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EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*)

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Abstract: *Phytoextraction is an emerging cost-effective solution for remediation of contaminated soils which involves the removal of toxins, especially heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs. The aim of the present investigation is to study the effects of EDTA and citric acid on accumulation potential of marigold (Tagetes erecta) to Zn, Cu, Pb, and Cd and also to evaluate the impacts of these chelators (EDTA and citric acid) in combination with all the four heavy metals on the growth of marigold. The plants were grown in pots and treated with Zn (7.3 mg l^{-1}), Cu (7.5 mg l^{-1}), Pb (3.7 mg l^{-1}) and Cd (0.2 mg l^{-1}) alone and in combination with different doses of EDTA i.e., 10, 20 and 30 mg l^{-1}. All the three doses of EDTA i.e., 10, 20 and 30 mg l^{-1} significantly increased the accumulation of Zn, Cu, Pb and Cd by roots, stems and leaves as compared to control treatments. The 30 mg l^{-1} concentration of citric acid showed reduced accumulation of these metals by root, stem and leaves as compared to lower doses i.e., 10 and 20 mg l^{-1}. Among the four heavy metals, Zn accumulated in the great amount (526.34 mg kg^{-1} DW) followed by Cu (443.14 mg kg^{-1} DW), Pb (393.16 mg kg^{-1} DW) and Cd (333.62 mg kg^{-1} DW) in leaves with 30 mg l^{-1} EDTA treatment. The highest concentration of EDTA and citric acid (30 mg l^{-1}) caused significant reduction in growth of marigold in terms of plant height, fresh weight of plant, total chlorophyll, carbohydrate content and protein content. Thus EDTA and citric acid efficiently increased the phytoextractability of marigold which can be used to remediate the soil contaminated with these metals.*

Key words: *Accumulation, Citric acid, EDTA, Heavy metals, Marigold, Phytoextraction*
PDF of full length paper is available online

Introduction

The increasing use of wide variety of heavy metals in industries and agriculture has caused a serious concern of environmental pollution. In higher concentration, these heavy metals cause severe damage to plants (Mohan and Hosetti, 2006; Sinhal, 2007; Sinhal *et al.,* 2007; Gupta *et al*., 2008a,b; Pandey *et al.,* 2007; Saxena *et al.,* 2008; Perez and Sharma, 2008; Agoramoorthy *et al.*, 2009; Handique and Handique, 2009). Phytoextraction is an environmentally friendly in situ technique for cleaning up metal contaminated land. Unfortunately, efficient metal uptake by remediation plants is often limited by low phyto availability of the targeted metals. Chelant assisted phytoextraction has been proposed to improve the efficiency of phytoextraction (Susan *et al.,* 2006). Phytoextraction accumulates toxic metals from contaminated soil into the above ground tissue of higher plants, which are then harvested and incinerated and/or buried (Garbisu and Alkorta, 2001; Thangavel and Subbhuraam, 2004). Ensley *et al.* (1999) described chemically induced phytoextraction as a two-step process in which plants first accumulate metals in their roots and then by application of an inducing agent, enhanced transfer of the metals to the shoots occurs. This transfer is due to disrupting the plant metabolism that regulates the transport of metal to the shoots. Wenzel *et al.* (2003) hypothesize that free protonated EDTA enters the roots, subsequently forming metal complexes that enhance metal transport to shoots. Turgut *et al.* (2004) found that lower concentration of citric acid significantly increased the metal uptake (Cd, Cr and Ni) while increasing concentration of citric acid posed a severe phytotoxic effect in selected plant species.

The objective of this study are (i) to assess the effects of adding EDTA and citric acid on the accumulation of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*) (ii) to evaluate the accumulation potential of marigold to different heavy metals (Zn, Cu, Pb, and Cd), (iii) to investigate the effects of heavy metals (Zn, Cu, Pb, and Cd) and chelating agents (EDTA and citric acid) on the growth of marigold and (iv) also to determine the hyper accumulating nature of marigold.

Materials and Methods

The seeds of marigold (*Tagetes erecta*, cultivar, Pusa basanti) were grown in 80´40´20 cm sized pots. In one set of experiment, some pots were supplied with different concentrations of Zn ($ZnSO_4$), Cu ($CuSO_4$), Pb [$Pb(NO_3)_2$] and Cd [$Cd(NO_3)_2$] *i.e.*, 7.3, 7.5, 3.7 and 0.2 mg l^{-1} respectively as reported in the city waste water of Bareilly city (Singh *et al.*, 2000), while in another set of experiment pots were supplied with EDTA and citric acid, each with concentration of 10, 20 and 30 mg l^{-1} in combination with each heavy metal *i.e.*, Zn, Cu, Pb and Cd. In each experiment, 7 litre of solution was used at the interval of 5 days for irrigation of pots. The pH value of soil (6.8) was determined using glass electrode in a soil to water ratio 1:1 (Mc Lean, 1982). The soil particle size distribution (sandy loam) was analysed using the pipette method (Gee and Bauder, 1986).Organic matter (21.14 g kg^{-1}), organic carbon (12.13 g kg^{-1}) and organic nitrogen (12 g kg^{-1}) were determined by the Walkley-Black wet combustion method (Nelson and Sommers, 1982).

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To determine the effects of different concentrations of EDTA and citric acid (10, 20 and 30 mg l^{-1}) in combination with each heavy metal concentration on plant height, five plants were randomly selected from each treatment. The plant height was measured in centimeters and fresh weight in grams. Chlorophyll content was measured by the method of Arnon (1949), carbohydrate by Morris (1948) and protein content by the method of Lowry *et al.* (1951). The amount of Zn, Cu, Pb, and Cd accumulated by marigold was determined by using atomic absorption spectrophotometer (GBC Avanta S AAS, Australia) after the samples were digested with concentrated HNO_3 and $HClO_4$. One way ANOVA was conducted to compare the means of different treatments at $p<0.05$ level of significance.

Results and Discussion

The results for the evaluation of the effect of EDTA and citric acid treatments on accumulation of Zn, Cu, Pb and Cd by marigold are presented in Fig. 1, which shows that all the concentrations of EDTA (10, 20 and 30 mg l^{-1}) significantly increased the accumulation of Zn, Cu, Pb and Cd by marigold than control treatment but maximum increase in accumulation of these metals was noticed with 30 mg l^{-1} concentration of EDTA. In case of citric acid, although all the concentrations (10, 20 and 30 mg l^{-1}) were found to increase the accumulation of Zn, Cu, Pb and Cd than control but maximum increase in accumulation was noticed with 20 mg l^{-1} concentration of citric acid. Among root, stem and leaves the maximum concentration of Zn, Cu, Pb and Cd *i.e.*, 526.34, 443.34, 393.16 and 332.62 mg kg^{-1} DW, respectively, was noticed in leaves with 30 mg l^{-1} concentration of EDTA while in case of citric acid, the maximum concentration of Zn, Cu, Pb and Cu *i.e.*, 230.66, 203.16, 181.54 and 143.38 mg kg^{-1} DW in leaves was noticed with 20 mg l^{-1} concentration. Thus, the accumulation of these heavy metals by marigold was in order of Zn>Cu>Pb>Cd under the influence of different concentrations of EDTA and citric acid as well as in control treatments in root, stem and leaves.

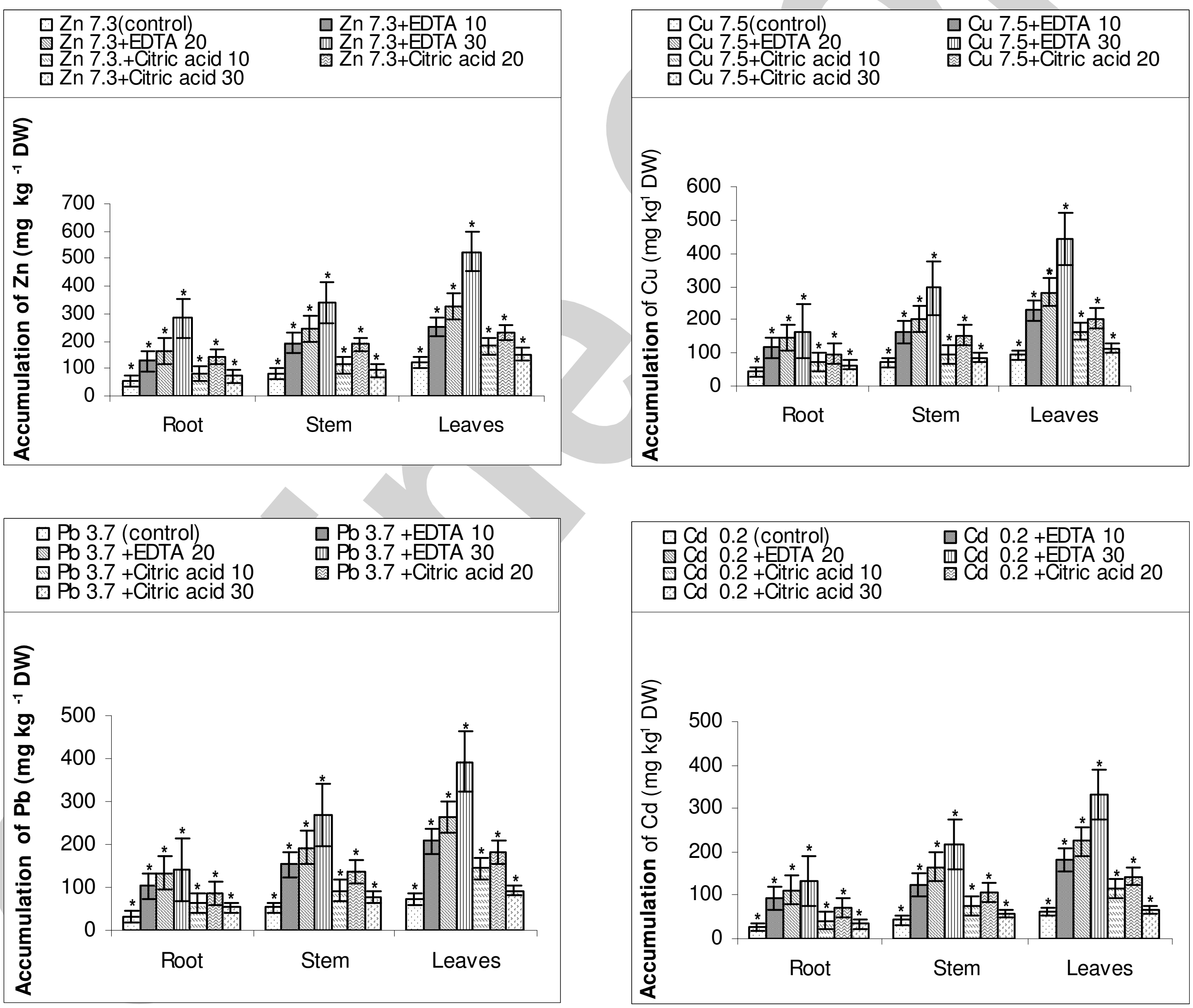

Fig 1: Accumulation of Zn, Cu, Pb, and Cd in root, stem and leaves of marigold (*Tagetes erecta*) with EDTA and Citric acid. Error bars indicate ± SE and * indicate probability level of significant difference at $p<0.05$. Replicate (n) = 3

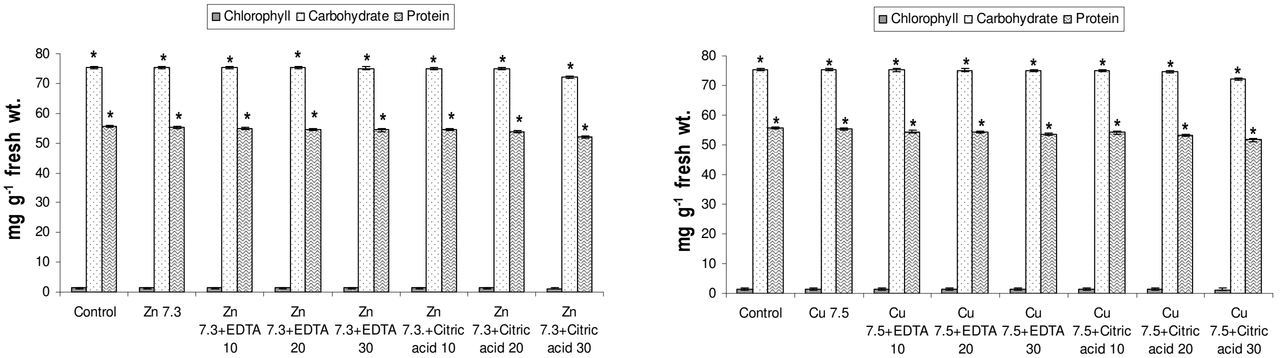

Fig. 2: Effect of Zn, and Cu alone in combination with EDTA and Citric acid on Chlorophyll, Carbohydrate and Protein content of marigold (*Tagetes erecta*). Error bars indicate ± SE and *indicate probability level of significant difference at $p<0.05$. Replicate (n) = 3

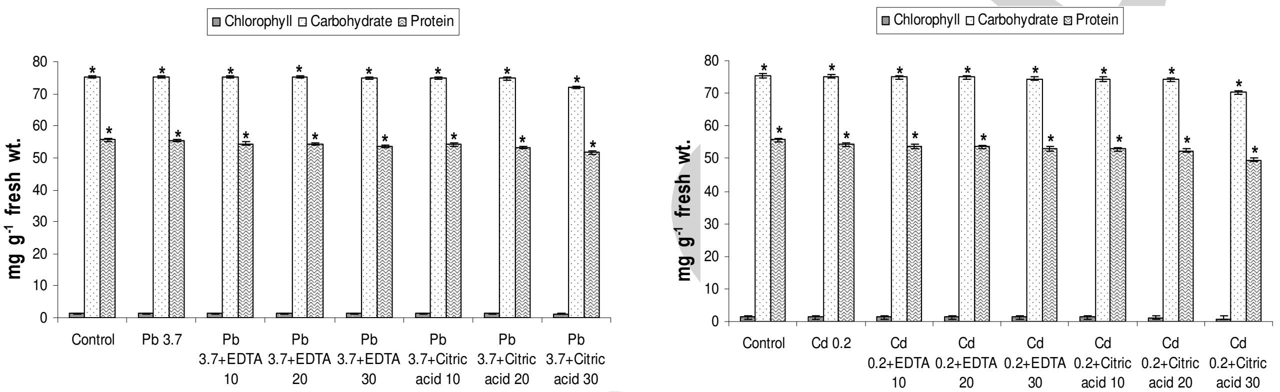

Fig 3: Effect of Pb and Cd alone in combination with EDTA and Citric acid on Chlorophyll, Carbohydrate and Protein content of marigold (*Tagetes erecta*). Error bars indicate ± SE and *indicate probability level of significant difference at $p<0.05$. Replicate (n) = 3

Phytoextraction is a potential, innovative and cost-effective technology that involves the removal of toxins, especially heavy metals and metalloids, by the roots of plants with subsequent transport to aerial plant organs (Salt *et al.,* 1998; Lombi *et al.,* 2001; Zhuang *et al.,* 2005). The results of the present study clearly indicate that marigold efficiently accumulated the Zn, Cu, Pb and Cd from treated soil and addition of EDTA and citric acid had many fold increased the accumulation of these metals than control treatments. EDTA is a well known chelating agent and has been tested to enhance the metal bio-availability and subsequent uptake and translocation in aerial organs of plant by various workers in different plant species (Turgut *et al.,* 2004; Turgut *et al.,* 2005; Zhuang *et al.,* 2005; Gupta *et al*., 2008a,b). Wenzel *et al.* (2003) hypothesized that free protonated EDTA enters the roots, subsequently forming metal complexes that enhance metal transport to shoots. Lai *et al.* (2004) also suggested that EDTA forms metal-EDTA complex in soil which becomes readily available for uptake and translocation to aerial parts of the plants. Turgut *et al.* (2005) investigated the effect of citric acid on heavy metals (Cd, Cr and Ni) uptake and translocation in *Helianthus annuus* and found that citric acid significantly increased the metal availability and enhanced the metal accumulation many folds in the shoots of plant. The results of the present study indicate that EDTA had greater efficiency to enhance the metal accumulation in marigold than citric acid. The results are also in conformity with that of Lesage *et al.* (2005), where they evaluated the effect of EDTA and citric acid on heavy metals (Cu, Pb, Zn and Cd) uptake by *Helianthus annuus* and found that EDTA and citric acid, both have the capacity to increase the metal accumulation, but more increase in metal accumulation was noticed with EDTA treatments, than citric acid treatments. The findings of the present study showed that the concentration of metals was more in leaves and stems than roots. This may be due to hyper accumulating nature of marigold because in hyper accumulator plants phytoextraction is completed in two steps, firstly, plants accumulate metals in their roots and secondly they transport these metals to aerial organs by protein transporters (Thangavel and Subburaam, 2004) while in non-accumulator plants, after the uptake of metal ions, they are sequestered in vacuoles by binding with appropriate ligands like organic acids, proteins, and peptides and presence of enzymes that can function at high level of metallic ions (Robinson *et al.,* 1994). Hyper accumulator plants usually have a shoot/root metal concentration ratio of >1, where as non-hyper accumulator plants have the ratio of >1, *i.e.*, non-hyper accumulator plants have higher metal concentrations in roots than in shoots (Shen *et al.,*

Table - 1: Effects of Zn, Cu, Pb, and Cd alone and in combination with EDTA and Citric acid on the growth of marigold (*Tagetes erecta*)

Treatment (mg l^{-1})	Plant height (in cm)	Fresh weight (in gm)
Control	**20.62 ± 0.031**	**19.81 ± 0.035**
Zn 7.3	20.59 ± 0.030	19.69* ± 0.037
Zn 7.3 + EDTA 10	20.50* ± 0.020	19.66* ± 0.030
Zn 7.3 + EDTA 20	20.13* ± 0.076	19.60* ± 0.028
Zn 7.3 + EDTA 30	19.97* ± 0.060	19.53* ± 0.014
Zn 7.3 + Citric acid 10	20.34* ± 0.101	19.52* ± 0.044
Zn 7.3 + Citric acid 20	19.75* ± 0.121	19.49* ± 0.042
Zn 7.3 + Citric acid 30	18.40* ± 0.132	18.40* ± 0.118
Cu 7.5	20.46* ± 0.039	19.71* ± 0.046
Cu 7.5 + EDTA 10	20.43* ± 0.063	19.67* ± 0.066
Cu 7.5 + EDTA 20	20.05* ± 0.026	19.66* ± 0.020
Cu 7.5 + EDTA 20	19.97* ± 0.085	19.45* ± 0.029
Cu 7.5 + Citric acid 10	20.34* ± 0.113	19.46* ± 0.024
Cu 7.5 + Citric acid 20	19.75* ± 0.104	19.43* ± 0.037
Cu 7.5 + Citric acid 30	18.32* ± 0.101	18.32* ± 0.078
Pb 3.7	20.45* ± 0.053	19.63* ± 0.088
Pb 3.7 + EDTA 10	20.19* ± 0.052	19.53* ± 0.145
Pb 3.7 + EDTA 20	19.95* ± 0.028	19.48* ± 0.072
Pb 3.7 + EDTA 20	19.81* ± 0.072	19.40* ± 0.057
Pb 3.7 + Citric acid 10	19.71* ± 0.171	19.38* ± 0.016
Pb 3.7 + Citric acid 20	19.48* ± 0.072	19.35* ± 0.048
Pb 3.7 + Citric acid 30	18.32* ± 0.094	18.21* ± 0.101
Cd 0.2	20.35* ± 0.031	19.50* ± 0.061
Cd 0.2 + EDTA 10	20.09* ± 0.023	19.48* ± 0.060
Cd 0.2 + EDTA 20	19.74* ± 0.097	19.43* ± 0.088
Cd 0.2 + EDTA 30	19.23* ± 0.035	19.36* ± 0.068
Cd 0.2 + Citric acid 10	19.33* ± 0.120	19.31* ± 0.044
Cd 0.2 + Citric acid 20	19.05* ± 0.074	19.25* ± 0.076
Cd 0.2 + Citric acid 30	17.93* ± 0.088	17.90* ± 0.100

Note: ± Value indicate standard error and *indicate probability level of significant difference at $p<0.05$. Replicate (n) = 3

1997). Thus present study suggests that marigold (*Tagetes erecta*) is a hyper accumulator plant. The results of present study also indicate that Zn accumulated in higher amount then Cu, Pb, and Cd in root stem and leaves. These results are also in accordance of the observations of Singh and Agrawal (2006) and Pandey (2006), where they found that Zn was accumulated in higher amount than Cu, Ni, Pb, Cr and Cd.

The data for the analysis of the effect of Zn, Cu, Pb and Cd, alone and in combination EDTA and citric acid on the growth of marigold have been presented in Fig. 2 and Fig. 3, which shows that different concentrations of these metals and chelating agents did not adversely affect the growth of marigold in terms of plant height, fresh weight, total chlorophyll, carbohydrate and protein contents but some minute non significant differences between treatment plants and control plants have been noticed. Although Zn, Cu, Pb and Cd in combination with 30 mg l^{-1} concentration of citric acid was found to have reduced level on plant height, fresh weight of plant, total chlorophyll, carbohydrate, and protein content. Phytotoxic effects shown by citric acid with 30 mg l^{-1} concentration, resulted into reduced growth and heavy metal accumulation, may be due to dissolution of the carbonates and compaction of the soil (Lesage *et al.,* 2005). The similar findings were also noticed by Turgut *et al.,* (2004) where they found that increasing concentration posed a severe phytotoxicity as evidenced by stunted growth and diminished uptake rates in *Helianthus annuus*. Thus, the overall view of the present investigation is that EDTA and citric acid are effective chelating agents and have ability to enhance the metal accumulation but at elevated level citric acid may adversely affect the plant growth and result into reduced accumulation. Further, marigold has greater accumulation potential to Zn rather than Cu, Pb and Cd and can be used as hyper accumulator plant to remediate the soil contaminated with Zn.

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