Caspian J. Env. Sci. 2015, Vol. 13 No.2 pp. 153~166

CJES
Caspian Journal of Environmental Sciences

[Research]

Effect of EDTA treatment method on leaching of Pb and Cr by *Phragmites australis* (Cav.) Trin. Ex Steudel (common reed)

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(Received: Oct. 27.2014. Accepted: Apr. 8.2015)

ABSTRACT

One of the major problems about the chelant-enhanced phytoextraction is the potential metal leaching associated with chelant application. A glasshouse pot experiment was conducted to investigate phytoextraction efficiency of common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) for Lead and Chromium and to determine EDTA (ethylenediaminetetraacetic acid) enhancement of the mobility and phytoextraction of Pb and Cr and the potential for leaching of metals during the phytoextraction process. The results revealed that the bioconcentration factors of underground organs of the plant species were relatively higher than the bioconcentration factors of shoots and metals concentrations in the plant organs decreased in the order of root› rhizome› leave› stem. Thus, *P. australis* would be applicable for Pb and Cr phytostabilization. Addition of EDTA (0, 2.5, 5, 10 mmol.kg^{-1}) to polluted pots with Pb and Cr significantly enhanced the mobility of soil metals and led to elevated soil solution concentrations of Pb and Cr. Positive correlation coefficients were found between treatment time and heavy metals concentrations in the plant organs. Optimum phytoextraction was observed when 5mmol. kg^{-1} EDTA was added in single dosage, 60 days for Pb and double dosage for Cr, after the plant cultivation and, consequently, soil Pb and Cr concentration decreased with the passage of time. It can be concluded that *P. australis* can remediate Pb-Cr contaminated soils and EDTA had potential to promote the uptake of Pb and Cr for common reed, but with respect to its environmental leaching risk to ground waters, low dose should be used.

Keywords: Chelating agent, Heavy metals, Leaching, Phytoremediation, *Phragmites australis*, Soil pollution.

INTRODUCTION

Aquatic macrophytes are the predominant organisms in the highly productive, littoral ecosystems, such as wetlands and sea beds (Brix & Schierup, 1989). They can absorb metals through their roots and rhizomes as well as through their leaves because the latter provide an expanded area to trap particulate matter, sorb metal ions, and accumulate and sequester pollutants (Levine *et al*., 1990; Ebrahimi *et al*., 2011). These plants can accumulate metals in concentrations 100,000 times greater than in the associated water and therefore they have been used for phytoextraction of variety of sources (Mishra & Tripathi, 2008; Bonanno & Lo Giudice, 2010). Although phytoextraction 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. Therefore, in order to enhance both the availability of metals in soil and translocation from root to shoot, synthetic chelating agents such as EDTA (ethylene diamine tetra acetic acid), diethylene trinitrilo pentaacetic acid (DTPA), nitrilo triacetic acid (NTA), pyridine-2,6-dicarboxylic acid (PDA), trans-l,2-diaminocyclohexane-N,N,N0,N0-tetraacetate (CDTA), or ethylenediamine disuccinate (EDDS) have been proposed (Wu *et al.,* 2004; Meers *et al.,* 2005). Among chelators, EDTA was found as the most efficient in increasing the

concentration of water-soluble heavy metals (Blaylock *et al.*, 1997). When EDTA is applied to soils, a large fraction of the total metals is dissolved and becomes available for phytoextraction (Elliott & Brown, 1989) without inducing a strong acidification of the growth medium (Evangelou *et al.,* 2006). Furthermore, when compared to other chemical compounds, EDTA can solubilize metals with fewer undesirable effects on the physico-chemical properties of the soil (Steele & Pichtel, 1998). This offers great prospects as a less invasive alternative for conventional ex situ soil washing procedures. But, in recent years, the use of persistent APCAs (aminopoly carboxylic acids) such as EDTA has been disfavored by several scientists (Alkorta *et al.*, 2004; Meers *et al.,* 2004). EDTA has been shown to persist for extended periods in soils because of its poor degradability (Nowack, 2002; Meers *et al.,* 2005). In addition, despite the possible usefulness of EDTA, some concerns have been reported regarding the potential risk of leaching of metals to groundwater because EDTA mobilized metals rapidly, and subsequently, their concentrations decreased slowly. High concentrations of heavy metals in the soil solution make an environmental risk in the form of groundwater contamination and EDTA greatly enhances metal leaching. Little work has been done on the extent of EDTA limitations. Therefore, to avoid possible metals chelate movement into ground water and the effect of remaining EDTA on soil microorganisms, the amount and process of *chelate application* are important to novel irrigation technique and time control of chelate application. This study aimed at defining whether there are differences in heavy metals concentration accumulated by the plant organs of *Phragmites australis* (Cav.) Trin. Ex Steudel (common reed) and assessing EDTA application in relation to chelator dosage, treatment time, and application mode.

MATERIALS AND METHODS

Soil sources and treatment the soil (sandy loam texture, hydrometer method) (Day, 1982) used in this study was taken from the greenhouse (Faculty of Agriculture, University of Zabol). The soil samples were sieved to 4mm and moisture contents were adjusted to 70% water-holding capacity.

Uncontaminated soil was used as the control variable throughout the study. Chemical analysis of the soil showed that total N_1, total P_2, total K_3, pH_4, EC_5, CEC_6, organic carbon$_7$, were 0.15%, 0.51%, 0.37%, 8.00, 3.67dSm^{-1}, 39.00 meq, 15.00%, 12.34% respectively. (Source: Kjeldahl method in Black ,1965; molybdenum blue method in Olsen & Sommers,1982; Flame photometry method in Berry *et al.*, 1946; 1:1 soil/ water ratio, Model 691, Metrohm AG Herisau Switzerland in Thomas, 1996; solid: deionized water = 1:2 w/v, Model DDS-307, Shanghai, China in Rhoades, 1996; Cation Exchange Capacity, Bower & Hatcher method (1966); Walkley-Black method in Nelson & Sommers, 1996 and CaCO3 equivalent, Black *et al.,* 19657.

After sieving (4mm), the soil was prepared by homogenizing aliquots of 100 kg in a concrete mixer with Pb ($PbNO_3$) 450 mg.kg^{-1} and Cr [$CrNO_3$]^3 550 mg.kg^{-1}. The soil samples were left to equilibrate for a period of two weeks before it was remixed and used for the experiments. This procedure was adopted to reproduce the process of metals sorption by the soil. 5 kg of dried soil were stored in plastic pots (diameter 20 × diameter 15 × height 60cm).

Rhizomes of *P. australis* (Cav.) Trin. Ex Steudel (common reed) were taken from plants growing along the banks of Hamoon protected area (30° 49′N-61° 14′E, Zabol, Iran). Cuttings of approximately 15cm were buried evenly throughout each pot at least 5 to 6cm from the edge and the pots were placed in the greenhouse at the University of Zabol, with environmental conditions of 24 ± 5°C during the day to 20 ± 5°C during the night without supplementary light. The humidity is 60% and with moisture content of 70% water-holding capacity until roots and shoots develop, then the seedlings were harvested after 30 days. At the end of growing trail (30 days of growth), the plant dry weight, tolerance index (dry weight

of the plants grown in heavy metal solution/dry weight of the plants grown in control solution, Wilkins, 1978), length of shoot and length of root were determined, and the changes in these parameters were used to evaluate heavy metals toxicity. The plants were harvested and separated into root, rhizome, leaf and stem with the aid of a knife. Plant organs were washed before analysis and samples were baked at 70°C to a constant weight for approximately 48hrs and ground into fine powder in an agate mortar. Metals were analyzed after mineralization of 400mg dry plant materials 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 25 ml final volume with deionized water, filtered (0.45 mm, millipore) and then analyzed for Pb and Cr using ICP/OES (GBC Avanta, Australia). Dried soil samples were passed through a 2mm diameter sieve. About 100mg dry soil was digested with HNO_3 and HCl (3:1) in a microwave oven. After mineralization, the samples were diluted, filtered and analyzed using ICP/OES. Metals concentrations of soil samples were measured as described for the plant samples. At second step, to recognize the effects of EDTA on phytoremediation efficiency of common reed, the seedlings of the plant were placed each pot and chelator solution was added to the soil. EDTA (disodium salt dehydrate of EDTA, C_{10} H_{14} N_2 Na_2 $O_8.2H_2O$) solutions was prepared at concentrations of 2.5, 5, 10 mmol.kg^{-1} soil. The control pots (uncontaminated soil) were prepared with no EDTA (C).

Plants were harvested after 30 days of adding chelator solutions and dissected in aboveground and belowground organs to recognize the different bioaccumulation capability and optimum of chelator dosage. At third step (treatment time dependent experiment), the plant was treated with the optimum dosage of chelating agent for the highest heavy metal uptake for 30, 60 and 90 days, respectively, and at the end of each period, the plants were harvested and trace elements analysis in the plants was performed with ICP/OES (GBC Avanta, Australia). At fourth step (addition methods dependent experiment), optimum dosage of EDTA (5 mmol.kg^{-1}) was added to the pots in three different ways: single (5 mmol.kg^{-1}) at day1, double (2.25 mmol.kg^{-1} soil each) at days 1 and 7 and triple (1.66 mmol.kg^{-1} soil each) at days 1, 7 and 14 successive doses. Finally, after the experiment, the plants were harvested 60 days after the first application of EDTA and soil was removed from 4/5 of length of the pots below the surface, air-dried, ground to <0.2 mm, and analyzed to investigate changes in total metals concentrations under different methods of application. In order to determine heavy metals concentrations in the plant organs and soil samples, the sequential extraction technique by Du Laing *et al.* (2003) was used. The methodology for metal concentrations in soil was referenced using the SRM 2711 (Institute of Standard and Technology, USA) and methodology for metals concentration in plant was referenced using BCR-060 (Institute for Reference Materials and Measurements, Belgium). All the analyses were performed in five replicates.

Phytoextraction Efficiency

The bioconcentration factor (BCF) and translocation factor (TF) were calculated to determine the heavy metal phytoextraction efficiency (Mattina *et al.*, 2003; Zayed *et al.*, 1998; Yoon *et al.*, 2006). The BCF expresses the ability of a plant to accumulate metal from soils and TF is the capacity of a plant to transfer metal from its roots to shoots. In the current study, the BCF and TF values for Pb and Cr are given by:

$BCF_{root} = (C_{root})/(C_{soil})$
$BCF_{leaf} = (C_{leaf})/(C_{soil})$
$BCF_{rhizome} = (C_{rhizome})/(C_{soil})$
$BCF_{stem} = (C_{stem})/(C_{soil})$
$TF_{stem/root} = (C_{stem})/(C_{root})$
$TF_{leaf/root} = (C_{leaf})/(C_{root})$

Where Croot, Cleaf, Crhizome and Cstem are the metal concentrations in the roots, leaf, rhizome and stems, respectively, and Csoil is

the metal concentration in the soil (Yoon *et al*. 2006).

Data Analysis

Parametric statistical tests require the data to be normally distributed (Davies, 1997). Therefore, data were log-transformed where needed, using the natural log (ln) to attain normal distribution. The statistical processing was mainly conducted by one-way analysis of variance (ANOVA). For one-way analysis of variance, Duncan t-test between means was calculated only if F-test was significant at the 0.05 level of probability. Correlation coefficients between treatment time and heavy metals contents in plant organs and soil were also calculated through the Pearson's r coefficient. A probability of 0.05 or lower was considered as significant. All statistical calculations were performed using SPSS release 19.0.

RESULTS AND DISCUSSION

Heavy metals tolerance and Morphological characteristics the reduction observed for all measured growth parameters was significant ($p<0.05$) (Table 1). It was evident that Pb and Cr negatively affected the plant growth but the plants grown on Cr treatment exhibited significantly higher dry weight than those determined for Pb treatment. The results showed that, the root length was the most sensitive parameter among all measured parameters. The root length reduction was 21.19% at Cr treatment, but reached 59.78% in Pb treatment. With respect to the control, the shoot growth was 12.33 for the Pb treatments, giving a 49.80% reduction of shoot length whilst the reduction was 30.34% in the Cr treatment. The tolerance index at Pb treatments showed that common reed was more sensitive to Pb than was Cr. The tolerance index of common reed was 100% in control treatment, whereas it was only 73% and 81% in Pb and Cr treatment, respectively. Different parameters, such as biomass, and rates of shoot, and root growth, have been used to evaluate metals toxicity in plants (Baker & Walker, 1989). However, for common reed, root elongation was more sensitive to Pb and Cr than were the rate of shoot growth or plant dry weight.

Similar results were also observed in *Sesamum indicum* (Kumar *et al.,* 1991), *Sinapis alba* (Fargasova, 1994), lettuce and radish (Nwosu *et al.,* 1995). The mechanisms underlying the phytotoxic effect of heavy metals are not fully understood**.** However, it seems that damage to the plasmalemma of roots cells constitutes the first effect of metals toxicity (Woolhouse, 1983), causing a loss of ions, such as K, and other solutes (Woolhouse & Walker, 1981).

Thus, the degree of metals tolerance may depend on the capacity of the plant to prevent this effect (Ait Ali, 2004) and one of the explanations for roots to be more responsive to toxic metals in environment might be that roots are the specialized absorptive organs so that they were affected earlier and subjected to accumulation of more heavy metals than any of the other organs. This could also be the main reason that root length was usually used as a measure for determining heavy metal tolerant ability of plant (Xiong, 1998). Decrease in shoot growth and dry weight in both Pb and Cr contaminated soil were evident as compared to the control treatment. Arduini *et al.* (1994) found the similar result in a research on morphology of *Pinus pinea* and Pinus pinaster using cadmium and copper.

Peralta *et al*. (2004) reported that reduction in chlorophyll could diminish the growth of aboveground organs and decrease in dry biomass might be due to the decrease in water absorption in plant tissues caused by toxic metals, thereby producing undesirable impacts in plant growth (Fuentes *et al*., 2006). Similar results have also been reported in the study of Inckot *et al.* (2011) and Papazoglou *et al*. (2005).

Heavy metals Content in plant tissues

Concentrations of heavy metals in the organs of common reed are shown in Table 2. The plant species had concentrations of metals in roots that were greater than concentrations in leaves, stems, or rhizomes. In general, the level of metals decreased in the order of: roots ›rhizomes ›leaves ›stems. In this study, the fact that roots showed high accumulation of elements could imply relatively high

availability in the soil. Higher contents of metals in roots were expected because the dominant uptake pathway of metals from the soil is via the rhizosphere system.

It is generally known that most metals tend to accumulate in the roots rather than in shoots (Fitzgerald *et al.,* 2003), which suggests that the plants adopt either external or internal exclusion mechanisms to hinder translocation of metals to the aerial tissues (Motesharezadeh & Savaghebi-Firoozabadi, 2011; Hansel *et al.,* 2001). On the other hand, stems (which consist mainly of vascular tissues) exhibit lower metabolic activity than leaves and, therefore, it is expected that they accumulate metals to a lesser extent than leaves (Sawidis *et al.,* 1995**)**. The relatively low accumulation of heavy metals in aboveground tissues was probably due to the need of plant to prevent toxicity to the photosynthetic apparatus as suggested by other authors (Stoltz & Greger 2002; Bragato *et al.,* 2006). BCFs were calculated to assess concentrations in roots to environmental loading. In this study, metals in common reed was accumulated in root with concentrations greater than was found in adjacent soil with BCF of ›1. Plants with BCFshoot values ‹1 are excluders (Baker, 1981). The results showed that the plant species had BCFshoot <1, indicating that it had the potential for use as an excluder and the BCFroot values of ›1 indicate high efficacy in the phytostabilization of metal-contaminated soils. Khan *et al*. (2009) in their study for the purification of industrial wastewater by macrophyte species reported the highest bioconcentration factor of chromium (3.5) for the common reed. These researchers introduced species with BCFroot of >1 suitable for the purification of contaminated soils.

An important characteristic as a hyperaccumulator is the translocation factor (TF). Usually, it can indicate the ability of metal transferring from roots to shoots of a plant. Plants with TF values ›1 are classified as high-efficiency plants for metal translocation from the roots to shoots (Ma *et al*. 2001).

The results evaluated that the plant species had TFs‹ values 1 for Pb and Cr, indicating that accumulation of heavy metals in the roots is higher than in the shoots (Table 3). The relatively low accumulation of heavy metals in shoot tissues was probably due to the need of plants to prevent toxicity to the photosynthetic apparatus as suggested by other authors (Stoltz & Greger, 2002; Bragato *et al.,* 2006). The roots of plant species are mainly responsible for heavy metals phytoextraction and plants species with TF ‹1 have the potential for phytostabilization (Yoon et al., 2006; Usman *et al*., 2012), because in this process the metal tolerant plant species immobilize heavy metals through absorption and accumulation by roots, adsorption onto roots or precipitation within the rhizosphere (Wong, 2003). This process also decreases metal mobility and reduces the likelihood of metals entering into the food chain. Therefore, the use of metal tolerant native flora represents an inexpensive long-term solution (Ashraf *et al.,* 2011).

The effect of chelating agent EDTA to enhanced heavy metals accumulation

A gradual increase in EC and available metals content were observed with increasing concentration of EDTA (Table 4). PH did fall slightly, from a weakly alkaline (8.0) to a weakly acid (6.80) (Table 4). Soil pH is an important parameter in determining the effectiveness of applied EDTA in enhancing metals uptake (Blaylock *et al.,* 1997). Corresponding results showing an increase in EDTA-extractable metals as a function of decreasing pH have been noted in soil washing studies. Mossop *et al.* (2009) in their study on the effects 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 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 dose of 10mmol.kg^{-1} had highly significant effects on soil pH and EC. Metal-extraction efficiency depends of several factors, such as the matrix characteristics (i.e., substrate structure, chemical composition, texture, grain size, etc.), metal properties, leachant characteristics (i.e., concentration, binding power, solubility, etc.), and the conditions of the process itself (e.g., pH, temperature, phase ratio, agitation, and extraction time (Peters, 1999). Among these factors, pH is one of the more important parameters since it governs speciation, complexation and solubility as well as bioavailability and transport of heavy metals (Liphadzi *et al.,* 2005; Nowack *et al.,* 2006), therefore metal uptake can be affected by application of EDTA due to low acidity.

In C treatment, levels of Pb and Cr were below the set detection limits and the increase in the level of metals uptake was quite significant from C to 10 EDTA but insignificant between 5 EDTA and 10 EDTA, showing that 5 EDTA was enough to avoid possible metal chelate movement into ground water and the effect of remaining EDTA on the soil microorganisms.

EDTA exhibited heavy metal extraction efficiency. The lead BCFs of the plant species varied between 0.67 and 3.78, with the lowest BCF in 2.5 EDTA for stem and the highest BCF in10 EDTA for root (Table 5). BCF values for Cr in common reed at 0.55 were the lowest and 1.80 the highest. TFs of the metals did not show significant difference ($p<0.05$).

The highest values (root to leaf) were recorded in 10 EDTA with around 0.30 and 0.55 for Pb and Cr, respectively. Epstein *et al*. (1999) found a positive correlation between Pb and EDTA in the xylem sap and leaves of *Brassica juncea*, either in solution or in a contaminated soil, suggesting that, in the presence of EDTA, all the metal was taken up and stored as a metal–chelate. Bareen and Tahira (2010) showed that the application of EDTA significantly enhanced chromium uptake in seven different cultivated plant species, but increasing the amount of EDTA did not show a significant effect. In a series of laboratory experiments, Wu *et al.* (2004) evaluated the effects of different EDTA dosages (0, 3, 6, 12 mmol.kg^{-1}) on leaching of different heavy metals (Cd, Cu, Pb, and Zn). The leaching of these heavy metals increased with increasing concentration of EDTA. Soil analyses showed a 4–78% loss in total Pb after application of EDTA at the different rates. A maximum loss of Pb from the soil was observed at a dose of 12 mmol.kg^{-1} EDTA.

The correlation between treatment time and extractable soil metals and the metals contents in the plant species

Positive correlation coefficients were found between treatment time and heavy metal concentrations in the plant organs (Table 6). Metals concentration in the plant organs of common reed increased significantly ($p<0.05$) with passage of time. The most significant increase in Pb concentration in the plant organs occurred on 90th days of EDTA application. However, the maximum Cr in the plant organs was observed on 90th day of chelating application, there was no significant difference between concentrations of Cr in the plant tissues on 60d and 90d (Table 6). The soil Pb and Cr concentrations was negatively related to harvest time which indicated that harvest time had positive influences on the soil metals reduction. The maximum reduction was measured on 90d but it could be seen that after 60 days of harvest, no significant decrease was observed between day of 90th and 60th. Plants should be harvested one or two weeks after applications of chelating agents (Huang *et al*., 1998; Römkens *et al*., 2002). In general, harvest time as suitable dose of chelating agents is a crucial factor in the effectiveness of phytoextraction and there is still a lack of information about the exact timing of the harvest after application of chelating agents (Wang *et al.,* 2009; Cooper *et al.,* 1999).

In this way, Chiu *et al*. (2005) reported that Cu intake in vetiver shoots under HEIDA application reached at its maximum on day 16th. At present study, treatment time dependent experiment showed that harvesting the shoots of plants on the 60th day after the first harvest could achieve the highest phytoextraction efficiency. Consequently, early harvest may not be effective in terms of removing maximum amount. Wang *et al.* (2009) reported that the shoots of *Sedum alfredii* on 14th day for low Pb soil and on 10th day for high Pb soil could achieve the highest phytoextraction effects. The authors cited EDDS addition may affect plant growth significantly with the passage of time, especially for high Pb soil because of higher available Pb in soil.

In the experiment of Wu *et al.* (1999), the concentration of DTPA-extractable Pb in soil decreased with increasing extraction time from 6 to 12hrs.

Table 1. Morphological characteristics for common reed at the end of growing trail (30 days of growth) in Pb-Cr treatments.

Treatment	Dry weight (g)	Root length (cm)	Shoot length (cm)	Tolerance index
Pb contaminated soil	7.74 ± 0.04[a]	12.11 ± 0.10[a]	12.33 ± 0.10[a]	0.73 ± 0.00[a]
Cr contaminated soil	9.21 ± 0.05[b]	23.73 ± 0.14[b]	17.11 ± 0.11[b]	0.81 ± 0.00[ab]
Control	15.98 ± 0.07[c]	30.11 ± 0.16[c]	24.56 ± 0.14[c]	1.00 ± 0.00[b]

Values (± SE) within a column followed by different letter are significantly different ($p<0.05$, post hoc Duncan test).

Table 2. Total concentration of Pb and Cr in plant organs (mgkg-1 Dw) and bioconcentration factors.

Plant organs	Pb mg kg-1	Cr mg kg-1	BCF Pb	BCF Cr
Root	100.23 ± 1.33[a]	78.11 ± 1.09[a]	5.11 ± 0.07[a]	4.78 ± 0.06[a]
Leaf	52.19 ± 1.20[b]	43.20 ± 0.90[b]	0.67 ± 0.03[b]	0.40 ± 0.02[b]
Stem	61.29 ± 1.11[c]	51.18 ± 0.92[b]	0.76 ± 0.03[b]	0.41 ± 0.02[b]
Rhizome	83.41 ± 1.17[d]	60.09 ± 0.90[c]	2.23 ± 0.06[c]	1.76 ± 0.04[c]

Values (±SE) within a column followed by different letter are significantly different ($p<0.05$, post hoc Duncan test).

Table 3. Translocation factors (TF) for common reed.

Metal	$TF_{stem/root}$	$TF_{leaf/root}$
Pb	0.60 ± 0.03[a]	0.66 ± 0.03[a]
Cr	0.53 ± 0.03[a]	0.55 ± 0.03[a]

Values (± SE) within a column followed by the same letter are not significantly different ($p<0.05$).

Table 4. Physico-chemical analysis of soil after treatment by EDTA

Treatments	pH	EC dS. m-1	Pb mg kg-1	Cr mg kg-1
C	8.00 ± 0.01[a]	3.67 ± 0.21[a]	ND (0.02›)	ND (0.002›)
2.5 EDTA	7.70 ± 0.01[ab]	4.50 ± 0.22[b]	131.19 ± 1.50[a]	94.72 ± 1.40[a]
5 EDTA	7.40 ± 0.01[bc]	4.87 ± 0.22[b]	152.55 ± 1.62[b]	111.06 ± 1.43[b]
10 EDTA	6.80 ± 0.01[c]	5.22 ± 0.30[c]	164.10 ± 1.67[b]	125.71 ± 1.62[b]

ND= NOT Detected/Below detectable range. Values shown are the means ± SE. Values within a column followed by different letter are significantly different ($p<0.05$, post hoc Duncan test).

Table 5. Pb and Cr extraction efficiency.

Metal	Treatments	BCF_{root}	BCF_{leaf}	BCF_{stem}	$BCF_{rhizome}$	$TF_{stem/root}$	$TF_{leaf/root}$
Pb	C	-	-	-	-	-	-
	2.5 EDTA	2.30 ± 0.10[a]	0.67 ± 0.01[a]	0.75 ± 0.01[a]	1.63 ± 0.10[a]	0.18 ± 0.01[a]	0.21 ± 0.01[a]
	5 EDTA	2.92 ± 0.20[a]	0.80 ± 0.01[a]	1.50 ± 0.09[b]	2.00 ± 0.10[ab]	0.24 ± 0.01[a]	0.26 ± 0.01[ab]
	10 EDTA	3.78 ± 0.24[b]	1.24 ± 0.09[b]	1.79 ± 0.09[c]	2.62 ± 0.20[b]	0.25 ± 0.01[a]	0.30 ± 0.02[b]
Cr	C	-	-	-	-	-	-
	2.5EDTA	1.00 ± 0.06[a]	0.55 ± 0.01[a]	0.57 ± 0.01[a]	0.87 ± 0.03[a]	0.28 ± 0.01[a]	0.50 ± 0.04[a]
	5EDTA	1.60 ± 0.06[b]	0.80 ± 0.02[ab]	1.00 ± 0.06[b]	1.21 ± 0.05[b]	0.35 ± 0.01[b]	0.54 ± 0.04[a]
	10EDTA	1.80 ± 0.06[b]	0.94 ± 0.02[b]	1.00 ± 0.06[b]	1.41 ± 0.06[b]	0.35 ± 0.01[b]	0.55 ± 0.04[a]

Values shown are the means ± SE. Values within a column followed by different letter are significantly different ($p<0.05$, post hoc Duncan test).

Table 6. Effect of treatment time of 5EDTA on heavy metals uptake and correlation coefficients.

		30d		60d		90d	
Metals		**r^2**	**Metal Content (mg.kg^{-1})**	**r^2**	**Metal Content (mg.kg^{-1})**	**r^2**	**Metal Content (mg.kg^{-1})**
Pb	Root	0.98**	161.21 ± 1.50^a	0.98**	193.21 ± 1.72^b	0.98**	257.94 ± 2.03^c
	Leaf	0.93**	87.88 ± 1.11^a	0.93**	110.22 ± 1.18^b	0.93**	101.09 ± 1.20^c
	Steam	0.94**	91.91 ± 1.21^a	0.94**	118.56 ± 1.24^b	0.94**	129.16 ± 1.30^c
	Rhizome	0.91**	117.03 ± 1.33^a	0.91**	157.77 ± 1.34^b	0.91**	196.66 ± 1.66^c
	Soil	-0.94**	135.46 ± 1.17^a	-0.88*	85.11 ± 1.06^b	-0.86*	75.52 ± 1.00^b
Cr	Root	0.97**	120.15 ± 1.22^a	0.98**	118.41 ± 1.30^b	0.98**	191.32 ± 1.66^b
	Leaf	0.96**	63.09 ± 1.10^a	0.93**	74.70 ± 1.13^b	0.93**	81.18 ± 1.15^b
	Steam	0.90**	70.22 ± 1.10^a	0.94**	82.16 ± 1.16^b	0.94**	99.26 ± 1.21^b
	Rhizome	0.90**	89.14 ± 1.13^a	0.91**	120.13 ± 1.22^b	0.91**	129.52 ± 1.34^b
	Soil	-0.97**	107.25 ± 1.10^a	-0.93**	76.09 ± 1.00^b	-0.89*	64.60 ± 1.00^b

Values shown are the means ± SE. Values within a row followed by different letter are significantly different ($p<0.05$, post hoc Duncan test).
*significant at the 0.05 probability level, **significant at the 0.01 probability level.

EDTA treatment methods and heavy metals uptake and correlation coefficient

The relations between the application methods of EDTA (5 mmol.kg^{-1}) and heavy metals concentrations are shown in Table 7. Negative correlations were obtained between Pb and Cr concentrations in the plant organs of common reed and methods of application, which indicated that there was reduction in metals content in the plant organs according to different application methods ($p<0.05$). The maximum Pb concentration in the organs of plant species was calculated at single dosage. It was seen that under three separate application methods, metals content in the plant tissues reached at its minimum concentration, while Cr concentration in the plant organs did not vary considerably when double and triple dosage were added ($p<0.05$). The liner relationship between the soil concentrations of heavy metals and the treatment mode was positive (Table 7). The amounts of soil Pb and Cr reached at their maximum at triple dosage. The results from the application methods of EDTA on the soil Pb and Cr concentration (Table 7) indicated that under single dosage application, heavy metals content in the soil reached at its minimum concentration. Pb content increased by 25.24% when double dosage was added. This increase was calculated in triple dosage 26.23%. However, different application methods influenced the Cr uptake. There was no significant effect between double and triple dosage on the Cr content of the soil. The results indicated that the multidose chelate application method could limit the solubility and migration potential of heavy metals in the soil. Increasing metal solubility is the major purpose of applying chelants to the soil, and a precondition to enhance metal uptake by plants. On the other hand, potential metal leaching associated with the application of chelants may be of concern for the chemical assisted phytoextraction (Wang *et al.*, 2012).

Lombi *et al*. (2001) found that most of the heavy metals in soil pore water are combined with EDTA. Thus, further efforts should focus on the methods of how to degrade or separate heavy metal-EDTA complexes and release heavy metal ions. Grčman *et al*. (2001) reported that single dose of 2.9g EDTA kg^{-1} enhanced 105-fold Pb accumulation in cabbage (*Brassica oleracea* L.) grown in a greenhouse, as compared with a 44-fold increase if the same amount of EDTA was split and added in four intermittent doses. The authors reported that if a soil has a high Pb retention capacity, application of EDTA in multiple doses could be ineffective in mobilizing and enhancing root to shoot translocation. In these conditions, application of the full rate of chelant in a single dose could constitute the more effective approach. Ebrahimi (2014) showed that addition of EDTA had virtually and significantly affected on uptake of the metals by *Echinochloa crus galii* and elevated Pb and Cr concentrations in plant organs. Optimum phytoextraction was observed when 5 mmol kg^{-1} EDTA was added

in single dosage 60 days after the plant cultivation and, consequently, soil Pb and Cr concentration decreased with the passage of time. Experiment of Wang *et al*. (2009) showed that if the EDDS addition was split into three or five doses, Pb concentration in the shoots of *Sedum alfredii* grown in Pb contaminated soils decreased significantly in comparison to those treated with a single dosage. But Shen *et al*. (2002) in assessing effect of EDTA treatment methods on efficiency of phytoremediation showed that application of EDTA with a triple dosage increased Pb uptake considerably comparing with single and double dosage. Wenzel *et al*. (2003) assessed the effects of dosage (up to 2.01g.kg^{-1}) and mode (single vs. split) of EDTA application on leaching of Cu, Pb and Zn during and after the harvest of *Brassica napus* L.

They reported that the metal concentrations in the leachates were related to the amount of EDTA applied, but the authors found no difference between applications of the same amount of EDTA in single or split doses.

Table 7. Effect of 5EDTA application methods on the concentration of Pb and Cr in tissues of

Metals		r²	Single	r²	Double	r²	Triple
Pb	Root	-0.87*	187.44 ± 1.10^{a}	-0.86*	156.44 ± 1.11^{b}	-0.86*	120.00 ± 1.21^{c}
	Leaf	-0.87*	105.44 ± 1.21^{a}	-0.85*	83.00 ± 1.14^{b}	-0.85*	71.29 ± 1.04^{c}
	Steam	-0.90**	113.23 ± 1.21^{a}	-0.83*	105.77 ± 1.31^{ab}	0.80*	100.08 ± 1.11^{b}
	Rhizome	-0.93**	150.88 ± 1.10^{a}	-0.90**	120.11 ± 1.34^{b}	-0.90**	90.33 ± 0.95^{c}
	Soil	0.88*	80.03 ± 1.00^{a}	0.91**	100.23 ± 1.00^{b}	0.90**	126.52 ± 2.33^{c}
Cr	Root	-0.80*	117.20 ± 1.32^{a}	-0.80*	110.43 ± 1.27^{ab}	-0.83*	102.87 ± 1.20^{b}
	Leaf	-0.83*	69.41 ± 1.07^{a}	-0.80*	59.05 ± 1.00^{a}	-0.80*	58.60 ± 1.00^{a}
	Steam	-0.88*	76.11 ± 1.22^{a}	-0.90**	60.23 ± 1.13^{b}	-0.90**	59.11 ± 1.04^{b}
	Rhizome	-0.90**	115.32 ± 1.19^{a}	-0.90**	107.44 ± 1.12^{ab}	-0.90**	95.44 ± 1.01^{b}
	Soil	0.90**	70.67 ± 1.51^{a}	0.93**	94.21 ± 1.65^{b}	1.00**	104.78 ± 2.42^{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).
*significant at the 0.05 probability level, **significant at the 0.01 probability level.

CONCLUSION

The results revealed that heavy metals concentrations in common reed depended significantly on the kind of plant organs. Belowground organs showed a greater capacity of accumulation as compared to the shoots. The significantly different levels of Pb and Cr found in the various organs may imply low metal mobility from roots to rhizomes and to shoots (leaves and stems) and the plant species would be applicable for Pb and Cr phytostabilization because it had BCFroot values > 1 and a relatively low TF value. Considering metals uptake, EDTA was efficient for extraction of Pb and Cr from the tissues of the plant species but the optimum dose of chelators for chelate-assisted phytoextraction must be investigated before the application of this technique. Present study concludes that EDTA should be added at the concentration of 5 mmol.kg^{-1} a single dosage for 60 days in Pb contaminated soils and a double dosage for Cr contaminated soil.

ACKNOWLEDGEMENTS

The author wish to acknowledge the Department of Range and Watershed Management, University of Zabol, for providing necessary facilities to undertake this study.

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تاثیر روش کاربرد EDTA در آبشویی سرب و کروم توسط گیاه نی (*phragmites australis* (Cav.) Trin. Ex Steudel)

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چکیده

یکی از مهم‌ترین مشکلات مربوط به گیاه‌استخراجی با استفاده از مواد بهساز، آبشویی فلز با کاربرد این مواد است. آزمایش گلدانی به‌منظور ارزیابی کارایی گیاه‌استخراجی فلزات سرب و کروم توسط گیاه نی (*Phragmites australis* (Cav.) Trin. Ex Steudel) و تعیین افزایش تحرک سرب و کروم و پتانسیل آبشویی این فلزات طی فرایند گیاه‌استخراجی انجام شد. نتایج نشان داد که فاکتورهای تجمع فلزات در اندام‌های زیرزمینی گونه گیاهی بیشتر از فاکتورهای تجمع فلزات در اندام‌های هوایی است و غلظت فلزات در اندام‌های گیاهی به‌ترتیب ریشه>ریزوم>برگ>ساقه بود. بنابراین، گونه گیاهی مورد مطالعه قابلیت استفاده برای گیاه‌تثبیتی سرب و کروم را دارا است. اضافه کردن EDTA ($mmol.kg^{-1}$ ۰، ۲/۵، ۵، ۱۰) به گلدان‌های آلوده به سرب و کروم به‌طور معنی‌داری باعث افزایش تحرک فلزات در خاک شد و غلظت سرب و کروم درمحلول خاک را افزایش داد. همبستگی مثبت بین زمان تیمار و غلظت فلزات سنگین در اندام‌های گیاهی به‌دست آمد. اپتیمم گیاه‌استخراجی سرب در کاربرد EDTA درغلظت $5mmol.kg^{-1}$ در کاربرد به‌صورت یکبار و برای کروم در شیوه کاربرد به صورت دوبار متوالی ۶۰ روز پس از کشت گیاه به‌دست آمد و غلظت سرب و کروم با گذشت زمان در خاک کاهش نشان داد. به‌طور کلی گونه گیاهی مورد مطالعه قابلیت پالایش خاک‌های آلوده به سرب و کروم را دارا است و EDTA پتانسیل افزایش جذب سرب و کروم را برای گونه نی دارد، اما باتوجه به خطر آبشویی آن به آب‌های زیرزمینی، غلظت‌های پایین آن باید استفاده شود.