



## Research Article

## Chromium stress impact on Lemongrass grown in Over Burden Soil of Sukinda Chromite Ore Mine (Odisha), India.

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**Abstract:** The pot culture experiments were carried out to study the toxicological changes in Lemongrass plants grown in varied percentage of Cr rich Over Burden Soil (OBS) with or without chelators such as Ethylene Diamine Tetra Acetic Acid (EDTA), Diethylene Triamine Penta Acetic Acid (DTPA), Citric Acid (CA), Salicylic Acid (SA) and metal ions (Zn and Mg). The toxicological impact of Cr on treated plants with reduced Translocation index ( $T_i$ ) values indicated more root Chromium accumulation as compared to shoot. Shoot translocation of Cr as depicted by transportation index ( $T_i$ ) values for different crops were enhanced with the application of chelating agents. The Bio-Concentration Factor (BCF) and Total Accumulation Rate (TAR) of Chromium were elevated with the supplementation of chelators and metal ions, thus, illustrated more metal bioavailability in plants.

**Keywords:** Lemon grass plants, Chelators, Metals ions and Bioavailability

### Introduction

Chromite is an oxide of chromium and iron. More than 93% resources of chromite are located in Odisha state (India), mostly in the Sukinda valley in Jajpur districts. Minor deposits are scattered over Manipur, Nagaland, Karnataka, Jharkhand, Maharashtra, Tamil Nadu and Andhra Pradesh. Chromium (Cr) is one of the major industrial wastes produced from many industries like textiles, tanneries, electroplating, metallurgical which causes health issues in humans and animals and also affects marine life (Ajmal *et al.*, 1996, Moncur *et al.*, 2005). Cr as a trace element is not ranked as an 'essential element' for plants (Huffman and Allaway 1973). With a view to tackle the severe problem of contamination of toxic hexavalent Chromium from contaminated sites several methods are adopted for alleviation of Chromium bearing wastes from chrome chemicals and possessing industries. But the conventional treatments methods are either not feasible or not economical. It is observed that there are some plants as well as microorganisms that can absorb and tolerate high dose of heavy metals in their biological system, which are now being used to remove metal pollutants from soil and aquatic system. Such methods are known as bioremediation technology. Phytoremediation is the direct use of living green plants for *in situ*, or in place, removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater.

The intent of this experiment was to alleviate the toxic impact of hexavalent Chromium in soil using Lemongrass by chelators and metal ion supplementation. The applications of different chelators for phytoextraction of Cr was based on

chelate based phytoremediation technology. Chelating agents such as EDTA, DTPA, CA etc. are mostly used to enhance the bioavailability of heavy metals and their enhance uptake by the plants.

### Materials and Methods

#### Plant materials and design of experiment

Pot culture experiments were conducted at Utkal University, Bhubaneswar, India. Lemongrass plantlets were collected from city forest division, Ghatikia, Bhubaneswar (Govt. of Odisha, India). Three plantlets of uniform height were planted in each polybag containing varied percentage of Cr rich Over Burden Soil (OBS) ( $T_0$ - 100% garden soil as control,  $T_1$ - 90% garden soil + 10% OBS,  $T_2$ - 80% garden soil + 20% OBS,  $T_3$ - 70% garden soil + 30% OBS,  $T_4$ - 60% garden soil + 40% OBS,  $T_5$ - 50% garden soil + 50% OBS,  $T_6$ - 30% garden soil + 70% OBS,  $T_7$ - 100% OBS). Out of several treatments as described above  $T_2$ : 80% garden soil + 20% OBS was chosen for future experimental study involved in the study of phytotoxicity assessment and phytoremediation of toxic  $Cr^{+6}$  available in the contaminated soil used in the designed pot culture experiment.

The plants grown in  $T_2$ : 80% garden soil + 20% OBS were further treated with organic and inorganic amendments/chelators such as Ethylene Diaminetetra Acetic Acid (EDTA), Diethylene Triamine Penta Acetic Acid (DTPA), Citric Acid (CA) and Salicylic Acid (SA) and metal ions (Zn and Mg). Different chelators, organic acids and metal ions were applied to  $T_2$ : 80% garden soil + 20% OBS in the equimolar ratio by calculating the total

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Cr content available in the 20% OBS. After 16 days of plantation of Lemongrass in Cr contaminated over burden soil (T<sub>2</sub>: 80% garden soil + 20% OBS), chelators, organic acids and metal ions were applied to as follows. Details of the treatments are (T<sub>0</sub>- 100% garden soil as control, T<sub>1</sub>- 20% OBS, T<sub>2</sub>- 20% OBS-EDTA, T<sub>3</sub>- 20% OBS-DTPA, T<sub>4</sub>- 20% OBS-Citric Acid, T<sub>5</sub>- 50% OBS- Salicylic Acid, T<sub>6</sub>- 20% OBS-Zn and T<sub>7</sub>- 20% OBS -Mg).

**Chromium tolerance indices**

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)

**BCF=**

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

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

**TAR =**

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

Where TAR is in mgkg<sup>-1</sup>day<sup>-1</sup>, biomass is in g dry mass, and concentration is in mgkg<sup>-1</sup> dry mass. Stress tolerance indices for different growth parameters were calculated using the following formulae (Wilkins, 1957).

**Root Length of Stress Tolerance Index (RLSTI)** = (Root length of stressed plant / Root length of control plant) × 100

**Shoot Length of Stress Tolerance Index (SLSTI)** = (Shoot length of stressed plant / Shoot length of control plant) × 100

**Root Fresh Stress Tolerance Index (RFSTI)** = (Root fresh weight of stressed plant / Root fresh weight of control plant) × 100

**Shoot Fresh Stress Tolerance Index (SFSTI)** = (Shoot fresh weight of stressed plant / Shoot fresh weight of control plant) × 100

**Root Dry Stress Tolerance Index (RDSTI)** = (Root dry weight of stressed plant / Root dry weight of control plant) × 100

**Shoot Dry Stress Tolerance Index (SDSTI)** = (Shoot dry weight of stressed plant / Shoot dry weight of control plant) × 100

Stress tolerance indices for various growth parameters were determined using the formulae of Wilkins (1957). The tolerance index was determined as follows (Wilkins, 1978, Wang *et al.*, 2014).

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

**Results and Discussion**

There was a significant deterioration in the growth of Lemongrass in higher percentage of Cr rich OBS (Plate 1).



**Plate No. 1.** Effect of Cr on growth of Lemongrass plants grown on Over Burden Soil of Sukinda Chromite Mine area of Odisha.

A decrease in root length and biomass were observed from T<sub>3</sub> to T<sub>7</sub>. Similar results have been reported by several other workers in different plants (Zayed *et al.*, 1998; Zayed and Terry 2003; Mohanty *et al.*, 2008; Mohanty and Patra 2011a; Patra *et al.*, 2018). The application of lower percentage of Cr rich OBS in T<sub>2</sub> the growth of Lemongrass was enhanced might be due to enhanced chlorophyll biosynthesis leading to high biomass and productivity. Cr accumulation and its translocation to the shoots can be interpreted from the values of TAR and Ti, as illustrated in (Table 1).

**Table 1.** Effect of Cr on Bioaccumulation Factor (BCF), Transpiration index (Ti), Total Accumulation Rate (TAR) & Tolerance index of 60 days Old Lemongrass grown on over burden soil of chromite ore mine.

Treatment	Bio-Concentration Factor (BCF)	Transportation index (Ti)	Total Accumulation Rate (TAR)	Tolerance index (TI)
T <sub>1</sub>	0.68	24.0	193.81	132.25
T <sub>2</sub>	0.85	36.33	185.24	175.80
T <sub>3</sub>	0.88	23.77	113.39	104.83
T <sub>4</sub>	0.91	34.72	142.29	108.06
T <sub>5</sub>	0.92	29.62	140.94	101.61
T <sub>6</sub>	0.81	28.98	81.14	29.03
T <sub>7</sub>	0.193	34.64	58.78	16.1

The maximum transportation index values for Cr were noted in the T<sub>4</sub> whereas maximum TAR values was noted in T<sub>2</sub>. The application of Cr at higher concentrations lowered the percentage of tolerance in Lemongrass.

The elevated amount of Root Length of Stress Tolerance Index (RLSTI), Shoot Length of Stress Tolerance Index (SLSTI), Root Fresh Stress Tolerance Index (RFSTI), Shoot Fresh Stress Tolerance Index (SFSTI), Root Dry Stress

Tolerance Index (RDSTI) and Shoot Dry Stress Tolerance Index (SDSTI) were observed 145.36%, 123.14%, 188.80% 169.30%,170.06% and 138.93% at T<sub>2</sub> respectively and the lowest value 18.96%, 47.55%, 43.22%, 21.39%, 27.21% and 7.31% at T<sub>7</sub> respectively (Table 2).

**Table 2.** Toxicological interpretation in 60 days old Lemongrass grown on over burden soil of chromite ore mine under Cr stress.

Treatment	Root Length of stress tolerance index (RLSTI)	Shoot Length of stress tolerance index (SLSTI)	Root fresh Wt. of stress tolerance index (RFSTI)	Shoot Fresh Wt. of stress tolerance index (SFSTI)	Root Dry Wt. of stress tolerance index (RDSTI)	Shoot Dry Wt. of stress tolerance index (SDSTI)
T <sub>1</sub>	116.95	108.36	132.49	118.45	129.25	118.82
T <sub>2</sub>	145.36	123.14	188.80	169.30	170.06	138.93
T <sub>3</sub>	81.87	94.50	105.77	107.10	122.44	95.0
T <sub>4</sub>	71.01	87.96	90.25	95.48	120.04	85.92
T <sub>5</sub>	61.48	85.0	76.89	92.48	74.82	82.26
T <sub>6</sub>	39.84	72.56	68.59	52.15	64.28	29.25
T <sub>7</sub>	18.96	47.55	43.32	21.39	27.21	7.32

However, application of chelating agents and metals ions were helpful for combating toxicological effects of Cr<sup>+6</sup> and growth stimulation in Lemongrass Plants (Plate 2).



**Plate No. 2.** Effect of Cr and chelating agents on growth of Lemongrass plants grown on OBS of Sukinda Chromite Mine area of Odisha.

The use of chelator and metals ions exemplified enhanced growth as compared to control and other treatments. The stimulation of growth was accompanied by an increase in plants biomass. Use of chelates will enhance the solubility and uptake of essential metals into the plants. (Patra *et al.*, 2018). Enhancement of plant growth is therefore due to the supply of nutrients by chelate mobilization of cations in the soil and the augmentation of uptake by their roots. Hexavalent chromium at toxic concentration affects plant growth and metabolism by decreasing nutrient uptake and photosynthetic abilities (Vazquez *et al.*, 1987; Bonet *et al.*, 1991; Barcelo *et al.*, 1993, Mohanty and Patra 2011a, 2011b). The root Chromium bioaccumulation was highest as compared to shoot of Lemongrass plants. An enhancement in Chromium amount occurred in the root of Lemongrass plant grown on soil treated with Citric acid, DTPA, Salicylic Acid and metals ions like Mg and Zn is resolved by BCF values. The uptake of metals from soil to roots of the plants enhanced with the application of chelators. This clearly indicates the efficiency of chelators in mobilizing metals in the rhizospheric region. An increase in the BCF and Ti values with chelator's treatment indicated the better mobilization of metals from soil to roots and subsequently to aerial parts (Table 3).

**Table 3.** Effect of Cr<sup>+6</sup> and chelating agents on Bio-concentration factor, Total accumulation rate, Transportation index and Tolerance index in 60 days old Lemongrass under Cr stress grown on over burden soil of chromite ore mine

Treatment	Bio-Concentration Factor (BCF)	Transportation index (Ti)	Total Accumulation Rate (TAR)	Tolerance index (TI)
T <sub>1</sub>	0.81	29.81	176.72	127.14
T <sub>2</sub>	0.88	32.09	211.82	137.14
T <sub>3</sub>	0.92	34.79	241.90	151.42
T <sub>4</sub>	0.94	37.75	264.48	158.57
T <sub>5</sub>	0.90	33.36	239.19	148.57
T <sub>6</sub>	0.98	37.90	278.21	161.42
T <sub>7</sub>	0.98	39.06	287.17	168.57

The addition of chelators significantly increased the tolerance potential of Lemongrass. The maximum amount of Root Length of Stress Tolerance Index (RLSTI), Shoot Length of Stress Tolerance Index (SLSTI), Root Fresh Stress Tolerance Index (RFSTI), Shoot Fresh Stress Tolerance Index (SFSTI), Root Dry Stress Tolerance Index (RDSTI) and Shoot Dry Stress Tolerance Index (SDSTI) were observed 156.45%, 127.52%, 287.5%, 172.84%, 266.66% and 141.81% at T<sub>7</sub> (OBS-Mg) respectively (Table 4).

**Table 4.** Toxicological interpretation in 60 days old Lemongrass plants grown in over burden soil of chromite ore mine under Cr stress and chelating agents

Treatment	Root Length of stress tolerance index (RLSTI)	Shoot Length of stress tolerance index (SLSTI)	Root fresh Wt. of stress tolerance index (RFSTI)	Shoot Fresh Wt. of stress tolerance index (SFSTI)	Root Dry Wt. of stress tolerance index (RDSTI)	Shoot Dry Wt. of stress tolerance index (SDSTI)
T <sub>1</sub>	147.58	121.28	220.83	158.94	166.66	116.36
T <sub>2</sub>	151.61	123.30	258.33	164.23	200	120
T <sub>3</sub>	154.03	123.48	262.5	166.88	226.66	130.90
T <sub>4</sub>	154.03	123.85	262.5	168.21	246.66	134.94
T <sub>5</sub>	151.61	123.30	262.5	165.56	226.66	127.27
T <sub>6</sub>	155.64	124.220	279.16	170.86	260	134.54
T <sub>7</sub>	156.45	127.52	287.5	172.84	266.66	141.81

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