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Effect of EDTA Application on Lead and Zinc Uptake and Germination of *Thlaspi caerulescens* L. in a Contaminated Soil

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ABSTRACT Pot experiment was carried out to investigate the effects of ethylenediaminetetraacetic acid (EDTA) on some morphological characteristics of *Thlaspi caerulescens* L., and also on the accumulation of lead (Pb) and zinc (Zn) in roots and shoots of *T. caerulescens* L.. Experiments were then set up in three treated pots with doses of 3, 6 and 9 mmol kg^{-1} of EDTA and control pots (C: uncontaminated soil and W: contaminated soil). The results indicated the significant effect of EDTA on morphological characteristics and accumulation of heavy metals in the plant ($P<0.05$). Data revealed that the maximum of germination (99.11 and 96.00%), maximum of root length (73.31 and 70.14 mm) and maximum of shoot length (51.64 and 44.14 mm) and maximum of biomass weight (61.31 and 52.18 mg) were achieved by C treatment followed by W treatment. The maximum bioconcentration factor (3.57) and translocation factor (0.89) was observed on 9 mmol kg^{-1} EDTA. In addition, the effect of EDTA on Tolerance Index (TI) showed that the TI decreased with increasing doses of EDTA. The findings indicated that the study species tolerated heavy metals concentration. EDTA had potential to promote the uptake of heavy metals for *T. caerulescens* L., but with respect to non-significant differences between 6 mmol kg^{-1} EDTA and 9 mmol kg^{-1} EDTA treatments. Thereore, low dose of EDTA suggested to be applied because of its environmental risk.

Key words: *Chelating agents, Heavy metals, Morphological characteristics, Phytoextraction*

1 INTRODUCTION

Heavy metals released from mine exploitation, vehicle emissions and irrational use of chemical fertilizers, seriously contaminate environment and soil (Chen *et al*., 2014; Shahid *et al*., 2014). This situation has become a critical environmental issue owing to the potential adverse ecological effects of the pollutants (Bisone *et al*., 2014). Compared with physical and chemical techniques of remediation such as vitrification, landfilling, electrokinetics, phytoremediation is a developing technology that aims to extract or inactivate metals and it has attracted much attention because it is an environmentally friendly and relatively cheap technique (Wang *et al*., 2012; Chen *et al*., 2014; Ebrahimi, 2014b). Phytoremediation can be categorized into two different approaches. First approach is phytoextraction which metal accumulating plants are planted on

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contaminated soil and later harvested in order to remove metals from the soil (Saifullah *et al*., 2009; Usman and Mohamed, 2009). Second approach is phytostabilization. In the second method metal-tolerant plants are used to reduce the mobility of metals, thus, the metals are stabilized in the substrate (Antosiewicz *et al*., 2008; Zhao and McGrath, 2009).

One of the plant species which has been considered to remediate the contaminated soil is *Thlaspi caerulescens* L. This plant is a low biennial or perennial plant that has small basal rosettes of stalked elliptic lanceolate leaves with entire margins. The one or more flowering stems have small stalkless, alternate leaves clasping the stem. The inflorescence is a dense raceme which continues to lengthen after flowering. The individual flowers are regular, with white or pinkish petals and are about 5 mm wide. Each flower has four sepals, four petals, six stamens with violet anthers and a single carpel (Alpine Pennycress, 2013). The species has been identified as a Zinc (Zn) and Nickel (Ni) hyper-accumulator (Reeves *et al*., 1983; Ingrouille and Smirnoff, 1986; Reeves and Baker, 2000). Many of the previous works on *T. caerulescens* L. has involved the collection of field samples and measurement of metal concentration in aboveground dry matter. Recently, Ghaderian and Nosouhi (2014) found mean shoot zinc, lead, chromium and nickel concentrations as high as 226.3, 637, 74 and 91.5 μg g^{-1}, for plants growing on metalliferous mine wastes at sites in Mobarakeh Steel Complex, Iran. Though phytoremediation can be applied for the reclamation of elevated concentrations of heavy metals present in contaminated soils, just a fraction of soil metal content is readily available for plant uptake and a large portion is generally present as insoluble compounds unavailable for absorption by roots, restricting absorption of hyper-accumulating plants (Wang *et al*., 2009). In order to enhance the availability of heavy metals in soil solution and its translocation from root to shoot, a variety of chelating agents have been investigated (Evangelou *et al*., 2007), but one of the most studied chelating agents is ethylenediaminetetraacetic acid, EDTA (Zaier *et al*., 2010; Zhao *et al*., 2011; Wang *et al*., 2012, Ebrahimi *et al*., 2015). Natural phytoaccumulation uses the natural ability of the plant to remediate metal polluted sites. In this method, only the number of plant growth repetitions is controlled (Turgut *et al.*, 2005; Zhao *et al*., 2011). While in chelate induced phytoextraction, artificial chelates are added to increase the uptake of metal contaminants (Salt *et al*., 1997; Wenzel *et al.*, 2003). In order to make this technology feasible, the plants must extract large concentrations of heavy metals into their roots and translocate the heavy metals to surface biomass (Vassil *et al.*, 1998; Chen *et al*., 2004; Usman and Mohamed, 2009).

The objectives of this study were (1) to investigate Pb and Zn uptake of *T. caerulescens* L. in contaminated soils; (2) to identify effects Pb and Zn on plant species morphological characteristics viz. germination, biomass, root and shoot lengths; and (3) to identify the ability of ethylenediaminetetraacetic acid (Na_2EDTA) to enhance the uptake and phytoextraction of Pb and Zn from contaminated soils by use of *T. caerulescens* L. under greenhouse conditions.

2 MATERIALS AND METHODS

2.1 Soil and pot preparation

Uncontaminated sandy loam texture soil (laser diffractometry method, Wang *et al*., 2012) was taken from the surface layer (0-15 cm) around Zabol City. All soil samples were sieved to 4 mm. The moisture contents were 70% water-holding capacity (WHC) (Ait Ali *et al*., 2004). The soil sample air-dried under room temperature and they ground to pass through a 2 mm sieve before analysis. Soil chemical analysis showed that total nitrogen (Kjeldahl method; Black *et al*., 1965), total phosphorous

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(molybdenum blue method; Olsen and Sommers, 1982), total potassium (Flame photometry method; Bery *et al*., 1946), pH (1:1 soil/ water ratio, pH meter, Model 691, Metrohm AG Herisau Switzerland) (Thomas, 1996), Electrical Conductivity EC_e (solid: deionized water = 1:2 w/v, EC meter, Model DDS-307, Shanghai, China) (Rhoades, 1996) and Cation Exchange Capacity (CEC, Bower and Hatcher, 1966) were 0.20%, 0.54%, 0.46%, 8.10, 2.01 dS m^{-1} and 37.00 meq, respectively.

After sieving (4 mm) the soil samples, three kg of dried soil were stored in plastic pots (20 cm×15 cm). Then, 50 pots were prepared for the experiments (25 for each metal). Two days later, the soil was spiked with Pb ($PbNO_3$) 450 mg kg^{-1} and Zn ($ZnSO_4$) 450 mg kg^{-1} and thoroughly mixed. The soil was then allowed to equilibrate for two weeks in the greenhouse. In all treatments, 15 seeds of the plants were buried evenly throughout each pot at least 1 to 2 cm from the edge and pots placed in the greenhouse located in the University of Zabol with, temperature of 23±5 °C, humidity of 60% and moisture content of 70% water-holding capacity.

EDTA was applied to the pots having contaminated soil in the form of sprinkling solutions. The solutions of EDTA were prepared from a disodium salt dehydrate of EDTA (C_{10} H_{14} N_2 Na_2 $O_8 \cdot 2H_2O$). The treatments comprised the following dosage with five replicates per treatment: (1) Control with no EDTA disodium salt (C); (2) Contaminated soil without EDTA (W); (3) Contaminated soil + 3 $mmolkg^{-1}$ EDTA; (4) Contaminated soil + 6 $mmolkg^{-1}$ EDTA; (5) Contaminated soil + 9 $mmolkg^{-1}$ EDTA. For each treatment, germination was monitored closely over 14 days of the trials. Germinated seeds were counted daily according to the method proposed by Maguire (1962) and two germination variables final germination percentage (number of germinated seeds in each pot) and germination rate (a measure of germination speed, with lower values indicating faster germination) were determined. Germination Rate was calculated as follows:

$$RG = \Sigma N_i D_i \quad (1)$$

where, N_i is the number of germinant daily, D_i is the number of days from the initial sowing. When the plants had been growing for 30 days, the seedlings were harvested at the end of growing trial. The plants were separated into root and shoot. The plant organs were washed before analysis and samples were baked at 70 °C to a constant weight for approximately 48 hours and ground into fine powder in an agate mortar. Metals were analyzed after mineralization of 400 mg dry shoot and root material in a microwave oven (MEMMERT UNB 400, Germany) with 5 ml of nitric acid (69% v/v), 5 ml deionized water and 2 ml H_2O_2 (30% v/v). The digest was made to 25 ml final volume with deionized water, filtered (0.45 mm, millipore) and then analyzed for Pb and Zn using ICP/OES (Inductively Coupled Plasma/Optical Emission Spectometry) (model: GBC Avanta, Australia). Dried soil samples were passed through a 2 mm diameter sieve. About 100 mg dry soil was digested with HNO_3: HCl (3:1) in a microwave oven. After mineralization, the samples were diluted, filtered and analyzed using ICP/OES. Metals concentrations of soil samples were measured as described for the plant samples (Du Laing *et al.*, 2003). The methodology for metal concentrations in soil was according to the SRM 2711 (Institute of Standard and Technology, USA). The methodology for metal concentrations in the plant was according to BCR-060 (Institute for Reference Materials and Measurements, Belgium).

2.2 Calculation of BCF, TF and TI

The bioconcentration factor (BCF), translocation factor (TF) and tolerance index

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(TI) were calculated to determine the heavy metals phytoextraction efficiency (Mattina *et al*., 2003; Yoon *et al*., 2006). The BCF expresses the ability of a plant to accumulate metal from soils and TF is the capacity of a plant to transfer metal from its roots to shoots. The TI based on the dry weight of plant (dry weight of the plants grown in heavy metal solution/dry weight of the plants grown in control solution) was chosen as indicator of the toxic effects of metals on plants under different dose of ETDA treatments. In the current study, the TF and BCF values for heavy metals are given by (Eqs. 2 to 4):

$$BCF_{shoot} = C_{shoot} / C_{soil} \quad (2)$$

$$BCF_{root} = C_{root} / C_{soil} \quad (3)$$

$$TF = C_{shoot} / C_{soil} \quad (4)$$

where C_{shoot} and C_{root} are the metal concentrations in the shoots and roots, respectively, and C_{soil} is the metal concentration in the soil (Yoon e*t al*., 2006).

2.3 Data analysis

Statistical analyses of the experimental data were performed using SPSS 18.0. All data were checked for their normality and homogeneity of variance, and where necessary, data were log-transformed before statistical analysis. All reported results are the means of five replicates and deviations were calculated as standard error of mean (SEM). The statistical processing was mainly conducted by analysis of variance (ANOVA). Duncan test was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as significant.

3 RESULTS and DISCUSSION

3.1 Germination and seed growth

Treatment of contaminated soil with EDTA significantly affected the growth of *T. caerulescens* L. in terms of germination, root length, shoot length and biomass ($P < 0.05$) (Table 1). EDTA treatments decreased the germination rates and percentages. The lowest germination rate (44.16%) and germination percentage (56.18%) have been observed when 9 mmol kg^{-1} EDTA was applied. Although response to the chelator dosage varied between roots and shoots length, it demonstrated an overall dosage dependent response to the EDTA. Addition of 9 mmol kg^{-1} EDTA appeared to show the most effect on the root and shoot length of the plant (Table 1). The lowest root (31.71 mm) and shoot length (26.19 mm) have been observed in 9 mmol kg^{-1} EDTA treatment.

The dry weight of *T. caerulescens* L. decreased with the increasing concentrations of EDTA and the application of EDTA at the dose of 9 mmol kg^{-1} produced the minimum dry weight (30.51 mg) of the plant. The seed germination of *T. caerulescens* L. declined with increase in EDTA dosage showing significant reduction and delay in seed germination at 9 mmol kg^{-1} concentration. These results might be considered that EDTA elevates the bioavailability of heavy metals in the soil. Some studies found that in a certain range of concentrations, EDTA strongly inhibited plant growth (Saifullah *et al*., 2009; Zhao *et al*., 2011). In this research, with increased EDTA dose, the biomass of *T. caerulescens* L. decreased compared to the control, which could be due to the high contents of heavy metals mobilized to the soil solution and to some extent, due to the toxicity of free EDTA, if present (Zaier *et al*., 2010; Bisone *et al*., 2014, Ebrahimi, 2015).

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Table 1 Results (means ± standard error) of seed germination, dry weight of biomass, roots and shoots length of *T. caerulescens* L.

Variables / Treatment	Germination rate (%)	Germination percentage	Root Length (mm)	Shoot length (mm)	Dry weight of biomass (mg)
Uncontaminated Soil	99.11±2.41^{a}	100.00±1.17^{a}	73.31±1.00^{a}	51.64±1.00^{a}	64.31±1.00^{a}
Contaminated Soil	96.00±2.43^{ab}	97.10±1.140^{ab}	70.14±1.00^{a}	44.17±1.00^{b}	52.18±1.00^{b}
3 mmol kg^{-1} EDTA	81.33±2.32^{b}	84.55±1.11^{b}	61.00±1.00^{b}	33.12±1.00^{c}	46.31±1.00^{c}
6 mmol kg^{-1} EDTA	63.14±2.32^{c}	70.12±1.00^{c}	54.00±0.93^{b}	33.10±1.00^{c}	40.10±1.00^{c}
9 mmol kg^{-1} EDTA	44.16±2.23^{c}	56.18±1.00^{d}	31.71±0.70^{c}	26.19±1.00^{c}	30.51±1.00^{d}
F ratio	4.490	5.23	4.91	4.09	4.07

Values within a column followed by the different letter are significantly different (P<0.05, Duncan test)

Reduction in above-ground net primary production would lead to a decrease in the total amount of metals removed by plants (Quiroz *et al.,* 2002; Turgut *et al.,* 2005). Our data showed that the lowest dry weights of the plant (30.51 and 40.10 mg) were observed in the 9 and 6 mmol kg^{-1} EDTA treatments, respectively, and there were no significant increase in Pb and Zn uptake of plant's organs between two treatments. However, during chelate-assisted Pb phytoextraction, there are several factors that influenced plant growth, among which the most important were chelate/Pb molar ratio (Vassil *et al.,* 1998; Zhao *et al.,* 2011; Ebrahimi, 2014b), mode and time of chelates application (Ebrahimi, 2014b; Wenzel *et al.,* 2003), plant species as well as type and concentration of other heavy metals (Lombi *et al.,* 2001; Evangelou *et al.,* 2006). Thus, the degree of metals tolerance may depend on the capacity of the plant to prevent this effect (Ait Ali *et al*., 2004). Plant performance during chelate-assisted phytoextraction may be influenced adversely by both the direct action of chelate and the increased bioavailability of heavy metals in the soil. The presence of free EDTA is toxic to plants because it can negatively affect the balance of minerals, e.g., zinc, copper, iron and calcium, leading to disturbances in cell metabolism and destabilization of biological membranes (Ruley *et al.,* 2006). Excess of free Pb (II) in growth the soil can severely reduce cell division and plant growth (Johnson and Petras, 1998). However, in the presence of EDTA the cytological impacts of free Pb ions are eliminated.

3.2 Heavy metals content in plant organs and soil

The distribution of heavy metals in the plant was significantly affected by the application of EDTA (Table 2). Compared to the control, the application of chelating agent significantly increased the concentrations of metals either in the roots or the shoots. With the increased EDTA dose, Zn content in the roots of the plant increased when the dose of EDTA was 9 mmol kg^{-1}. Pb contents in the plant tissues had the same variation with the doses of EDTA increasing. Considering the dry matter yield of the plant, heavy metals concentration of underground part was higher (maximum BCF=3.78) than that in the aboveground (maximum BCF= 3.42) part. It seemed from the results that the root cells of *T. caerulescens* L.

[DOR: 20.1001.1.23222700.2015.3.4.1.1]

were able to accumulate more Zn and Pb. The decreasing trend of metal concentrations in both root and shoot was Zn> Pb. A slight increase in the soil Pb and Zn was observed with the addition of chelating doses to the soil (Table 2). The contents of metals in ranged in the order of root> shoot>soil, respectively.

Several studies have tried to elucidate the mechanism behind the enhanced uptake of heavy metals by chelates (Jarvis and Leung, 2002; Hong and Jiang, 2005; Ruley *et al.*, 2006). As yet the mechanism has not been completely described as it is dependent on the metal and the plants used. Majority of metals taken up by roots are bound to carboxyl groups of mucilage uronic acids (Morel *et al*., 1986). According to Jarvis and Leung (2002), metal retention in the roots is based on the binding of metal to ion exchange sites on the root cell walls and extracellular precipitation, mainly in the form of metal-carbonates. 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 to as the bioavailable fraction. The bioavailability of heavy metals within these pools can be enhanced upon application of mobilizing agents such as EDTA (Hong and Jiang, 2005).

Analyses of the soil used for the pot experiment and contaminated soil with different EDTA treatments are given in Table 2. A gradual increase in available Pb and Zn contents were observed with the increasing concentration of EDTA. A slight decrease in pH was observed with the application of EDTA doses to the soil. Soil pH is one of the effective mechanism in increasing the uptake of metals from the soil by plant (Yang *et al*., 2006). Therefore, the efficiency of chelates on metal solubilization and accumulation can be enhanced by lowering the soil pH. Ebrahimi (2014a, b) showed an increase in the availability of metals after EDTA supply, possibly due to the low lixiviation rate of the soil. Bareen and Tahira (2010) studied efficiency of seven different cultivated plant species for phytoextraction of toxic metals from Tannery effluent contaminated soil using EDTA and showed that addition of EDTA to the soil at dose of 10 mmol kg^{-1} had significant effect on the pH and EC. Soil pH is an important factor for Pb adsorption and desorption in the soil. Ebrahimi *et al*. (2015) reported that Pb and Zn extraction by EDTA depended on soil pH and showed a strong positive relation up to a soil pH of 8.5.

With respect to non-significant difference between 6 mmol kg^{-1} EDTA and 9 mmol kg^{-1} EDTA treatments, low dose (6 mmol kg^{-1}) should be used. It should be considered that long-lived chelating agents, such as EDTA are inappropriate for use in enhanced phytoextraction; its longevity will cause elevated metal mobility, even after harvesting plants (Kos and Leŝtan, 2003). Hence, although the concentration of metals increased with increasing EDTA concentration, application of higher dose of EDTA to metals-contaminated soils may be of environmental concern because of the increased risk of groundwater contamination via metal leaching (Meers *et al*., 2005). Grčman *et al*. (2003) reported that 38% of the initial Pb was leached out of the soil column soon after treatment with 10 mmol kg^{-1} EDTA.

[DOR: 20.1001.1.23222700.2015.3.4.1.1]

Table 2 Results (means ± standard error) of heavy metals concentration in the soil and seedling organs of *T. caerulescens* L.

Treatment	Zn mg kg^{-1}			Pb mg kg^{-1}			
	pH	Root	Shoot	Soil	Root	Shoot	Soil
Uncontaminated Soil	8.10±0.02^{A}	ND	ND	ND	ND	ND	ND
Contaminated Soil	8.2±0.02^{A}	201.13±7.13$^{C\text{-}a}$	83.11±1.39$^{C\text{-}b}$	72.62±2.12$^{D\text{-}c}$	73.12±1.00$^{C\text{-}a}$	51.21±0.50$^{B\text{-}b}$	44.13±2.09$^{D\text{-}c}$
3 EDTA	7.7±0.02^{B}	220.18±7.17$^{BC\text{-}a}$	96.18±1.31$^{C\text{-}b}$	83.02±2.10$^{C\text{-}c}$	81.14±1.09$^{BC\text{-}a}$	64.13±0.60$^{B\text{-}b}$	50.08±2.13$^{C\text{-}c}$
6 EDTA	7.5±0.02^{BC}	237.41±8.33$^{B\text{-}a}$	101.51±2.27$^{B\text{-}b}$	145.62±5.11$^{B\text{-}c}$	85.16±1.09$^{AB\text{-}a}$	73.12±0.62$^{A\text{-}b}$	60.61±2.21$^{B\text{-}c}$
9 EDTA	7.3±0.02^{C}	259.16±9.64$^{A\text{-}a}$	116.74±2.52$^{A\text{-}b}$	177.29±5.71$^{A\text{-}c}$	99.53±1.21$^{A\text{-}a}$	81.65±1.31$^{A\text{-}b}$	70.36±2.60$^{A\text{-}c}$
F ratio	4.47	4.47	4.09	4.41	4.91	5.42	4.82

Different capital letters in each column indicate significant differences between treatments. Different lower case letters in each row indicate significant differences among organs and soil ($p<0.05$, Duncan test). ND= not detected/below detectable range.

3.3 Phytoextraction efficiency of the plant

Results showed that all dose of EDTA treatments enhanced BCF in the roots and the shoots of the plant (Table 3). Accumulation of heavy metals in the plant organs were maximum in the roots of 9 mmol kg^{-1} EDTA-treatment followed by 6 and 3 mmol kg^{-1} EDTA, and W treatments; however the differences were not always significantly ($P<0.05$). The maximum bioconcentration factor (3.57) was observed on 9 mmol kg^{-1} EDTA.

Application of 9 mmol kg^{-1} EDTA enhanced the translocation factor, but there was no significant ($P<0.05$) increase in TF for all treatments (Table 4). The values of TI normally ranged from 1 to 0.54 (Table 4). Application of EDTA showed relatively decrease in TI value. The lowest value of TI was recorded in 9 mmol kg^{-1} EDTA-treated. Maximum TI was found in the control treatment that showed significant difference at 5% level.

In particular, it was found that EDTA enhanced BCF in the roots and the shoots, but BCF_{root} values was higher than BCF_{shoot}, indicating that accumulation of heavy metals in the roots is higher than the shoots. Plants with BCF_{shoot} values >1 are accumulators, while plants with BCF_{shoot} values <1 are excluders (Baker, 1981). The results showed that the plant species had the potential for use as an excluder and the BCF_{root} values of >1 indicate high efficacy in the phytostabilization of metal-contaminated soils. In addition to the bio concentration factors, high doses of EDTA increased the translocation factor. The maximum TF (0.89) was observed in 9 mmol kg^{-1} EDTA treated. Also, effect of EDTA on TI showed that the index decreased with increasing doses of EDTA. The value of TI is equal to one when there is no influence of treatment on the growth, higher than one when there is a favorable effect of sludge on the growth and lower than one 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 heavy metals from soil, EDTA applied at larger rates could result in contamination of ground water due to enhanced solubilization and leaching of metals as well as metal–EDTA complexes (Saifullah *et al*., 2009).

[DOR: 20.1001.1.23222700.2015.3.4.1.1]

Table 3 Results (means ± standard error) of the effect of EDTA on bioconcentration factor (BCF)

Treatments	BCF_{Zn} root	BCF_{Zn} shoot	BCF_{Pb} root	BCF_{Pb} shoot
Uncontaminated Soil	-	-	-	-
Contaminated Soil	$1.16\pm0.10^{C-a}$	$0.93\pm0.20^{C-b}$	$1.92\pm1.13^{B-a}$	$1.23\pm0.10^{C-b}$
3 EDTA	$2.50\pm0.10^{B-a}$	$1.42\pm0.20^{B-b}$	$2.67\pm1.21^{B-a}$	$1.42\pm0.10^{C-b}$
6 EDTA	$2.68\pm0.10^{B-a}$	$1.66\pm0.20^{A-b}$	$2.83\pm1.20^{A-a}$	$2.35\pm0.10^{B-b}$
9 EDTA	$3.53\pm0.10^{A-a}$	$1.71\pm0.20^{A-b}$	$3.78\pm1.30^{A-a}$	$3.42\pm0.10^{A-b}$
F value	3.09	3.16	4.07	3.55

Different capital letters in each column indicate significant differences between treatments. Different lower case letters in each row indicate significant differences between organs ($p<0.05$, Duncan test).

Table 4 Results (means ± standard error) of the effect of EDTA on translocation factor (TF) and tolerance index (TI)

Treatments	TF_{Zn}	TF_{Pb}	TI
Uncontaminated Soil	-	-	-
Contaminated Soil	0.50 ± 0.00^{a}	0.80 ± 0.10^{a}	1.00 ± 0.00^{a}
3EDTA	0.57 ± 0.100^{a}	0.82 ± 0.10^{a}	0.66 ± 0.00^{b}
6 EDTA	0.67 ± 0.00^{b}	0.84 ± 0.10^{a}	0.62 ± 0.0^{b}
9 EDTA	0.70 ± 0.00^{b}	0.89 ± 0.10^{a}	0.54 ± 0.10^{b}
F value	2.33	2.14	2.05

Values within a column followed by the different letter are significantly different ($p<0.05$, Duncan test)

4 CONCLUSION

The results of present pot experiment showed that Pb and Zn treatments decreased the plant's germination and biomass. However, EDTA treatments enhanced the uptake of Pb and Zn, *T. caerulescens* L. can accumulate high concentration of Zn and Pb, so if application of EDTA is along with planting some plants such as *T. caerulescens* L., it can be useful for remediation of contaminated soils with Zn and Pb. In this study, 6 mmol kg^{-1} EDTA is suggested to enhance efficiency of phytoremediation in the same conditions. The results suggested that high doses of EDTA have deleterious effects on the plant growth. It is clear that the total amounts of extracted metals will be more elevated in the presence of EDTA because this chelating enhanced Pb and Zn concentration but with respect to leaching risk it is needed to use the low dosage of EDTA to remediate the contaminated soils.

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تأثیر کاربرد EDTA بر جذب روی و سرب و جوانه‌زنی *Thlaspi caerulescens* L. در یک خاک آلوده

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چکیده آزمایش گلدانی به‌منظور مطالعه تاثیر EDTA بر برخی خصوصیات موفولوژیکی گونه *Thlaspi caerulescens* L. و هم‌چنین میزان جذب سرب و روی در ریشه و اندام هوایی *T. caerulescens* L. انجام شد. آزمایش با غلظت‌های متفاوت EDTA (۳، ۶، ۹ میلی‌مول در کیلوگرم) در خاک شاهد (C) و آلوده (W) انجام شد. نتایج نشان داد که کاربرد EDTA تاثیر معنی‌داری ($p<۰/۰۵$) بر خصوصیات مورفولوژیک و تجمع فلزات سنگین در اندام‌های گیاه داشت. حداکثر جوانه‌زنی (۹۹/۱۱ و ۹۶ درصد)، حداکثر طول ریشه (۷۱/۳۱ و ۷۰/۱۴ میلی‌متر) و حداکثر ساقه (۵۱/۶۴ و ۴۴/۱۴ میلی‌متر) و حداکثر زیست‌توده (۶۱/۳۱ و ۵۲/۱۸ میلی‌گرم) گیاه به‌ترتیب در تیمارهای شاهد C و W به‌دست آمد. حداکثر عامل تجمع (BCF) و عامل انتقال (TF) در تیمار ۹ میلی‌مول در کیلوگرم به‌دست آمد. علاوه بر این تاثیر EDTA بر شاخص تحمل (TI) نشان داد که این شاخص با افزایش غلظت EDTA کاهش یافت. نتایج نشان داد که گونه مورد مطالعه می‌تواند غلظت فلزات سنگین را تحمل نماید. نتایج نشان داد EDTA موجب افزایش جذب فلزات سنگین در گونه *T. caerulescens* L. شده است. اما با توجه به عدم تفاوت معنی‌دار بین تیمارهای ۶ و ۹ میلی‌مول در کیلوگرم و به‌دلیل خطرات محیط زیستی، غلظت‌های کم این ماده بهتر است مورد استفاده قرار گیرد.

کلمات کلیدی: خصوصیات مورفولوژیکی، عوامل کلات‌کننده، فلزات سنگین، گیاه استخراجی

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