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Physiological and Pigment variations in *Talinum triangulare* (Jacq.) Willd. grown on Chromium polluted Soil treated with Chelants

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Abstract

A study was conducted in an artificially polluted soil to evaluate the growth of Talinum triangulare under varying concentrations of two different chelants- EDTA and citric acid either separately or in combination. The performance of these chelants in promoting plant growth under chromium pollution was also studied which would help in selection of the chelant to be employed for any further chelant assisted phytoremediation techniques. It was found that increase in concentration of chelants above 500mg/kg in case of EDTA, 750mg/kg of mixed chelants produced toxicity symptoms in the plant. At a specific concentration of each chelant, growth was induced and later decreased showing that higher concentration of chelants may itself pose toxicity to the plants which are already facing metal stress. Therefore selection of the chelant and also the concentration to be employed becomes a necessary part of chelant assisted phytoremediation. From this study it can be stated that a safer removal of chromium can be brought about by employing citric acid which does not lead to any toxicity.

Keywords: Chelant, phytoremediation, phytotoxicity, photosynthetic pigments, Talinum triangulare.

Introduction

Increasing consumption and exploitation of the earth's raw materials (fossil fuel and minerals) coupled with the exponential population growth over the past years have resulted in environmental degradation. Very little attention is paid to the treatment of industrial effluents and it has led to the build up of waste products of which metals are of great concern[1,2]. Traces of certain heavy metals may act as dietary constituents but if they accumulate in living systems they may pose serious hazards. Metal ions have both beneficial and harmful effects on human physiology; some of them are essential for life while some others are toxic at higher concentrations[3]. In recent years, soil polluted by heavy metals has increased due to human activities and removal of such pollutants has gained much importance[4]. Apart from the essential elements such as Cu, Fe, Ni and Zn, there are some non-essential heavy metals which play a major role due to their presence in areas of heavy metal pollution such as chromium (Cr), mercury (Hg) and lead (Pb) [5]. Among these heavy metals, chromium (Cr) which is one of the abundant elements on Earth exists in the range of approximately 100 µg/g [6]. It has been classified by the International Agency for Research on Cancer (IARC) as a Group I human carcinogen[7].

Chromium exists in two stable states, i.e. hexavalent chromium (Cr^{+6}) and trivalent chromium (Cr^{+3}) of which Cr^{+6} is the most toxic form. Chromium compounds have been known to be highly toxic in biological systems and act as strong allergens to some individuals. Soil is considered to be a dynamic component of the ecosystem characterized by seasonal changes. Due to extreme poor regeneration capacity of the soil, nutrient recycling is affected which becomes a constraint for plant growth. Vegetables contaminated by heavy metal pollution may pose a serious threat to human health[8,9]. Chromium toxicity affects plant growth and metabolism to a considerable extent[10,11]. It has been reported that chromium caused reduction in root length, shoot length, decreased the number of branches and induced biochemical and physiological changes in crops[12-14].

A study was carried out to determine the growth performance of *Talinum triangulare* plant under Cr stress in the presence of two different chelants. Effects of chelants on photosynthetic pigments were also studied.

Material and Methods

Plant description: *Talinum triangulare* is an edible plant with medicinal properties. It is a succulent herb that grows in shallow soil in rocky outcrops. It is capable of accumulating chromium in its roots than in shoot which makes it a metal excluder. It is shown that Cr treatment altered growth parameters of the plant[15,16].

Experimental set up: Soil was collected from fallow agricultural lands in Coimbatore, Tamilnadu, INDIA. Location of the soil sampling area is given in figure 1. It was thoroughly dried, powdered and passed through a 2 mm sieve and was used for further studies. Soil characteristics were studied prior to the experimental work[17]. 5 kg of soil was taken in a set of 12 polythene bags containing four concentrations (250, 500, 750 and 1000 mg/kg) of chelants- EDTA, Citric acid and Mixed

(EDTA + Citric acid). The experiment was carried out in triplicates with each set containing 12 bags as mentioned above. All the bags were mixed with 25 mg/kg potassium dichromate as a source of Cr (VI) and allowed to equilibrate for three days prior to addition of chelants. Soil alone served as control.

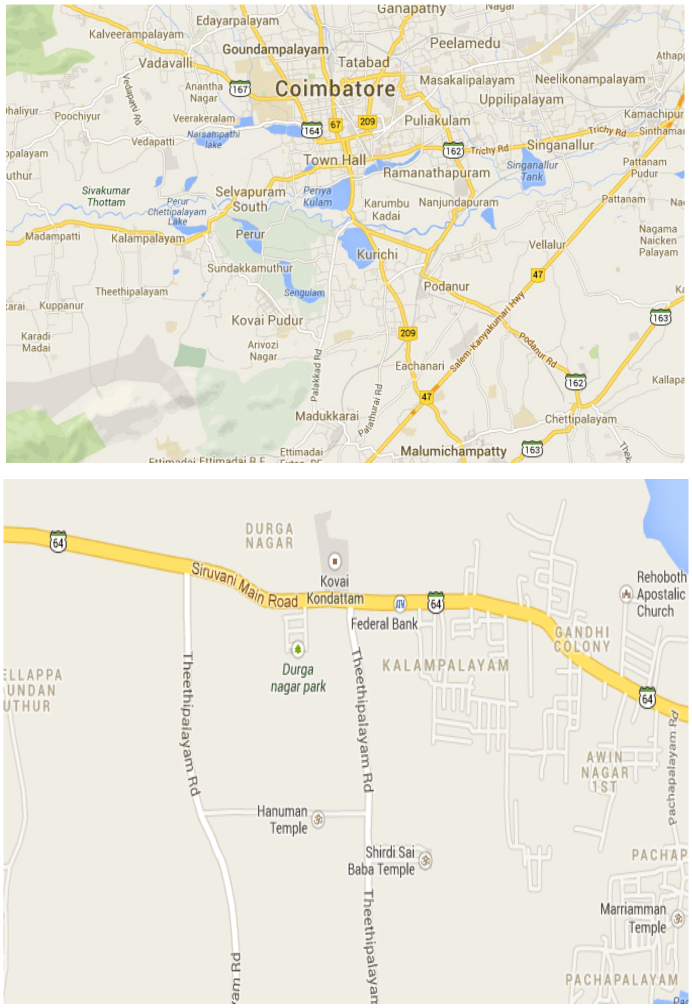

Figure-1
Location of the soil sampling area (area marked with arrow shows the sampling spot)

Seeds were sown in a tray containing soil mixed with sand in the ratio of 3:1 and adequate water was supplied. After 2 weeks, the plantlets were carefully transferred to field and allowed to grow for 50 days. Then even sized cuttings were procured from these plants and planted in appropriately labelled bags. The plants were grown in a shade house under 50% sunlight for 50 days after treatment. They were irrigated on alternate days.

Growth parameters: Growth parameters of the plant such as leaf count, shoot length, girth and appearance were observed at an interval of 10 days until the end of treatment period (50 days). Root length, fresh and dry weight were measured after harvest[14].

Photosynthetic pigments: Fresh leaves were thoroughly washed with distilled water and used for analysis. Photosynthetic pigments such as chlorophyll a, b and carotenoids were analyzed according to the method of Radic *et al.,* 2010[18].

Results and Discussion

Treatment of the plants grown in chromium polluted soil altered their growth pattern with visible differences in their pigmentation too. Previous studies have also reported that chromium causes reduction in root length, shoot length and decreased the number of branches and induced biochemical changes in crops[14]. Orhue and Ekhomun have also shown that chromium treatment altered growth parameters of the plant[15].

Previous studies suggest that chromium toxicity affects plant growth and metabolism to a considerable extent, which includes stunted growth, chlorosis, reduced crop yield, delayed germination, senescence, premature leaf fall, biochemical lesions, enzymatic changes and reduced biosynthesis. It is also demonstrated that application of chelating agents minimized the toxicological effects of chromium and induced better growth when compared to control[10,19]. Similar results have been noticed in the present study. Compared to control plants, flowering was earlier in citric acid treated plants irrespective of the concentration. Initial girth of the cuttings was 4 cm. At the end of the treatment period, girth of the plants increased to a certain extent when compared to untreated control plants (5.9 cm). Girth was larger (6.9 cm) in EDTA treated plants at a concentration of 250 mg/kg. This was followed by 500 mg/kg EDTA (6.7 cm) and then both citric acid and mixed treatment of 500 mg/kg (6.1 cm). In all other cases increase in girth corresponded to the height of plants irrespective of the treatment. It has been stated that *in-situ* successful application of hyper- accumulator has been limited because of their low biomass and specific growth needs[4]. In case of *T.triangulare* it was seen that the plant was capable of tolerating hexavalent chromium pollution and produced high biomass under chelant treatment. Increase in biomass will obviously lead to increase in plant area for accumulation of pollutants and therefore this becomes an important criterion for selection of a suitable accumulator plant. In this manner *T.triangulare* becomes a plant much suited for accumulation studies. Growth parameters of the plant such as leaf count, shoot length, root length and biomass yield are discussed below:

Growth parameters: Soil characteristics: The physicochemical properties of the soil used in this study are presented in table 1. The soil used for this study was of sandy loam type with moderate quantity of calcium carbonate (lime) and phosphorus, and was slightly basic in nature. Nitrogen and zinc content was less than normal but potassium and other micronutrients such as Fe, Mn and Cu were in higher levels.

Table-1
Physicochemical properties of the test soil used in the study

S. No.	Parameter	Results
1	Soil texture	Sandy loam
2	Electrical conductivity (dSm^{-1})	0.26
3	pH	8.3
4	Macronutrients (Kg/acre)	
(i)	Nitrogen	42
(ii)	Phosphorus	7.5
(iii)	Potassium	330
5	Micronutrients (ppm)	
(i)	Ferrous	5.8
(ii)	Manganese	2.4
(iii)	Zinc	1.06
(iv)	Copper	1.8

Leaf count: Plants subjected to EDTA and mixed treatment showed maximum leaf count at 250 mg/kg concentration (figures 2-4). Leaf count followed the order M250> E250> Control> CA250, with increase in chelant concentration posing a negative impact on the number of leaves. Though citric acid treatment did not give rise to higher number of leaves compared to other treatments, the toxicity symptoms were less. Mixed treatment did not show much toxicity but EDTA treatment led to severe reduction in leaf count on increasing concentrations.

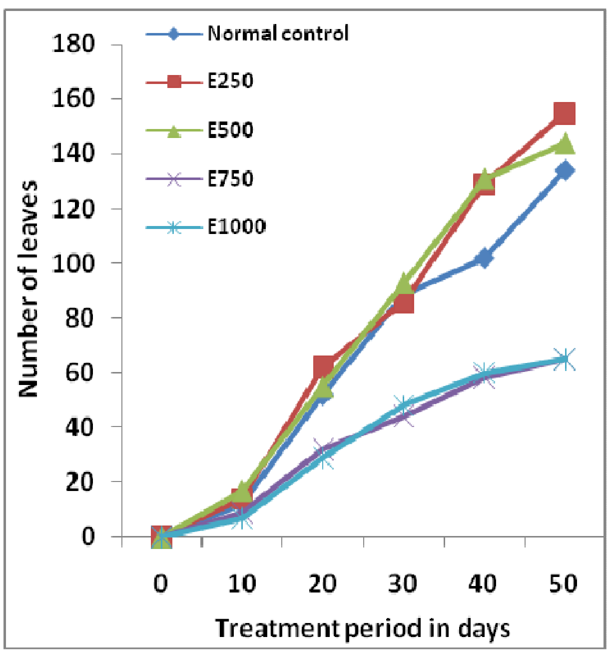

Figure-2
Leaf count of EDTA treated plants

Shoot length: Maximum growth (48.5 cm) of plants was observed at 750 mg/ kg concentration of citric acid following the order CA750> M500> E500>Control (figures 5-7). Citric acid treatment of all four concentrations showed better growth than EDTA treatment. Mixed treatment resulted in maximum growth (47.2 cm) at 500 mg/kg concentration. Growth was more or less similar to that of control at 250 and 750 mg/kg of mixed chelants with 1000 mg/kg showing stunted growth. EDTA treated plants also showed stunted growth at 1000 mg/kg concentration. Stunted growth, chlorosis and necrosis have been shown to be some of the visible symptoms indicating severe metal phytotoxicity[14]. Therefore it can be said that high concentrations of chelants also induce phytotoxicity in a similar way as that of metals.

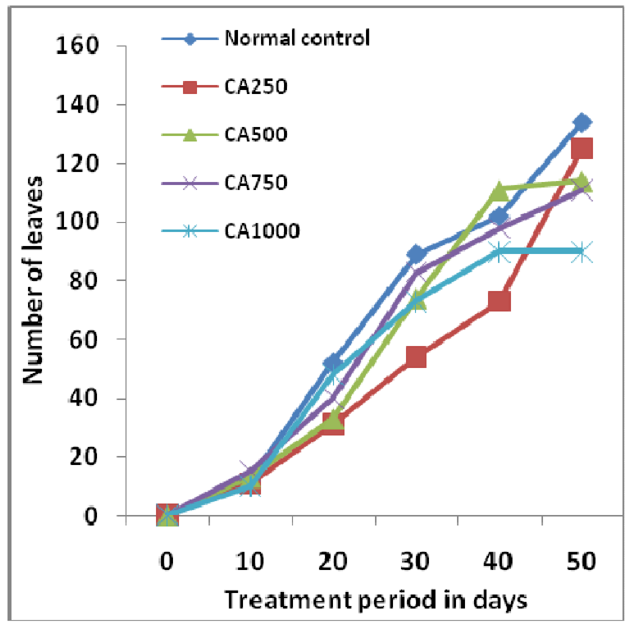

Figure-3
Leaf count of Citric acid treated plants

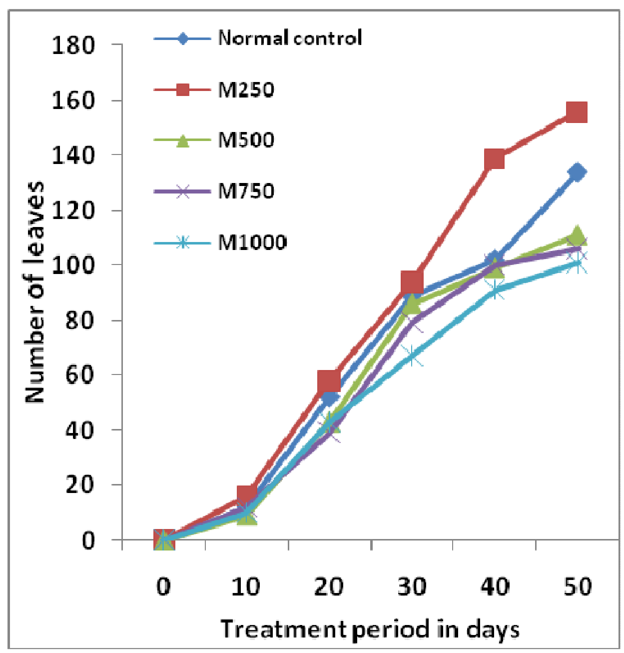

Figure-4
Leaf count of mixed treatment plants

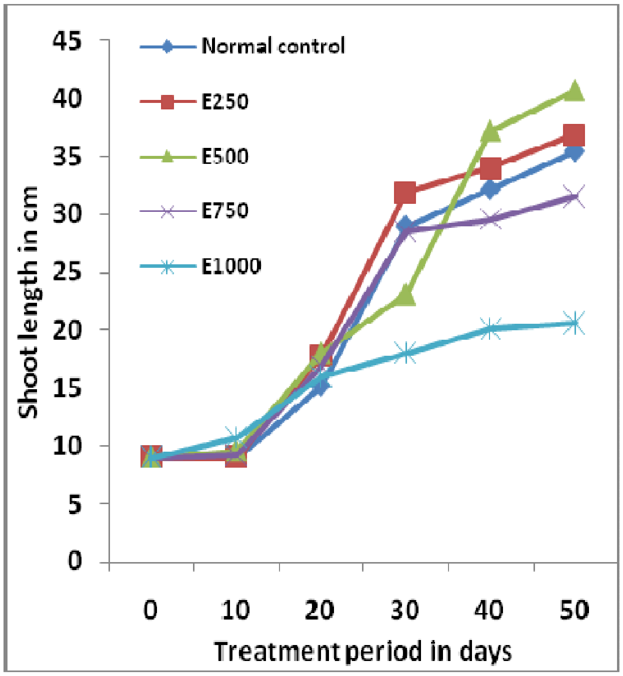

Figure-5
Shoot length of EDTA treated plants

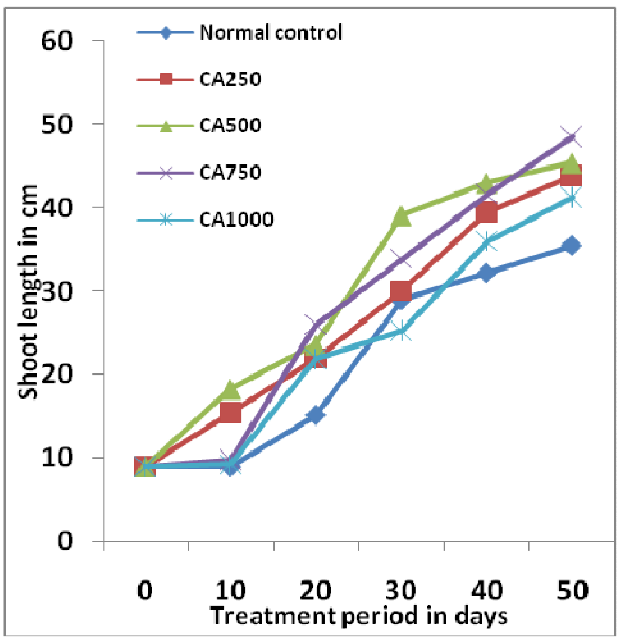

Figure-6
Shoot length of Citric acid treated plants

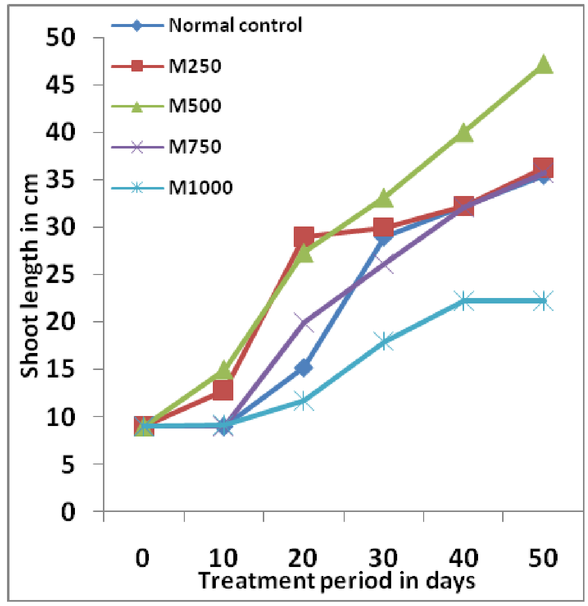

Figure-7
Shoot length of mixed treatment plants

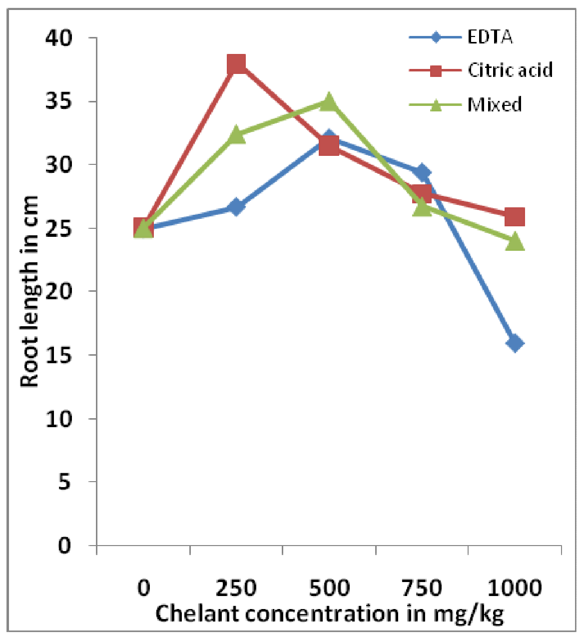

Figure-8
Root length of treated plants

Root length: Root length was better than control in all treatments which indicated a stimulatory effect of chelants on root elongation. Root length was in the order CA250> M500> E750> Control, with all concentrations of the three treatments showing better root length than control (figure 8). 1000 mg/kg concentration of EDTA alone showed much reduced root length (16 cm) compared to control (48.5 cm) indicating toxicity at this level.

Biomass yield: Biomass yield was high (194.9 g fresh wt. and 25.8 g dry wt.) at a concentration of 250 mg/kg of EDTA and this reduced below control values (164.3 g fresh wt. and 20.3 g dry wt.) with increase in concentration. Sun *et al* (2009) has observed similar effects in an experiment employing *Solanum nigrum* for Cd accumulation. It was found that higher rates of EDTA (0.5 g/kg) could reduce the plant biomass and the total amount of Cd removed[20]. All other treatments led to reduction in biomass yield compared to control plants (figure 9).

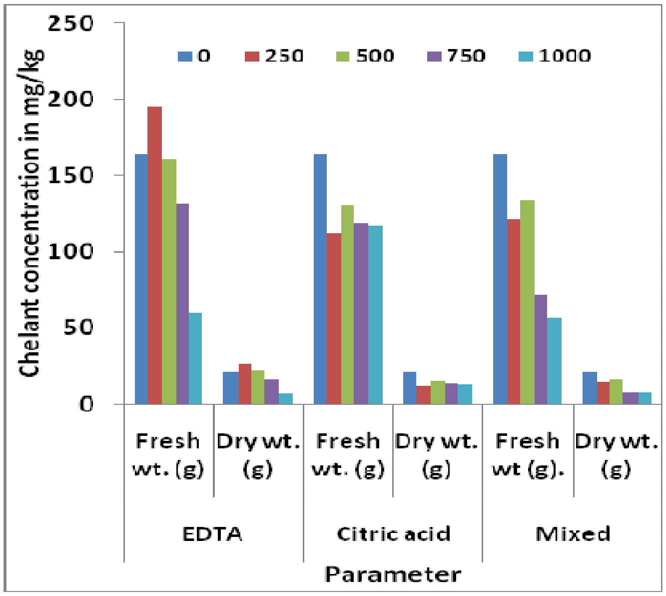

Figure-9
Fresh and dry weight of treated plants

Photosynthetic pigments: Chlorophyll content*:* On mixed application of chelants, chlorophyll concentration increased to 0.174 mg/kg at 1000 mg/kg concentration, while control plants had 0.057 mg/kg (figure 12). An increase in concentration of EDTA also resulted in an increase in the chlorophyll content of plants from 0.057 mg/kg (control) to 0.141 mg/kg at 1000 mg/kg concentration (figure 10). In citric acid treatment, both 500 and 750 mg/kg concentration showed high chlorophyll concentration (0.072 mg/kg) whereas at 1000 mg/kg the levels decreased (0.048 mg/kg) suddenly (figure 11). Mixed treatment showed highest concentration of total chlorophyll followed by EDTA and then citric acid.

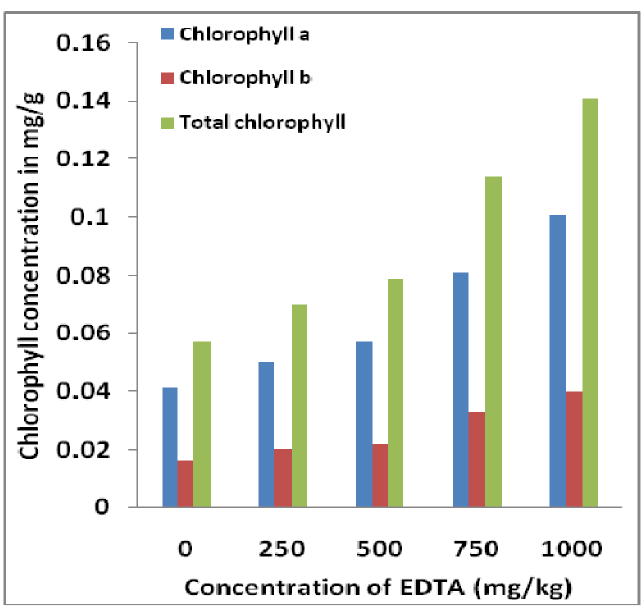

Figure-10
Chlorophyll content in EDTA treatment

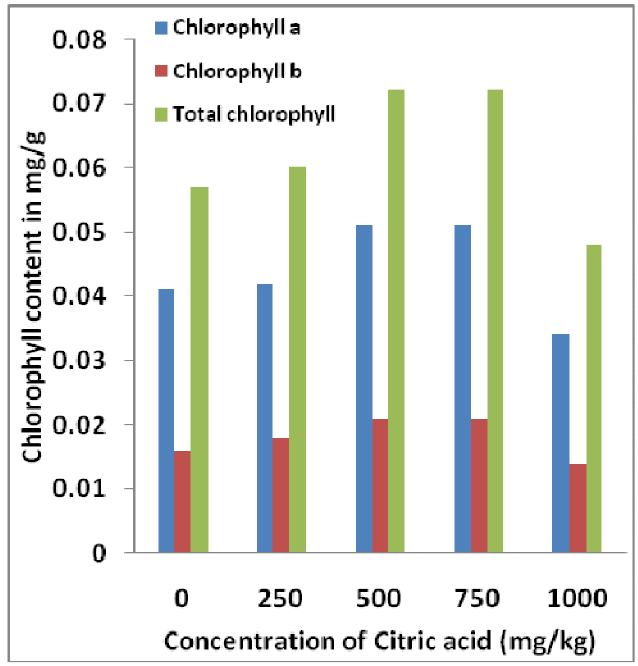

Figure-11
Chlorophyll content in citric acid treatment

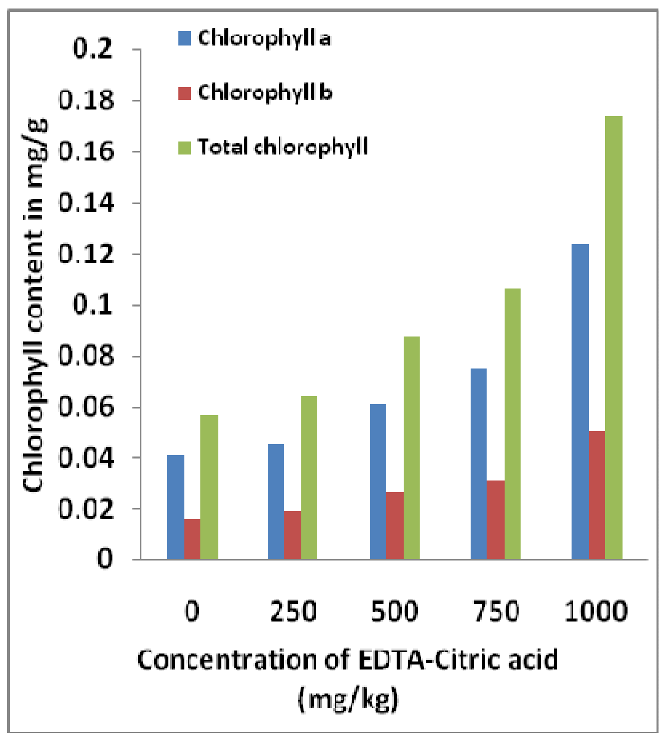

Figure-12
Chlorophyll content in mixed treatment

Carotenoid content: The carotenoid content in mixed treatment was much higher (307.07 mg/kg) than that of control (107.97 mg/kg). Next to mixed treatment, EDTA and then citric acid showed elevated levels of carotenoids (figure 13).

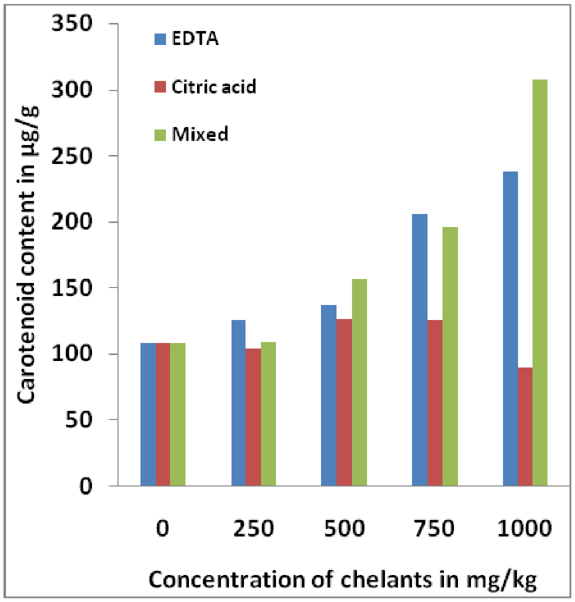

Figure-13
Carotenoid content in chelant treated plants

Conclusion

This study demonstrated that citric acid can be considered as a good candidate chelant for environmentally safe phytoextraction of heavy metals in contaminated soil. Citric acid alone or in combination with EDTA (mixed chelant) led to increase in all growth parameters except biomass yield which was seen to be high in case of EDTA application. Photosynthetic pigments were more in mixed treatment and EDTA treatment. Though EDTA and mixed application gave good results, they hindered growth at higher concentrations. Citric acid showed much lesser toxicity symptoms compared to EDTA. Usage of either citric acid alone or other chelants (EDTA or EDTA- citric acid) in a moderate level can help in achieving better growth in plants which would obviously result in effective removal of pollutants. Therefore it can be concluded that citric acid can be used to minimize pollution in a safer way. For effective results a combination of citric acid with EDTA in a specified range can be applied.

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References

1. Ayeni, O. O., Ndakidemi, P. A., Snyman, R. G. and Odendaal, J. P., Metal contamination of soils collected from four different sites along the lower Diep River, Cape Town, South Africa, *Int. J. Phys. Sci.*, **5(13)**, 2045-2051 **(2010)**

2. Venkateswarlu, P., Venkata Ratnam, M., Subba Rao, D. and Venkateswara Rao, M., Removal of chromium from an aqueous solution using Azadirachta indica (neem) leaf powder as an adsorbent, *Int. J. Phys. Sci.,* **2 (8)**, 188-195 **(2007)**

3. Abdullahi, M. A., Mohammed, S. S. and Mshelia, M. S., Distributions of Cr, Zn and Pb in soil along the Bank of River Kaduna, Nigeria, *Int. Res. J. Environment Sci.,***2(7)**, 64-68 **(2013)**

4. Grace Esohe Uwumarongie-Ilori and Felix Ebhodaghe Okieimen, Phytoremediation of chromated copper arsenate contaminated soil by maize (Zea mays L.), *J. Biodiversity and Environ. Sci.,* **1(2)**, 1-6 **(2011)**

5. John Jason Mellem, Phytoremediation of Heavy Metals using Amaranthus dubius-Thesis submitted in partial fulfilment of the Degree of Master of Technology (Biotechnology) in the Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa, **(2008)**

6. Vemula Madhavi, Ambavaram Vijay Bhaskar Reddy, Kalluru Gangadhara Reddy, Gajulapalle Madhavi and Tollamadugu Naga Krishna Vara Prasad, An overview on research trends in remediation of chromium, *Res. J. Recent Sci.,***2(1)**, 71-83 **(2013)**

7. Panichev, N., Mandiwana, K., Kataeva, M. and Siebert, S., Determination of Cr (VI) in plants by electrothermal atomic absorption spectrometry after leaching with sodium carbonate, *Spec. Acta B,* **60**, 699-703 **(2005)**

8. Rakesh Sharma, M. S. and Raju, N. S., Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by cluster analysis, *Int. Res. J. Environmen. Sci.,***2(10)**, 22-27 **(2013)**

9. Chiroma, T. M., Ebewele, R. O. and Hymore, F. K., Levels of heavy metals (Cu, Zn, Pb, Fe and Cr) in Bushgreen and Roselle irrigated with treated and untreated urban sewage water, *Int. Res. J. Environment Sci.,***1(4)**, 50-55 **(2012)**

10. Mohanty, M. and Patra, H. K., Attenuation of Chromium toxicity in mine waste water using water hyacinth, *J. Stress Phy. & Biochem*., **7(4)**, 335-346 **(2011)**

11. Lakatos, G. Y., Csatári, I., Igaz, T., Gyulai, I. and Mészáros, I., Studies on phytoremediation in a chromium contaminated area in Hungary, *Int. Rev. Appl. Sci. Eng.,* **2(2)**, 111–116 **(2011)**

12. Virendra Kumar Mishra and Tripathi, B.D., Accumulation of chromium and zinc from aqueous solutions using water hyacinth (Eichhornia crassipes), *J. Haz. Mat.,* **164**, 1059-1063 **(2009)**

13. Rajalakshmi, K., Kumar, P., Saravanakumar, A., Aslam, A., Shahjahan, A. and Ravikumar, R., Arachis bioassay for soil contaminated with hexavalent chromium, *Rec. Res. Sci. Tech.,* **2(6)**, 110-115 **(2010)**

14. Perumal Sundaramoorthy, Alagappan Chidambaram, Kaliyaperumal Sankar Ganesh, Pachikkaran Unnikannan and Logalakshmanan Baskaran, Chromium stress in paddy: (i) Nutrient status of paddy under chromium stress; (ii) Phytoremediation of chromium by aquatic and terrestrial weeds, *C R Biol.,* **333(8)**, 597-607 **(2010)**

15. Orhue, E.R. and Ekhomun, A.M., Chromium effects on early growth of water leaf (Talinum triangulare) in an Ultisol, *American- Eurasian J. Agric. and Environ. Sci.,* **7 (5)**, 586-590 **(2010)**

16. Abhay Kumar, Prasad, M.N.V. and Oksana Sytar, Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically, *Chemosphere*, **89**, 1056- 1065 **(2012)**

17. Quan- Ying Wang, Dong- Mei Zhou, Long Cang and Tian-Ran Sun, Application of bioassays to evaluate a copper contaminated soil before and after a pilot-scale electrokinetic remediation, *Environmental pollution*, **157**, 410-416 **(2009)**

18. Sandra Radic, Marija Babic, Dragan Skobic, Vibor Roje and Branka Pevalek- Kozlina. Ecotoxicological effects of aluminium and zinc on growth and antioxidants in Lemna minor L., *Ecotox. Environ. Safety,* **73**, 336-342 **(2010)**

19. Monalisa Mohanty and Hemanta Kumar Patra. Effect of chelate- assisted hexavalent chromium on physiological changes, biochemical alterations, and chromium bioavailability in crop plants- an *in vitro* phytoremediation approach, *Bioremediation journal*, **16(3)**, 147-155 **(2012)**

20. Sun, Y., Zhou, Q. and Wang, L., Liu, W. The influence of different growth stages and dosage of EDTA on Cd uptake and accumulation in Cd- hyperaccumulator (*Solanum nigrum L.*), *Bull. Environ. Contam. Toxicol.,* **83**, 348-353 **(2009)**