Environ. Process. (2016) 3:735–746
DOI 10.1007/s40710-016-0180-0

ORIGINAL ARTICLE

EDTA-Enhanced Phytoextraction by *Tagetes sp*. and Effect on Bioconcentration and Translocation of Heavy Metals

Syed Yakub Ali[1] · **Shibani Chaudhury**[1]

Received: 28 March 2016 / Accepted: 24 July 2016 / Published online: 1 August 2016

Abstract Phytoextraction is one of the processes of phytoremediation, in which heavy metals are extracted by plant roots and shoots. For enhanced phytoextraction efficiency use of some chelating agent such as ethylenediaminetetraacetic acid (EDTA) is an effective approach to remove heavy metals from contaminated soils. In the present study, the effect of EDTA on the uptake of Zn, Cd, Pb, and Cu by *Tagetes sp*. was examined. The application of EDTA had inhibitory effects on the growth of the plants, but effectively increased the mobility of Zn, Cd, Pb, and Cu in soils, and significantly enhanced the accumulation of these heavy metals in aerial parts of the plants. The portions of Zn extraction from soil were increased by 1.71, 1.86 and 1.9 times, Cd extraction from soil was increased by 2.33, 2.44 and 2.46 times, Pb extraction from soil was increased by 1.65, 1.93 and 2.01 times and Cu extraction from soil was increased by 1.93, 1.95 and 2 times, under the treatments of 5 (E5), 10 (E10) and 15 mg EDTA/kg of soil (E15), respectively, compared to the control. The highest bioconcentration factor of shoot (BCF_{shoot}) and the translocation factor (TF) were observed in E15 and highest remediation factor (RF) was observed in E10. But 5 mmol EDTA/kg of soil is the best treatment for selected heavy metals remediation because it has less toxic effects on plant as well as it is economical both cost wise and for the decorative flowers.

Keywords Phytoextraction · Phytoremediation · Heavy metals · Chelating agent · *Tagetes sp.* · Bioconcentration factor of shoot · Translocation factor

1 Introduction

Heavy metals create environmental pollution which affects the ecosystem. Anthropogenic activities are the main sources of heavy metals in the environment. Seepage from rocks into water, volcanic activity, forest fires etc. are the natural sources of heavy metals, whereas the

✉ Shibani Chaudhury
shibani.chaudhury@visva-bharati.ac.in

[1] Department of Environmental Studies, Siksha-Bhavana, Visva-Bharati, Santiniketan, India

anthropogenic sources of the heavy metal pollution are from sewage sludge, mines wastes, fossil fuels, phosphate fertilizers, industrial wastes and urbanization.

According to Suruchi and Khanna (2011), heavy metals in soil are very hazardous pollutants as they are non-biodegradable and extremely toxic at low concentrations, and changes of the mobility of metals occur under changing physical-chemical conditions. The living organisms accumulate heavy metals from the environment, particularly from soils, via ground water or through the food chain (Kamnev and Lelie 2000). According to He et al. (2005), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni) and Zinc (Zn) are some of the essential heavy metals. A few of these metals are used by various enzymes for regulation of osmotic pressure in cells (Bruins et al. 2000). Other heavy metals, such as Cadmium (Cd), Lead (Pb), Mercury (Hg), Aluminum (Al), Gold (Au) and Silver (Ag), have no biological functions (Chang et al. 1996).

Phytoremediation is a cleanup technique to remove or destroy heavy metals or organic pollutants from the environment. It is being investigated and/or used commercially to treat a variety of contaminants (Prasad 2003) as it is cost effective and eco-friendly. Phytoextraction is one of the processes of phytoremediation, in which heavy metals are extracted by plant roots and shoots which are subsequently harvested to remove the contaminants from the environment (Jadia and Fulekar 2009).

According to Turan and Esringü (2007), some metals are immobile in soil and their availability and phytoextraction rate are restricted due to solubility and diffusion at the root surface which might obstruct plant remediation efficiency. Application of chelating agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitriletriacetic acid (NTA), pyridine-2,6-dicarboxylic acid (PDA), trans-l,2-diaminocyclo hexane-N,N,N',N'-tetraacetate (CDTA) or ethylenediamine disuccinate (EDDS), or organic chemicals such as citric acid, malic acid or oxalic acid are used to overcome this problem (Blaylock et al. 1997; Huang et al. 1997; Ebbs and Kochian 1997; Wu et al. 1999; Epstein et al. 1999). EDTA is the most effective and popular reagent, as it is a resistant and biostable chelating agent having the property for enhancement of phytoextraction of heavy metals from soil (Hong et al. 1999). When excessive EDTA is present, the amounts of cations extracted are usually pH-independent, but when EDTA is in short supply, the amounts of cations extracted show a complex behavior in relation to pH (Ghestem and Bermond 1998). EDTA significantly elevates the extractability of Pb, Zn, Cd and Ni in unpolluted and metal-contaminated soils and increases the mobility of Pb, Zn, Cd and Ni (Li and Shuman 1996; Sun et al. 2009).

The phytoextraction of heavy metals by application of EDTA to soil significantly enhances metal accumulation by plants (Sun et al. 2009; Barrutia et al. 2010; Ramamurthy and Memarian 2014; Suthar et al. 2014), and the application of EDTA to soil also increases the translocation of heavy metals from soil to plants parts. Sun et al. (2009) noted that the addition of chelators effectively increased the mobility of target heavy metals (Cd, Cu, Pb and Zn) in soils, and significantly enhanced the accumulation of these heavy metals in aerial parts of the *S. alfredii*. Suthar et al. (2014) reported that the Cd and Pb concentrations in plants increased linearly with increasing rate of EDTA. They observed that by applying EDTA (5 mM/kg) to soil, significantly increased the soluble fraction of Cd and Pb by 3.1 and 13.1 fold respectively, compared to control. They also observed that the application of EDTA (2 mmol/kg) to soils significantly increased the Cd (1.8 times in root and 1.9 times in shoot compared to control) and Pb concentration (2.1 times in root and 1.7 times in shoot compared to control) in Indian mustard, which depends on plant type. However, according to Jean et al. (2008), biomass reduction caused by EDTA decreased the metal accumulation capacity. Some studies also

reported that the EDTA could not significantly enhance or reduce the metals uptake by plants (Walker et al. 2003; Robinson 1997).

Tagetes sp. is one of the well-known ornamental plants grown all over the world. In south Asia, its flowers are used to decorate religious statues and buildings, funerals, wedding and other ceremonies. It is also an effective herb for treatment of skin problems, piles, kidney troubles, muscular pain, ulcers, and wounds (Dixit et al. 2013). *Tagetes sp*. is also used in the phytoextraction of heavy metals because of its high biomass yield and early production rate, as well as because it can be grown in all climatic conditions with minimum care. *Tagetes sp*., a non-edible ornamental plant, may be used as an accumulator plant in heavy metal contaminated areas (Mani et al. 2015) and as such this plant does not affect the food chain.

Earlier studies were conducted to investigate EDTA on phytoextraction of Zn, Cd, Pb and Cu by *Tagetes sp*. (Sinhal et al. 2010; Wei et al. 2012; Ali et al. 2016). In the present study, higher concentration of EDTA (5, 10 and 15 mmol EDTA/kg of soil) were used, and plant growth and dry biomass were determined using *Tagetes sp*. compared to the earlier phytoextraction studies using the same plant. The earlier studies (Sinhal et al. 2010; Wei et al. 2012; Ali et al. 2016) did not report on blooming of the *Tagetes sp*. exposed to EDTA nor was there any mentioning of the accumulation of heavy metals in the flowers.

The objectives of the present study were to: (i) asses the effect of EDTA on the growth and biomass of *Tagetes sp*. exposed to heavy metals; (ii) test the null hypothesis that application of EDTA to the experimental plants did not significantly increase the heavy metal accumulation; (iii) study the distribution of heavy metals in the various part of the *Tagetes sp*.; and (iv) identify the most suitable concentration of EDTA for phytoextraction which would be efficient as well as economic.

2 Materials and Methods

2.1 Pot–Culture Experiment

Soil samples were collected at 20 cm depth from field, dried, sieved and were then thoroughly mixed. Various soil parameters such as soil texture, pH, EC, organic carbon, water soluble sodium, potassium, calcium, exchangeable sodium, potassium, calcium, total nitrogen, available phosphorus and cation exchange capacity were analyzed. Grain size of the soil was analyzed by Pipette method. Soil pH was determined by potentiometric method using pH meter (Systonic 361) in 1:2.5 water suspensions. Electrical conductivity was measured in 1:2 soil:water extract using conductivity meter (Thermo Scientific conductivity cell, Orion 013605MD), organic carbon was determined by Walkley and Black (1934). Total nitrogen was determinate using Kjedahl instrument (Plican Kelplus-Distyl) as followed by Subbiah and Asija (1956), and available phosphorus was measured by Olsen's method (Olsen et al. 1954). Water soluble anion and cation were determined using Ion Chromatography (Metrohm 797 VA Computrace). Exchangeable sodium, potassium, and calcium of soil samples were extracted by ammonium acetate and measured by flame photometry (ELICO CL 361). Cation exchangeable capacity of the soil was determined by following the method of Harada and Inoko (1980).

Pots for the experiments were prepared as follows: in several plastic pots, 1 kg soil/pot were taken and mixed with cow dung and urea. Aqueous solution of Lead nitrate [$Pb(NO_3)_2$], Cadmium nitrate [$Cd(NO_3)_2.4H_2O$], Zinc sulphate [$ZnSO_4.\ 7H_2O$], Copper sulphate [$CuSO_4.\ 5H_2O$] were separately used to artificially contaminate the soil to get a concentration 100 mg of

a single metal/pot. The humidity of the soil was maintained by using water 3–4 times per week.

After soil preparation, one marigold (*Tagetes sp.*) plant was transplanted into each pot and allowed to grow. After 30 days, EDTA was added to the pots as aqueous solution at concentration of 5 mmol/kg of soil (E5), 10 mmol/kg of soil (E10) and 15 mmol/kg of soil (E15). Respective control plants of *Tagetes sp.* (with metals exposure) were grown against each treatment without the addition of EDTA.

2.2 Collection and Sample Preparation

After 3 months, the plants were collected from the pots, thoroughly washed with running tap water and rinsed with deionized water to remove any soil particles attached to the plant surfaces. Then, the plant samples were dried at 70 °C for 48 h (Turan and Esringü 2007). The dried plant samples were weighed and ground for analysis of metals concentration.

2.3 Heavy Metals Analysis of Soil and Plants

Soil and plant samples (1 g) were digested separately with 15 mL of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ at 5:1:1 ratio) at 80 °C until a transparent solution was obtained (Allen et al. 1986). After cooling, the digested samples were filtered using Whatman No. 42 filter paper and the filtrate was finally made up to 50 mL with distilled water. Metals were analyzed by using Anodic Stripping Voltammetry (Metrohm VA 797 Computraces).

2.4 Phytoextraction Efficiency of the Plants

The dry biomass and plant growth are some of the common indicators related to plant tolerance to toxicity imparted to metals and chelates (Ramamurthy and Memarian 2014). Several indicators describe the efficiency of phytoextraction of heavy metals from soil.

The bioconcentration factor of shoot (BCF_{shoot}) is calculated as the ratio of the element concentration in plant shoot to the initial concentration of the element in the soil. The bioconcentration factor of root (BCF_{root}) is calculated as the ratio of the element concentration in plant root to the initial concentration of the element in the soil (Ebrahimi 2013):

$$\text{Bioconcentration factor of shoot } (\mathrm{BCF_{Shoot}}) = \frac{\text{element concentration in plant shoot}}{\text{element concentration in soil}}$$

$$\text{Bioconcentration factor of root } (\mathrm{BCF_{root}}) = \frac{\text{element concentration in plant root}}{\text{element concentration in soil}}$$

The translocation factor (TF) is calculated as the ratio of metal concentrations in aerial part of the plant to those in roots, indicating the ability of the plant to translocate metals from the roots to the shoots (Roongtanakiat 2009):

$$\text{Translocation factor } (\mathrm{TF}) = \frac{\text{element concentration in aerial part of the plant}}{\text{element concentration in root of the plant}}$$

The remediation factor (RF) is defined as the ratio of metal accumulation in shoots to that in soil (Sun et al. 2009), calculated by the following formula:

$$\text{Remediation factor (RF)} = \frac{\text{metal concentration of shoot of the plants} \times \text{dry plant biomass (above ground)}}{\text{metal content of soil} \times \text{soil mass}}$$

3 Statistical Analysis

All treatments were replicated three times in the experiments. Means and standard errors (SE) were calculated by the Microsoft Office Excel 2013. One-way ANOVA and Duncan test were carried out using SPSS20.

4 Result and Discussion

4.1 Basic Characteristics of the Soil

The physico-chemical characteristics and heavy metal concentrations of soil are listed in Table 1. The soil texture was sandy loam with a moderate cation exchange capacity of 7.2 meq/100 g soil. The metal concentrations in the soil were 80.16 mg Zn/kg, 20.55 mg Pb/kg and 54.85 mg Cu/kg. Cd was not found in the tested soil. After artificial addition of metal solution, the final concentrations of Zn, Cd, Pb and Cu were 191.61 ± 8.1 mg/kg, 107.7 ± 2.6 mg/kg, 124.09 ± 4.2 mg/kg and 160.8 ± 5.4 mg/kg, respectively.

4.2 Response of EDTA on Plant Growth and Biomass

The application of different concentrations of EDTA had inhibitory effects on plant growth and reduced the dry biomass, indicating phytotoxicity of EDTA on the *Tagetes sp*. (Fig. 1). The plants biomass decreased by 20.5, 28.6 and 56 % under the treatments of 5 mmol EDTA/kg, 10 mmol EDTA/kg and 15 mmol EDTA/kg of soil, respectively, when compared to control. The application of EDTA (5, 10 and 15 mmol/kg) decreased the growth in different parts of the plants: up to 25.28, 31.81, and 40.34 % reduction in root length, and 9.4, 11.3, and 37.81 % reduction for the shoot length were noted compared to the control.

Various toxic effects, such as growth reduction, chlorosis etc., after application of EDTA were probably due to high amount of heavy metals mobilized in the soil solution (Huang et al. 1997). The mobilization of metals can reduce cell division (Johnson and Petras 1998), transpiration rate, viscosity and elasticity of cell wall (Ma 2004), and damage the plasma membranes, which are normally stabilized by Ca^{2+} and Zn^{2+} (Kaszuba and Hunt 1990). Huang et al. (1997) reported that addition of 0.5 g HEDTA/kg of soil increased the soluble Pb concentration which exceeded approximately 200 mg Pb/kg of soil and caused death of corn and pea plants within 1 week. Wu et al. (2004) showed that EDTA addition to the soil resulted in numerous brown dots on the leaves of Indian mustard which turned yellow with subsequent death of the plant within 2–4 days. Similarly in the present study, experimental plant species exhibited visible chlorosis and necrosis symptoms at concentrations 10 mmol and 15 mmol EDTA/kg of soil. Luo et al. (2005) found that, compared to control, the dry biomass of shoots was reduced up to

Table 1 Physico-chemical characteristics of soil

Soil Parameters	Type/ Values
Soil type	Sandy loam
pH	6.5 ± 0.3
Electron conductivity (mS/cm)	1.12 ± 0.23
Organic Carbon (%)	1.09 ± 0.33
Water soluble Na (meq/100 g)	0.20 ± 0.065
Water soluble Ca (meq/100 g)	0.08 ± 0.02
Water soluble K (meq/100 g)	0.15 ± 0.09
Exchangeable Na (meq/100 g)	0.92 ± 0.12
Exchangeable Ca (meq/100 g)	3.84 ± 1.66
Exchangeable K (meq/100 g)	0.04 ± 0.007
Phosphorus (mg/kg)	15.66 ± 2.76
Available Nitrogen (mg/kg)	209.5 ± 15.23
Cation Exchange Capacity (meq/100 g)	7.2 ± 1.4
Zn (mg/kg)	80.16 ± 10.77
Cu (mg/kg)	54.85 ± 5.12
Pb (mg/kg)	20.55 ± 6.87
Fe (g/kg)	1.05

60 % for corn, and 76 % for beans on the 14th day after the application of EDTA. Sun et al. (2009) found that the dry weights of *S. alfredii* showed significant reduction after application of EDTA. In comparison with the growth of the control plants, those treated with EDTA showed reduction in the dry biomass: up to 64.9 and 84.0 % decrease for the stems, 49.3 and 85.7 % decrease for the leaves, and 55.6 and 85.0 % decrease for the shoots at the concentration of 5 mmol EDTA/kg of soil and 8 mmol EDTA/kg of soil, respectively (Sun et al. 2009). According to Sun et al. (2011), the application of EDTA (1 g/kg) decreased the height and shoot biomass by 17.1 and 39.6 %, respectively, in pre-flowering stage of *Rorippa globosa*, relative to the control.

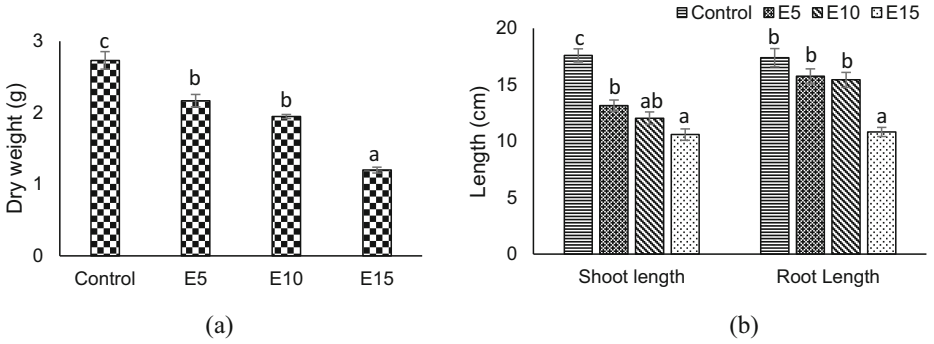

Fig. 1 The dry biomass (**a**) and shoot length, root length (**b**) of *Tagetes sp.* Same alphabet indicates no statistically significant difference according to Duncan test at $p < 0.05$

4.3 Effects of EDTA on Metal Accumulation of *Tagetes sp.*

EDTA application to the soil enhanced the uptake of heavy metals by *Tagetes sp*. compared to the control (Fig. 2), which indicates the bioaccumulation of the metals by the experimental plants. The addition of different concentrations of EDTA (5 mmol EDTA/kg, 10 mmol EDTA/kg and 15 mmol EDTA/kg of soil) resulted in increased Zn accumulation by 1.29, 2.25 and 2.82 times for shoot and 9.37, 9.68 and 10.11 times for leaves, respectively (Fig. 2a). Cd accumulation increased by 2.01, 2.51 and 3.58 times for the shoots and 5.84, 6.65, and 12.02 times for leaves at concentrations 5 mmol EDTA/kg, 10 mmol EDTA/kg and 15 mmol EDTA/kg of soil, respectively (Fig. 2b). Similarly, Cu accumulation increased by 1.28, 1.56 and 1.74 times in shoot and 4.28, 4.55 and 4.55 times in leaves, respectively (Fig. 2d). Pb is less bioavailable and less mobile than the other metals (Prusty et al. 1994), as is evident from the present study where highest Pb concentration was observed in root and very little amount of Pb was found in aerial part of control plants (Fig. 2c). But application of EDTA significantly enhanced the Pb accumulation at the rate of 2.65, 3.74 and 3.9 times for shoot and 21.1, 28.3 and 44.1 times for leaves at concentrations 5 mmol EDTA/kg, 10 mmol EDTA/kg and 15 mmol EDTA/kg of soil, respectively (Fig. 2c). There are good linear relationships ($R^2 = 0.89–0.99$) between tested metal concentration in the shoots of *Tagetes sp*. and the difference in EDTA concentrations of soil (Fig. 3). Significant increase of the selected metals were noted in the flowers at 5 mmol EDTA/kg treated soil compared to the control plants. Highest concentration was found for Zn followed by Cd, Cu and Pb. However, no buds or flower appeared at higher concentrations of EDTA in other treated plants (Fig. 2). Thus, 5 mg EDTA/kg of soil was effective among all the treatments because flower bloomed only in this treatment. On the other hand, the application of EDTA resulted in significant decrease in concentration of the tested metals in the roots of *Tagetes sp*. (Fig. 2). A similar result was found by Grčman et al. (2001), where single addition of 10 mmol EDTA/kg decreased the concentration of Zn, Cd and Pb in root of the tested plants by 71, 69 and 41 %, respectively, compared to control plants.

It is evident from the present study that application of EDTA could significantly increase the heavy metal concentrations in various part of the *Tagetes sp*. Hence the null hypothesis was rejected. Earlier studies were conducted to investigate EDTA on phytoextraction of Zn, Cd, Pb and Cu by *Tagetes sp*. (Sinhal et al. 2010; Wei et al. 2012). Sinhal et al. (2010) observed that the application of EDTA significantly increased the accumulation of Zn, Cu, Pb and Cd in roots, stems and leaves of *Tagetes sp*. as compared to control plants. They also observed that the Zn accumulated at higher amounts (526.34 mg/kg dry weight [DW]) followed by Cu (443.14 mg/kg DW), Pb (393.16 mg/kg DW) and Cd (333.62 mg/kg DW) in leaves with 30 mg/L EDTA treatment. According to Wei et al. (2012), the rate of accumulation of Cd by French marigold was dependent on Cd concentration of soil. The concentration of Cd in the shoot of French marigold significantly increased to 213 mg/kg and 324 mg/kg at the treatment of 20 mg Cd/kg and 40 mg Cd/kg of soil, respectively. Another study conducted by Ali et al. (2016) revealed that the application of 2 and 4 g EDTA/kg of soil significantly increased the accumulation of Cd and Pb concentration in *Tagetes sp*. by 2.41 to 15.7 times and 2.28 to 6.01 times, respectively. These results are in line with the present findings, where there were significant increases of heavy metal uptake by *Tagetes sp*. exposed to different concentrations of EDTA (Fig. 2).

EDTA addition increased the release of heavy metals in the soil solution, and also increased their mobility and bioavailability. According to Meers et al. (2008), the binding constant of EDTA for metals are very high (Cd: 18.1, Pb: 19.7, Zn: 18 and Cu: 20.49), which helps the

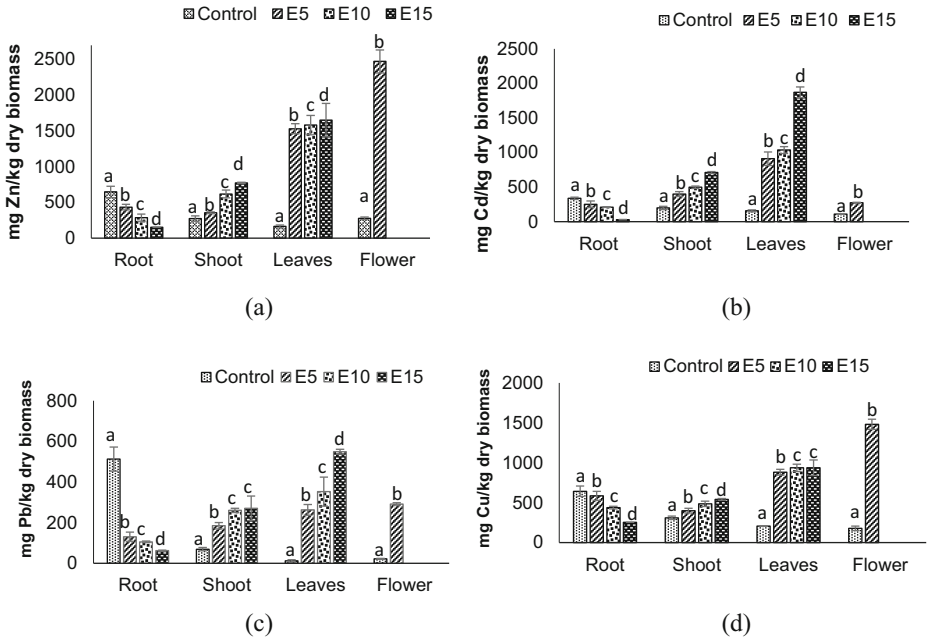

Fig. 2 Effects of application of chelators on the uptake of Zn (**a**), Cd (**b**), Pb (**c**) and Cu (**d**) in *Tagetes sp*. Same alphabet indicates no statistically significant difference according to Duncan test at $p < 0.05$

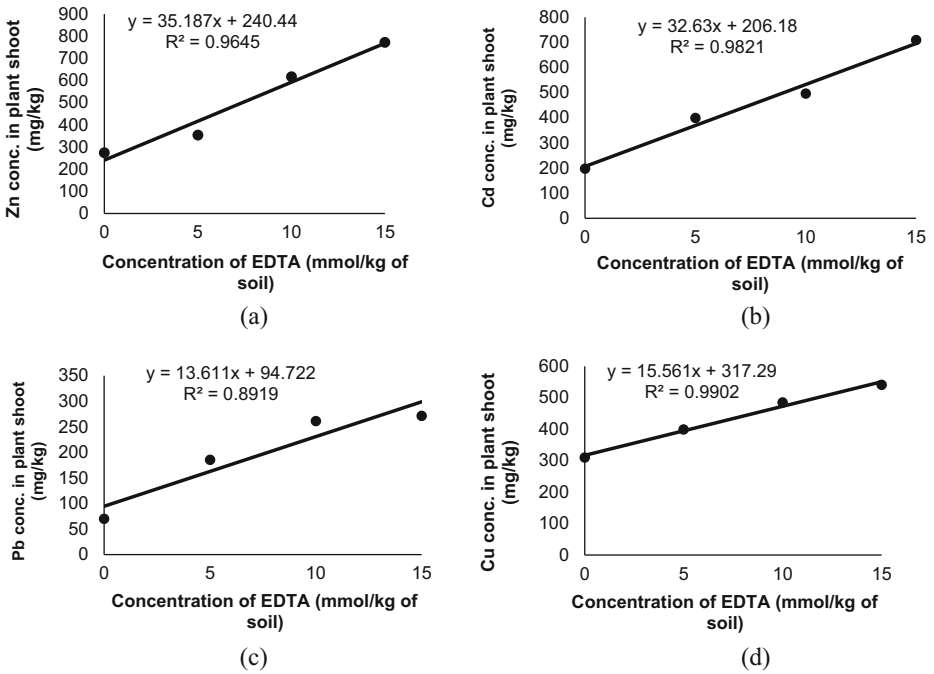

Fig. 3 The linear relationship between Zn (**a**), Cd (**b**), Pb (**c**) and Cu (**d**) concentration in shoot and the concentration of EDTA

dissolution of heavy metals from soil mainly bound with carbonate and exchangeable fraction of soil (Ramos-Miras et al. 2011), and thus, enhanced the mobility in the soil solution. Reports from various studies (Wu et al. 2004; do Nascimento 2006), have shown that Pb concentration in soil solution increased from 217 to 600 times after addition of EDTA compared to the soil without EDTA. Muhammad et al. (2009) reported that EDTA application significantly increased the concentration of water-soluble Cd, Cu, Pb and Cr in soil to about 118, 18, 94 and 4-fold higher than those in the control soil (treated with water). Another study conducted by Munn et al. (2008) noted that the mobile fraction of Cd increased from 1.4 mg/kg to 1.56 mg/kg after addition of EDTA.

4.4 EDTA Effects on the Bioconcentration, Translocation and Remediation Factor

In the present study, the BCF_{root} values of the plant for all the metals in no EDTA treated soil were greater than 1. This value decreased with increasing EDTA concentration (Table 2). The highest BCF_{root} value was observed for Pb, followed Cu, Zn and Cd. On the other hand, the value of BCF_{shoot} increased with increasing EDTA concentration. Highest BCF_{shoot} value was observed for Cd at the concentration 15 mmol EDTA/kg of soil, followed by Zn, Cu and Pb. This result indicates that the application of EDTA increases the mobility of metals from soil to the plants.

It was also observed in this study that the distribution of heavy metals in the aerial parts of the plant significantly increased with increasing EDTA concentrations. High TF values indicated that plants could take up metals from the soil and store them in their aerial parts (NorleelaSelamat et al. 2014). Only Cadmium among the four selected metals in the present study showed that the TF value was greater than 1 in control plants. The highest TF values was observed for Cd at 15 mmol EDTA/kg of soil, followed by Zn, Pb

Table 2 Bioaccumulation and Translocation Factors of Zn, Cd, Pb and Cu in the *Tagetes sp*

Heavy Metals	Treatment	BCF_{shoot}	BCF_{root}	TF	RF
Zn	Zn- control	1.52	3.58	0.67	4.16
	Zn- E5	1.96	2.40	4.35	4.26
	Zn- E10	3.43	1.58	7.69	6.59
	Zn- E15	4.28	0.84	15.95	5.14
Cd	Cd-control	1.98	3.37	1.04	5.41
	Cd-E5	3.99	2.51	5.21	8.67
	Cd-E10	4.96	2.08	7.35	9.69
	Cd-E15	7.09	0.29	88.45	8.51
Pb	Pb-control	0.57	4.25	0.16	1.58
	Pb-E5	1.53	1.08	3.41	3.33
	Pb-E10	2.16	0.88	5.78	4.22
	Pb-E15	2.25	0.52	13.09	2.70
Cu	Cu-control	2.00	4.15	0.94	5.48
	Cu-E5	2.58	3.77	2.03	5.60
	Cu-E10	3.13	2.82	3.25	6.11
	Cu-E15	3.49	1.63	5.83	4.19

and Cu, indicating that the application of EDTA can enhance the mobility of metals in the following sequence: Cd > Zn > Pb > Cu. It is clear from the present study that EDTA not only enhances mobility of heavy metals in soil, but it also increases uptake and translocates heavy metals into the plants tissues, as it was noted by Tandy et al. (2006). A number of studies reported several-fold enhanced translocation of heavy metals from roots to aerial parts by EDTA (Wang et al. 2007; Sun et al. 2009; Wei et al. 2012; Ramamurthy and Memarian 2014).

Sun et al. (2009) reported that after application of EDTA the bioconcentration factor of Zn, Cd, Pb and Cu in *S. alfredii* increased by 8.4, 4.16, 6.3 and 4.95 times, respectively, whereas the translocation factors of Zn, Cd, Pb and Cu increased by 3.32, 2.2, 1.16 and 2.95 times. Another study by Wei et al. (2012) noted that the BCF value of Cd in French marigold decreased 1.1 fold after application of EDTA, whereas TF value increased 3.1 fold.

Translocation occurs primarily by the equilibrium driving force between the liquid water phase in the leaves and the water vapour phase present in the atmosphere and humidity. This process is regulated by the plant with the help of the production of abscisic acid, which signals to the microscopic leaf pores, the stomata, to alter their aperture size (Trejo et al. 1995).

The phytoextraction efficiency of plants depends not only on metal concentration of plants part but depends also on the biomass yield of the plants (Komárek et al. 2008). As shown in Table 2, among the chelator induced treatments, the RF values of Zn, Cd, Pb and Cu were in the order of E10 > E15 > E5, E10 > E5 > E15, E10 > E5 > E15 and E10 > E5 > E15, respectively. So, the highest RF values for the tested elements were obtained in the treatment of 10 mmol EDTA/kg of soil followed by E5 and E15 (except Zn). The accumulation of heavy metals was higher in plants treated with 15 mmol EDTA/kg of soil, though the RF value was lower because of low biomass yield. So, adding EDTA at 5 and 10 mmol/kg to soil would be promising phytoextraction for soils contaminated by Zn, Cd, Pb and Cu.

5 Conclusions

EDTA application to artificially metal contaminated soil enhanced the metal bioavailability and accumulation in *Tagetes sp*. The concentrations of Zn, Cd, Pb and Cu in aerial parts of the plants was significantly increased after the treatments with EDTA compared to the control (without EDTA) plants. However, the addition of EDTA inhibited the growth and also reduced the dry biomass of plants. The treatment of 15 mg EDTA/kg of soil was observed to have the highest bioconcentration and translocation factor of Zn, Cd, Pb and Cu. The treatment of 10 mg EDTA/kg of soil showed the highest phytoextraction efficiency of heavy metal followed by that of 5 mg EDTA/kg of soil, while the lowest phytoextraction efficiency was found with 15 mg EDTA/kg of soil (except Zn). So, in view of phytoremediation efficiency, the treatment of 10 mg EDTA/kg of soil is the best treatment, but in economical view, the treatment of 5 mg EDTA/kg of soil is better than the other two treatments because flower bloomed only under this treatment. So, plantation of *Tagetes sp*. in heavy metal contaminated soil with 5 mmol EDTA/kg of soil may help in phytoremediation, as well as the flowers of this plant species will support the economy.

References

Ali SY, Paul M, Chaudhury S (2016) EDTA-enhanced phytoextraction of Cd and Pb in spiked soil with Marigold and associated potential leaching risk. Int J Environ Agric Res 2:114–118

Allen SE, Grimshaw HM, Rowland AP (1986) Chemical analysis. In: Moore PD, Chapman SB (eds) Methods in plant ecology. Blackwell, Scientific Publication, Oxford, London, pp 285–344

Barrutia O, Garbisu C, Hernandez-Allica J, Garcia-Plazaola JI, Becerril JM (2010) Differences in EDTA-assisted metal phytoextraction between metallicolous and non-metallicolous accessions of *Rumex acetosa* L. Environ Pollut 158:1710–1715

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31: 860–865

Bruins M, Kapil S, Oehme F (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Saf 45:198–207

Chang L, Magos L, Suzuki T (1996) Toxicology of metals. CRC Press, Boca Raton, FL

Dixit P, Tripathi S, Verma KN (2013) A brief study on marigold (*Tagetes species*): a review. Int Res J Pharm 4: 43–48

do Nascimento CWA, Amarasiriwardena D, Xing B (2006) Comparison of natural organic acids synthetic chelates at enhancing phytoextraction of metal from a multi-metal contaminated soil. Environ Pollut 140: 114–123

Ebbs SD, Kochian LV (1997) Toxicity of zinc and copper to brassica species: implications for phytoremediation. J Environ Qual 26:776–719

Ebrahimi M (2013) Effect of EDTA application on heavy metals uptake and germination of *Echinochloa crus galii* (L.) Beave in contaminated soil. Int J Agric Crop Sci 6:197–202

Epstein AL, Gussman CD, Blaylock MJ, Yermiyahu U, Huang JW, Kapulnik Y, Orser CS (1999) EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. Plant Soil 208:87–94

Ghestem JP, Bermond A (1998) EDTA extractability of trace metals in polluted soils: a chemical–physical study. Environ Technol 19:409–416

Grčman H, Velikonja-Bolta Š, Vodnik D, Kos B, Lestan D (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation leaching and toxicity. Plant Soil 235:105–114

Harada Y, Inoko A (1980) The measurement of the cation-exchange capacity of composts for the estimation of the degree of maturity. Soil Sci Plant Nutr 26:127–134

He Z, Yang X, Stoffella P (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19:125–40

Hong AP, Li C, Banerji SK, Regmi T (1999) Extraction, recovery, and biostability of EDTA for remediation of heavy metal contaminated soil. J Soil Contam 8:81–103

Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environ Sci Technol 31:800–805

Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8:921–928

Jean L, Bordas F, Gautier-Moussard C, Vernay P, Hitmi A, Bollinger JC (2008) Effect of citic acid and EDTA on chromium and nickel uptake and translocation by *Datura innoxia*. Environ Pollut 153:555–563

Johnson CE, Petras RJ (1998) Distribution of zinc and lead fraction within a forest spodosol. Soil Sci Soc Am J 62:782–789

Kamnev AA, van der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20:279–258

Kaszuba M, Hunt GRA (1990) Protection against membrane damage: A 1H-NMR investigation of the effect of Zn^{2+} and Ca^{2+} on the permeability of phospholipid vesicles. J Inorg Biochem 40:217–25

Komárek M, Tlustoš P, Száková J, Chrastny V (2008) The use of poplar during a two year induced phytoextraction of metals from contaminated agricultural soils. Environ Pollut 151:27–38

Li ZB, Shuman LM (1996) Extractability of zinc, cadmium, and nickel in soils amended with EDTA. Soil Sci 161:226–232

Luo CL, Shen ZG, Li XD (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59:1–11

Ma JF (2004) Role of organic acids in detoxification of aluminum in higher plants. Plant Cell Physiol 41:383–390

Mani D, Kumar C, Patel NK, Sivakumar D (2015) Enhanced clean-up of lead-contaminated alluvial soil through *Chrysanthemum indicum* L. Int J Environ Sci Technol 12:1211–1222

Meers E, Tack FMG, Van Slycken S, Ruttens A, Du Laing G, Vangronsveld J, Verloo MG (2008) Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals. Int J Phytoremediation 10:390–414

Muhammad D, Chen F, Zhao J, Zhang G, Wu F (2009) Comparison of EDTA and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. Int J Phytoremediation 11:558–574

Munn J, January M, Cutright TJ (2008) Greenhouse evaluation of EDTA effectiveness at enhancing Cd, Cr, and Ni uptake in *Helianthus annuus* and *Thlaspi caerulescens*. J Soil Sediments 8:116–122

NorleelaSelamat S, Abdullah RSS, Idris M (2014) Phytoremediation of lead (Pb) and arsenic (As) by melastomamalabathricum L. from contaminated soil in separate exposure. Int J Phytoremediation 16:694–703

Olsen S, Cole C, Watanabe F, Dean L (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. GPO, Washington D.C

Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–700

Prusty B, Sahu K, Godgul G (1994) Metal contamination due to mining and milling activities at the Zawar zinc mine, Rajasthan, India. Contam Stream Sediments: Chem Geol 112:275–292

Ramamurthy AS, Memarian R (2014) Chelate enhanced phytoremediation of soil containing a mixed contaminant. Environ Earth Sci 72:201–206

Ramos-Miras J, Roca-Perez L, Guzmán Palomino M, Boluda R, Gil C (2011) Background levels and baseline values of available heavy metals in Mediterranean greenhouse soils (Spain). J Geochem Explor 110:186–192

Robinson BH (1997) The phytoextraction of heavy metals from metalliferous soils. Ph.D. Thesis. Massey University, New Zealand

Roongtanakiat N (2009) Vetiver phytoremediation for heavy metal decontamination. Tech Bull 1–20

Sinhal VK, Srivastava A, Singh VP (2010) EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). J Environ Biol 31:255–259

Subbiah BV, Asija GL (1956) A rapid procedure for the determination of available nitrogen in soil. Curr Sci 25: 259–260

Sun Y, Zhou Q, An J, Liu W (2009) Chelator-enhanced phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with the hyperaccumulator plant (*Sedum alfredii* Hance). Geoderma 150: 106–112

Sun Y, Zhou Q, Xu Y, Wang L, Liang X (2011) The role of EDTA on cadmium phytoextraction in a cadmium-hyperaccumulator *Rorippa globosa*. J Environ Chem Ecotoxicol 3:45–51

Suruchi, Khanna P (2011) Assessment of heavy metal contamination in different vegetables grown in and around urban areas. Res J Environ Toxicol 5:162–179

Suthar V, Memon KS, Mahmood-ul-Hassan M (2014) EDTA-enhanced phytoremediation of contaminated calcareous soils: heavy metal bioavailability, extractability, and uptake by maize and sesbania. Environ Monit Assess 186:3957–3968

Tandy S, Schulin R, Nowack B (2006) The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62:1454–1463

Trejo CL, Clephan AL, Davies WJ (1995) How do stomata read abscisic acid signals? Plant Physiol 109:803–811

Turan M, Esringü A (2007) Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn. Plant. Soil Environ 53:7–15

Walker DJ, Clemente R, Roig A, Bernal MP (2003) The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environ Pollut 122:303–312

Walkley A, Black I (1934) An estimation of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 34:29–38

Wang HQ, Lu SJ, Li H, Yao ZH (2007) EDTA-enhanced phytoremediation of lead contaminated soil by *Bidens maximowicziana*. J Environ Sci 19:1496–1499

Wei JL, Lai HY, Chen ZS (2012) Chelator effects on bioconcentration and translocation of cadmium by hyperaccumulators. *Tagetes patula* and *Impatiens walleriana*. Ecotoxicol Environ Saf 84:173–178

Wu J, Hsu FC, Cunningham SD (1999) Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. Environ Sci Technol 33:1898–1904

Wu LH, Luo YM, Xing XR, Christie P (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agric Ecosyst Environ 102:307–318