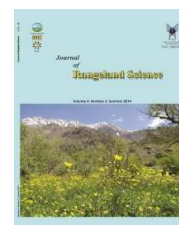




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Use of *Festuca ovina* L. in Chelate Assisted Phytoextraction of Copper Contaminated Soils

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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 mmolkg⁻¹, control (C: uncontaminated soil without EDTA) and W (contaminated soil without EDTA). Results showed that the application of 1.5 mmolkg⁻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 mmolkg⁻¹ of EDTA. A sharp increase in root Cu concentration was observed when 3 mmolkg⁻¹ of EDTA was applied. The highest amount of Cu extracted for the plant tissues was achieved at the doses of 6 mmolkg⁻¹ and 3+3 mmolkg⁻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 mmolkg⁻¹, respectively. Application of EDTA showed a relatively decrease in TI (Tolerance Index) value and the lowest value of TI was recorded in 6 mmolkg⁻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

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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), DiethyleneTriaminePentaacetic Acid (DTPA), EDTA, Ethylenebis (Oxyethylenetrinitrilo) 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 *Festuca 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-hyper-accumulator being able to accumulate at least 1000 mgkg⁻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 *Festuca ovina* as a Pb-hyper-accumulator 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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (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 \times diameter 10 cm \times 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_4NO_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):

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

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

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 ($\text{C}_{10} \text{H}_{14} \text{N}_2 \text{Na}_2 \text{O}_8 \cdot 2\text{H}_2\text{O}$). 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 $\text{HNO}_3/\text{HClO}_4$ (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):

$$\text{RF} = \frac{\text{Cu}_{\text{plant}} \times \text{B}_{\text{plant}}}{\text{Cu}_{\text{soil}} \times \text{W}_{\text{soil}}} \times 100\% \text{ (Equation 3)}$$

Where

Cu_{plant} is the content of Cu in the plant dry biomass (mgkg^{-1}), B_{plant} the dry weight plant's biomass yield (g), Cu_{soil} the total content of Cu in the soil (mgkg^{-1}) and W_{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. Tolerance Index (TI) based on the dry weight of the plant was chosen as the indicator of toxic effects of metal on the plant under different dose of EDTA treatments (Wilkins, 1978).

Table 1. Chemical and physical characteristics of soil

| Texture | CEC (meq) | K_{tot} (%) | P_{tot} (%) | N_{tot} (%) | OC (%) | EC (dSm^{-1}) | pH | CaCO_3 (%) | Cu (mgkg^{-1}) |
|-----------|--------------|--------------------------------|--------------------------------|--------------------------------|-----------|-----------------------------|------|------------------------|------------------------------|
| Clay loam | 39.00 | 0.37 | 0.51 | 0.15 | 0.15 | 3.43 | 8.00 | 12.34 | 5.13 |

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.

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

| Treatment | Germination Rate (%) | Germination Percentage | Dry Weight (g) | Root Length (mm) | Shoot Length (mm) | Tolerance Index |
|-------------------|--------------------------|--------------------------|------------------------|-------------------------|-------------------------|------------------------|
| Control | 100.00±4.00 ^a | 100.00±4.02 ^a | 8.51±0.62 ^a | 94.45±3.30 ^a | 72.24±4.10 ^a | 1.00±0.03 ^a |
| Contaminated soil | 61.70±3.00 ^b | 54.32±2.30 ^b | 5.31±0.07 ^b | 56.14±2.10 ^b | 55.50±3.10 ^b | 0.62±0.01 ^b |

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 mgkg⁻¹ were obtained for control and contaminated soil treatments, respectively. The values are normal for the plant species.

The application of 1.5 mmolkg⁻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 $3+3 \text{ mmol kg}^{-1}$ 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} of 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_{shoot} and Cu_{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 *et al.*, 1986) and once absorbed by roots, Pb is rather immobile showing very limited translocation into above-ground foliage (Wilde *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 *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 *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 $6 \text{ mmol EDTA kg}^{-1}$ 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 *et al.*, 1998).

Turgut *et al.* (2005) investigated the use of two EDTA concentrations for enhancing the bioavailability of cadmium, chromium and nickel in three natural soils (Ohio, New Mexico and Colombia). They reported that the EDTA level resulted in a higher total metal uptake but 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 *et al.*, 2003; Luo *et al.*, 2006).

Neugschwandtner *et al.* (2007) showed that although the phytoextraction of Pb and Cd using single EDTA and split EDTA 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 (mgkg^{-1}) and dry weight(g) at the end of growing trial

| Treatments | Cu_{shoot} (mgkg^{-1}) | Cu_{root} (mgkg^{-1}) | Seedling Dry Weight (g) |
|-------------------|--|---|-----------------------------|
| Control | $8.77 \pm 0.55^{\text{d-B}}$ | $14.97 \pm 1.76^{\text{d-B}}$ | $25.00 \pm 1.24^{\text{a}}$ |
| Contaminated soil | $30.90 \pm 3.00^{\text{c-B}}$ | $62.73 \pm 3.21^{\text{c-A}}$ | $13.60 \pm 1.10^{\text{b}}$ |
| 1.5 EDTA | $36.10 \pm 3.20^{\text{c-B}}$ | $73.83 \pm 4.10^{\text{c-A}}$ | $13.24 \pm 1.12^{\text{b}}$ |
| 3.0 EDTA | $90.09 \pm 6.10^{\text{b-B}}$ | $187.25 \pm 6.10^{\text{b-A}}$ | $12.99 \pm 1.00^{\text{b}}$ |
| 6.0 EDTA | $154.0 \pm 7.00^{\text{a-B}}$ | $249.11 \pm 7.0^{\text{a-A}}$ | $7.25 \pm 0.70^{\text{c}}$ |
| 1.5+1.5 EDTA | $40.22 \pm 3.20^{\text{c-B}}$ | $82.38 \pm 3.20^{\text{c-A}}$ | $12.52 \pm 1.11^{\text{b}}$ |
| 3.0+3.0 EDTA | $111.6 \pm 7.20^{\text{b-B}}$ | $194.17 \pm 7.12^{\text{b-A}}$ | $8.62 \pm 0.75^{\text{c}}$ |

Values shown are the means \pm 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)

| | Dry Weight | Cu_{shoot} | Cu_{root} |
|----------------------------|----------------------|----------------------------|---------------------------|
| Cu_{shoot} | $-0.32^{\text{n.s}}$ | | |
| Cu_{root} | $-0.35^{\text{n.s}}$ | $0.18^{\text{n.s}}$ | |
| EDTA | -0.52^* | 0.77^{**} | 0.80^{**} |

^{n.s} 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 mmolkg^{-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 $\text{TI}=1$ 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

| Treatments | RF(%) | TI (%) |
|------------|------------------------|------------------------|
| C | - | 1.00±0.20 ^a |
| W | 0.04±0.01 ^b | 0.84±0.10 ^b |
| 1.5EDTA | 0.04±0.01 ^b | 0.83±0.10 ^b |
| 3EDTA | 0.04±0.01 ^b | 0.80±0.10 ^b |
| 6EDTA | 0.08±0.01 ^a | 0.51±0.10 ^c |
| 1.5+1.5 | 0.05±0.01 ^b | 0.80±0.10 ^b |
| 3+3 | 0.07±0.01 ^a | 0.62±0.10 ^c |

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 the amounts 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

Abdel-Ghani, N. T., Hefny, M. and El-Chagbaby, G. A. F., 2007. Removal of lead from aqueous solution using low cost abundantly available adsorbents. *Jour. Envir. Sci. Tech.* 41: 67-73.

Abrisqueta, C. and Romero, M., 1969. Digestion húmeda rápida de suelos y materiales orgánicos. *Jour. Anales Edafol. Agrobiol.* 27: 855-867.

Ait Ali, N., Pilar Bernal, M. and Mohammed, A., 2004. Tolerance and bioaccumulation of cadmium by *Phragmites australis* grown in the presence of elevated concentrations of cadmium, copper, and zinc. *Jour. Aquat. Bot.* 80: 163-176.

Álvarez, E., Fernández Marcos, M. L., Vaamonde, C. and Fernández-Sanjurjo, M. J., 2003. Heavy metals in the dump of an abandoned mine in Galicia (NW Spain) and in the spontaneously occurring vegetation. *Jour. Sci. Total Environ.* 313: 185-197.

Antosiewicz, D.M., Escude-Duran, C., Wierzbowska, E. and Skłodowska, A., 2008. Indigenous plant species with the potential for the phytoremediation of arsenic and metals contaminated soil. *Jour. Water Air Soil Pollution.* 193: 197-210.

Archambault, D. J. and Winterhalder, K., 1995. Metal tolerance in *Agrostis scabra* from the Sudbury Ontario area. *Jour. Can Bot.* 73: 766-775.

Baker, A. J. M. and Brooks, R. R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery.* 1: 81-126.

Baker, A. J. M. and Walker, P. L., 1989. Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem. Spec. Bioavail.* 1: 7-17.

Berry, J. W., Chappell, D. G. and Barnes, R. B., 1946. Improved Method of Flame Photometry. *Ind. Eng. Chem. Anal. Ed.* 18(1): 19-24.

Black, C. A., 1965. Methods of soil chemical analysis and microbiological properties. *Agronomy No. 9.* American Society of Agronomy, Madison.

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- Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D. and Raskin, I., 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Jour. Environ. Sci. Technol.* 31, 860–865.
- Bower, C. A. and Hatcher, J. T., 1966. Simultaneous determination of surface area and cation-exchange capacity. *Jour. Soil Sci. Soc. Am.* 30: 525–527.
- Cunningham, S. D. and Ow, D. W., 1996. Promises and prospects of phytoremediation. *Jour. Plant Physiol.* 110: 715–719.
- Ebrahimi, M., 2014. The Effect of EDTA Addition on the Phytoremediation Efficiency of Pb and Cr by *Echinochloa crus-galli* (L.) P. Beauv. and Associated Potential Leaching Risk. *Jour. Soil and Sediment Contam.* 23 (3): 245–256. (In Persian).
- Fargasova, A., 1994. Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapis alba* seeds. *Bull. Environ. Contam. Toxicol.* 52: 452–456.
- Fuentes, D., Disante, K. B., Valdecantos, A., Cortina, J. and Vallejo, V. R., 2006. Response of *Pinus halepensis* Mill. Seedling to biosolids enriched with Cu, Ni and Zn in three Mediterranean forest soils. *Jour. Environ. Pollut.* (Oxford, U. K.). 20: 1–8.
- Grčman, H., Vodnic, D., Velikonja-Bolta, S. and Leštan, D., 2003. Ethylenediamine disuccinate as a new chelate for environmentally safe enhanced lead phytoremediation. *Jour. Environ. Qual.* 32: 500–506.
- Hong, P. K. A. and Jiang, W., 2005. Factors in the selection of chelating agents for extraction of lead from contaminated soil: effectiveness, selectivity and recoverability. In: Nowack B, Van Briesen J, eds. *Biogeochemistry of Chelating Agents*, ACS Symposium Series, vol. 910. American Chemical Society. p. 421–431.
- Huang, J. W., Chen, J., Bert, W. R. and Cunningham, S. D., 1997. Phytoremediation of lead contaminated soils: role of synthetic chelates in lead phytoextraction. *Jour. Environ. Sci. Technol.* 31: 800–805.
- Inckot, R. C., Santos, G. O., Souza, L. A. and Bona, C., 2011. Germination and development of *Mimosa pilulifera* in petroleum-contaminated soil and bioremediated soil. *Jour. Flora.* 206: 261–266.
- Joner, E. J. and Leyval, C., 2001. Influence of Arbuscular mycorrhiza on clover and ryegrass grown together in a soil spiked with polycyclic aromatic hydrocarbons. *Jour. Mycorrhiza.* 10: 155–159.
- Jones, P. W. and Williams, D. R., 2001. Chemical speciation used to assess [S,S0]-Ethylene Diamine Dissuccinic Acid (EDDA) as a readily-biodegradable replacement for EDTA in radiochemical decontamination formulations. *Jour. Appl. Radiat. Isot.* 54: 587–593.
- Komárek, M., Tlustoš, P., Száková, J., Chrastný, V. and Ettler, V., 2007. The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. *Chemosphere.* 67: 640–651.
- Kumar, G. P., Yadav, S. K., Thawale, P. R., Singh, S. K. and Juwarkar, A. A., 2008. Growth of *Jatropha curcas* on heavy metal contaminated soil amended with industrial wastes and *Azotobacter* – a greenhouse study. *Jour. Bioresource Tech.* 99: 2078–2082.
- Leštan, D., Luo, C. L. and Li, X. D., 2008. The use of chelating agents in the remediation of metal-contaminated soils: a review. *Jour. Environ. Pollut.* 153: 3–13.
- Lui, J., Dong, Y., Xu, H., Wang, D. and Xu, U., 2005. Accumulation of Cd, Pb and Zn by 19 wetland plant species in constructed wetland. *Jour. Hazard Mater.* 147: 947–953.
- Luo, C. L., Shen, Z. G., Li, X. and Baker, A. J. M., 2006. Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS. *Chemosphere.* 63: 1773–1784.
- Maguire, J. D., 1962. Speed of germination: Aid in selection and evaluation of seedling emergence and vigor. *Jpn. Jour. Crop Sci.* 2: 176–177.
- Morel, J. L., Mench, M. and Guckert, A., 1986. Measurement of Pb^{2+} , Cu^{2+} and Cd^{2+} binding with mucilage exudates from Maize (*Zea mays* L.) roots. *Jour. Biol. Fert. Soils.* 2: 29–34.
- Nelson, D. W. and Sommers, L. E., 1996. Total carbon, organic carbon, and organic matter. Methods of soil analysis. In: Bartels JM, ed. *Chemical methods—SSSA book series no. 5. Soil Science Society of America*. Madison: WI. p. 961–1010.
- Neugschwandtner, R. W., Tlustoš, P., Komárek, M. and Száková, J., 2007. Phytoextraction of lead and cadmium from a contaminated agricultural soil using EDTA application regimes: Laboratory versus field scale measures of efficiency. *Geoderma.* 144: 446–454.
- Nwosu, J. U., Harding, A. K. and Linder, G., 1995. Cadmium and lead uptake by edible

- crops grown in a silt loam soil. *Bulletin of Environmental Contamination and Toxicology*. 54:570-578.
- Olsen, S. R. and Sommers, L. E., 1982. Phosphorus. In: Page, A. L., Miller, R. H., Keeney, D. R. (Eds.), *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*, second ed. Agronomy No. 9. ASA, SSSA, Madison, WI, pp. 403-430.
- Papassiopi, N., Tambouris, S. and Kontopoulos, A., 1999. Removal of heavy metals from calcareous contaminated soils by EDTA leaching. *Jour. Water Air Soil Pollut.* 109: 1–15.
- Papazoglou, E.G., Karantounias, G.A., Vemmos, S.N. and Bouranis, D.L., 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Jour. Environ. Int.* 31: 243-249.
- Peralta, J. R., De la Rosa, G., Gonzalez, J. H. and Gardea-Torresdey, J. L., 2004. Effect of the growth stage on the heavy metal tolerance of alfalfa plants. *Jour. Adv. Environ. Res.* (Oxford, U. K.). 8: 679-685.
- Prasad, M. N. V. and De Oliveira-Freitas, H. M., 2003. Metal hyper accumulation in plants-Biodiversity prospecting for phytoremediation technology. *Jour. Electr. Biotech.* 6: 285–321.
- Reeves, R. D., 2006. Hyper accumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N, editors. *Phytoremediation of Metal-contaminated Soils*. NATO Sciences Series 68. Springer, New York. 25–52.
- Rhoades, J. D., 1996. Salinity: Electrical conductivity and total dissolved solids. In: *Methods of soil analysis*, American Society of Agronomy, pp. 417-435 (Page, A.L., Ed). Madison, WI.
- Saifullah Meers, E., Qadir, M., de Caritat, P., Tack, F. M. G., Du Laing, G. and Zia, M. H., 2009. EDTA-assisted Pb phytoextraction (review). *Chemosphere*. 74: 1279–1291.
- Salt, D. E., Blaylock, M., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I. and Raskin, I., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Jour. Biotechnology*. 13: 468–474.
- Samantaray, S., Rout, G. R. and Das, P., 1996. Root growth of *Echinochloa colona*: Effects of heavy metals in solution culture. *Fresenius Environ. Bull.* 5: 469-473.
- Sauve, S., McBride, M. and Hendershot, W., 1998. Soil solution speciation of lead. (II): effect of organic matter and pH. *Jour. Soil Sci. Soc. Am.* 62: 618-621.
- Terry, N. and Bañuelos, G. S., 2000. *Phytoremediation of contaminated soil and water*. CRC Press, Lewis Publ, Boca Raton.
- Thomas, G. W., 1996. Soil pH and soil acidity. *Methods of soil analysis*. In: Bartels JM, ed. *Chemical methods-SSSA book series no. 5. Soil Science Society of America*. Madison: WI. p. 475–490.
- Turgut, C., Katie, M. and Teresa, J. C., 2005. The effect of EDTA on *Helianthus annuus* uptake, selectivity, and translocation of heavy metals when grown in Ohio, New Mexico and Colombia soils. *Chemosphere*. 58: 1087–1095.
- Usman, A. R. A. and Mohamed, H. M., 2009. Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. *Chemosphere*. 76: 893–899.
- Vamerali, T., Bandiera, M. and Mosca, G., 2010. Field crops for phytoremediation of metal contaminated land. A review. *Environ. Chem. Lett.* 8: 1–17.
- Vassil, A.D., Kapulnik, Y., Raskin, I. and Salt, D. E., 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol.* 117: 447–453.
- Vysloulilová, M., Tlustoš, P. and Száková, J., 2003. Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. *Jour. Plant Soil Environ.* 49: 542–547.
- Wang, A., Chunling, L., Renxiu, Y., Yahua, C., Zhenguo, S. and Xiangdong, L., 2012. Metal leaching along soil profiles after the EDDS application-A field study. *Jour. Envir. Pollution*. 164: 204-210.
- Wilkins, D. A., 1978. The measurement of tolerance to edaphic factors by means of root growth. *New Phytol.* 8: 623-633.
- Wilde, E. W., Brigmon, R. L., Dunn, D. L., Heitkamp, M. A. and Dagnan, D. C., 2005. Phytoextraction of lead from firing range soil by Vetiver grass. *Chemosphere*. 61: 1451–1457.
- Woolhouse, H. W. and Walker, S., 1981. The physiological basis of copper toxicity and tolerance in higher plants. In *Copper in Soils and Plants*. (eds. Loneragan, J. F. Robson, A. D. and Graham, R. D). pp. 235–262. Academic Press, Inc. New York.
- Woolhouse, H. W., 1983. Toxicity and tolerance in the responses of plant to metals. In *Encyclopedia of Plant Physiology*. (eds. Lange, O.L. Nobel, P.S. Osmond, C. B. and Ziegler, H) 12 C. pp. 245–300. New series, Springer-Verlag. Berlin.

- Wu, J., Hsu, F.C. and Cunningham, S. D., 1999. Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. *Jour. Environ. Sci. Technol.* 33: 1898–1904.
- Xiong, Z. T., 1998. Lead Uptake and Effects on Seed Germination and Plant Growth in a Pb Hyperaccumulator *Brassica pekinensis* Rupr. *Bull. Environ. Contam. Toxicol.* 60: 285-291.
- Yoon, J., Cao, X., Zhou, Q. and Ma, L. Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Jour. Sci. Total Envir.* 368: 456–464.
- Zaier, H., Tahar, G., Kilani, B.R., Abdelbasset, L., Salwa, R. and Fatima, J., 2010. Effects of EDTA on phytoextraction of heavy metals (Zn, Mn and Pb) from sludge-amended soil with *Brassica napus*. *Bioresour. Technol.* 101: 3978–3983.
- Zhao, F. J. and McGrath, S. P., 2009. Biofortification and phytoremediation. *Current Opinion. Jour. Plant Biology.* 12: 373-380.

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کاربرد *Festuca ovina* L. در گیاه استخراجی خاک‌های آلوده به مس با استفاده از مواد کلات‌کننده

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چکیده *Festuca ovina* L. یکی از گیاهان مرتعی بیش‌اندوز است که با توجه به قابلیت این گیاه در پالایش خاک‌های آلوده مورد توجه بسیار می‌باشد. تحقیق حاضر جهت ارزیابی پتانسیل *F. ovina* L. در خاک‌های آلوده به مس و تعیین کارایی غلظت‌های متفاوت اتیلن دی آمین تترا استیک اسید (EDTA) در جذب مس انجام گرفت. بذرها در خاک‌های غیرآلوده و آلوده به مس (به‌شکل مصنوعی آلوده به ۱۵۰ میلی‌گرم در کیلوگرم مس) کاشته شدند. نتایج نشان داد که مس تاثیر منفی بر رشد گیاه و شاخص‌های بردباری *F. ovina* داشت و طول ریشه حساس‌ترین پارامتر گیاهی در میان فاکتورهای مورد اندازه‌گیری بود. تیمارهای مورد استفاده در ارزیابی کارایی EDTA شامل ۱/۵، ۳، ۶، ۱/۵+۱/۵، ۳+۳ میلی‌مول بر کیلوگرم، کنترل (غیرآلوده بدون EDTA) و W (آلوده بدون EDTA) بود. نتایج نشان داد که غلظت ۱/۵ میلی‌مول بر کیلوگرم تاثیر معنی‌داری بر استخراج مس نداشت و بین استفاده از این تیمار در غلظت‌های یک‌بار و دوبار مجدد تفاوتی وجود نداشت. افزایش معنی‌دار در غلظت مس در ریشه گیاه در استفاده از تیمار ۳ میلی‌مول در کیلوگرم مشاهده شد. بیشترین فاکتورهای پالایش (۰/۰۸ و ۰/۰۷ درصد) به‌ترتیب در تیمارهای ۶ و ۳+۳ میلی‌مول در کیلوگرم مشاهده شد. کاربرد EDTA به‌طور نسبی شاخص تحمل گیاه (TI) را کاهش داد و حداقل مقدار این شاخص در تیمار ۶ میلی‌مول بر کیلوگرم اندازه‌گیری شد. به‌طور کلی EDTA کارایی استخراج مس با کاربرد گونه *F. ovina* را دارد اما، بررسی‌های بیشتری در مقدار و نحوه کاهش خطر آبخویی این ماده به آب‌های زیرزمینی لازم است.

کلمات کلیدی: EDTA، تحمل فلز، گیاه‌پالایی، آلودگی خاک