EDTA Enhanced Phytoextraction Capacity of Scirpus Maritimus L. Grown on Pb-Cr Contaminated Soil and Associated Potential Leaching Risks

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Received 01 August 2014; Accepted 11 September 2014

Abstract. A pot experiment was conducted to study phytoextraction efficiency of Scirpus maritimus L. 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. The results revealed that the bioconcentration factors of underground organs were relatively higher and metals concentrations in the plant organs decreased in the order of roots› rhizomes› leaves› stems. Thus, the plant species would be applicable for Pb and Cr phytostabilization. Addition of EDTA (2.5, 5, 10 mmol kg⁻¹) to polluted pots 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 metals concentrations in the 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 metals concentration decreased with the passage of time. It can be concluded that of Scirpus maritimus L can remediate Pb-Cr contaminated soils and EDTA (at low doses) had potential to promote the uptake of Pb and Cr for the plant species in eco-friendly manner.

Keywords: Chelating agent, heavy metal, leaching, phytoremediation, Scirpus maritimus L.

1. INTRODUCTION

Recently phytoremediation, as a potential method for removal of metals from polluted areas, has attracted great attention. It is an emerging technology driven by solar energy that uses green plants to remove toxic metals from contaminated aquatic and terrestrial sites (Suresh and Ravishanker, 2004). The main features of this green technology are that it is cost-effective and environmentally sound unlike the chemical remediation technology (Macek et al., 2000).

In present study, Scirpus maritimus L. was evaluated to investigate lead and chromium phytoextraction in the Lia contaminated area (35°27'N-48°50'E), an important industrial area of Qazvin (Northern Iran). It has a total area of 108 ha with 151 industrial factories. The main human activities are agriculture especially greenhouse cultivation, smelting, sewage sludge treatment and electronic industries. Industrialization in this area has exposed the soil to various effluent inputs including heavy metals. Several locations of Lia area suffer from metals contamination (Ebrahimi, 2014).

S. maritimus L. is a facultative halophyte. This plant belongs to the family Cyperaceae and it is a metal-accumulator plant, which can accumulates elevated values of heavy metals in its rhizomes, leaves and stems (Carranza-Álvarez 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. Therefore, in order to enhance both the availability of metals in the soil and translocation from root to shoot, many chelants have been studied (Arwidsson et al., 2007). Among them, EDTA (ethylenediaminetetraacetic acid) has been widely examined which was found as the most efficient in increasing the concentration of water-soluble heavy metals. When EDTA is applied to the soils, a large fraction of the total metals is dissolved and becomes available for phytoremediation without inducing a strong acidification of the growth medium (Evangelou et al., 2006). Furthermore, as compared to other chemical compounds, EDTA can solubilize metals with fewer undesirable effects on the soil physico-chemical properties (Steele and Pichtel, 1998). This offers great prospects as a less invasive alternative for
conventional *ex situ* soil washing procedures. However, due to its high level of persistence, EDTA has been disfavored by several scientists (Arwidsson et al., 2007).

In recent years, the use of EDTA has been disfavored. EDTA has been shown to persist for extended periods in soils because of its poor degradability (Nowack, 2006). It therefore increases the activity of trace metals in the soil solution for extended periods and might cause enhanced toxicity towards plants and soil organisms. Moreover, accelerated, uncontrolled release and leaching of metals from sparingly soluble metal compounds is likely to increase groundwater pollution (Grêman et al., 2001; Wenzel et al., 2003).

Little work has been done on the extent of EDTA limitations. Therefore to avoid possible metals chelate movement into groundwater and the effect of remaining EDTA on the soil microorganisms, the amount and method of chelate application are important to novel irrigation technique and time control of chelate application.

The objectives of this study were: (1) to investigate the remediation ability of *S. maritimus* L. in Pb-Cr contaminated soils; (2) to identify influence of application of different concentrations of EDTA on phytoextraction efficiency of the plant species and recognize optimum of chelator dosage; and (3) to consider effect of treatment time and application mode on the phytoextraction of Pb and Cr contaminated soils.

### 2. MATERIALS AND METHODS

#### 2.1. Soil source characterization and treatments

The soil used in this study was taken from Lia area. Uncontaminated soil was used as the control throughout the study. The results of heavy metals concentration and physico-chemical properties of the soil such as Total N (Kjeldahl method) (Black, 1965), total P (molybdenum blue method) (Olsen and Sommers, 1982), total K (Flame photometry method) (Berry et al., 1946), pH (1:1 soil/water ratio, Model 691, Metrohm AG Herisau Switzerland) (Thomas, 1996), EC (solid: deionized water =1:2 w/v, Model DDS-307, Shanghai, China) (Rhoades, 1996), organic carbon (Walkley-Black method) (Nelson and Sommers, 1996) and CaCO₃ equivalent (Black et al., 1965) were depicted in Table 1.

After sieving (4mm), 5 kg of dried soil were stored in plastic pots (diameter 20×diameter 15xheight 60cm). Seeds of *S. maritimus* L. were obtained commercially in Pakanbazar Company, Esfahan, Iran. Seeds of the plant were buried evenly throughout each pot at least 1 to 2 cm from the edge and pots placed in the greenhouse with the environmental conditions, temperature 24±5°C (day) to 20±5°C (night) without supplementary light, humidity 60% and moisture content 70% water-holding capacity until roots and shoots had developed, then the seedlings were harvested (30 days of growth) at the end of growing trial, 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 the shoot and length of the root were determined, and the changes in these parameters were used to evaluate heavy metals toxicity.

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 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 H₂O₂ (30% v/v). The digest was made to 25ml final volume with deionized water, filtered (0.45mm, 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 HNO₃ and HCl (3:1) in a microwave oven (MEMMERT UNB 400). After mineralization, the samples were diluted, filtered and analyzed using ICP/OES (GBC Avanta, Australia). Metals concentrations of the soil samples were measured as described for the plant samples (Du Laing et al., 2003).

In second step, to recognize effect of EDTA on phytoremediation efficiency of *S. maritimus* L. seedlings of the plant were placed throughout each pot and chelator solution was added to the soil. EDTA (disodium salt dehydrate of EDTA, C₁₀H₁₄N₂Na₂O₆₂H₂O) solutions were prepared at concentrations of 2.5, 5, 10mmolkg⁻¹ soil. The control pots (contaminated soil) prepared with no EDTA (C). Plants were harvested after 30 days of adding chelator solutions and dissected in root, leaf, stem, and rhizome organs to recognize the different bioaccumulation capability and optimum of chelator dosage.

In third step (treatment time dependent experiment), the plant was treated with the most 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 metals analysis in the plants was performed with ICP/OES (GBC Avanta, Australia).

In forth 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) at day1, double (2.25 mmol/kg soil each) at...
days 1 and 7 and triple (1.66 mmol/kg 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 the soil was removed from 4/5 of length of the pots below the surface, air-dried, ground to <0.2mm, and analyzed to investigate changes in total metals concentrations under different methods of application.

The methodology for metals concentrations in the soil was referenced using the SRM 2711 (Institute of Standard and Technology, USA) and methodology for metals concentration in the plant was referenced using BCR-060 (Institute for Reference Materials and Measurements, Belgium). All the analyses were performed in five replicates.

2.2. Determination of bioconcentration factor (BCF) and translocation factor (TF)

Bioconcentration factor (BCF), represents the ratio of metal concentration dry weight (DW) in the plant to the metal concentration DW in the soil. BCF is an indication of the magnification of contaminants from a lower to a higher trophic level. For plants, the BCF has been used as a measure of the metal accumulation efficiency, whereby value greater than 1 is an indication of plants potential to phytoextraction (Yoon et al., 2006; Santillan et al., 2011). To analyze the total metal concentration in DW taken by the upper parts of the plants from ground level, translocation factor (TF) was calculated (Yoon et al., 2006; Santillan et al., 2011). In the current study, the BCF and TF values for Pb and Cr are given by:

\[
\begin{align*}
BCF_{\text{Root}} & = \frac{C_{\text{root}}}{C_{\text{soil}}} \quad (1) \\
BCF_{\text{Leaf}} & = \frac{C_{\text{leaf}}}{C_{\text{soil}}} \quad (2) \\
BCF_{\text{Rhizome}} & = \frac{C_{\text{rhizome}}}{C_{\text{soil}}} \quad (3) \\
BCF_{\text{Stem}} & = \frac{C_{\text{stem}}}{C_{\text{soil}}} \quad (4) \\
TF_{\text{stem/root}} & = \frac{C_{\text{stem}}}{C_{\text{root}}} \quad (5) \\
TF_{\text{leaf/root}} & = \frac{C_{\text{leaf}}}{C_{\text{root}}} \quad (6)
\end{align*}
\]

Where \( C_{\text{root}}, \ C_{\text{leaf}}, \ C_{\text{rhizome}} \) and \( C_{\text{stem}} \) are the metal concentrations in the root, leaf, rhizome and stem, respectively, and \( C_{\text{soil}} \) is the metal concentration in the soil (Yoon et al., 2006).

2.3. Statistical analysis

Parametric statistical tests require the data to be normally distributed. 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, mode of EDTA application with 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.

3. RESULTS

3.1. Metals accumulation and plant tolerance

Results shown in Table 2 indicated the reduction for the measured growth parameters. For \( S. \ maritimus \) L. root elongation was more sensitive to Pb and Cr than the rate of shoot growth or plant dry weight. The root length reduction was 13.90% whilst the shoot reduction was 10.21%. The tolerance index of the plant species was 100% in the control treatment, whereas it was only 80% in the contaminated soil. Decrease in shoot growth and dry weight in Pb-Cr contaminated soil was evident as compared to the control treatment.

Heavy metals concentrations in the organs of \( S. \ maritimus \) L. are shown in Table 3. The plant species had root concentrations of metals that were greater than concentrations in leaves, stems, or rhizomes. In general, the metals level decreased in the order of: roots \( \approx \) rhizomes \( \approx \) leaves \( \approx \) stems.

Bioconcentration factors in the studied plant were from 0.54 to 4.62 for Pb and 0.37 to 3.19 for Cr (Table 3). In this study, metals in \( S. \ maritimus \) L. were accumulated in the root with concentrations greater than was found in adjacent soil with BCF of \( > 1 \).

The results showed that the plant species had BCF\(_{\text{root}}\) values of \( > 1 \) indicate high efficacy in the phytostabilization of metal contaminated soils and the plant has adopted an exclusion strategy and there is less risk of metals entering the food chain if the plant is consumed. The results evaluated that the plant species had TF\(_{\text{root}}\) values 1 for Pb and Cr, indicating that accumulation of heavy metals in the roots is higher than in the shoots (Table 4).
### 3.2. The effect of EDTA on heavy metals accumulation

The pH values after EDTA addition are shown in Table 5. A gradual increase in EC and available metals content were observed with increasing concentration of EDTA (Table 5). pH did fall slightly, from a weakly alkaline (8.00) pH to a weakly acid (6.76) pH (Table 5).

A decrease in pH increases Pb desorption from soil constituents resulting in increased Pb concentration in the soil solution. 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 10EDTA but insignificant between 5EDTA and 10EDTA, showing that 5EDTA was enough to avoid possible metal chelate movement into groundwater and the effect of remaining EDTA on the soil microorganisms.

EDTA exhibited heavy metals extraction efficiency. The lead BCFs of the plant species varied between 0.57 and 3.56, with the lowest BCF in 2.5EDTA for stem and the highest BCF in 10EDTA for the root (Table 6). BCF values for Cr in *S. maritimus* L. 0.45 were the lowest and 2.23 the highest. TFs of the metals are presented in Table 6. The highest values (root to leaf) were recorded in 10EDTA with around 0.30 and 0.28 for Pb and Cr respectively.

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

The results showed a significant correlation between treatment time and heavy metal concentrations in the plant organs (Table 7). Metals concentration in the plant organs of *S. maritimus* L. increased significantly (p<0.05) with passage of time. The most significant increase in Pb and Cr concentration in the plant organs occurred on 90th days of EDTA application. However the maximum Pb and Cr in the plant organs was observed on 90th day of chelating application, there was no significant difference between concentrations of heavy metals in the plant tissues on 60d and 90d (Table 7).

The soil Pb and Cr concentrations were 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.

At present study, treatment time dependent experiment showed that harvesting the shoots of the plant on 60 day after the first harvest could achieve highest phytoextraction efficiency. Consequently, early harvest may not be effective in terms of removing maximum amount.
Table 5: Physio-chemical analysis of soil after treatment by EDTA

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>EC (dS/m)</th>
<th>Pb (mg/kg)</th>
<th>Cr (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8.00±0.01*</td>
<td>3.60±0.21*</td>
<td>ND (0.02)</td>
<td>ND (0.002)</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>7.60±0.01b</td>
<td>4.66±0.22b</td>
<td>120.29±1.22b</td>
<td>73.42±1.220b</td>
</tr>
<tr>
<td>5 EDTA</td>
<td>7.42±0.01bc</td>
<td>4.76±0.22b</td>
<td>139.07±1.45b</td>
<td>121.34±1.43b</td>
</tr>
<tr>
<td>10 EDTA</td>
<td>6.76±0.01c</td>
<td>5.42±0.30c</td>
<td>143.71±1.24c</td>
<td>132.46±1.56c</td>
</tr>
</tbody>
</table>

*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, Duncan test)

Table 6: Pb and Cr extraction efficiency

<table>
<thead>
<tr>
<th>Metal</th>
<th>Treatments</th>
<th>BCFroot</th>
<th>BCFstem</th>
<th>BCFleaf</th>
<th>BCFrhizome</th>
<th>TFroot</th>
<th>TFstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>1.24±0.100b</td>
<td>0.57±0.01b</td>
<td>0.65±0.01b</td>
<td>1.04±0.10b</td>
<td>0.21±0.01b</td>
<td>0.15±0.01b</td>
<td></td>
</tr>
<tr>
<td>5 EDTA</td>
<td>2.50±0.15b</td>
<td>0.64±0.01b</td>
<td>1.44±0.07a</td>
<td>1.70±0.10b</td>
<td>0.27±0.01ab</td>
<td>0.20±0.01a</td>
<td></td>
</tr>
<tr>
<td>10 EDTA</td>
<td>3.56±0.20b</td>
<td>1.11±0.07a</td>
<td>1.59±0.09a</td>
<td>2.02±0.22b</td>
<td>0.30±0.01a</td>
<td>0.25±0.01a</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>0.92±0.01b</td>
<td>0.45±0.01b</td>
<td>0.57±0.01b</td>
<td>0.77±0.03b</td>
<td>0.18±0.01b</td>
<td>0.15±0.04b</td>
<td></td>
</tr>
<tr>
<td>5 EDTA</td>
<td>1.42±0.01a</td>
<td>0.72±0.02ab</td>
<td>1.13±0.06a</td>
<td>1.33±0.05b</td>
<td>0.23±0.01ab</td>
<td>0.23±0.04b</td>
<td></td>
</tr>
<tr>
<td>10 EDTA</td>
<td>2.23±0.01a</td>
<td>0.80±0.02a</td>
<td>1.72±0.06a</td>
<td>1.50±0.06b</td>
<td>0.28±0.01a</td>
<td>0.27±0.04b</td>
<td></td>
</tr>
</tbody>
</table>

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

Table 7: Effect of treatment time of 5EDTA on heavy metals uptake and correlation coefficients

<table>
<thead>
<tr>
<th>Metals</th>
<th>30d</th>
<th>60d</th>
<th>90d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>0.82**</td>
<td>96.34±2.77a</td>
<td>0.76</td>
</tr>
<tr>
<td>Steam</td>
<td>0.85**</td>
<td>53.21±1.56a</td>
<td>0.76</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.86**</td>
<td>62.18±1.78a</td>
<td>0.90</td>
</tr>
<tr>
<td>Rhizome</td>
<td>0.90**</td>
<td>87.54±2.17a</td>
<td>0.85</td>
</tr>
<tr>
<td>Soil</td>
<td>-0.90**</td>
<td>130.46±2.94a</td>
<td>-0.69</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>0.85**</td>
<td>79.64±1.24a</td>
<td>0.96</td>
</tr>
<tr>
<td>Steam</td>
<td>0.76**</td>
<td>49.32±1.14a</td>
<td>0.90**</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.88**</td>
<td>57.68±1.17a</td>
<td>0.88**</td>
</tr>
<tr>
<td>Rhizome</td>
<td>0.93**</td>
<td>70.34±1.09a</td>
<td>0.96</td>
</tr>
<tr>
<td>Soil</td>
<td>-0.90**</td>
<td>114.12±2.42a</td>
<td>-0.79</td>
</tr>
</tbody>
</table>

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

*The probability level of p<0.05; **The probability level of p<0.01

3.4. Effect of EDTA treatment methods on heavy metals uptake

The relations between the application methods of EDTA (5mmol/kg) and heavy metals concentrations are shown in Table 8. Generally, the correlation coefficients between the application method of EDTA and Pb concentration in the plant organs and the soil are better than those in Cr.

Negative correlations were obtained between Pb and Cr concentrations in the plant organs of S. maritimus L. and methods of application, which indicated that there was reduction in the metals content in the plant organs according to different application methods (p<0.05). The maximum Pb and Cr concentration in the organs of the plant species was calculated at single dosage. It was seen that during three separate application methods, metals content in the plant tissues reached at its minimum concentration, while Pb concentration in the plant organs did not vary considerably when single and double dosages were added (p<0.05).

The liner relationship between the soil concentrations of heavy metals and the treatment mode was positive (Table 8). 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 8) indicated that under single dosage application, heavy metals content in the soil reached at its minimum concentration. Pb and Cr content increased by 39.36 % and 27.01% respectively when double dosage was added. This increase was calculated in triple dosage 56.83% and 49.86% for Pb and Cr respectively. However, different application methods influenced the Pb uptake, there was no significant effect between double and triple dosage on the Pb content of the soil. The results indicated that the chelate application method could limit the solubility and migration potential of heavy metals in the soil.
4. DISCUSSION

Results indicate the reduction for the measured growth parameters and the root elongation was more sensitive to metals. An excess of particular metal above the critical level of accumulation may impair the health of plant or may even kill it. Metals would have a toxic effect on the normal metabolism of the plant. Plant growing on soils with higher metals value, exhibit signs of unhealthy growth (Rout and Das, 2003). The morphology of the plants differs to some extent from the plants growing on normal soil. Leaves of these plants are greenish yellow in color, less glossy, more fragile and have crenulations of higher amplitudes along the leaf margins compared to the leaves of the similar species growing on soils without any metal enrichment. The roots of these plants that grow on high metals content soil are smaller dimensions with relatively thinner barks and in lesser quantities than from stems of those plants that growing on normal soils (Ashraf et al., 2011). It seems that damage to the plasmalemma of roots cells constitutes the first effect of metals toxicity, causing a loss of ions, such as K, and other solutes ( Woolhouse and Walker, 1981). Thus, the degree of metals tolerance may depend on the capacity of the plant to prevent these effects and one of the explanations for roots to be more responsive to toxic metals in environment might be that roots were 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 the plant (Xiong, 1998).

In this study, decrease in shoot growth and dry weight in contaminated soil was evident. Peralta et al. (2004) reported that reduction in chlorophyll could diminish the growth of above ground organs and decreasing in dry biomass might be due to toxic metals decrease water absorption in plant tissues causing undesirable impacts in plant growth.

*S. maritimus* L. accumulates metals in root. In this study, the fact that roots showed high accumulation of elements could imply relatively high availability in the soil. Although higher root metals contents were expected, as the dominant uptake pathway of the 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 the shoots, which suggests that the plants adopt either external or internal exclusion mechanisms to hinder translocation of metals to the aerial tissues (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. 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 (Macek et al., 2000).

In this study, metals in *S. maritimus* L. were accumulated in the root with BCF of >1. Plants can tolerate high heavy metals concentration from soil by two basic strategies. The first strategy is called accumulation strategy where metal can accumulate in plants at both high and low concentration from soil. These plants are capable of rendering the metals in various ways, for instance by binding them to cell walls, compartmentalizing them in vacuoles or complexing them to certain organic acids or proteins (Reeves and Baker, 2000). The second strategy is called exclusion strategy, where transport of heavy metals in shoots and leaves is limited over a wide range of metal concentrations in the soil. Some of the plants make stable metal complexes in the root cells to prevent metal translocation from the roots to aboveground tissues (Tordoff et al., 2000).

The results showed that the plant species had BCFroot values of >1 indicate high efficacy in the phytostabilization of metal contaminated soils and the plant has adopted an exclusion strategy and there is less risk of metals entering the food chain if the plant is consumed.

An important characteristic as a hyperaccumulator is the translocation factor (TF). Usually, it can indicate the ability of metal transferring from the 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 (Yoon 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), 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 reduce the likelihood of metals entering into the food chain. Therefore, the use of metal tolerant native flora represents an inexpensive long-term solution.

A gradual increase in EC and available metals content were observed with increasing concentration of EDTA and pH did fall slightly. Soil pH is an important parameter in determining the effectiveness of applied EDTA in enhancing metals uptake (Bareen and Tahira, 2010). Therefore, the efficiency of EDTA on the metal solubilization and accumulation can be enhanced by lowering the soil pH (Bareen and Tahira, 2010). 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 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, of course, the conditions of the process itself (e.g., pH, temperature, phase ratio, agitation, extraction time) (Liphadzi and Kirkham, 2005). Among these factors, pH is one of the most important parameters since it governs speciation, complexation and solubility as well as bioavailability and transport of heavy metals (Nowack et al., 2006), therefore metal uptake can be affected by application of EDTA due to low acidity.

EDTA exhibited heavy metals extraction efficiency. Wu et al. (1999) evaluated the effects of different EDTA dosages (0, 3, 6, 12mmol/kg) on leaching of different heavy metals (Cd, Cu, Pb, 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 12mmol/kg EDTA.

The results showed a significant correlation between treatment time and heavy metal concentrations in the plant organs and metals concentration in the plant organs of S. maritimus L. increased with passage of time. Harvest time had positive influences on the soil metals reduction.

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).

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 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.

The amounts of soil metals reached at their maximum at triple dosage and under single dosage application, heavy metals content in the soil reached at its minimum concentration. The results indicated that the 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 for enhanced 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.

Grčman et al. (2001) reported that single dose of 2.9g EDTA/kg 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 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. Wenzel et al. (2003) assessed the effects of dosage (up to 2.01g/kg) 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 metals 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.

5. CONCLUSION

The results revealed that heavy metals concentrations in S. maritimus L. depend significantly on the kind of plant organs. Underground 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 phytoextraction because it had BCF\text{root} values \(\geq 1\) 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 chelator 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 5mmol/kg a single dosage for 60 days in Pb and Cr contaminated soils.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Range and Watershed Management, University of Zabol (Iran) for providing necessary facilities to undertake this study.

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