soil of Sukinda chromite mine in presence of chelators and metals ions. The plants were grown in pots having 20% over burden soils with different chelators and metals ions. The plants were harvested after 60 days of plantation for measurement of root and shoot growth. Chlorophyll content, proline bioaccumulation, protein biosynthesis, oil content, antioxidant enzyme activities and chromium bioavailabilty in plants were examined. The results indicated that plant growth, chromium bioaccumulation, activities of antioxidant enzymes, proline and oil content in plants (T_1) under chromium stress were less as compared to plants (T_2 to T_7) grown in presence of chelators and metals ions. Experimental evidences suggested that the application of chelators and metals ions enhanced the chromium bioaccumulation which mitigated the harmful effects of chromium on plants. The increase in the Bio-concentration factor and transportation index values in the presence of chelator's and metals ions showed enhanced the mobilization of chromium from soil to shoot via root accumulation and transportation. The experiment with chelate based phytoextraction method for decontamination of chromium contaminated mine soil using lemongrass plants can be considered as a suitable option in future.

Key words: antioxidant; chromium; decontamination; Phytoextraction.

Introduction

Chromium is one of the most abundant minerals resources of Odisha, India. Chromium is a key element used for built-up of stainless steel. It is commonly used in metallurgy and refractory industries. Odisha has about 98% of the country's chromium ore resources, of which 97% found in Sukinda valley occupy an area about 200 sq. km., belongs to Jajpur district (Das *et al*., 2010). The valley is rich in chromite deposit and it is the largest open cast mines in the world and contamination caused due to mining activities is the major problems for inhabitants (Blacksmith institute report, 2007). Remediation of chromium contaminated soil from sites of contamination is a challenging job. The objective of remediation by phytoextraction process aims at eliminating the toxic metals from the contaminated soil by root and their accumulation in shoot. The Phytoextraction has been extensively studied, mainly due to the potential for high efficiency and possible economic value (in metal recovery, energy production) (Patra *et al*., 2020a). The plant based remediation of heavy metals from mining sites has attracted a great deal of investigation in last few years. Remediation of chromium contaminated soil using hyper accumulator species is well known. However, an alternative method for using plants with high biomass for improvement of metal uptake has been proposed by way of chelator application (Marschner, 1995). The mobility of metals from contaminated soil by using chelators has been known through the phytoextraction method. The applications of chelators in enhancing the metal uptake in plants and restraining the leaching of toxic metals from contaminated soil have become an attractive field of investigation (Meers *et al*., 2005). A number of studies have been carried out for remediating chromium from contaminated sites by using chelators and metals ions. For instance, Patra *et al*., (2018c) reported that the

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chelators and metals ions effectively mobilised chromium from Cr^{+6} contaminated soil by using lemon grass. The improvement of chromium bioavailability in crop plants has also been reported by the application of chelating agent.

In the present context, additional efforts have been taken to evaluate and reconfirm the phytoextraction potentiality of lemon grass grown in chromium contaminated over burden mine soil assisted by chelators and metal ions. The objectives of this study were to examine (1) the Phytoextraction ability of lemon grass for chromium decontamination from over burden mine soil by the applications of chelator and metal ion, (2) effect of chelators and metal ions on antioxidant defense system of lemon grass and (3) to assess the effect of chelate-assisted chromium on oil production in lemon grass plants.

The plants used for phytoextraction process should possess the following features (a) tolerance to high concentrations of toxic metals; (b) the ability to accumulate the metals in roots; (c) fast growth; (d) high biomass production; (e) profuse fibrous root system; (f) be easy to cultivate and harvest. (Ali *et al*., 2013). Lemon grass plant is well known for its faster growth, extensive fibrous root system and high biomass as compared to other grass species used in experimental research. The plants are tolerant and resistant to heavy metal applications and the production of oil is not affected in the presence of metals (Patra *et al*., 2019). The essential oil extracted from lemon grass plant is known to be free from the threat of heavy metals extractable from plant parts. (Zheljazkov *et al*., 2006). Therefore, choosing lemon grass plant is a better option for phytoremediation research.

Materials and Methods

Plant materials and design of experiment

Lemon grass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson plantlets were collected from city forest division, Ghatikia, Bhubaneswar (Govt. of Odisha, India) on 4th December 2017. Three plantlets of invariable weight with similar diameter and age were implanted in each polybag on the same day containing garden soil and over burden soil in different percentage (80% garden soil and 20% over burden soil). Then, the plantlets were grown for 16 days and treated with chelators such as ethylene diaminetetra acetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), citric acid (CA) and salicylic acid (SA) and metal ions (Mg and Zn). Eqimolar ratio of chelators/metal ions with respect to existing total chromium content in 20% over burden soil (OBS) were applied to 16 days grown plants. The chelators and metal ions were supplement to soils of different pots in the following manner *i.e.*, T_0 Control, T_1- 20% OBS only, T_2- 20% OBS-EDTA, T_3- 20% OBS- DTPA, T_4-20% OBS- CA, T_5-20% OBS- SA, T_6-20% OBS-Zn and T_7-20% OBS-Mg. The Zn and Mg was supplied in the form of $ZnSO_4.7H_2O$ and $MgCl_2.6H_2O$. The plantlets were grown up to 60 days and after that sampling were done on 2nd February 2018. All parameters were performed in triplicates.

Physico–chemical properties of soil samples

The physico–chemical studies of soil samples were performed in the laboratory of soil science department of Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. Soil pH and EC (Electrical Conductivity) were determined with the help of pH and EC meter. For measurement of pH and EC, the proportion of soil to water was 1: 2.5 (Jackson, 1973). Organic Carbon (Walkey and Black, 1934), Cation exchange capacity (Jackson, 1973), Ca, Na, K and Mg (Gupta, 2000), available soil N (Subbiah and Asija, 1956), K (Jackson, 1967) and P (Bray and Kurtz, 1945) were analyzed as adopted by the methods described above. Water holding capacity (WHC%) was analyzed by Chaturvedi *et al*., (2012).

Growth parameters

After sixty days of plantation lemon grass plants were collected for the study of root and shoot length, fresh and dry weights of roots and shoots. For the investigation of dry biomass, the plant samples were kept in an oven at 80°C for 3 days for obtaining steady biomass.

Analysis of chlorophyll, protein and proline content

Total Chlorophyll, protein and proline contents were investigated by Porra, (2002); Lowry *et al*., (1951) and Bates *et al*., (1973) respectively.

Activity of antioxidant enzymes

The activities of oxidative inhibitor enzymes *i.e.*, Catalase, peroxidase and superoxide dismutase were evaluated by Chance and Maehly, (1955) and Marshall and Worsfold, (1978) respectively.

Estimation of available chromium content in soil and its bioavailability in lemon grass

The soil samples were air dried for 10 days and grinded with the help of mortar and pestle then digested by nitric acid and hydrochloric acid in the proportion of 1:3. The total Cr contents were analysed in roots and shoots of lemon grass plants after 60 days of plantation. The roots and shoots of plant of various treatments were desiccated by hot air oven at 80°C for 3 days till steady biomass was achieved and grinded into fine particles, then digested with nitric acid and perchloric acid in the proportion of 10:1. Then the acid mixed plant samples were digested and extracted for metal content using MDS-8 (Microwave Digestion Unit, MDS-8; Shanghai Sineo Microwave Chemistry Technology Co. Ltd., Shanghai, China). The acid digested solutions were sieved and the final volume was made up to 100 ml. The available Cr content in soil and total Cr content in plant materials were calculated by examining those extracted liquid samples in atomic absorption spectrophotometer (A Aanalyst 200 Perkin Elmer).

Extraction of essential oil

The leaves from control (T_0) and treated plants (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) of lemon grass were collected in triplicates and the essential oils were extracted using the hydro-distillation technique in a Clevenger's apparatus by following the method of Guenther (Guenther, 1972). 100g of leaves were weighed and washed properly with water to remove soil and then chopped into small pieces. The chopped leaves were then inserted into a round bottom flask and distilled water was added to it. The flask containing the plant materials were heated up to 30-40°C for 4-5h and the condensed vapour was separated using water separator. The oil deposited at the upper layer was collected in the eppendroff tube. Then the distilled essential oils were dried over anhydrous sodium sulphate. The oil was stored at -4°C in borosil glass vial for constituent analysis.

Gas chromatography analysis

Gas chromatography analysis was done with nitrogen as carrier gas flowing at a rate of 1 ml/min. Flame ionizing detector (FID) supplied with air (350 ml/min) and hydrogen (35 ml/min) was used. The oil sample was injected at a volume of 0.02µl. Initial oven temperature was 50°C. The major constituents were analyzed using Gas chromatography system (GC Agilent technologies 6890 system, USA).

Statistical analysis

The treatments were carried out in triplicates and the data are furnished in figure and table with mean ± SEM. Multiple comparison tests are carried to compare the control group and different treatment conditions. Dunnett test was used for present experimental set up to compare the treated mean with control mean and this method is appropriate when there is a control group (Dunnett, 1955). In our analysis we observed that the control mean is statistically significant ($p<0.05$) from all the treated mean of all the parameters. The Spearman non-parametric correlation coefficients (r) values were calculated to see the effect of availability of chromium in roots and shoots with respect to other parameters. All the calculations were done using graphpad PRISM 7 software.

Results and Discussion

Physico-chemical analysis of soil samples

The physico-chemical properties of over burden soil (OBS) were analysed. It was found that OBS was having varied combinations of clay, minerals and rock. The pH of the soil was 5.48. The available NPK, Fe and S were found extremely low. The cation exchange capacity and water holding capacity were observed as 12.65 (meq/gm) and 37.0 (%) respectively. The available chromium content was 92.5 mg/kg. But the physico-chemical properties of OBS was improved by the addition of chelators and metals ions. The chelating agents increased the electrical conductivity, cation exchange capacity and water holding capacity of soil as observed earlier (Patra *et al*., 2018c). In chelate assisted OBS the value of available Cr content was observed low which may be due to uptake of Cr by lemon grass plants with chelate assistance (Fig. 1). The applications of chelators were based on ideas for improving heavy metal uptake in plants and restraining the discharge of heavy metals from contaminated soil.

Growth impairment in lemon grass in response to Cr Stress

There was an enhancement of plant growth in T_1 soil as compared to control. There was promotional growth of lemon grass plants due to further applications of chelators and metals ions to T_1 soil. However, the applications of DTPA, citric acid and metals ions (Mg, Zn) as compared to other chelators like EDTA, Salicyclic acid were found more effective on plant growth (Fig. 2). The applications of chelating agents have been shown to

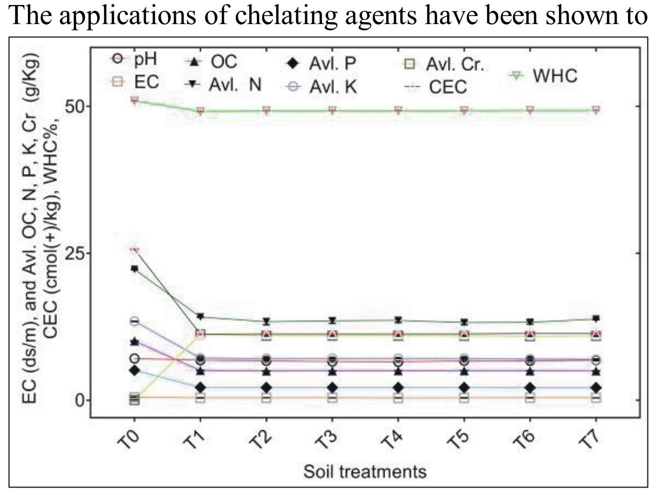

Fig. 1: Physico-chemical characteristics of over burden soil amended with chelators and metals ions collected from chromite mining site.

improve the solubility and uptake of essential nutrients in plants. For example, Fe is accessible to the plant in the chelated form (Fe-EDTA), which helps the plants for increased chlorophyll biosynthesis leading to high biomass. Chelators are helpful for the mobilization of nutrients, which provide plants with vital metal complexes. 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 (Bonet *et al*., 1991). The use of chelating agents such as EDTA, DTPA, citric acid, salicyclic acid etc. are known to augment the bioavailability of heavy metals for plant uptake. (Turnau, 1998; Wu *et al*., 2003). The applications of chelators (EDTA, DTPA, CA and SA) and metal ions (Mg and Zn) to over burden soil (OBS) was advantageous for stimulation of plant growth due minimization of toxic chromium level in contaminated soil. The Spearman's rank correlation coefficient analysis shows that the plant growth is positively correlated with the available chromium in mine soil and they are statistical significant ($p<0.05$).

Effect of Cr on biochemical parameters in lemon grass

The results demonstrated that the chlorophyll and protein contents increased further in leaves of lemon grass when grown in Cr contaminated OBS assisted by chelating agents (T_2, T_3, T_4 and T_5) and metals ions. (T_6 and T_7) in comparison to T_1 soil (Fig. 3). The plants were treated with chelating agents showed high pigment biosynthesis. Similar reports on the impacts of metal toxicity in different plants are available (Raskin *et al*., 1997; Zayed *et al*., 1998). A noticeable rise in proline amount was also observed in leaves when Cr contaminated OBS were assisted by the chelators (DTPA and CA) and metal ions (Mg^{+2}). Proline which is a known protein stabiliser and osmoprotectant, was found to be increase in lemon grass plants in the presence of chelators and ions. The increase in proline amount is deliberated to assist the cells in osmoprotection as well as regulating the redox potential, scavenging reactive oxygen species in the protection against denaturation of several biomolecules (Khan *et al*., 2002). The total chromium at root and shoot is positively correlated with total chlorophyll and protein (p-value <0.05). Proline is positively correlated ($r=0.7847$, $p<0.05$) with total chromium at root but negatively correlated with total chromium at shoot ($r=-0.3095$, $p=0.4618$). The Spearman's correlation coefficient with p-values.

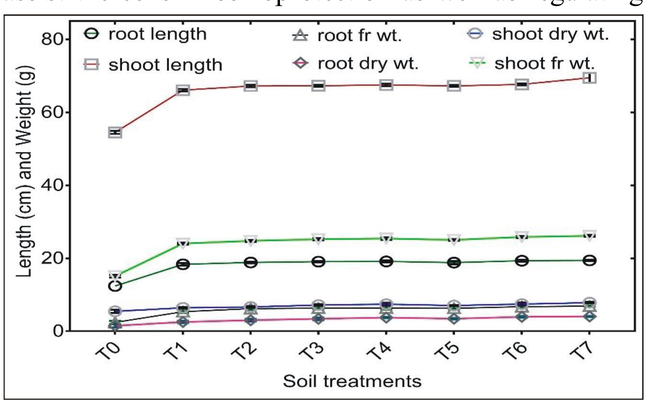

Fig. 2: Changes in growth parameters of lemon grass plants grown in over burden soil amended with chelators and metals ions.

Effect of chelators and metals ions on antioxidant enzymes

The application of chelating agents and metals ions significantly augmented the activities of antioxidant enzymes namely peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD). The activities of enzymes were more in roots than shoot of lemon grass. The applications of metals ions (Mg and Zn) to OBS exhibited maximum activity in both roots and shoots of lemon grass whereas the chelators (EDTA, DTPA, CA and SA) were found more effective for increasing the activity of enzymes. Due to chromium bioavailability in lemon grass, the inhibitory action of bio-available metal ions on antioxidant enzymes was reported earlier (Patra *et al*., 2018a; Patra *et al*., 2020b). To cope with oxidative stress, the activities of the enzymes were elevated in presence of chelators and metals ions. The finding of the present study are in conformity with those of Mahmud *et al*., 2018 who reported an increase in activity of antioxidant enzymes with chelators supplementation in *B. juncea* under Cd stress. All the enzymes activities are highly positively correlated with the total chromium at roots and shoot (Fig. 4). The Spearman's rank correlation coefficients with p-values. In all cases the correlation is statistically significant ($p<0.05$).

Chromium bioavailability in lemon grass

The uptake of chromium from contaminated mine soil to roots of the plants increased in the presence of chelators and metals ions. The results shows the efficacy

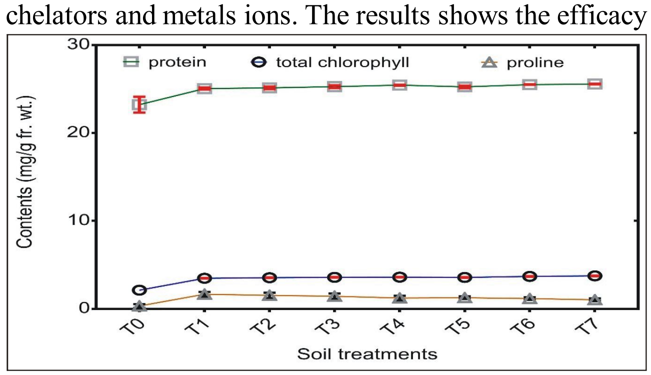

Fig. 3: Changes in chlorophyll, protein and proline content of lemon grass plants grown amended with chelators and metals ions in over burden soil.

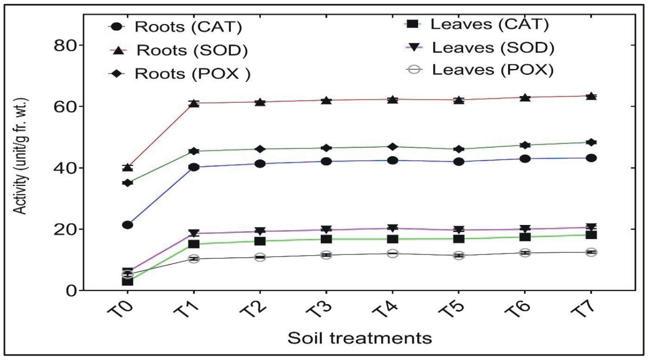

Fig. 4: Changes in the catalase, peroxidase and superoxide dismutase activities (unit/g fresh wt.) of lemon grass plants grown in over burden soil amended with chelators and metals ions.

of chelators in mobilizing chromium in the rhizospheric region of the plants. Using both the Bio Concentration Factor (BCF) and the Translocation Factor (TF) it is possible to assess the phytoextraction capacity of the plant. CA and Mg were found to translocate Cr most effectively as compared to EDTA, DTPA and SA respectively. An upsurge in the BCF and TF values in the presence of chelator's and metals ions showed enhanced the mobilization of chromium from soil to roots and subsequently to shoots (Patra *et al*., 2018b). The concentrations of chromium in plant organs were affected by types of chelators and metals ions. The chelators like CA and metals ions like Mg and Zn were found to be more effective for the uptake of metals from the contaminated soil. The chelators hold the toxic metals from contaminated soil by forming chelate-metal-complexes, which can be taken away from the contaminated sites by plants through chelate assisted phytoextraction.The bioavailability of chromium in roots and shoots of lemon grass plants showed a high level difference (Fig. 5).

Role of chromium on oil biosynthesis in lemon grass

The lemon grass oil is commercially significant and the citral content of the oil indicates its quality. Both quality and quantity of lemon grass oil were affected with the

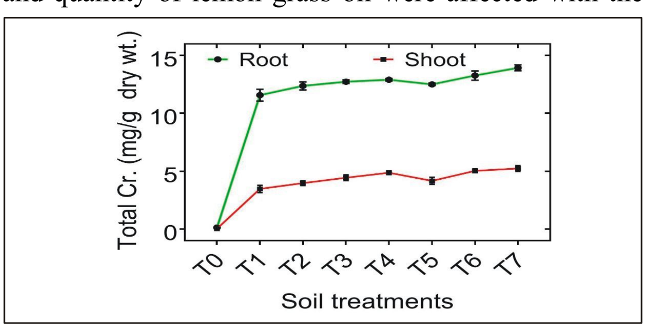

Fig. 5: Changes in total Chromium content of root and shoot of lemon grass plants grown in over burden soil amended with chelators and metal ions.

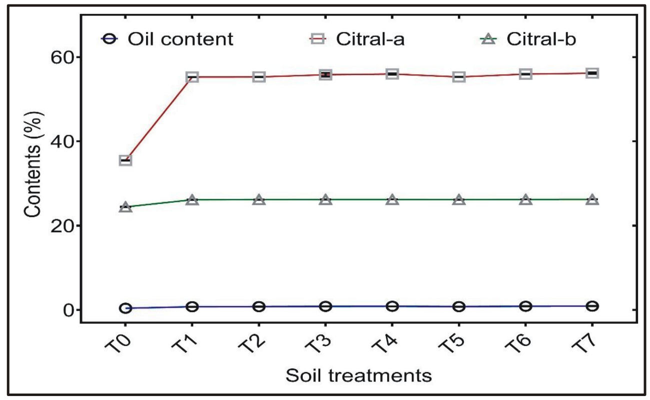

Fig. 6: Changes in oil, citral-a and citral –b content lemon grass plants grown in over burden soil amended with chelators and metal ions.

applications of chelators and metals ions to OBS (Fig. 6). The essential oil and citral-a and citral-b content steadily increased with increase in the concentration of chromium in OBS as reported earlier (Patra *et al*., 2019). The applications of chelating agents and metals ions increased essential oil, citral-a and citral-b content of lemon grass plants. However the applications of CA, DTPA and Mg were more effective to increase both quality and quantity of lemon grass oil in plants grown in chromium rich over burden soil. The Spearman's rank correlation coefficient analysis shows that the oil contents, citral-a and citral-b are positively highly correlated with the total chromium and they are statistical significant ($p<0.05$).

Conclusion

From the present study it may be concluded that chelators and metals ions assist the lemon grass plants growing under chromium stress to recover growth, proteins and chlorophyll synthesis. In addition, the plants under chromium stress promoted the antioxidant system defense system by enhancing the activities of catalase, peroxidase and superoxide dismutase and by facilitating the production of non-enzymatic antioxidant like proline. The chelators and metals ions improved the uptake of chromium from contaminated sites without any significant deteriorative role on oil production and morpho-physiological damage to the plant. The outcome of the present experiment using lemon grass plants will help to prescribe the evolved phytoremediation technology for attenuation of chromium toxicity and its practical application under field conditions. The lemon grass plants can serve as an effective green tools for combating chromium stress in contaminated sites particularly in over burden chromite mining location in Sukinda areas of India. The developed innovative chelate based methods of phytoremediation by using lemon grass as green tools will contribute knowledge substantially to the scientific community for future research and development.

Highlights

• Chromium contamination is a serious environmental problem and attenuation of chromium toxicity via phytostabilisation plan using lemon grass in contaminated over burden soil is the focus of the current study

• Phytoextraction method using chelators is one of the options for clean-up Cr from contaminated soil.

• Chelating agents and metal ions in Cr contaminated soil induce mobilization and extraction of potentially toxic Cr

• Stimulation of proline biosynthesis leading to protective stress tolerance in plants and improvement of antioxidant defence activity

• Enhancement of oil biosynthesis, citral-a and citral-b contents in lemon grass.

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