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Use of *Festuca ovina* L. in Chelate Assisted Phytoextraction of Copper Contaminated Soils

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Abstract. *Festuca ovina* L. is a hyperaccumulating plant which has aroused considerable interest with respect to its possible use for phytoremediation of contaminated soils. This study has been conducted to evaluate the potentials of *F. ovina* L. to serve as a phytoremediation plant in the cleaning up of Cu in the polluted soils and to identify extraction efficiency of Ethylene Diamine Tetraacetic Acid (EDTA) for desorbing copper in relation to chelator dosage. Seeds have been sown in control and Cu contaminated pots (artificially contaminated with 150 mg kg\(^{-1}\) Cu). Results revealed that Cu negatively affected growth and tolerance indices of *F. ovina* and the root length was the most sensitive parameter among all measured parameters. The treatments used for assessing EDTA efficiency were 1.5, 3, 6, 15+1.5, 3+3 mmol kg\(^{-1}\), control (C: uncontaminated soil without EDTA) and W (contaminated soil without EDTA). Results showed that the application of 1.5 mmol kg\(^{-1}\) of EDTA did not significantly improve the phytoextraction of Cu and statistically, there was no significant difference in Cu uptake between single and split applications of 1.5 mmol kg\(^{-1}\) of EDTA. A sharp increase in root Cu concentration was observed when 3 mmol kg\(^{-1}\) of EDTA was applied. The highest amount of Cu extracted for the plant tissues was achieved at the doses of 6 mmol kg\(^{-1}\) and 3+3 mmol kg\(^{-1}\) EDTA, respectively. Higher Remediation Factors (RF) were obtained for the plants grown in contaminated soil and the highest RFs (0.08% and 0.07%) were recorded after the addition of 6 and 3+3 mmol kg\(^{-1}\), respectively. Application of EDTA showed a relatively decrease in TI (Tolerance Index) value and the lowest value of TI was recorded in 6 mmol kg\(^{-1}\) EDTA treatment. According to the experiment, EDTA has appeared to be an efficient amendment when Cu phyto-extraction with *F. ovina* was addressed. But further studies would be needed on investigating the reduction of percolation risk by the amount and process of chelate application.

**Key words:** EDTA, Metal tolerance, Phytoremediation, Soil pollution
Introduction
Contamination of soil with Potentially Toxic Elements (PTE) is a worldwide concern. Many methods including removal, incineration and removal followed by thermal desorption have been used for the cleanup of contaminated soils (Joner and Leyval, 2001), but most of them are expensive and technically complicated and cause additional adverse side effects on the environment (Cunningham and Ow, 1996).

Therefore, phytoremediation is a promising technology in cleanup of polluted sites using plants to restore the deteriorated soils, ground water or surface water due to less destructive effects, low cost and environmentally friendly nature (Wang et al., 2012; Zhao and McGrath, 2009).

It can be categorized into two different approaches: i) phytoextraction: metal accumulating plants are planted in contaminated soil and later harvested in order to remove metals from the soil (Salt et al., 1995; Yoon et al., 2006; Usman and Mohamed, 2009) and ii) phytostabilization; metal-tolerant plants are used to reduce the mobility of metals. Thus, metals can be stabilized in the substrate (Salt et al., 1995; Abdel-Ghani et al., 2007; Antosiewicz et al., 2008).

Among all types of phytoremediation addressed for metals’ pollution, phytoextraction has received an increasing attention starting from the discovery of hyper-accumulator plants that are able to concentrate high levels of specific metals in the harvestable biomass. Few plant species may be discussed as hyper-accumulators of various metals (Vamerali et al., 2010) and these plants can accumulate very high concentrations of metals in their tissues besides normal levels found in most species (Baker and Brooks, 1989).

Although hyper-accumulators 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, chelant-assisted phytoextraction is proposed as an alternative in metal phytoextraction by applying chelant and using high biomass plants to enhance metal removal (Leštan et al., 2008; Lui et al., 2005). Among chelators, EDTA (Ethylene Diamine Tetra Acetic Acid) was found as the most efficient one in increasing the concentration of water-soluble heavy metals (Ebrahimi, 2014; Wu et al., 1999; Blaylock et al., 1997). Huang et al. (1997) further proved that among five chelating agents such as (trans-1, 2-Cyclohexylene Ditrilo Tetraacetic Acid (CDTA), Diethylene Triamine Pentaacetic Acid (DTPA), EDTA, Ethylenebis (Oxyethylenetirinitrilo) Tetraacetic Acid (EGTA), Nitrilo Triacetic Acid (NTA), EDTA was the most efficient one in increasing shoot lead concentration in both peas and corns.

In this way, the ability of the plant species Festa ovina L. to accumulate Pb in its tissue has been well documented (Terry and Bañuelos, 2000; Prasad and De Oliveira-Freitas, 2003; Reeves, 2006). Indeed, F. ovina is a Pb-hyperaccumulator being able to accumulate at least 1000 mg kg⁻¹ Pb in its shoot dry matter (Álvarez et al., 2003). However, available data about its natural ability to accumulate copper are currently very few.

This study has been done in order to investigate the effects of Cu on morphological characters (germination, biomass, root and shoot length) of Festa ovina as a Pb-hyper-accumulotor plant and the ability of EDTA (sodium salt) in enhancing the uptake and phytoextraction of copper under greenhouse conditions.

Materials and Methods
Soil preparation
Soil (clay loam) of research farm of agricultural faculty located near Sistan
dam (25 km far from Zabol, Iran) was used as substrate for the plant in this study. The soil was air-dried, homogenized and sieved through a 4 mm stainless sieve before analysis. Chemical analyses of soil have included total N (Kjeldahl method), total P (molybdenum blue method), total K (Flame photometry method), pH (1:1 soil/ water ratio, Model 691), EC (solid: the deionized water= 1:2 w/v, Model DDS-307), CEC (Cation Exchange Capacity), organic carbon (Walkley-Black method) and CaCO$_3$ equivalent (Black, 1965; Olsen and Sommers, 1982; Berry et al., 1946; Thomas, 1996; Rhoades, 1996; Bower and Hatcher, 1966; Nelson and Sommers, 1996; Black et al., 1965) as they have been shown in Table 1. Concentration of copper extractable with 1M ammonium acetate-EDTA (pH 4.60) was 5.13 mg kg$^{-1}$ Cu. The value is normal for uncontaminated soil in the area.

Pot experiments were performed during March–April in greenhouse conditions (university of Zabol, Iran). After sieving (2mm), the soil was prepared by homogenizing aliquots of 100 kg in a concrete mixer with CuSO$_4$ 5H$_2$O (150 mg kg$^{-1}$). Soil samples were left to equilibrate for a period of two weeks before being remixed and used for the experiment. This procedure was adopted in order to reproduce the process of metal sorption by the soil.

The pots (diameter 15 cm × diameter 10 cm × height 40 cm) were filled with 5 kg of air-dried soil and then, they were brought to 2/3 of field capacity with the deionized water. Subsequently, seeds of F. ovina were sown in the pots. Each treatment consisted of 15 seeds in five replicates. Seeds’ surfaces were sterilized by soaking in a 5% of sodium hypochlorite solutions for five minutes prior to us; then, they were rinsed three times and soaked in the distilled water for 5 minutes. The pots were irrigated during the germination period. The necessary light for the growth of the plants was obtained from the sun. The pots were placed behind the glass windows of the greenhouse and received the solar light during the experiment. Temperature was ranged 21 to 26°C. Considering the duration of the pot experiments, all pots were fertilized with a mineral fertilizer solution to avoid limiting nutritional conditions. The nutrient solution contained 1.00 g of N (2.86 g of NH$_4$NO$_3$) per pot. In each pot, 4 uniform seedlings were retained and the others were harvested. Final germination percent (number of germinated seeds in each pot), germination rate (a measure of germination speed with lower values indicating faster germination) (Maguire, 1962) (Equation 1), the plant dry weight, tolerance index formed the following equation (Wilkins, 1978)(Equation 2):

$$RG = \frac{\sum ND}{\sum N} \quad \text{(Equation 1)}$$

$$\text{Tolerance index}= \frac{\text{dry weight of the plants grown in heavy metal solution}}{\text{dry weight of the plants grown in control solution}} \quad \text{(Equation 2)}$$

Length of shoots and length of the roots were determined, and the changes in these parameters were used to evaluate Cu toxicity.

In second step, EDTA was applied to the pots having uniform seedlings grown in contaminated soil in the form of sprinkling solutions (1.5, 3, 6, 15+1.5, 3+3 mmol kg$^{-1}$), control C (uncontaminated soil without EDTA) and W (contaminated soil without EDTA). 1.5+1.5 of EDTA and 3+3 of EDTA received second application for 10 days after the initial treatment. The solutions of EDTA were prepared from a disodium salt dehydrate of EDTA (C$_{10}$ H$_{14}$ N$_2$ Na$_2$ O$_8$ 2H$_2$O). At the end of the experiment (after 2 weeks), the shoots were separated from the roots. The plant roots and shoots were washed twice with the distilled water (acidified to pH 4.0 with HCl) and then, they were washed with the deionized water. The samples were oven-dried (MEMMERT UNB 400) at 70°C for 24h to obtain the dry weight, and then
ground to a fine powder. For analysis, dry plant material was digested in a mixture of HNO₃/HClO₄ (3/1, v/v) at 150 °C for 2h and 210 °C for 1h and then dissolved in HCl (0.5 N) (Abrisqueta and Romero, 1969). The concentration of Cu in the extracts was analyzed by flame atomic absorption spectrometry (KONIK (WON 300) BURKE). The methodology for metal concentrations in the soil was referred using the SRM 2711 (Institute of Standard and Technology, USA) and methodology for metal concentrations in the plant was referred using BCR-060 (Institute for Reference Materials and Measurements, Belgium). In order to compare the phytoextraction efficiency of the studied plant after the addition of different EDTA concentrations, the Remediation Factor (RF) (Vysloužilová et al., 2003) was calculated as follows (Equation 3):

\[
RF = \frac{\text{Cu}_{\text{plant}} \times B_{\text{plant}}}{\text{Cu}_{\text{soil}} \times W_{\text{soil}}} \times 100% \quad \text{(Equation 3)}
\]

Where

\( \text{Cu}_{\text{plant}} \) is the content of Cu in the plant dry biomass (mg kg\(^{-1}\)), \( B_{\text{plant}} \) the dry weight plant’s biomass yield (g), \( \text{Cu}_{\text{soil}} \) the total content of Cu in the soil (mg kg\(^{-1}\)), and \( W_{\text{soil}} \) the amount of soil in the pot (g). The RF reflects the amount of Cu extracted by the plant from the soil during one cropping season.

### Statistical analysis

Statistical analyses of the experimental data were performed using the SPSS18. All reported results are the means of five replicates and deviations were calculated as the Standard Error of the Mean (SEM). The statistical processing was mainly conducted by the analysis of variance (ANOVA) and T-test. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as a significant one. Correlations between amendment concentration, dry weight production and tissues heavy metal concentrations were evaluated using Pearson’s correlation coefficient.

### Results and Discussion

#### Cu tolerance and growth

The reduction observed for all measured growth parameters before EDTA application was significant (p<0.05) (Table 2). Significant differences were found in seed germination rate and percent in the studied plant species. The presence of Cu contamination treatment significantly (p<0.05) decreased the germination of plant (Table 2). It was evident that Cu negatively affected the plant growth and the plants grown in the control treatment exhibited significantly higher dry weight than those determined for Cu treatment.

Results showed that the root length was the most sensitive parameter among all the measured ones. The root length was 94.45 mm in the control treatment, but reached 56.14 mm in Cu treatments (40.56% reduction). With respect to the control, the shoot growth was 55.50 mm for the Cu treatment giving a 23.17% reduction of the shoot length. The tolerance index showed that the plant species was sensitive to Cu and it was 100% in the control treatment whereas it was only 62% in Cu treatment.

### Table 1. Chemical and physical characteristics of soil

<table>
<thead>
<tr>
<th>Texture</th>
<th>CEC (meq)</th>
<th>K(_{\text{sat}}) (%)</th>
<th>P(_{\text{sat}}) (%)</th>
<th>N(_{\text{sat}}) (%)</th>
<th>OC (%)</th>
<th>EC (dSm(^{-1}))</th>
<th>pH</th>
<th>CaCO(_3) (%)</th>
<th>Cu (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay loam</td>
<td>39.00</td>
<td>0.37</td>
<td>0.51</td>
<td>0.15</td>
<td>0.15</td>
<td>3.43</td>
<td>8.00</td>
<td>12.34</td>
<td>5.13</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Statistical analyses of the experimental data were performed using the SPSS18. All reported results are the means of five replicates and deviations were calculated as the Standard Error of the Mean (SEM). The statistical processing was mainly conducted by the analysis of variance (ANOVA) and T-test. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as a significant one. Correlations between amendment concentration, dry weight production and tissues heavy metal concentrations were evaluated using Pearson’s correlation coefficient.
Germination tests are used to quickly indicate the plant response to environmental factors (Archambault and Winterhalder, 1995). The present study showed that seeds had a lower germination percent in the soil containing Cu; very high percent of germination was recorded in Cu free soil. Similar observations were found by Archambault and Winterhalder (1995) in Agrostis scabra where they found that the germination of seeds from control treatment was drastically reduced on contaminated soil. Samantaray et al. (1996) reported high concentration of metals like chromium and nickel hampered seed germination of Echinochloa colona in solution culture.

Some parameters such as biomass and rates of shoot and root growth have been used to evaluate metal toxicity in plants (Baker and Walker, 1989). However, for F. ovina, root elongation was more sensitive to Cu than the rate of shoot growth or plant dry weight. Similar results have also been observed in Sesamum indicum (Kumar et al., 2008), Sinapis alba (Fargasova, 1994) and lettuce and radish (Nwosu et al., 1995).

The mechanisms underlying the phytotoxic effects of heavy metals are not fully understood. However, it seems that damage to the plasma lemma of roots cells constitutes the first effect of metals toxicity (Woolhouse, 1983) 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 from this effect (Ait Ali et al., 2004). One of the explanations for the roots to be more responsive to toxic metals existing in the environment might be the fact that roots were the specialized absorptive organs so that they were affected earlier and subjected to the accumulation of more heavy metals than any of other organs. This could also be the main reason that root length was usually used as a scale for determining heavy metal tolerant ability of plant (Xiong, 1998).

Decrease in shoot growth and dry weight in contaminated soil was evident as compared to the control treatment. Peralta et al. (2004) reported that the reduction in chlorophyll could diminish aboveground organs growth and decrease in dry biomass might be due to toxic metals’ decreased water absorption in plant tissues causing undesirable impacts on 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).

### Table 2. Morphological characteristics for F. ovina at the end of growing trail before EDTA application

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination Rate (%)</th>
<th>Germination Percentage</th>
<th>Dry Weight (g)</th>
<th>Root Length (mm)</th>
<th>Shoot Length (mm)</th>
<th>Tolerance Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00±4.00</td>
<td>100.00±4.02</td>
<td>8.51±0.62</td>
<td>94.45±3.30</td>
<td>72.24±4.10</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>Contaminated soil</td>
<td>61.70±3.00</td>
<td>54.32±2.30</td>
<td>5.31±0.07</td>
<td>56.14±2.10</td>
<td>55.50±3.10</td>
<td>0.62±0.01</td>
</tr>
</tbody>
</table>

Values (±SE) within a column followed by the different letter are significantly different according to the T-test (p<0.05)

### Cu content in the plant organs

Concentrations of Cu in shoots and roots are shown in Table 3. The lowest extractable Cu in plant organs with the average values of 8.77 and 30.90 mg kg⁻¹ were obtained for control and contaminated soil treatments, respectively. The values are normal for the plant species.

The application of 1.5 mmol kg⁻¹ of EDTA did not significantly improve the phytoextraction of Cu regarding the plant species. It may be speculated that the treatment was insufficient to break down the uptake barriers of the plant under the experiment conditions and there was no statistically significant difference in Cu uptake between single and split
applications of 1.5 mmol kg\(^{-1}\) EDTA. A sharp increase in root Cu concentration was observed when 3 mmol kg\(^{-1}\) EDTA was applied. The highest amount of Cu extracted for both root and shoot was achieved at the doses of 6 and 3+3 mmol kg\(^{-1}\) EDTA, respectively. Considering the dry matter yield of the plant, Cu concentration of underground part was higher than that in aboveground part. It seemed from the results that the root cells of \(F.\ ovina\) were able to accumulate more Cu.

The plant dry biomass yield after two weeks growth in the pots was supplemented with various contents of EDTA (Table 3) when no chelate was added to the soil (control). Plant showed normal development without visual symptoms of toxicity but the plant grown in contaminated soil and A1 (1.5 mmol kg\(^{-1}\) EDTA) treatments produced half of biomass yields as compared to the plants grown in uncontaminated soil control.

Dry weight did not significantly change after 1.5+1.5 and 3 mmol kg\(^{-1}\) EDTA addition as compared to the 1.5 mmol kg\(^{-1}\) EDTA. However, the addition of 3+3 and especially, 6 mmol kg\(^{-1}\) EDTA significantly decreased biomass yields of the plant and the dry weight decreased to 65.52 and 71% of the control plants, respectively. Serious growth suppression upon EDTA addition at higher doses indicates that the plant was subjected to copper stress.

Correlations between amendment concentration, dry weight production and tissues heavy metal concentrations were evaluated using Pearson’s correlation coefficient (Table 4). A negative correlation was obtained between dry weight and both Cu\(_{\text{shoot}}\) and Cu\(_{\text{root}}\) concentration (Table 4). However, it was not significant. Effects of EDTA on the plant growth were visible through the negative and significant correlations between the EDTA and the dry weight production of the plant species (Table 4).

Majority of metals taken up by roots are bound to carboxyl groups of mucilage uronic acids (Morel \textit{et al.}, 1986) and once absorbed by roots, Pb is rather immobile showing very limited translocation into above-ground foliage (Wilde \textit{et al.}, 2005).

Treatment of soil with EDTA increased the mobility of Cu in the soil solution and the maximum extractable metal was observed in 6 mmol kg\(^{-1}\) EDTA treatment. The efficiency of removing heavy metals using plant-based remediation strategies depends on the availability of target heavy metals in the soil solution also referred as the bioavailable fraction. The bioavailability of heavy metals within these pools can be enhanced upon the application of mobilizing agents such as EDTA (Papassiopi \textit{et al.}, 1999; Hong and Jiang, 2005). Soil pH is one of the effective mechanisms in increasing the uptake of metals from the soil by plant (Sauve \textit{et al.}, 1998). Some soil properties such as pH and total metal concentration may affect the efficiency of a chelating agent (Jones and Williams, 2001).

Application of EDTA showed a relatively slow growth in the plant at high doses. The growth reduction after the 3+3 and 6 mmol EDTA kg\(^{-1}\) treatment is probably due to high contents of Cu mobilized in the plant organs (Table 3) and to some extent, the toxicity of free EDTA, if present (Vassil \textit{et al.}, 1998).

Turgut \textit{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 high concentrations of EDTA are toxic for the 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 (Grčman \textit{et al.}, 2003; Luo \textit{et al.}, 2006).
Neugschwandtner et al. (2007) showed that although the phytoextraction of Pb and Cd using single EDTA and split EDTA applications in an agricultural field increased the mobility of target heavy metals in the soil solution and metal uptake by Zea mays, dry biomass production was significantly reduced.

**Table 3.** Effects of the application of chelator on concentration of Cu in the plant tissues (mg kg\(^{-1}\)) and dry weight (g) at the end of growing trial

<table>
<thead>
<tr>
<th>Treatments</th>
<th>(\text{Cu}_{\text{shoot}}) (mg kg(^{-1}))</th>
<th>(\text{Cu}_{\text{root}}) (mg kg(^{-1}))</th>
<th>Seedling Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.77 ±0.55 d(^B)</td>
<td>14.97 ±1.76 d(^B)</td>
<td>25.00 ±1.24 a</td>
</tr>
<tr>
<td>Contaminated soil</td>
<td>30.90±3.00 c(^B)</td>
<td>62.73±3.21 c(^A)</td>
<td>13.66±1.10 b</td>
</tr>
<tr>
<td>1.5 EDTA</td>
<td>36.10±3.20 c(^B)</td>
<td>73.83±4.10 c(^A)</td>
<td>13.24±1.12 b</td>
</tr>
<tr>
<td>3.0 EDTA</td>
<td>90.09±6.10 b(^B)</td>
<td>187.25±6.10 b(^A)</td>
<td>12.99±1.00 b</td>
</tr>
<tr>
<td>6.0 EDTA</td>
<td>154.0±7.00 a(^B)</td>
<td>249.11±7.0 a(^B)</td>
<td>7.25±0.70 c</td>
</tr>
<tr>
<td>1.5+1.5 EDTA</td>
<td>40.22±3.20 c(^B)</td>
<td>82.38±3.20 c(^A)</td>
<td>12.52±1.11 b</td>
</tr>
<tr>
<td>3.0+3.0 EDTA</td>
<td>111.6±7.2 b(^B)</td>
<td>194.17±7.12 b(^A)</td>
<td>8.62±0.75 c</td>
</tr>
</tbody>
</table>

Values shown are the means±SE. Different capital letters in each rows indicate significant differences between organs. Different lower case letters in each column indicate significant differences between treatments (p<0.05, Duncan test).

**Table 4.** Pearson’s correlation coefficients between chelating concentrations, Cu concentration in the plant tissues and Dry Weight (DW)

<table>
<thead>
<tr>
<th></th>
<th>Dry Weight</th>
<th>(\text{Cu}_{\text{shoot}})</th>
<th>(\text{Cu}_{\text{root}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(_{\text{shoot}})</td>
<td>-0.32(^*)</td>
<td>-0.35(^*)</td>
<td>0.18(^*)</td>
</tr>
<tr>
<td>Cu(_{\text{root}})</td>
<td>-0.52(^*)</td>
<td>0.77(^**)</td>
<td>0.80(^**)</td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) not significant, \(^*\) significant at the 0.05 probability level, \(^**\) significant at the 0.01 probability level.

**Phytoextraction efficiency of the plant species**

Higher Remediation Factors (RF) were obtained for the plants grown in contaminated soils compared to control one due to higher Cu contents in the plant organs (Table 5). The highest RFs (0.08% and 0.07%) were recorded after the addition of 6 and 3+3 mmol kg\(^{-1}\) EDTA, respectively. However, this phytoextraction efficiency is not high enough to remediate Cu contaminated soil in a reasonable time and without any unwanted side effects such as the increased leaching of heavy metals–EDTA complexes into the ground water, successfully. Therefore, any further increases of EDTA concentrations would have rather negative effects such as downward leaching of heavy metals–EDTA complexes, higher toxicity for plants and micro-organisms (Komárek et al., 2007).

Application of EDTA showed a relatively decrease in TI (Tolerance Index) value. The lowest value of TI was recorded in 6 EDTA-treated and it might be the greater toxic effects of Cu and EDTA on the plant. Maximum TI was found in the control treatment that showed significant difference at 5% level. The value of TI=1 when there is no influence of treatment on the growth; it is higher than 1 when there is a favorable effect of sludge on the growth and lower than 1 when the growth is affected negatively by the treatment (Zaier et al., 2010). However, the concentration of EDTA enhanced significantly root and shoot accumulations of Cu from the soil while EDTA applied at larger rates could result in the contamination of ground water due to the enhanced solubilization and leaching of metals as well as metal–EDTA complexes (Saifullah et al., 2009).
Table 5. Remediation Factors (RF) and Tolerance Index (TI) of *F. ovina* grown on the studied soils

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RF (%)</th>
<th>TI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.00±0.20 a</td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.04±0.01 b</td>
<td>0.84±0.10 b</td>
</tr>
<tr>
<td>1.5EDTA</td>
<td>0.04±0.01 b</td>
<td>0.83±0.10 b</td>
</tr>
<tr>
<td>3EDTA</td>
<td>0.04±0.01 b</td>
<td>0.80±0.10 b</td>
</tr>
<tr>
<td>6EDTA</td>
<td>0.08±0.01 a</td>
<td>0.51±0.10 c</td>
</tr>
<tr>
<td>1.5+1.5</td>
<td>0.05±0.01 b</td>
<td>0.80±0.10 b</td>
</tr>
<tr>
<td>3+3</td>
<td>0.07±0.01 a</td>
<td>0.62±0.10 c</td>
</tr>
</tbody>
</table>

Values shown are the means±SE. Different letters in each column indicate significant differences between treatments (p<0.05, Duncan test).

Conclusion

*F. ovina* chosen for this work can be adapted to a soil having relatively high levels of available Cu but Cu caused serious growth suppression of *F. ovina*. Pot experiment tried to overcome the phytoextraction limitations by adding EDTA to Cu polluted soil and results showed that increasing theamouts of EDTA resulted in an increase in root and shoot metal concentrations leading to the assumption that the plant suffered from Cu-EDTA stress. The maximum amount of extracted Cu was achieved by the applications of 6 and 3+3 mmol kg⁻¹ EDTA. The data suggest that high dose of EDTA has deleterious effects on plants growth. It is clear that total amounts of extracted metal will be more elevated in the presence of EDTA because this chelator enhanced metal concentration but we must apply the low dosage of EDTA (with respect to leaching risk). Further studies would be needed on investigating the reduction of percolation risk by the amount and process of chelate application and the use of more degradable alternatives to EDTA.

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Literature Cited


چکیده
یکی از گیاهان مرتعی بیشترندوز است که به توجه به قابلیت این گیاه در پالایش خاکهای آلوده مورد توجه بسیار می‌باشد. تحقیق حاضر جهت جستجوی کارایی پتانسیل F. ovina (EDTA) در خاکهای آلوده به مس و تبعیض کارایی غلظت‌های متفاوت ایالین در آمیز تری استیک اسید (EDTA) در جذب مس انجام گرفت. بدنه‌تر در خاکهای غیرآلوده و آلوده به مس (به‌شکل مصنوعی آلوده به 0/15 میلی‌گرم در کیلوگرم مس) کاشته شدند. نتایج نشان داد که مس تاثیر منفی بر رشد گیاه و شاخه- های برداری F. ovina های برداری مورد استفاده در ارزیابی کارایی EDTA الکل شامل 0/15/01/01/05/05/00/05 میلی‌مول بر کیلوگرم، کنترل (غیرآلوده بدون EDTA و (آلوده بدون EDTA بود. نتایج نشان داد که غلظت 0/15 میلی‌مول بر کیلوگرم تأثیر معنی‌دار بر استخراج مس نداشت و بین استفاده از این تیمار در غلظت- های یکی‌بار و دوبار مجدد تفاوت وجود نداشت. افزایش معنی‌دار در غلظت مس در ریشه گیاه در استفاده از تیمار 0/3 میلی‌مول در کیلوگرم مشاهده شد. بهترین فاکتورهای پالایش (0/07 و 2/07 درصد) به- ترتیب در تیمارهای 6 و 3 میلی‌مول در کیلوگرم مشاهده شد. کاربرد EDTA به‌طور مثبتی نسبی شاخص تولید گیاه (TIA) کاهش داد و حداقل مقدار این شاخص در تیمار 6 میلی‌مول بر کیلوگرم اندام‌گیری F. ovina شد. بطور کلی اعداد کارایی استخراج مس با کاربرد گونه اکستراید اما، بررسی‌های بیشتری می‌تواند مقدار و نحوه کاهش خطر آلودگی این ماده به آب‌های زیرزمینی لازم است.

کلمات کلیدی: F. ovina, الولگی خاک, تحلیل فلز، گیاه بالایی، آلولگی خاک

کاربرد و استخراج خاکهای آلوده به مس با استفاده از مواد کلات غنده
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