J. Mater. Environ. Sci. 7 (6) (2016) 2221-2228
ISSN : 2028-2508
CODEN: JMESCN

Tolerance potential and physiological responses of *Helianthus annuus* L. exposed to varying doses of hexavalent chromium

M. Mohanty* and H.K. Patra

Laboratory of Environmental Biotechnology

Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, India

.

Received 02 May 2015, Revised 02 May 2016, Accepted 05 May 2016

**Corresponding author. E-mail: 18.monalisa@gmail.com ; Tel: (+919861077321)*

Abstract

The present investigation assesses the physiological and biochemical response of sunflower plants (*Helianthus annuus* L.) to increased doses of hexavalent chromium (Cr^{6+}) at different growth periods (15, 30 and 45 days) under *in vivo* condition. Treatment with 500 mg Cr^{6+}/Kg dry soil showed 80% inhibition in Seed germination. The Seedlings after 30 and 45 days of Cr^{6+} treatment (100 mg/Kg dry soil) showed negligible difference in their shoot length values, but it was significantly decreased using 200 mg Cr^{6+}/Kg dry soil treatment. Notable increase in root length was observed at 50 mg Cr^{6+}/Kg soil treatment after 45 days of plant growth. Plant biomass, chlorophyll and carotenoid contents of 45 days treated seedlings were significantly reduced at 200 mg Cr^{6+}/Kg soil. The increase in total free amino acid accumulation was noted up to 30 days using different concentrations of Cr^{6+} with maximum at 200 mg Cr^{6+}/Kg dry soil but on 45 days it showed a decline value with some minor deviations using different concentrations of Cr^{6+}. The higher level of Chromium accumulation in shoot in comparison to roots indicates the tolerance potential of *H. annuus* towards application of toxic doses of hexavalent chromium even through the survival capacity of the plants reduced with increasing Cr^{6+} supply. This leads to evolved phytoremediation techniques using *in situ* application of the plant as a green tool.

Keywords: Chromium, sunflower, photosynthetic pigments, total free amino acids, bioaccumulation

Introduction

Widespread use of chromium in several industrial and mining activities leads to the release of huge quantities of toxic hexavalent chromium to environment. Hexavalent chromium (Cr^{6+}) stress is one of the major abiotic stress problems in chromite mining area of Orissa. The two stable forms of chromium (Cr) i.e., hexavalent (Cr^{+6}) and trivalent (Cr^{+3}) differs in terms of their mobility, bioavailability and toxicity. Various mutagenic, toxic and carcinogenic effects have been imposed by chromium compounds in biological systems [1, 2, 3, 4]. Cr is found in all phases of the environment, including air, water, and soil with varying ranges of Cr^{6+} [3]. As per the detail literature survey and research on Cr induced phytotoxicity and potential hyperaccumulation in several plants carried out by eminent researchers [5, 6, 7, 8].

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, Sunflower is chosen as the experimental material to evaluate its hyper-accumulation potential and tolerance by growing the plants in Cr contaminated soil under *in vivo* designed pot culture experiments.

Sunflower (*Helianthus annuus* L.) from the family of Asteraceae is the world's fourth largest oil-seed crop [9]. Besides its ornamental use, the seeds of sunflower are also used as food and dried stalk as fuel and parts of this plant are used in making dyes for textile industry, body painting, and other decorations. Sunflower meal is a potential source of protein for human consumption due to its high nutritional value and lack of anti-nutritional

factors [9]. As sunflowers have highly efficient root systems, they can be grown in areas which are too dry for many crops, they could be used as a salt tolerant hyperaccumulator species for several heavy metals as reported by researchers [9]. Plants are quite drought-resistant except during flowering. It has also proved to be extremely tolerant of a wide range of heavy metal contaminated sites (Ni, Pb, and Cd). The present study was conducted to determine the phytotoxic responses of sunflower and its ability of take away the Cr from the contaminated soil. The toxic effects of Cr^{6+} at physiological and biochemical levels in growing sunflower plants were also discussed.

The plant was tested for its Cr phytoaccumulation ability that could be used 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.

2. Materials and methods

2.1. Experimental design and Plant material

Pot culture experiments were conducted in completely randomised design. Dry graded seeds of sunflower (*Helianthus annus L.*) were procured from Govt. Nursery, Unit-2, Bhubaneswar and were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes [4].

2.2 Germination study

The pretreated uniform healthy sunflower seeds were germinated in petriplates 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}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}, 300 mg L^{-1} and 500 mg L^{-1} along with a control for two days inside BOD incubator at 25 ± 2 ^{0}C. After 48 hours (two days) the number of germinated seeds under each treatment of Cr^{6+} was counted. The germination percentage and germination index (IG %) of seeds were calculated.

2.3 Growth of sunflower seedlings

The seeds were germinated in earthen pots (size: height 30cm and diameter 15cm) containing 5 kg garden soil (control: Cr^{6+}-0 mg L^{-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}) and were grown at the nursery site of Department of Botany, Utkal University for 15, 30 and 45 days. Pots supplemented with half strength Hoagland nutrient solution ([10] were taken as control treatment.

2.4 Analysis of Biochemical Parameters

Analysis of seedling growth, pigment content (Chlorophyll and carotenoid), total free amino acid was conducted using 15, 30 and 45 days grown sunflower seedlings. The extraction of chlorophyll was made using cold alkaline acetone (80% v/v) and calculated as per the methods of Arnon [11] with a little modification [12]. Total free amino acid content of seedlings at different growth periods and exposed to varying concentrations of Cr^{6+} were also calculated [13].

2.5 Total Cr bioavailability in plant tissues

Sunflower seedlings grown after 45 days in different treatment of Cr^{6+} were analyzed for total Cr content in roots and shoots [14, 15]. Before analysis of total Cr content, the roots were rinsed with 0.01N HCl followed by washing with distilled water for removing mixed Fe and Cr hydroxides, which may have precipitated on the root surfaces [7]. Root, stem and leaves of 45 days treated sunflower seedlings from different treatment pots of Cr^{6+} were oven dried and grinded separately to fine powders. The 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 [14]. 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 and the final volume was made up to 100 ml. Total Cr bioaccumulation in different parts

of plants were estimated by analyzing those extracted liquid samples in an Atomic Absorption Spectrophotometer (Perkin Elmer, AAanalyst 200, USA).

2.6 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.

3. Results and discussion

3.1. Seed Germination affected by Cr stress

The seed germination test under increasing concentrations of Cr^{6+} (ranging from 10 to 500 mgL^{-1}) showed gradual inhibition. Seed germination is the first physiological process affected by Cr. Seeds germinated under different concentration of Cr^{6+} showed significant reductions in germination percentage as compared to control (Fig. 1). The germination % ranged from 20-90% with decreasing concentrations of Cr^{6+} treatments (Fig. 1). The reduction in germination of sunflower seeds may be attributed to increased protease activity under chromium stress and depressive effect of Cr on the subsequent transport of sugars to the embryo axis [15, 16]. However, germination percentage was significantly affected at 50 and 100 ppm by 40 % and 50% reduction in germination, indicating that higher level of chromium produced toxic effect in seed germination. Similar results were reported by several researchers [9, 17] in other plants

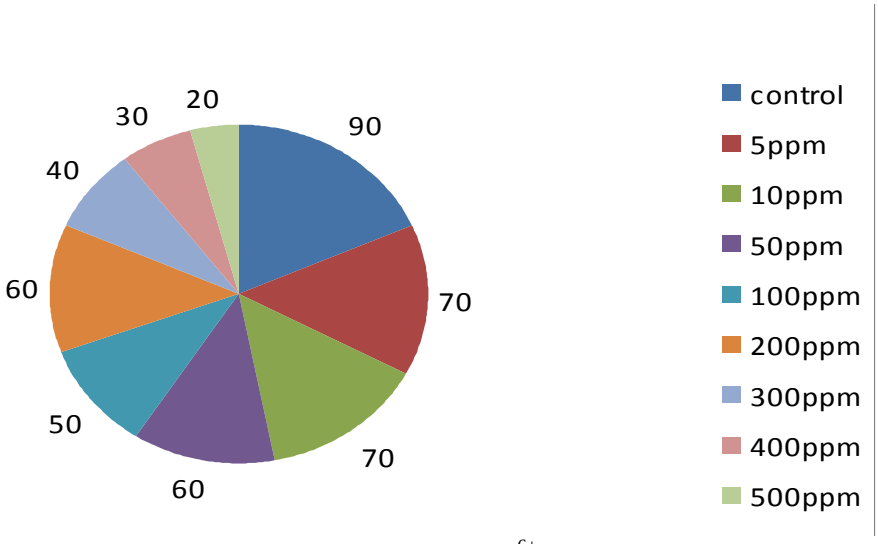

Figure 1: Effect of different concentrations of Cr^{6+} on germination of sunflower seeds.

3.2 Analysis of growth indices impairment in response to Cr^{6+} stresses

Heavy metals influence the growth and tissue ionic concentration. According to Paiva *et al*. 2000), dry weight of shoot and root showed a significant reduction with increase in heavy metals level. Such reduction becomes more damaging at high levels of heavy metals; therefore, plants show greater variation ranging from morphological characters to physiochemical characters. In the present study, growth rate, increase in plant height and fresh weight were significantly reduced. Although there were remarkable reductions in various growth parameters, the effect was more pronounced with high Cr levels, which inhibit growth more than control. It was noticed that the fresh and dry weight parameters were better in control than that under Cr stress. This is probably due to the reason that toxicity of heavy metals significantly inhibited root vitality, preventing plant from absorbing inorganic nutrients and leading to inhibit plant growth [18].

The growth parameter studies of 45 days-treated sunflower seedlings under different treatments of Cr^{6+} showed significant deterioration in seedling growth and survival with increasing supply of Cr^{6+} (Table 1).

Table 1. Effect of Cr^{6+} on germination and seedling survivability of sunflower.

Treatments of Cr^{+6}(mg Kg^{-1})	Mean No of plants survived after 45 days
Control (0)	**15 ± 0.298**
10	**11 ± 0.596**
50	**11 ± 0.516**
100	**9 ± 0.298**
200	**6 ± 0.298**

Root and shoot length of sunflower seedlings were not significantly affected with toxic concentrations of Cr^{6+} among the different treatments in comparison to control after 15, 30 and 45 days of growth. The effect of Cr^{6+} (10, 50 and 100 mgKg^{-1}) on root was found stimulatory as length of the roots have been increased as compared to control (Fig 2).

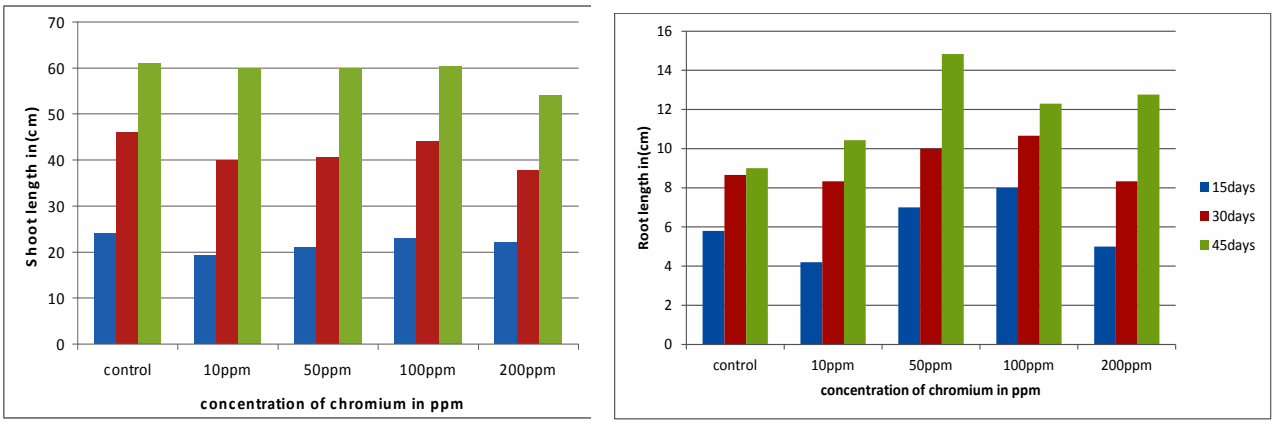

Figure 2. Effect of different concentrations of Cr^{6+} on the shoot and root length of 15, 30 and 45 days treated *Helianthus annuus* seedlings.

Toxic effect was initially detected at early stages of plant growth which gradually disappeared at mature age of plant in context of root and shoot length which reveals plant's tolerance mechanism to treatment of Cr concentration at 100mg Kg^{-1}.

At early stage of plant growth, Cr toxicity inhibits root cell division/root elongation or the extension of cell cycle in the roots, thereby inhibits root growth while roots directly contact with Cr in the medium, causing a collapse and subsequent inability of the roots to absorb water from the medium [9, 19]. But at maturity, the plants develop tolerance mechanism through increased antioxidative enzyme synthesis and other factors.

The deleterious effect was more pronounced in root and shoot biomass of 45 days treated seedlings supplemented with increasing supply of Cr^{6+} (200 mg Kg^{-1}) (Fig 3).

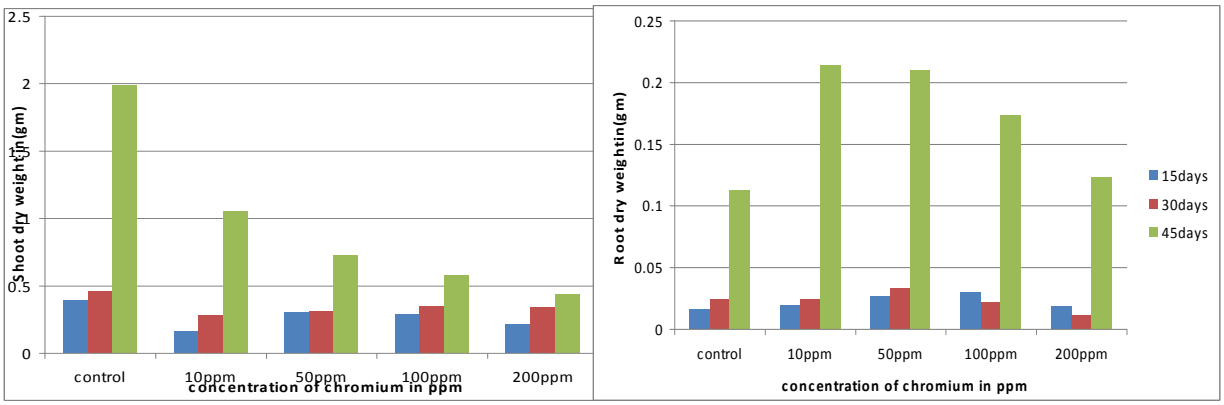

Figure 3. Effect of different concentrations of Cr^{6+} on the shoot and root dry weight of 15, 30 and 45-days treated *Helianthus annuus*.

Root growth inhibition is a primary toxic effects of heavy metals and this parameter is an ideal index to measure the degree of tolerance. Reduction of growth parameters was scored for high concentration of Cr^{6+} tested; at low concentration of Cr^{6+} (10 mg Kg^{-1}) growth stimulation was observed (Fig 4).

Figure 4. Photograph showing comparative growth of sunflower at different treatment concentrations of Cr^{6+} after 45 days of plant growth [Left to right: Control, Cr^{6+}(10), Cr^{6+} (50), Cr^{6+} (100), Cr^{6+} (200)]

Highest growth rate in control and decreased by increasing Cr levels was observed at all the three phases of plant growth.
Interactions of Cr with uptake and accumulation of other inorganic nutrients have received maximum attention by researchers. There was a decreasing trend in Na, K, P, and N contents of roots and shoots by increasing Cr level which mostly contributes to reduce plant growth as reported by several researchers [20, 21].

3.3 Toxic effects of Cr^{6+} on Biochemical Parameters

3.3.1 Effect of Cr^{6+} on Chlorophyll and carotenoid content

A significant deterioration in chlorophyll and carotenoid content of sunflower leaves were observed after 45 days treatment (Fig. 5).

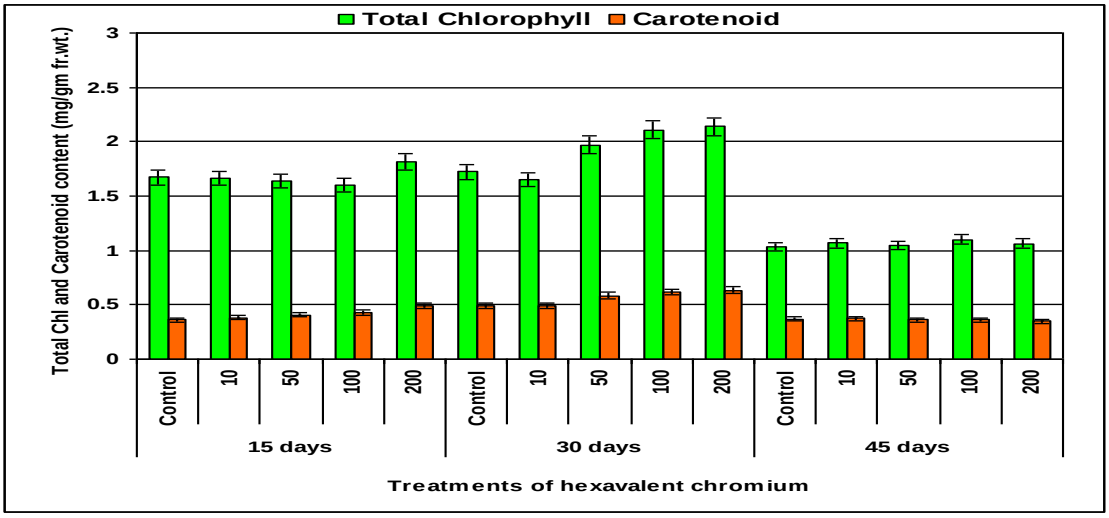

Figure 5. Effect of different concentrations of Cr^{6+} on the total chlorophyll and carotenoid content of 15, 30 and 45-days treated *Helianthus annuus* seedlings.

Negligible reduction in chlorophyll and carotenoid content was observed with increased Cr stress revealing the Cr tolerant nature of the plant. Very little increase in total chlorophyll content was observed at 30 days growth period with increased supply of Cr^{6+} treatment indicated by better growth of seedlings. It can be explained by that the higher source size and increased photosynthetic process were found to be the basis for the building up of organic substances and dry matter production under heavy-metal stress in general and Cr in particular [9, 22]. Decreased content of chlorophyll is a common symptom of heavy metal toxicity. This reflects the inhibitory effect of this metal on biosynthesis of pigments, which may be a metal specific action [23]. Moreover, it can also block the photosynthetic electron transport chain and thus degrade chlorophyll pigment [24, 25].

3.3.2 Effect of Cr^{6+} on Total free amino acid content

Total free amino acid content in 15, 30 and 45- days old plants of *Helianthus annuus* increased with increasing the dose of Cr in soil (Fig 6).

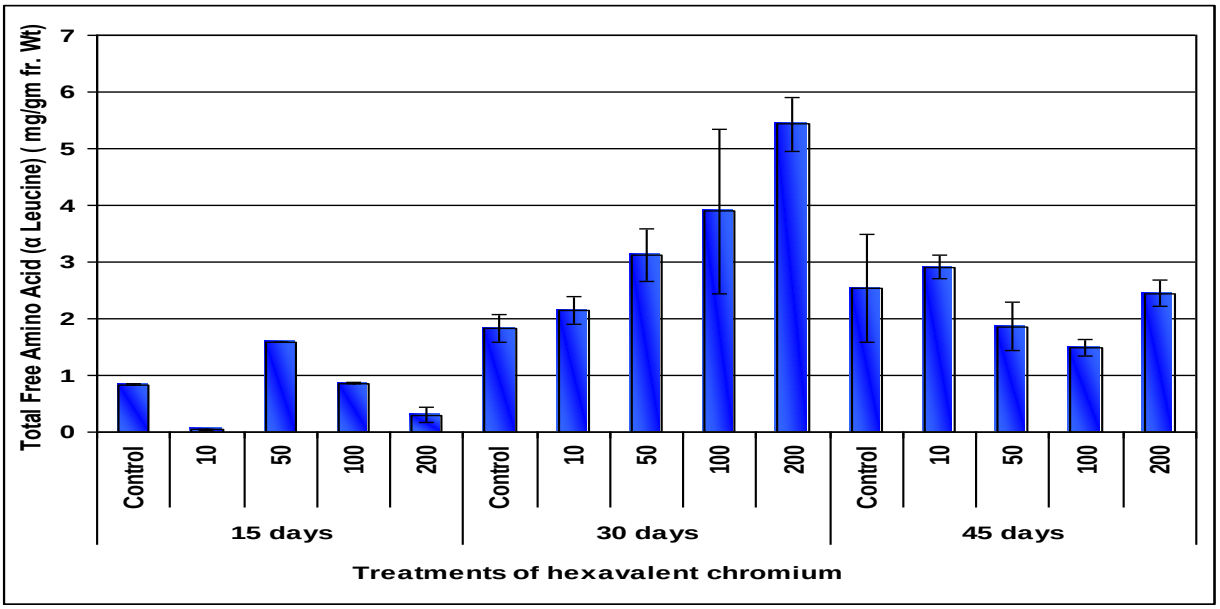

Figure 6. Effect of different concentrations of Cr^{6+} on total free amino acid content of 15, 30 and 45-days treated *Helianthus annuus* seedlings.

It was found that Maximum Total free amino acid biosynthesis was observed in *Helianthus annuus* plants treated with Cr^{6+} (100ppm). The order of increase in reducing sugar biosynthesis was as follows-

15 days	Control > Cr^{6+} (10ppm) < Cr^{6+} (50ppm) > Cr^{6+} (100ppm) > Cr^{6+} (200ppm).
30 days	Control < Cr^{6+} (10ppm) < Cr^{6+} (50ppm) < Cr^{6+} (100ppm) <Cr^{6+} (200ppm).
45 days	Control < Cr^{6+} (10ppm) > Cr^{6+} (50ppm) > Cr^{6+}(100ppm) < Cr^{6+} (200ppm).

3.4 Chromium bioaccumulation in sunflower

Chromium bioaccumulation in root and shoot gradually increases with supply of elevated concentrations of Cr upto 50 mg Kg^{-1} Cr treatment in root but the translocation of Cr to shoot increases with increased Cr concentration up to 200 mg Kg^{-1} Cr treatment. (Fig. 7). Heavy Cr accumulation in plant tissues and shoot translocation at high Cr concentration enable the plant to be a potential accumulator of Cr as evident from the figure 7.

The experimental results clearly illustrates that Cr is toxic at different degrees at different stages of plant growth and development [26]. It also explains that the toxicity is concentration and age dependent. A study on stress tolerance suggests that mechanism of tolerance helps plant to maintain growth even in the presence of potentially toxic metal concentrations used the root and shoot

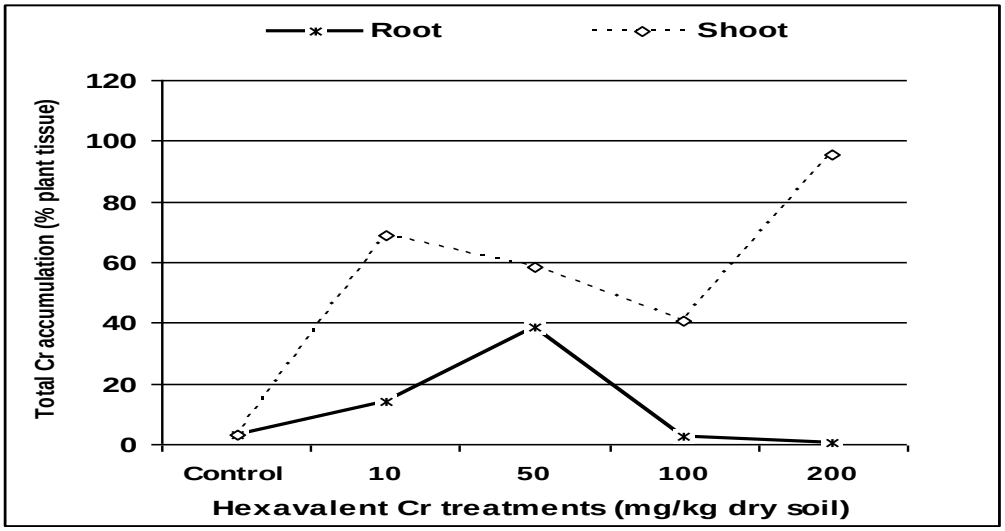

Figure 7. Effect of different concentrations of Cr^{6+} ($mg\,Kg^{-1}$) on total Cr bioaccumulation (in terms of % of plant mass) in 45 days grown sunflower seedlings.

Conclusion

1. The above studies on phytotoxic effects explain the severity of Cr pollution and cater to the need for safe removal of toxic chromium from the contaminated environment by using hyper accumulator species like sunflower.
2. The experimental results clearly showed that Cr is found relatively less non toxic at different degrees at different stages of plant growth and development even at high concentration which suggests further implication of the plant as a green tool of phytoremediation technology for Cr phytoextraction purpose.
3. This preliminary report contributes in exploring and finding the tolerance limit of *Helianthus annuus* at different concentration of chromium.
4. Results of present investigation are useful indicators of chromium tolerance to some extent for plantation of *H. annuus* in chromium contaminated areas.
5. However, further research is needed to determine the effect of different level of other metals in the environment and various parts of the plant through an *in situ* approach.
6. A field study is also recommended to find effect of natural variables (temperature, pH, light, soil quality, etc.) on the above laboratory based results.
7. The findings of the present research investigation will help to prescribe the evolved chromium phytoremediation technology for practical application under field condition.
8. Suitable plant based remediation techniques for sustainable ecosystem and stabilizing mining environment are given priority now-a-days.

Acknowledgements-The authors are thankful to CSIR for providing financial assistance to Dr. H .K. Patra (CSIR-ES) under CSIR-Emeritus Scientist Scheme, Post Graduate Department of Botany, Utkal University.

References

1. Mohanty M., and Patra H.K. Chelate assisted phytoextraction of chromium– an *in vitro* study in rice. In: Environmental stress impacts on plants (Patra H.K., Eds.). *Proceedings of National Seminar*, UGC-DRS (SAP-II). Utkal University, India. (2010) pp. 85-95.
2. Mohanty M., Pattanaik M.M., Misra, A.K. and Patra, H.K., Chromium Bioaccumulation in Rice grown in Contaminated Soil and Irrigated Mine Waste Water - A Case Study At South Kaliapani Chromite Mine Area, Orissa, India. *Int. J. Phytoremediat.* 13(2011) 397-409.
3. Mohanty M. and Patra H.K., Attenuation of chromium toxicity by bioremediation technology. *Reviews of Env. Contam. Toxicol.* 210 (2011a) 1-34.

4. Mohanty M. and Patra H.K., Effect of Cr^{+6} and chelating agents on growth, pigment status, proline content and chromium bioavailability in rice seedlings. *Int. J. Biotech. Appl.* 3, 3 (2011b) 91-96.
5. Zayed A.M., Terry N. Chromium in the Environment: Factor affecting biological remediation. Plant. Soil. 249(2003) 139–156.
6. Shanker A.K., Cervantes C., Loza-Tavera H., Avudainayagam S., Chromium toxicity in plants. Environ Int 31 (2005a) 739–753.
7. Mohanty, M., Pradhan, C. and Patra, H.K., Chromium translocation, concentration and its phytotoxic impacts in *in vivo* grown seedlings of *Sesbania sesban* L. Merrill. Acta Biol. Hungarica 66, 1 (2015) 80-92
8. Mohanty, M. & Patra, H.K., Hexavalent Chromium induced toxicological, physiological and biochemical alterations in *Sesbania sesban* L. Seedlings. *J Plant Physiol Pathol* 2, 3 (2014.) 1-6. http://dx.doi.org/10.4172/2329-955X.1000129
9. Andaleeb F., Anjum Zia M., Ashraf M., Mahmood Khalid Z., Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.). *J. Env. Sci.* 20 (2008) 1475–1480
10. Hoagland D.R., Arnon D.I., The water culture method for growing plants without soil. *Calif. Agric. Exp. Station Cir.* 347 (1938) 1-39.
11. Arnon D.I., Copper enzymes in chloroplast polyphenol oxidase in Beta vulgaris. *Plant Physiol.* 24 (1949)1-15.
12. Porra R.J., The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynth. Res.* 73(2002) 149 -156.
13. Moore S., Stein W.H., Photometric ninhydrin method for use in the chromatography of amino acids. *J Biol. Chem.,* 176(1948) 367 388.
14. Bonet A., Poschenrieder C. and Barcelo J., Chromium III-iron interaction in Fe-deficient and Fe-sufficient bean plants. I. Growth and nutrient content. *J. Plant Nutr.* 14 (1991) 403-414.
15. Mohanty M., Patra H.K., Effect of Chelate assisted Hexavalent Chromium on Physiological changes, Biochemical alterations and Cr Bioavailability in Crop Plants - An *in vitro* Phytoremediation Approach. *Bioremediat J* 16,3 (2012) 147–155.
16. Datta J.K., Bandhyopadhyay A., Banerjee, A. and Mondal N.K., Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars under laboratory condition. *J. Agric. Techy.*, 7, 2 (2011) 395-402.
17. Amin H., Arain B.A., Amin F., Surhio M.A., Phytotoxicity of Chromium on Germination, Growth and Biochemical Attributes of *Hibiscus esculentus* L. *American Journal of Plant Sciences*, 4, (2013) 2431-2439
18. Shu W.S., Lan C.Y., and Zhang Z.Q., Analysis of major constraints on plant colonization at Fankou Pb/Zn Mine tailings. Chinese Journal of Applied Ecology, 8, 3 (1997) 314-318
19. Barcelo J., Paschenrieder Ch. and Gunse B., Water relations of chromium treated bushbean plants (*Phaseolus vulgaris* L. c.v. contender) under both normal and water stress conditions. *J. Exp. Bot.* 37 (1986) 178-187.
20. Moral R., Pedreno N., Gomez I. and Matrix J., Effect of chromium on nutrient element content and morphology of tomato. *J. Plant Nutr.*, 18 , 4 (1995) 175-183.
21. Sujatha P., Gupta A., Tannery effluent characteristics and its effects on toxicity and Cr-P interactions in rye grass (Lolium perenne). *Acta. Agric. Scand.*, 39 (1996) 139–147.
22. Bishnoi N.R., Dua A., Gupta V.K., Sawhney S.K., Effect of chromium on seed germination seedling growth and yield of peas. *Agric. Ecosys. Environ.* 47, 1 (1993) 47–57.
23. Panda S.K., Choudhury S., Chromium stress in plants. *Braz. J. Plant Physiol.* 17, 1 (2005) 95–102.
24. Quartacci MF, Cosi E, Navari-Izzo F., Lipids and NADPH dependent superoxide production in plasma membrane vesicle from roots of wheat grown under copper deficiency or excess. *J. Exp. Bot.* 52,(2001)77-84.
25. Patsikka E., Kairavuo M., Sersen F., Aro E.M. and Tyystjarvi E.,. Excess copper predisposes photosystem-II to photoinhibition in vivo by out competing iron and causing decrease in leaf chlorophyll. *Plant Physiol.,* 129 (2002) 1359-1367.
26. Mohanty M., Pattanaik M.M., Misra A.K. and Patra H.K. Bioconcentration of chromium – An *in situ* phytoremediation study at South Kaliapani chromite mining area of Orissa, India. *Env. Monit. Assess.* 184, 2 (2012) 1015–1024.