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Original Research Article

Chromium toxicity, physiological responses and tolerance potential of lemon grass (*Cymbopogon flexuosus* Nees ex steud*.* wats.)

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Abstract: The present investigation describes the physiological and biochemical response of Lemon grass (*Cymbopogon flexuosus* Nees ex Steud*.*Wats.) to increaseing doses of hexavalent chromium ($Cr^{6+)}$ grown under *in vivo* pot culture experiments. Plantlets of lemon grass were transplanted in poly bags and grown for 10 days in uncontaminated 5 kg garden soil. After 10 days the plantlets were grown for 60 days supplemented with different concentrations of Cr^{+6}(10 mg Kg^{-}1, 50 mg $Kg^{-1,}$ 100 mg Kg^{-1} and 200 mg Kg^{-1}respectively). The plants grown in terms of length and biomass was analysed for toxicological impacts using varying degrees of Cr^{+6} by soil contamination. Although the plant survival was 100% (using all the treatments upto 50 ppm application of Cr^{+6}), the root and shoot toxicity, root and shoot tolerance values decreased significantly with increasing concentrations of $Cr^{+6.}$ The toxicological values were clearly correlated with the growth measures induced by Cr^{+6} ions in contaminated soil. The leaf pigments (chlorophyll & carotenoids), total protein, catalase and different carbohydrate fractions exhibited declining trend with increasing Cr^{+6} ions. However, accumulation of proline and enhanced peroxidase activity was due to plant protection device under chromium stress. One of the most important observations was that the lemon grass do not tolerate beyond toxic 50 ppm concentration of Cr^{+6} beyond which the effects becomes chronic for which the plants could not survive upto 60 days growth. This observation suggests the thriving potentiality of lemon grass at sub toxic levels which can be exploited in future for phytoremediation experiments and field application in mine waste soil for reclamation.

Key words: Lemon grass, Toxicity, Physiological and biochemical lesions

Introduction

Chromium pollution results largely from industrial and mining activities, but other natural and anthropogenic sources also contribute to the problem. Plants that are exposed to environmental contamination by chromium are affected in diverse ways, including a tendency to suffer metabolic stress. Hexavalent chromium (Cr^{6+}) stress is one of the major abiotic stress problems in chromite mining sites. Plants that are exposed to environmental contamination by chromium are affected in diverse ways, including a tendency to suffer metabolic stress. There has been a long history of attempts to successfully mitigate the toxic effects of chromium-contaminated soil on plants and other organisms (Salt *et al.,* 1998, Zayed and Terry, 2003; Panda and Patra, 1997; Mohanty and Patra, 2011, Chaturvedi *et al.,* 2015). One common approach, the shifting of polluted soil to landfills, is expensive and imposes environmental risks and health hazards of its own (Chaturvedi *et al.,* 2015). Sources of new native hyperaccumulator plants for use at contaminated sites are needed and constitute a key goal of ongoing phytoremediation research programs. Such new plants are needed to enhance the attractiveness of phytoremediation as an effective, affordable, and eco-friendly. Phytodetoxification is an *in situ* process, which involves detoxification of heavy metals through plant-based chelation, reduction, and oxidation mechanisms. (Panda and Patra, 1997; Mohanty and Patra, 2011, 2012, Mohanty *et al.,* 2014). The use of different plant species for cleaning contaminated soils and waters, named as phytoremediation, has gained increasing awareness since last decade as an emerging cheaper technology.

In this context, *Cymbopogon flexousus* var. Martinea is chosen here as the experimental material to evaluate its tolerance potential by growing the plants in Cr contaminated soil under *in vivo* designed pot culture experiments. *Cymbopogon flexuosus* is commonly known as lemon grass. Lemongrass

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is a drought tolerant, perennial, aromatic grass. The essential oil of lemongrass is widely used in soaps, and perfumes. The crop can be cultivated throughout the year in poor and marginal soils, waste lands and alkaline soils (pH-9.6) at overburdens in mining sites. Considering the economic importance, easy cultivation and drought tolerance nature it can be effectively used as a tool for phytoremediation of iron ore tailings at mining sites. The plant is also tried successfully for its growth in contaminated soil (Mohanty *et al.,* 2010).

Materials and Methods

Plant materialand Growth

Six weeks-old saplings of *Cymbopogon flexousus var*. martinea (Nees ex steud) (Lemon grass) were transported from Ekamra nursery centre, Bhubaneswar, odisha, India. The grass plant lets were transplanted in the experimentally designed poly bags containing soil and green manure (3:1). The plant lets of uniform height (60 cms each) were transplanted in each poly bags containing 5kg garden soil and after 10 days of plant growth, the plantlets were supplemented with selected concentrations of Cr^{+6} (10 mg $Kg^{-}1$, 50 mg $Kg^{-1,}$ 100 mg $Kg^{-1}$and 200 mg Kg^{-1}). The plant lets were grown up to 60 days and harvested for growth and biochemical analysis.

Toxicological analyses

The toxicological effects of Cr^{+6} were expressed in terms of % phytotoxicity, and tolerance index (TI), which were calculated by the methods described by Iqbal and Rahmati (1992), Labra *et al.,* (2006) and Datta *et al.,* (2011).

Analysis of Biochemical Parameters

Analysis of pigment content (chlorophyll and carotenoids) was conducted by extracting pigments from leaves of the same age (3rd nodal position) from 60 days grown paragrass plantlets (Arnon *et al.,* 1949, Patra and Mishra, 1979, Pora, 2002) Protein and Proline estimation was done as done previously (Patra and Mishra, 1979, Bates *et al.,* 1973). The extraction and quantitative estimation of reducing sugar was done by Nelson and Somogyi method and extraction of total sugar was done by Anthrone method.

Extraction and assay of enzymes (catalase and peroxidase) were followed by the method of Patra *et al.,* (1978) and Patra and Mishra (1979). Chilled tissues (leaves/roots) were cut into small pieces and homogenized with cold 0.1M phosphate buffer, pH 6.8 (100 mg tissue : 5ml buffer) in a pre-chilled glass mortar and pestle. The extract was centrifuged at 4°C for 15 min. at 17, 000 rpm in a refrigerated centrifuge. The clear supernatant was used for catalase activity by absorbancy at 470 by Lowry method. For peroxidase, pre-chilled tissues (leaves / roots) were cut into small pieces and homogenized withcold 0.1 M phosphate buffer of pH 6.8 (100 mg fresh tissues: 2ml buffer) in a pre-chilled glass mortar and pestle. Then, the extract was centrifuged at 4°C for 15 min. at 17,000 r.p.m. in a refrigerated centrifuge and the final volume of the clear supernatant was made up to 20ml using phosphate buffer (pH 6.8). The enzyme assay procedure was followed as done previously (Patra and Mishra, 1979). Peroxidase activity was determined from the absorbency at 420nm. One unit of peroxidase activity was expressed in terms of purpurogallin formed, which increased the absorbency by 0.1 O.D. per minute under the assay condition.

Statistical analyses

The experiments were conducted in triplicates and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates.

Results and Discussion

Growth responses and physiological implications to Cr^{+6} application

The growth parameter studies of 60 days-old lemon grass plants using different concentration of Cr^{+6}(10 mg $Kg^{-}1$, 50 mg $Kg^{-1,}$ 100 mg Kg^{-1} and 200 mg Kg^{-1}) showed significant deterioration in growth with increasing supply of Cr^{+6}(Table 1, Plate 1, Plate 2 and Plate 3). Root and shoot length of lemon grass were decreased using concentrations of Cr^{+6} (10 and 50 ppm) in comparison to control after 60 days of growth. Similar results have been reported by several other workers in other plants (Bonnet *et al.,* 1991, Zayed and Terry 2003). The effect was also pronounced in root and shoot biomass of 60 days grown plants supplemented with increasing levels of Cr^{+6}. Root growth inhibition is a primary toxic effects of heavy metals and this parameter is an ideal index to measure the degree of tolerance (Wong and Bradshaw, 1982). 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 2).

Hexavalent chromium affects plant growth and metabolism by decreasing nutrient uptake and photosynthetic abilities (Bonet*et al.,* 1991, Barcelo *et al.,* 1985, 1986). Reduction of growth parameters was scored at higher concentrations of Cr^{+6} tested. However, at low concentration of Cr^{+6} (10ppm) the damage was not fatal.

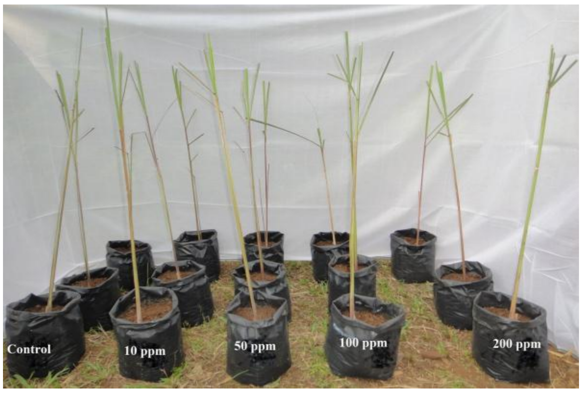

Plate 1: Lemon grass plant lets initally planted in poly pots. (Left to right: Control, Cr^{6+}-10 ppm), Cr^{6+}-50 ppm), Cr^{6+}-100 ppm and Cr^{6+}-200 ppm)

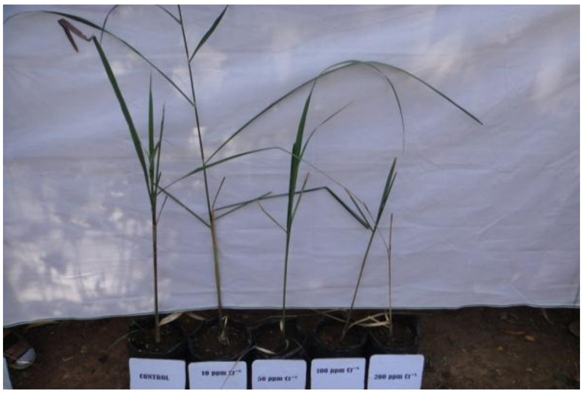

Plate 2: Photograph showing comparative growth of 30 days old lemon grass at different concentrations of Cr^{6+} (Left to right: Control, Cr^{6+}-10 ppm), Cr^{6+}-50 ppm), Cr^{6+}-100 ppm and Cr^{6+}-200 ppm)

Plate 3: Sixty days old Lemon grass in polypod culture. Photograph showing comparative growth of lemon grass at different concentrations of Cr^{6+} (Left to right: Control, Cr^{6+}-10 ppm), Cr^{6+}-50 ppm), Cr^{6+}-100 ppm and Cr^{6+}-200 ppm)

Table 1: Effect of different concentration of Cr^{+6} on growth of 60 days old plants of lemon grass *flexuosus*.

Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (gm)		Dry weight (gm)		
			Root	Shoot	Root	Shoot	Root/shoot
Control	36.666±0.715	127.5±1.242	17.487±0.441	27.631±0.594	5.133±0.299	9.413±0.345	0.545
10 ppm	34.9±0.533	135±0.937	14.258±0.328	26.464±0.276	4.233±0.228	9.608±0.069	0.440
50 ppm	22.333±0.631	85.833±0.585	8.432±0.193	20.445±0.368	1.014±0.030	4.867±0.358	0.208
100 ppm	NS*	NS*	NS*	NS*	NS*	NS*	NS*
200 ppm	NS*	NS*	NS*	NS*	NS*	NS*	NS*

NS*- Plants not survived beyond 30 days

Toxicological Analysis

The % phytotoxicity to roots and shoot of 60 days grown lemon grass under different Cr^{+6} treatments showed an increasing trend with increasing Cr^{+6} concentration (Table 2). The highest phytotoxicity values of root (39.1%) and shoot (32.7%) was found in plants supplied with 50 ppm of Cr^{+6}. The plants are prone to toxicity beyond 50 ppm supply with death of the plants beyond 30 days of growth. Results from the tolerance studies showed that root, shoot and plant tolerance index (TI) decreased with increasing Cr^{+6} supply treatment. Shoot tolerance has been found better as compared to root tolerance which may be due to direct contamination of chromium at the rhizosperic atmosphere affecting the root growth.

Table 2: Toxicological interpretation in 60 days old lemon grass under Cr^{+6} stress.

Treatment	% of survival after 30 days growth of plant	% phytotoxicty to root	% Phytotoxicity to shoot	Root tolerance index	Shoot tolerance index	Plant tolerance index
Control	100	0	0	100	100	100
10 ppm	100	4.816	0	95.184	105.882	103.493
50 ppm	100	39.091	32.680	60.909	67.320	65.888
100 ppm	NS*	NS*	NS*	NS*	NS*	NS*
200 ppm	NS*	NS*	NS*	NS*	NS*	NS*

NS*- Plants not survived beyond 30 days

Toxic effects of Cr^{+6} and Biochemical Lesions

The toxicological values for growth measures induced by Cr ions in contaminated soil were clearly correlated with the biochemical lesion (Fig. 1, 2, 3 and 4). The leaf pigments (chlorophyll & carotenoids), total protein, catalase and different carbohydrate fractions exhibited declining trend with increasing Cr^{+6}applications. However, accumulation of proline and enhanced peroxidase activity was due to plant protection device under chromium stress. (Mohanty *et al.,* 2014, Mohanty *et al.,* 2015). Proline accumulation is an important parameter to recognize the stress impact on plants (Mohanty and Patra, 2011). Proline accumulation may also help in non-enzymic free radical detoxification. The overall investigation shows that Cr^{+6} at higher concentration had deletorious effects on growth parameters and boichemical lessions. One of the most important observations was that the lemon grass do not tolerate beyond toxic 50 ppm concentration of Cr^{+6} (50 mg of Cr^{+6}/kg of soil) for which the effects becomes chronic and the plants could not survive up to 60 days growth. This observation suggests the thriving potentiality of lemon grass at sub toxic level which can be exploited in future for phytoremediation experiments and field application in mine waste soil for reclamation.

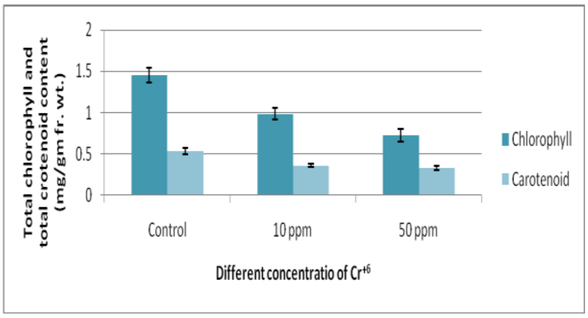

Figure 1: Effect of different concentration of Cr^{+6} on total chlorophyll and total carotenoid content of 60 days old lemon grass.

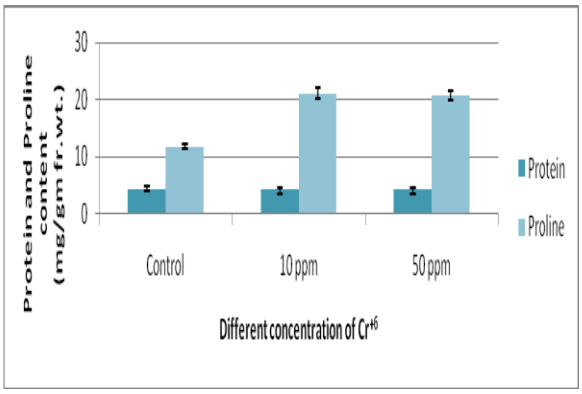

Figure 2: Effect of different concentration of Cr^{+6} on proteins and proline content 60 days old lemon grass.

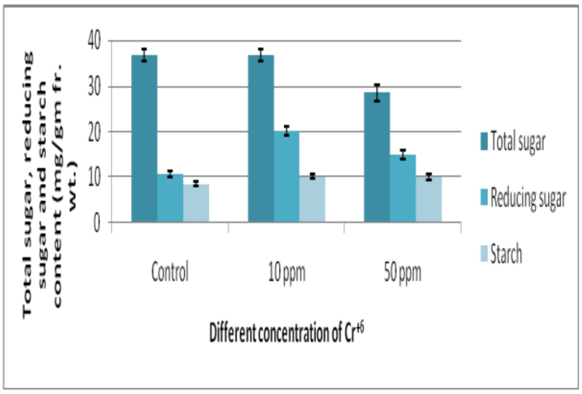

Figure 3: Effect of different concentration of Cr^{+6} on total sugar, reducing sugar and starch content 60 days old lemon grass.

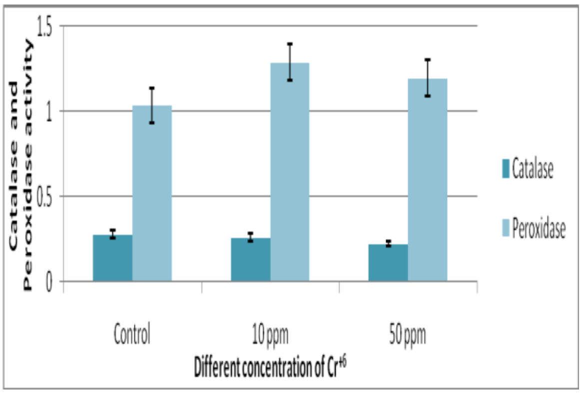

Figure 4: Effect of different concentration of Cr^{+6} on catalase activity (catalase unit/gm fr.wt.) and peroxidase activity (peroxidase unit /gm fr.wt.) of 60 days old lemon grass.

Conclusion

The present study indicates that the fibrous root system of lemon grass could be helpful for undertaking the on-going study to establish the dose dependent stress impacts of Cr application on growth and metabolisim of graminaceious plants. Future phytoremediation strategies will be carried out for decontamiantion of Cr^{+6} from polluted soil. This study will further suggest the measures for the farmers who grow crops at the contaminated sites.

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References

1. Arnon DI, Copper enzymes in chloroplast polyphenol oxidase in *Beta vulgaris* Plant Physiol, 1949, 24, 1-15.
2. Barcelo J, Poschenrieder CH, Gunse B, Effect of Cr (VI) on mineral element composition of bush beans J Pl Nut, 1985, 8(3), 211-217.
3. Barcelo J, Poschenrieder MD, Gunse B, Chlorophyll and carotenoid contents of Phaseolus vulgaris L. in relation to mineral nutrient disorders induced by Cr (VI) supply Photosynthetica, 1986, 20, 249-255.
4. Bates LS, Waldren RP, Teare ID, Rapid determination of free proline for water stress studies. Plant Soil, 1973, 39, 205–207.
5. Bonet A, Poschenrieder C, Barcelo J, Chromium III-iron interaction in Fe-deficient and Fe-sufficient bean plants. I. Growth and nutrient content. J Plant Nutr 1991, 14, 403-414.
6. Chaturvedi N, Dhal N, Patra HK, EDTA and Citric acid mediated phytoextraction of heavy metals from iron ore tailings using Andrographispaniculata: a comparative study. Intl J Min Reclamat Environ. 2015, 29 (1), 33-46.
7. Datta JK, Bandhyopadhyay A, Banerjee A, Mondal NK, Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars under laboratory condition. J Agr Tech 2011, 7, 395-402.
8. Iqbal MZ, Rahmati K, Tolerance of Albizialebbeck to Cu and Fe application. Ekologia, 1992, 11, 427-430.
9. Labra M, Gianazza E, Waitt R, Eberini I, Sozz, A, *Zea mays* L. protein changes in response to potassium dichromate treatments. Chemosphere, 2006, 62, 1234-1244.
10. Mohanty, M, Patra HK, Attenuation of Chromium Toxicity by Bioremediation Technology. Rev Environ Contam Toxicol, 2011, 210, 1-34.
11. Mohanty M, Pradhan C, Patra HK, Chromium translocation, bioconcentration and its phytotoxic impacts in *in vivo* grown seedlings of Sesbaniasesban L. Seedlings. Acta Biologica Hungarica, 2014, 66(1), 80-92.
12. Mohanty M, Patra HK, Effect of Chelate assisted Hexavalent Chromium on Physiological changes. Biochemical alterations and Cr Bioavailability in Crop Plants-An in vitro Phytoremediation Approach. Bioremediat J, 2012, 16(3), 147–155.
13. Panda SK, Patra HK, Physiology of Chromium Toxicity in Plants - A Review. Plant Physiology & Biochemistry, 1997, 4(1), 10-17.
14. Patra HK, Mishr, D, Pyrophosphatase, peroxidase and polyphenoloxidase activities during leaf development and senescence. Plant Physiol, 1979, 63, 318-323.
15. Patra HK, Kar M, Mishra D, Catalase activity in leaves and cotyledons during plant development and senescence, Biochem Physiol Pflanzen, 1978 172, 385-390.
16. Porra RJ, The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth Res, 2002, 73, 149 -156.
17. Salt DE, Smith RD, Raskin I, Phytoremediation, Annu Rev Plant Physiology, 1998, 49, 643-68.
18. Wong MH, Bradshaw AD, A comparison of the toxicity of heavy metals, using root elongation of rye grass, Lolium perenne. New Phytologist, 1982, 91(2), 255-261.
19. Zayed AM, Terry N, Chromium in the Environment: Factor affecting biological remediation. Plant and Soil, 2003, 249, 139–156.

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