Effect of EDTA application on heavy metals uptake and germination of Echinochloa crus galii (L.) Beave in contaminated soil

Mahdieh Ebrahimi

Assistant Professor, College of Water and soil, University of Zabol.

Corresponding author email: maebrahimi2007@uoz.ac.ir

ABSTRACT: Pot experiment was carried out to investigate the effects of EDTA (ethylenediaminetetraacetic acid) on morphological characters of Echinochloa crus galii L. (germination, biomass, root and shoot lengths), and also accumulation of Zn, Cu and Fe in roots and shoots of the plant species. Pots were treated with different EDTA concentrations (2.5, 5, 10 mmolkg⁻¹) and control pots (C: uncontaminated soil and W: Contaminated soil) were not treated with EDTA. The results showed that EDTA application had significant (p<0.05) effect on morphological characters and accumulation of heavy metals in the plant. The data revealed that maximum germination, root and shoot length and biomass was achieved by C treatment followed by W treatment, but the maximum bioconcentration factors (BCF) and translocation factor (TF) was observed under 10 mmolkg⁻¹ EDTA, in addition effect of EDTA on TI (Tolerance Index) showed that the index decreased with increasing doses of EDTA. The results indicated that the study species can tolerate heavy metals concentration and EDTA had potential to promote the uptake of heavy metals for E. crus galii, but with respect to non significant difference between 5EDTA and 10EDTA treatments, low dose should be used because of its environmental risk.

Key words: EDTA, Phytoremediation, Soil remediation, Morphological characters.

Abbreviations: EDTA=ethylenediaminetetraacetic acid, BCF=Bioconcentration factor, TF=Translocation factor, TI=Tolerance index.

INTRODUCTION

Contamination of soil with potentially toxic elements (PTE) is a worldwide concern. Various strategies exist for contaminated land remediation (Khan et al., 2004; Mulligan et al., 2001). Phytoremediation that uses metal-accumulating plants to extract the heavy metals from contaminated soils, groundwater or surface water is a promising technology in cleanup of polluted sites due to the characters of less destructive, low cost and environmentally friendly nature (Zhao and McGrath 2009). Among various types of phytoremediation, phytoextraction is the best approach for removing pollutants primarily from soil without damaging soil structure and fertility. Phytoextraction is a new technology that uses metal-accumulating plants to extract the metals from contaminated soils, groundwater or surface water, and has been proposed as an effective and affordable solution to clean up heavy metal contamination (Pulford and Watson 2003; Xu et al., 2009). It is also referred as phytoaccumulation (Rulkens et al., 1998).

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 is ethylene diamine tetraacetic acid, EDTA (Grčman et al., 2001). 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 (Salt et al., 1997). While in chelate induced phytoextraction, artificial chelates are added to increase the uptake of metal contaminants (Salt et al., 1997). 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, (Chen et al., 2004).

The aim of the present study was to investigate the ability of ethylenediaminetetraacetic acid (Na₂EDTA) in enhancing the uptake and phytoextraction of Zn, Cu and Fe from heavy metal contaminated soils by use of Echinochloa crus galii (L.) Beave under greenhouse conditions and its effects on morphological characters (germination, biomass, root and shoot lengths).
MATERIALS AND METHODS

Soil Preparation

Soil was selected from Lia industrial city (35° 27’N-48° 50’E), which located in Qazvin county (Northern), Iran. The area is influenced by industrial wastewater then the soil is highly polluted with heavy metals. Soil sampling was obtained from the depth of 0–40 cm and mixed. All soil samples were sieved to 4 mm and moisture contents were adjusted to 70% water-holding capacity (WHC). The most relevant characteristics of the soil were (mean values) as follows, pH 8.5 (1:1 soil/water ratio, Model 691, Metrohm AG Herisau Switzerland) (Thomas, 1996), EC 3.46 dS m⁻¹ (solid: deionized water = 1:2 w/v, DDS-307, Shanghai, China) (Rhoades, 1996), Total organic carbon (TOC) 1.5% (Nelson and Sommers 1996). Total nitrogen (Ntot) 15% (Bremner, 1996), CEC 48.69 meq (Cation Exchange Capacity) (Bower and Hatcher, 1966), total Zn 725.23 mg kg⁻¹, total Cu 280.16 mg kg⁻¹ and total Fe 1127.72 mg kg⁻¹ (ICP/OES, GBC Avanta, Australia). After sieving, 3 kg of dried soil were added to plastic pots (diameter 10 cm x height 40 cm).

A filter of paper was placed at the bottom of each pot to prevent soil escaping from the drainage holes. 15 seeds of the plant were buried evenly throughout each pot at least 1 to 2 cm from the edge. The pots were watered from the top during the germination period so that, soil moisture was kept near 70% field capacity. The necessary light for the growth of the plants was obtained from the sun. The samples were put behind the glass windows of the greenhouse (university of Zabol, faculty of agriculture) and received the solar light during the experiment. The minimum and maximum temperature was 23 and 28°C, respectively. 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 (C₁₀H₁₄N₂Na₂O₈·2H₂O). 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+2.5 mmol kg⁻¹ EDTA; (4) Contaminated soil+5 mmol kg⁻¹ EDTA; (5) Contaminated soil+10 mmol kg⁻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 parameters were determined: (1) final germination percentage (number of germinated seeds in each pot); (2) germination rate (a measure of germination speed, with lower values indicating faster germination).

Plants were harvested after 30 days of adding chelator solutions. The roots were carefully washed with deionized water to get rid of the sand. The plants were separated into root and shoot and dried in a microwave oven (MEMMERT UNB 400) at 70°C for 48 h. Then the biomass (dry weight) was determined. Dried samples were ground, digested in concentrated HNO₃ for 14 h, 12 h at room temperature first, then 2 h at 60°C, and further digested with concentrated HNO₃·HClO₄ (3:2 v/v) for 3 h at 140-160°C. After cooling, the extract was diluted with NHCl and made up to 25 mL.

The methodology for metal concentrations in soil was referenced using the SRM 2711 (Institute of Standard and Technology, USA) and methodology for metal concentrations in plant was referenced using BCR-060 (Institute for Reference Materials and Measurements, Belgium).

Phytoextraction Efficiency

The bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI) were calculated to determine the heavy metals phytoextraction efficiency (Wilkins, 1978; Zayed et al., 1998, 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. Tolerance index (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:

BCFshoot = \frac{C_{\text{shoot}}}{C_{\text{soil}}}

BCFroot = \frac{C_{\text{root}}}{C_{\text{soil}}}

TF = \frac{C_{\text{shoot}}}{C_{\text{root}}}

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

Statistical Analysis

Statistical analyses of the experimental data were performed using the SPSS 18.0. 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 analysis of variance (ANOVA). 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 significant.
RESULTS AND DISCUSSION

Effects of EDTA on E. crus gallii germination and growth

Statistically EDTA affected the growth of E. crus gallii 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 (57.12%) and germination percentage (54.19%) have been observed when 10mmolkg⁻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. Decline in root length ranged from 40.90% (with 2.5mmolkg⁻¹) to 72.38% (with 10mmolkg⁻¹). Similarly shoot height was also declined from 41.43% (with 2.5mmolkg⁻¹) to 65.66% (with 10mmolkg⁻¹), but the effects of dosage higher than 5mmolkg⁻¹ were not statistically significant (Table 1). The dry mass yields of E. crus gallii decreased with the increasing concentrations of EDTA and the application of EDTA at the dose of 10 mmolkg⁻¹ produced the minimum biomass of the plant (29.00 mg/plant). The seed germination of E. crus gallii declined with increase in EDTA dosage showing significant reduction and delay in seed germination at 10mmolkg⁻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 (Lian et al., 2007). In this paper, with increased EDTA dose, the biomass of E. crus gallii 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 (Vassil et al., 1998).

In spite of reported successes in increasing the bioavailability fraction of heavy metals using EDTA, researchers have expressed concerns about EDTA-assisted phytoextraction due to excessive levels of heavy metals in soil solution and dissolution of soil-bound metals. Plants exposed to high levels of both free ion and free EDTA produce low biomass due to low seed germination, leaf wilt, chlorosis and necrosis, abscission, shoot desiccation and reduced transpiration (Römken et al., 2002; Du et al., 2005; Nascimento et al., 2006).

Turgut et al. (2005) investigated 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 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 of above 10mmolkg⁻¹ chelant (Grčman et al., 2003; Luo et al., 2006).

Table 1. Effects of the application of EDTA on seed germination, biomass (DW), roots and shoots length of E. crus gallii at five different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination Rate (%)</th>
<th>Germination percentage (%)</th>
<th>Root Length (mm)</th>
<th>Shoot Length (mm)</th>
<th>Biomass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>100.00±0.00</td>
<td>100.00±1.00</td>
<td>86.30±1.11</td>
<td>49.51±1.00</td>
<td>63.67±1.00</td>
</tr>
<tr>
<td>W</td>
<td>97.23±0.00</td>
<td>95.13±1.30</td>
<td>70.42±1.10</td>
<td>37.00±2.00</td>
<td>49.72±1.30</td>
</tr>
<tr>
<td>2.5EDTA</td>
<td>85.24±1.70</td>
<td>86.33±1.55</td>
<td>51.00±2.30</td>
<td>29.00±2.10</td>
<td>40.55±2.22</td>
</tr>
<tr>
<td>5EDTA</td>
<td>65.17±1.11</td>
<td>63.64±1.42</td>
<td>30.50±1.29</td>
<td>18.10±1.00</td>
<td>32.00±1.21</td>
</tr>
<tr>
<td>10EDTA</td>
<td>57.12±1.44</td>
<td>54.19±1.44</td>
<td>23.85±1.00</td>
<td>17.00±1.00</td>
<td>29.00±1.09</td>
</tr>
</tbody>
</table>

C: Control, uncontaminated soil without EDTA; W: Contaminated soil without EDTA; 2.5 EDTA: Contaminated soil+EDTA (2.5mmolkg⁻¹); 5 EDTA: Contaminated soil+EDTA (5mmolkg⁻¹); 10 EDTA: Contaminated soil+EDTA (10mmolkg⁻¹). Values shown are the means±SE. Values within a column followed by the same letter do not differ significantly (p<0.05, post hoc Duncan test).

Heavy Metals Content in the Plant Organs

Concentrations of heavy metals, in shoots and roots are shown in Table 2. Compared to the control, the application of chelating agent significantly increased the concentrations of metals either in the roots or in the shoots. With the increased EDTA dose, Zn content in the roots of the plant increased when the dose of EDTA was 10 mmolkg⁻¹. Zn content in roots and shoots reached the maximum of 223.70mgkg⁻¹ and 94.40mgkg⁻¹ respectively. Cu and Fe 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 than that in aboveground part. It seemed from the results that the root cells of E. crus galli were able to accumulate more Zn, Cu and Fe. The decreasing trend of metal concentrations in both root and shoot was Zn>Cu>Fe. Zn and Cu showed the highest concentrations in plant tissues respectively.

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 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. Treatment of soil with EDTA increased the mobility of target metals in the soil solution and the maximum extractable metals were observed in 10EDTA 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 to as the bioavailable fraction. The bioavailability of heavy metals within these pools can be enhanced upon application of mobilizing agents such as EDTA (Papassioi 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).

With respect to non significant difference between 5EDTA and 10EDTA treatments, low dose (5mmolkg⁻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 Leštan 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 10mmolkg⁻¹ EDTA.

**Table 2. Heavy metals concentration (mgkg⁻¹) in seedling roots and shoots tissues of E. crus galiimat the end of growing trial.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zn Root</th>
<th>Zn Shoot</th>
<th>Cu Root</th>
<th>Cu Shoot</th>
<th>Fe Root</th>
<th>Fe Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>W</td>
<td>172.12±11.00c⁻a</td>
<td>76.20±4.27c⁻b</td>
<td>65.00±3.00c⁻a</td>
<td>43.33±2.09c⁻b</td>
<td>65.14±2.00c⁻a</td>
<td>44.40±1.60c⁻b</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>185.23±11.00c⁻a</td>
<td>84.00±4.00c⁻b</td>
<td>75.67±5.20c⁻a</td>
<td>50.00±4.00c⁻b</td>
<td>70.65±2.00c⁻a</td>
<td>50.20±1.22c⁻b</td>
</tr>
<tr>
<td>5 EDTA</td>
<td>205.52±13.24c⁻a</td>
<td>93.12±8.33c⁻a</td>
<td>90.09±7.21c⁻a</td>
<td>65.00±4.74c⁻b</td>
<td>83.09±3.02c⁻a</td>
<td>65.15±2.00c⁻b</td>
</tr>
<tr>
<td>10 EDTA</td>
<td>223.70±12.00c⁻a</td>
<td>94.40±9.65c⁻a</td>
<td>93.00±7.00c⁻a</td>
<td>68.00±5.55c⁻a</td>
<td>83.53±3.00c⁻a</td>
<td>68.00±2.04c⁻b</td>
</tr>
</tbody>
</table>

Values shown are the means±SE. 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, post hoc Duncan test). ND= NOT Detected/Below detectable range.

**Effects of EDTA on Phytoextraction Efficiency**

Treatment means showed that all levels of EDTA treatments enhanced BCF in roots and shoots of the plant (Table 3). Accumulation of heavy metals in plant organs were maximum in roots of 10 EDTA-treatment followed by 5EDTA, 2.5EDTA, and W treatments; however the differences were not always significantly (p>0.05). Regarding heavy metals, bioaconcentration factor decreased according to the order of Zn>Cu>Fe. Application of 10EDTA enhanced the translocation factor, but there was no significant (p>0.05) increase in TF for all treatments (Table 4). The observed maximum and minimum TF was 0.82 and 0.44 for Fe and Zn respectively. The values of Ti normally ranged from 1 to 0.57 (Table 7). Application of EDTA showed relatively decrease in Ti value. The lowest value of Ti was recorded in 10EDTA-treated and it might be the greater toxic effects of metals and EDTA on the plant. 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 roots and shoots, but BCF values were higher than BCF values indicating that accumulation of heavy metals in the roots is higher than the shoots. Plants with BCF values >1 are accumulators, while plants with BCF values <1 are excluders (Baker, 1981). The results showed that the plant species had the potential for use as an excluder and the BCF values of >1 indicate high efficacy in the phytoextraction of metal-contaminated soils. In addition to the bioconcentration factors, high doses of EDTA increased the translocation factor. The maximum Ti was observed in 10 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 1 when there is no influence of treatment on the growth, 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 Zn, Cu and Fe 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).

**Table 3. The effect of EDTA on Bioconcentration Factor (BCF).**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BCF Zn Root</th>
<th>BCF Zn Shoot</th>
<th>BCF Cu Root</th>
<th>BCF Cu Shoot</th>
<th>BCF Fe Root</th>
<th>BCF Fe Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.11±0.30c⁻a</td>
<td>0.90±0.10b⁻b</td>
<td>0.95±0.20c⁻a</td>
<td>0.80±0.10b⁻b</td>
<td>0.95±0.27c⁻a</td>
<td>0.80±0.10c⁻b</td>
</tr>
<tr>
<td>W</td>
<td>2.04±0.30b⁻a</td>
<td>1.20±0.10b⁻b</td>
<td>1.58±0.34a⁻a</td>
<td>0.93±0.10b⁻b</td>
<td>1.50±0.30a⁻a</td>
<td>0.91±0.10b⁻b</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>2.77±0.40a⁻a</td>
<td>1.26±0.10b⁻b</td>
<td>2.28±0.30a⁻a</td>
<td>1.20±0.30a⁻a</td>
<td>2.00±0.30a⁻a</td>
<td>1.00±0.35b⁻a</td>
</tr>
<tr>
<td>5 EDTA</td>
<td>2.90±0.40b⁻a</td>
<td>1.70±0.20a⁻b</td>
<td>2.54±0.30a⁻a</td>
<td>1.25±0.32c⁻a</td>
<td>2.28±0.30a⁻a</td>
<td>1.20±0.39a⁻b</td>
</tr>
</tbody>
</table>

Values shown are the means±SE. 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, post hoc Duncan test).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>$TF_{Zn}$</th>
<th>$TF_{Cu}$</th>
<th>$TF_{Fe}$</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.44±0.10a</td>
<td>0.67±0.10b</td>
<td>0.68±0.10a</td>
<td>1.00±0.23a</td>
</tr>
<tr>
<td>2.5 EDTA</td>
<td>0.45±0.10a</td>
<td>0.67±0.10b</td>
<td>0.73±0.10b</td>
<td>0.85±0.20b</td>
</tr>
<tr>
<td>5 EDTA</td>
<td>0.45±0.20a</td>
<td>0.72±0.20ab</td>
<td>0.79±0.20b</td>
<td>0.60±0.20c</td>
</tr>
<tr>
<td>10 EDTA</td>
<td>0.45±0.20a</td>
<td>0.74±0.20a</td>
<td>0.82±0.20a</td>
<td>0.57±0.20c</td>
</tr>
</tbody>
</table>

Values shown are the means±SE. Values within a column followed by the same letter do not differ significantly (p<0.05, post hoc Duncan test).

**CONCLUSION**

The results of present pot experiment showed that EDTA treatments enhanced the uptake of metals and E. crus galli can accumulate high concentration of Zn, Fe and Cu, so if application of EDTA is along with planting some plants such as E. crus galli, it can be useful for remediation of contaminated soils with Zn, Fe and Cu. In this study, 5mmolkg⁻¹ EDTA is suggested to enhance efficiency of phytoremediation in the same conditions. The results suggest that high does of EDTA has deleterious effects on the plants growth. It is clear that the total amounts of extracted metals will be more elevated in the presence of EDTA because this chelator enhanced metals concentration but we must apply the low dosage of EDTA (with respect to leaching risk). Further studies would be needed on investigating deeply 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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