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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

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 *etal*. (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-accumulatorbeing 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-accumultor 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 acetateEDTA (pH 4.60) was 5.13 $mgkg^{-1}$ 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 $CuSO_4$ $5H_2O$ (150 $mgkg^{-1}$). 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 treatmentconsisted of 15 seeds in five replicates.Seeds' surfaces were sterilized by soakingin a 5% of sodium hypochlorite solutions for fiveminutes 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):

$RG = \sum ND/\sum N$ (Equation 1)

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

(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 $mmolkg^{-1}$), 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. $2H_2O$). 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 (acidifiedto 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 $HNO_3/HClO_4$ (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):

$$RF = \frac{Cu_{plant} \times B_{plant}}{Cu_{soil} \times W_{soil}} \times 100\% \text{(Equation 3)}$$

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 ETDA 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 $SPSS_{18}$. 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 andtissues 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 andcontaminated 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 andthere was no statistically significant difference in Cuuptake between single and split

applications of 1.5 mmolkg^{-1} EDTA. A sharp increase in root Cu concentration was observed when 3 mmolkg^{-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 mmolkg^{-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 biomassyield 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 mmolkg^{-1} EDTA) treatments produced half of biomass yields as compared to the plants grown in uncontaminated soil control.

Dry weight did not significantly change after1.5+1.5 and 3 mmolkg^{-1} EDTA addition as compared to the 1.5 mmolkg^{-1} EDTA. However, the addition of 3+3 and especially, 6 mmolkg^{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 andtissues 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 mmolkg^{-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 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 ±0.55 $^{d-B}$	14.97 ± 1.76 $^{d-B}$	25.00± 1.24 ^{a}
Contaminated soil	30.90±3.00 $^{c-B}$	62.73 ± 3.21 $^{c-A}$	13.60± 1.10 ^{b}
1.5 EDTA	36.10±3.20 $^{c-B}$	73.83 ± 4.10 $^{c-A}$	13.24± 1.12 ^{b}
3.0EDTA	90.09±6.10 $^{b-B}$	187.25± 6.10 $^{b-A}$	12.99± 1.00 ^{b}
6.0 EDTA	154.0±7.00 $^{a-B}$	249.11± 7.0 $^{a-A}$	7.25 ± 0.70 ^{c}
1.5+1.5 EDTA	40.22±3.20 $^{c-B}$	82.38 ± 3.20 $^{c-A}$	12.52± 1.11 ^{b}
3.0+3.0 EDTA	111.6±7.20 $^{b-B}$	194.17± 7.12 $^{b-A}$	8.62 ± 0.75 ^{c}

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)

	Dry Weight	Cu_{shoot}	Cu_{root}
Cu_{shoot}	$-0.32^{n.s}$		
Cu_{root}	$-0.35^{n.s}$	$0.18^{n.s}$	
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

Phytoextractionefficiency 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 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).

Table5. 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 theamounts 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 wasachieved 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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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 ازگیاهان 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 ترا استیک اسید (EDTA) در جذب مس انجام گرفت. بذرها در خاک‌های غیرآلوده و آلوده به مس (به‌شکل مصنوعی آلوده به ۱۵۰میلی‌گرم در کیلوگرم مس) کاشته شدند. نتایج نشان داد که مس تاثیر منفی بر رشد گیاه و شاخص-های بردباری*F. ovina* داشت و طول ریشه حساس‌ترین پارامتر گیاهی در میان فاکتورهای مورد اندازه-گیری 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 ۱/۵میلی‌مول بر کیلوگرم تاثیر معنی‌داری بر استخراج مس نداشت و بین استفاده از این تیمار در غلظت-های 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) به-ترتیب 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. به‌طورکلی EDTA کارایی استخراج مس با کاربرد گونه *F. ovina*رادارد اما، بررسی‌های بیشتری در مقدار و نحوه کاهش خطر آبشویی این ماده به آب‌های زیرزمینی لازم است.

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