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Effect of EDTA and DTPA on Phytoremediation of Pb-Zn Contaminated Soils by *Eucalyptus camaldulensis* Dehnh and Effect on Treatment Time

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Abstract

In this research a pot experiment was carried out to investigate the remediation ability of *E. camaldulensis* Dehnh in Lead-Zinc (Pb-Zn) contaminated soil. The study also investigated the effects of ethylenediaminetetraacetic acid (EDTA) and diethylenetrinitrilopentaacetic acid (DTPA) on the phytoremediation efficiency of the plant species, and harvest time as a suitable dose of chelating agents was considered. When the plants had grown for 30 days, the seedlings were harvested to determine the concentration of metals in plant tissues and soil. In general, Pb level decreased in the order of: shoot > soil > root, whilst Zn content decreased in the sequence shoot > root > soil. As a second step, contaminated soils were treated with EDTA (1.5, 3, 6, 9mmolkg^{-1}) and DTPA (1.5, 3, 6, 9mmolkg^{-1}). The results demonstrated that chelating agents enhance metal content in *E. camaldulensis*. The greatest bioconcentration factor in EDTA treatments (3.94) was observed in 9EDTA treatment followed by 6EDTA treatment (3.41). Similarly this was observed for 9DTPA (2.30) and 6DTPA (2.23) respectively. With respect to non-significant difference between 9EDTA and 6EDTA treatments and between 6DTPA and 9DTPA, low doses (6mmolkg^{-1}) were used in the third step for the highest heavy metal uptake over 30, 60 and 90 days. Results reveal that the concentration of metal soil solution decreases gradually with the passage of time. The results indicate that *E. camaldulensis* has the potential for the phytoextraction of metal-contaminated soils but should not be used unless the biomass containing such accumulated metals is removed for disposal. Significant improvement over current chelate-assisted phytoextraction of metals may be possible but should be implemented cautiously because of the environmental risks.

Keywords: Phytoextraction; Environmental Pollution; Lead; Zinc; Chelating Agents

1. Introduction

Heavy metal contaminants are a serious environmental problem because of their adverse effects on human life (Ok et al. 2011) and threat to groundwater quality (Zehtabian, 2013). Compared with the physical and chemical techniques of remediation, phytoremediation is a cost-effective and environmental friendly green technology that utilizes the capacity of hyperaccumulator plants to extract heavy metals from soil (Krämer, 2005; McGrath et al. 2006; Wang et al. 2012). It can be categorized into two different approaches: (i) phytoextraction, whereby metal accumulating plants are planted on contaminated soil and later harvested in order to remove metals from the soil (Yoon et al. 2006; Usman and Mohamed, 2009), and ii) phytostabilization, whereby metal-tolerant plants are used to reduce the mobility of metals, thus stabilizing them in the substrate (Abdel-Ghani et al. 2007; Antosiewicz et al. 2008).

Although phytoremediation can be applied for the reclamation of elevated concentrations of heavy metals present in contaminated soils, just a fraction of soil metal content is readily available for plant uptake, and a large portion is generally present as insoluble compounds unavailable for absorption by roots, so restricting absorption by hyperaccumulating plants (Wang et al. 2009).

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A commonly used approach for enhancing phytoremediation has employed chelating agents such as ethylenediaminetetraacetic acid (EDTA) and diethylenetrinitrilopentaacetic acid (DTPA) (Blayloc et al. 1997; Huang and Cunningham, 1996; Luo et al. 2006). However, excessive addition of chelating agents in field conditions may pose secondary pollution of soils, and the leaching of chelating agents may risk ground water contamination by uncontrolled metal solubilization and leaching as well as increasing the cost of phytoremediation (Robinson et al. 2006).

The biodegradation and toxicity of the chelating agents and their metal complexes in soils needs careful assessment and evaluation (Grčman et al. 2001) to avoid possible metal chelate movement into ground water. The effect of their presence on soil microorganisms, and the appropriate quantity and process of chelate application, are important to novel irrigation techniques and time control of application. A comprehensive approach to phytoremediation should consider strategies in relation to the potential risk that may affect the ecosystem (McGrath et al. 2006).

However some plants such as *E. camaldulensis* appear to have potential for effective wind erosion control, on some hazardous waste sites that have large areal expanses of contaminated and severely degraded soil. Reclamation and vegetation of these soils will reduce wind and water erosion and subsequent dispersal of contaminated soil, as well as promote restoration of the local ecosystem (Smith and Bradshaw, 1979). The objectives of this study were: 1. to investigate the remediation ability of *E. camaldulensis* in Pb-Zn contaminated soils; 2. to identify the influence of application of different concentrations of EDTA and DTPA on the phytoextraction efficiency of the plant species and recognize the optimal chelator dosage; and 3. to consider the effect of treatment time on the phytoextraction of Pb- and Zn- contaminated soils.

2. Materials and Methods

2.1. Soil characterization

Uncontaminated soil (sandy loam texture, hydrometer method) (Day, 1982) was taken from farmland from the surface layer (0-30cm) at the University of Zabol. The soil sample was air-dried under room temperature, and ground to pass through a 2mm sieve before analysis. Chemical analysis of the soil showed that total N (Kjeldahl method; Black, 1965), total P (molybdenum blue method; Olsen and Sommers, 1982), total K (Flame photometry method; Bery et al. 1946), pH (1:1 soil/ water ratio, Model 691, Metrohm AG Herisau Switzerland) (Thomas, 1996) and EC (solid: deionized water = 1:2 w/v, Model DDS-307, Shanghai, China) (Rhoades, 1996) were 0.14%, 0.49%, 0.33%, 8.20, 3.55dSm^{-1} respectively.

2.2. Pot preparation

After sieving (4mm), 1.5kg of dried soil was stored in plastic pots (20cm×15cm). Two days later, the soil was spiked with Pb ($PbNO_3$) 450mgkg^{-1} and Zn ($ZnSO_4$) 450mgkg^{-1} and mixed thoroughly. The soil was then allowed to equilibrate for two weeks in the greenhouse.

The seeds of the plant were purchased from the institute of Pakanbazr, Esfahan province. In all treatments, ten seeds were buried evenly throughout each pot at least 1 to 2cm from the edge, and the pots placed in the greenhouse (University of Zabol) with the environmental conditions of temperature 25±5°C, humidity 60% and a moisture content of 70% water-holding capacity. When the plants had been growing for 30 days, the seedlings were harvested at the end of growing trial. The plants were separated into root and shoot. Plant organs were washed before analysis and samples were baked at 70°C to a constant weight for approximately 48h and ground into fine powder in an agate mortar. Metals were analysed after mineralization of 400mg dry shoot and root material in a microwave oven (MEMMERT UNB 400) with 5ml of nitric acid (69% v/v), 5ml deionized water and 2ml H_2O_2 (30% v/v). The digest was made to 25ml final volume with deionized water, filtered (0.45mm, Millipore) and then analysed for Pb and Zn using ICP/OES (GBC Avanta, Australia). Dried soil samples were passed through a 2mm diameter sieve. About 100mg dry sediment was digested with HNO_3 and HCl (3:1) in a microwave oven. After mineralization, the samples were diluted, filtered and analysed using ICP/OES. Metals concentrations of soil samples were measured as described for the plant samples.

As a second step, to recognize effect of EDTA and DTPA on phytoremediation efficiency of *E. camaldulensis*, seedlings of the plant were placed throughout each pot and two chelator solutions were added to the soil. EDTA (disodium salt dehydrate of EDTA (C_{10} H_{14} N_2 Na_2 $O_8.2H_2O$) and DTPA ($(HO_2C_2H_2)_2NC_2H_4$)-$NC_2H_3O_2$) solutions were prepared at concentrations of 1.5, 3, 6, 9mmolkg^{-1}soil.

Control pots were prepared at the same levels of spiked heavy metal concentration with no EDTA or DTPA (C). Plants were harvested after 30 days of adding chelator solutions and dissected in roots and shoots to recognize the different bioaccumulation capabilities and optimal chelator dosage.

As a third step, the plant was treated with the optimal dosage of chelating agents for the highest heavy metal uptake for 30, 60 and 90 days, respectively. At the end of each period, the plants were harvested and trace element analysis in the plants was performed with ICP/OES (GBC Avanta, Australia). In order to determine heavy metal concentrations in plant organs and soil samples, the sequential extraction technique by Du Laing et al. (2003) was used. The methodology for metal concentration in soil was referenced using the SRM 2711 (Institute of Standard and Technology, USA) and methodology for metal concentration in plants was referenced using BCR-060 (Institute for Reference Materials and Measurements, Belgium). Every analysis was performed in five replicates.

2.3. Calculation of BCF and TF

The bioconcentration factors (BCF) and translocation factors (TF) were calculated to determine heavy metal phytoextraction efficiency (Zayed et al. 1998; Mattina et al. 2003): BCF=heavy metal concentration in harvested plant material (mg kg^{-1})/heavy metal concentration in the soil (mg kg^{-1}), TF= heavy metal concentration in the aerial plant (mg kg^{-1})/heavy metal concentration in the root (mg kg^{-1}) (Dickinson and Pulford, 2005).

2.4. Statistical analysis

All experimental results were statistically analysed using the SPSS 18 package. Data in the text was expressed as mean ±standard error. The statistical significance of the differences between groups was evaluated by analysis of variance (ANOVA). Duncan t-test between means was calculated only if F-test was significant at the 0.05 level of probability. A probability of 0.05 or lower was considered as significant.

3. Results and Discussion

3.1. Concentration of heavy metals in the plant organs (mgkg^{-1}), bioconcentration factor and translocation factor before application of chelating agents.

Plant organs demonstrate a different affinity to the uptake of heavy metals (Fig. 1). *E. camaldulensis* had shoot concentrations of metals that were greater than concentration in the root. In general, the Pb level decreased in the order of: shoot > soil > root. The plant species was able to translocate Pb to the shoot.
The level of Zn in *E. camaldulensis* shoot exceeded the level of Zn in the roots, while the level of Zn in the root was significantly higher than in the soil. Zn content occurred in the sequence shoot > root > soil (Fig.1). The decreasing trend of metal concentrations in both root and shoot was Zn>Pb.

The bioconcentration factor (BCF) of Pb in the shoot and the root was 0.87 and 0.51 and the bioconcentration factor of Zn in the shoot and root was 2.02 and 1.16 respectively (Table 1). In particular, BCF_{shoot} values were higher than that of BCF_{root}, indicating that accumulation of heavy metals in the shoot is higher than in the root. Plants with BCF_{shoot} values >1 are accumulators, while plants with BCF_{shoot} values <1 are excluders (Baker, 1981). The results show that the plant species has the potential for use as an accumulator and the BCF_{shoot} values of >1 indicate high efficacy in the phytoextraction of metal-contaminated soils. However, the concentration of Pb accumulated by the plant was too low to consider phytoremediation of Pb (30-300 mgkg^{-1} Pb). Zn concentration was above the phytotoxic range (100-400mgkg^{-1} Zn) in the plant species (Kabata-Pendias, 2001).
An important characteristic as an accumulator is the translocation ability of a plant. Usually, the translocation factor (TF) can indicate the ability of metal transfer from roots to shoots of a plant (Dickinson and Pulford, 2005). If TF>1, it shows that the accumulation of heavy metals in the shoot is higher than that in the root. The TF values measured for Pb (1.69) and Zn (1.67) indicate that *E. camaldulensis* would be effective as an accumulator.

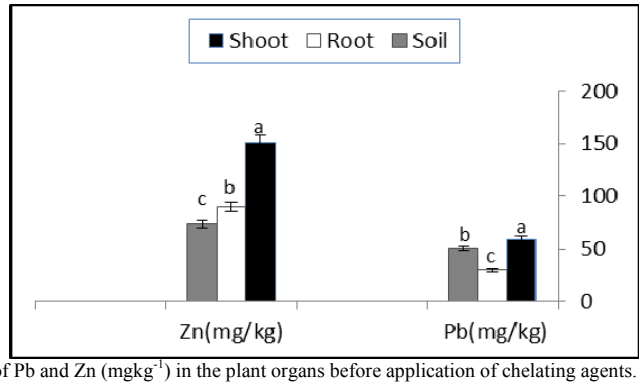

Fig. 1. Concentration of Pb and Zn (mgkg^{-1}) in the plant organs before application of chelating agents. Vertical bars show ±SE (Standard Error)

Table 1. Bioconcentration factor and translocation factor of *E. camaldulensis* before application of chelating agents

Metals	BCF_{shoot}	BCF_{root}	TF
Pb	0.87±0.03^{a}	0.51±0.02^{b}	1.69±0.09^{a}
Zn	2.02±0.06^{a}	1.16±0.06^{b}	1.67±0.09^{a}

Mean values are reported with SE (Standard Error). Values of BCFs within a row followed by different letters indicate significant difference and values of TFs in a column followed by the same letter do not differ significantly ($p<0.05$, post hoc Duncan test).

3.2. Effects of chelating agents on metal concentrations in plant organs and soil

Different effects were observed according to different concentrations of chelating agents (Table 2). A gradual increase in EC and available metal content was observed with increasing concentrations of EDTA and DTPA. A slight decrease in pH was observed with the addition of chelating doses to the soil. The ability of chelating agents to increase concentration of metals in soil solution is influenced by a number of factors, including concentration of metals and chelating agents; presence of competing cations; soil pH; adsorption of free and complexed metals onto charged soil particles and the formation constant of metal–ligand complexes. When chelating agents are applied at high concentrations, they have the potential to affect the release of metals from solid phases by forming dissolved complexes. The formation of metal–chelating agent complexes in soil solution may shift precipitation and sorption equilibria toward increased dissolution of metals (Dushenkov et al. 1997).

In addition, root exudates of plants and some chelating agents significantly enhance the mobilization of metals by plants (Knight et al. 1997), therefore metal uptake can be affected by the application of chelating agents due to low acidity. Some studies have illustrated that pH and EC are important in the extraction and uptake of metals by plants. Tandy et al. (2004) reported that Pb extraction by EDTA, at low chelant-to-metal ratio, depends on soil pH and shows a strong positive correlation up to a soil pH of 6.0.

Turgut et al. (2004), worked out that EDTA at dose of 1.5 and 3mmolkg^{-1} decreased the pH of the soil. Mossop et al. (2009) in their study on the effect of EDTA on the fractionation and uptake by *Taraxacum officinale* showed that the pH of the soil leachates was initially lower than that of the EDTA solution added (pH=7.0) due to buffering by the soil. Bareen and Tahira (2010) studied the efficiency of seven different cultivated plant species for phytoextraction of toxic metals from Tannery Effluent contaminated soil using EDTA and showed that addition of EDTA to the soil at a dose of 10mmol kg^{-1} had highly significant effects on soil pH and EC, but 1 and 5mmol kg^{-1} EDTA did not show significant effects on soil pH and EC.

The bioaccumulation factors for Pb and Zn in the root and shoot of the plant species are shown in Table 2. Treatment means showed that all levels of chelating agents significantly enhanced BCF in root and shoot of the plant. The greatest bioaccumulation capacity in EDTA treatments (3.94) was observed in 9EDTA treatment followed by 6EDTA treatment (3.41). Similarly this was observed for 9DTPA (2.30) and 6DTPA (2.23) respectively. However the increase in the level of metals BCF was observed from 1.5EDTA to 9EDTA, and the increase was not always significant. This was also found for DTPA.

In this study, application of chelating agents decreased the translocation factor. TF of the metals showed significant difference upon the addition of 1.5mmol kg^{-1} EDTA. The same

trend was noted for DTPA treatments. The minimum TF was calculated for both metals in 9DTPA treatment and the maximum (1.69) TF was observed for the control treatment in case of Pb.

In most hyperaccumulators of metals the harvested plant material-to-soil ratio of metal concentration is often greater than 1 (McGrath and Zhao, 2003). In the study, this ratio was greater than 1 or nearly 1 in those plant species found to be better metal accumulators. McGrath and Zhao (2003) have calculated that a plant with a bioconcentration factor of 40 can have the concentration of metal in the top 20cm of soil if it produces 5tons ha^{-1} $crop^{-1}$, whereas a plant with a bioconcentration factor of 20 must produce at least 10tones ha^{-1} $crop^{-1}$ to have the same effect. In addition to the bioconcentration factors, one of the important factors for selecting accumulator species is translocation factor. Low levels of the factor show the potential of a plant to accumulate metals in underground organs. However, data obtained for TF showed that high doses of EDTA and DTPA reduced the factor; the difference was not always significant.

Baylock et al. (1997) found that DTPA and EDTA could enhance Pb and Cd accumulation in shoot of *Brassia juncea* (1.6% and 1.0% respectively). Zhao et al. (2011) reported that EDTA and DTPA had approximately the same effect on the Pb content in shoots of ryegrass. Shen et al. (2002) found that EDTA increase more effectively the solubility of lead compared with the same amount of other mobilizing agents as HEDTA, NTA and nitric acid. Peñalosa et al. (2007) showed that increasing doses of the complexing agent EDTA significantly increased the concentration of soluble element (Pb). Kabata-Pendias (2004), and Madrid and Kirkham, (2002) showed that extractable amounts of Fe, Cu and Mn rise with the addition of EDTA to the soil.

Treatment of soil with chelating agents increased the mobility of target metals in soil solution (Table 2) and the maximum extractable metals were observed in 9EDTA and 9DTPA treatments. With respect to non-significant difference between 9EDTA and 6EDTA treatments and between 6DTPA and 9DTPA, low doses (6mmolkg^{-1}) were used in the third step of the pot experiment. It should be considered that long-lived chelating agents, such as EDTA, are inappropriate for use in enhanced phytoextraction; its longevity will cause elevated metal mobility, even after harvesting plants (Kos and Leštan, 2003). Hence, although the concentration of metals increased with increasing chelating agents concentration, application of higher dose of EDTA/DTPA to metal-contaminated soils may be of environmental concern because of the increased risk of groundwater contamination via metal leaching (Meers et al. 2005; Grčman et al. 2003).

3.3. Effect of treatment time and concentration on plant dry weight

Mean values of total dry weights subjected to different concentrations and treatment time (30d, 60d and 90d after the chelating agents application) are reported in Table 3 and Table 4. It was evident that EDTA and DTPA application negatively affected plant growth but plants grown on DTPA-amended soil exhibited significantly higher dry weights than those determined for EDTA treatments. However, this was not always statistically significant. Jesús et al. (2007) evaluated the effects of EDTA and DTPA on the autochthonous vegetation of a soil polluted with Cu, Zn and Cd. They showed that both chelating agents did not significantly affect dry weight of the plants.

Vassil et al. (1998) reported that the addition of 3 and 6mmol EDTA kg^{-1} did not significantly influence the biomass production of maize grown on studied soils compared to the control. The only statistically significant decrease in maize biomass yield was observed in soils after the addition of 9mmol EDTA kg^{-1}. They suggested that the growth reduction after the 9mmol EDTA kg^{-1} treatment is probably due to the high contents of heavy metals mobilized to the soil solution and, to some extent, due to the toxicity of free EDTA, if present.

Turgut et al. (2005) investigated the use of two EDTA concentrations for enhancing the bioavailability of cadmium, chromium, and nickel in three natural soils (Ohio, New Mexico and Colombia). They reported that the EDTA level resulted in a higher total metal uptake but that high concentrations of EDTA are toxic for plants and ultimately reduce plant biomass and concentrations of metals in the shoot. Cell membranes of the root tissues might be damaged by the chelants at a threshold concentration of above 10mmol chelant kg^{-1} (Grčman et al. 2003; Luo et al. 2006). Neugschwandtner et al. (2007) showed that although the phytoextraction of Pb and Cd using single EDTA and split EDTA application in an agricultural field increased the mobility of target heavy metals in soil solution and metal uptake by *Zea mays*, dry biomass production was significantly reduced.

Table 2. Physicochemical analysis of soil, bioconcentration factor (BCF) and translocation factor (TF) after application of chelating agents.

Treatments	pH	EC dSm^{-1}	Pbsoil $mgkg^{-1}$	Zn_{soil} $mgkg^{-1}$	BCF_{Zn} Shoot	BCF_{Zn} Root	BCF_{Pb} Shoot	BCF_{Pb} Root	TF Zn	TF Pb
C	8.20±0.01^{a}	3.55±0.01^{b}	64.41±3.27^{d}	76.17±3.12^{d}	1.12±0.02^{c}	0.84±0.01^{d}	0.65±0.02^{d}	0.45±0.01^{d}	1.65±0.01^{a}	1.69±0.01^{a}
1.5EDTA	8.00±0.01^{a}	3.64±0.01^{b}	150.03±17.23^{c}	118.73±5.00^{c}	2.30±0.03^{b}	0.93±0.01^{bc}	0.95±0.02^{c}	0.64±0.01^{c}	0.89±0.01^{b}	0.76±0.01^{b}
3EDTA	7.80±0.01^{ab}	4.73±0.01^{a}	200.73±7.80^{b}	230.37±7.64^{b}	2.80±0.03^{b}	1.15±0.02^{b}	1.34±0.02^{b}	0.67±0.01^{c}	0.80±0.01^{b}	0.73±0.01^{b}
6EDTA	7.60±0.01^{bc}	4.80±0.01^{a}	251.93±9.00^{a}	260.00±9.70^{b}	3.41±0.04^{a}	1.51±0.02^{a}	1.77±0.02^{a}	0.92±0.01^{b}	0.73±0.01^{cd}	0.64±0.01^{c}
9EDTA	7.30±0.01^{c}	4.89±0.01^{a}	277.71±9.22^{a}	300.21±10.25^{a}	3.94±0.04^{a}	1.64±0.02^{a}	1.90±0.02^{a}	1.23±0.01^{a}	0.70±0.01^{d}	0.62±0.01^{c}
C	8.20±0.01^{a}	3.55±0.01^{b}	64.12±5.12^{d}	76.56±4.12^{d}	1.00±0.01^{c}	0.82±0.01^{b}	0.60±0.01^{c}	0.43±0.01^{c}	0.90±0.01^{a}	0.82±0.01^{a}
1.5DTPA	8.00±0.01^{a}	3.70±0.01^{b}	117.46±5.50^{c}	134.73±4.67^{c}	1.44±0.01^{b}	0.84±0.01^{b}	0.85±0.01^{b}	0.50±0.01^{b}	0.72±0.01^{b}	0.65±0.01^{b}
3DTPA	7.90±0.01^{a}	3.82±0.01^{ab}	205.00±5.44^{b}	214.31±5.11^{b}	1.60±0.01^{b}	0.91±0.01^{b}	0.97±0.01^{ab}	0.58±0.01^{b}	0.62±0.01^{c}	0.60±0.01^{bc}
6DTPA	7.90±0.01^{a}	4.33±0.01^{a}	222.32±6.50^{a}	234.71±6.25^{a}	2.23±0.01^{a}	1.37±0.02^{a}	1.00±0.01^{a}	0.64±0.01^{a}	0.59±0.01^{c}	0.54±0.01^{c}
9DTPA	7.50±0.01^{b}	4.65±0.01^{a}	230.64±7.42^{a}	245.66±6.25^{a}	2.30±0.01^{a}	1.40±0.02^{a}	1.12±0.01^{a}	0.66±0.01^{a}	0.50±0.01^{c}	0.53±0.01^{c}

Mean values are reported with SE (Standard Error). Values within a column followed by the same letter do not differ significantly ($p<0.05$, post hoc Duncan test)

Table 3. Effect of treatment concentration on plant dry weight.

Treatment concentration		Dry weight (g)		Treatment concentration	Dry weight (g)
EDTA	C	10.63±2.31^{a}	DTPA	C	13.33±3.23^{a}
	1.5	9.22±2.10^{a}		1.5	11.32±3.17^{ab}
	3	6.33±1.27^{b}		3	9.50±2.76^{b}
	6	4.73±1.09^{c}		6	7.60±2.52^{c}
	9	4.20±1.00^{c}		9	6.42±2.11^{c}

Mean values are reported with SE (Standard Error). Different letters in each column indicate significant differences between treatment concentrations ($p<0.05$, post hoc Duncan test)

Table 4. Effect of treatment time on plant dry weight.

	Treatment time		
	30d Dry weight (g)	60d Dry weight (g)	90d Dry weight (g)
6EDTA	4.73±1.09^{a}	3.60±1.12^{b}	3.21±1.11^{b}
6DTPA	7.60±2.52^{a}	5.77±1.25^{b}	4.40±0.90^{b}

Mean values are reported with SE (Standard Error). Different letters in each row indicate significant differences between treatment times ($p<0.05$, post hoc Duncan test)

Dry weight of the plant species (Table 4) decreased significantly ($P<0.05$) with the passage of time. However the maximum dry weight was observed 30 days after application (4.73 and 7.60g for EDTA and DTPA treatments respectively), and no significant difference was seen between the dry weights of the plant on the 60th and 90th days. In general, harvest time as a suitable dose of chelating agents is a crucial factor in the effectiveness of phytoextraction (Wang et al. 2009) and there is still a lack of information about the exact timing of the harvest after application of chelating agents. In this way, Chiu et al. (2005) reported that Cu intake in vetiver shoots under HEIDA application reached at its maximum on day 16. At present, treatment time dependent experiments have shown that harvesting the shoots of plants on the 60th day after the first harvest could achieve the highest phytoextraction efficiency. Wang et al. (2009) reported that the shoots of *Sedum alfredii* on the 14th day for low Pb soil and on the 10th day for high Pb soil could achieve the highest phytoextraction effects. The authors cited that EDDS addition may affect plant growth significantly with the passage of time, especially for high Pb soil because of its higher available Pb.

3.4. Effects of treatment time on metal concentrations in soil and plant organs

Effects of chelating treatment time on metal uptake are summarized in Table 5. Metals concentration in the root and shoot of the plant species increased significantly ($P<0.05$) with the passage of time. However the maximum Pb and Zn in the plant organs was observed on the 90th day of chelating application, with no significant difference seen between concentrations of Pb and Zn in plant tissues on days 60 and 90 (Table 5). It was found that the concentration of Pb and Zn soil solution decreased gradually with the passage of time. The maximum reduction was measured on day 90, but no significant decrease was always observed between days 90 and 60. Similarly, it was found for DTPA and with the passage of time, that the metal concentrations in the soil solution decreased (Table 5). In experiments by Wu et al. (1999), the concentration of DTPA-extractable Pb in soil decreased with increasing extraction time from 6h to 12h.

Table 5. Effects of treatment time on metal concentrations in soil and tissues of *E. camaldulensis*

Treatments	Metals	Soil/Plant organs mgkg^{-1}	Day 30	60	90
6EDTA	Zn	shoot	342.26±10.21^{b}	362.31±9.70^{a}	371.36±9.55^{a}
		root	300.30±11.60^{b}	327.55±9.74^{a}	332.45±8.67^{a}
		soil	264.90±12.23^{a}	240.00±12.53^{b}	221.76±12.64^{c}
	Pb	shoot	275.22±10.45^{c}	291.11±9.15^{b}	303.00±10.21^{a}
		root	130.20±11.22^{b}	151.71±8.33^{a}	159.15±7.42^{a}
		soil	250.50±12.15^{a}	217.90±12.50^{b}	200.27±14.39^{b}
6DTPA	Zn	shoot	256.12±10.28^{b}	281.71±10.62^{a}	288.71±10.07^{a}
		root	236.65±9.67^{b}	251.81±12.23^{a}	263.31±12.25^{a}
		soil	230.20±11.32^{a}	208.40±9.44^{b}	200.15±9.75^{b}
	Pb	shoot	231.21±8.71^{b}	247.17±9.40^{a}	240.90±10.41^{a}
		root	160.11±9.26^{c}	180.61±10.27^{b}	231.17±11.63^{a}
		soil	218.56±10.45^{a}	191.67±8.45^{b}	180.00±8.50^{b}

Values shown are the means ±SE. Values within a row followed by the same letter do not differ significantly ($p<0.05$, post hoc Duncan test)

4. Conclusions

This research examined the phytoremediation efficiency of *E. camaldulensis* in Pb-Zn contaminated soil. The results revealed that plant species had shoot concentrations of metals that were greater than the concentration in the root. In particular, BCF_{shoot} values were higher than BCF_{root}, indicating that the plant species had the potential for use as an accumulator and had a high efficacy in the phytoextraction of metal-contaminated soils. However, the concentration of Pb accumulated by the plant was too low to consider phytoremediation of Pb, whilst Zn concentration was above the phytotoxic range in the plant species.

With respect to the results of this study, application of EDTA- and DTPA-enhanced metal uptake in *E. camaldulensis* is possible due to their greater bioavailability. However at higher doses, chelating agents may cause contamination of groundwater resources and may also exhibit phytotoxic effects. It is therefore suggested that they may be applied only specifically -for cleaning of metals from soil, and in any case lower doses should be used. As there was no significant difference between 6EDTA and 9EDTA treatments, the 6mmol dose of EDTA seemed optimal for

enhancing the efficiency of the plant species. Similarly it was demonstrated for DTPA that 6DTPA was the most effective dose of the treatment for increasing the solubility of Pb and Zn in contaminated soils. Treatment time-dependent experiments showed that harvesting the tissues of the plant on day 60 could achieve the best phytoextraction effects. A strong relationship may exist between the treatment time and remediation of contaminated soils, and the maximum remediation can be done 60 days after plant cultivation.

These results show that *E. camaldulensis* has the potential for phytoextraction of metal-contaminated soils. The use of phytoextraction, however, raises concerns about the transfer of contaminants to the broader ecosystem; thus *E. camaldulensis* should not be used because it increases diffusion of heavy metals through grazing animals and wind erosion due to its considerable BCF in organs above ground, unless the biomass containing the accumulated metals is removed for disposal.

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