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ORIGINAL PAPER

Chelate-assisted phytoextraction of cadmium from a mine soil by negatively charged sunflower

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Abstract The effects of some chelating agents and electricity on cadmium phytoextraction from a mine soil were examined in pot culture of sunflower to achieve more remediation efficiency. At the beginning of the flowering stage, ethylene-diamine-tetra-acetic acid (EDTA) as a chemical chelator, cow manure extract (CME) and poultry manure extract (PME) as organic chelators were applied (2 g kg^{-1} soil) during irrigation. Seven days later, *Helianthus annuus* was negatively charged by inserting a stainless steel needle in the lowest part of the stem with 10 and 30 V direct current electricity for 1 h each day for 14 days. Afterward, concentration of cadmium in roots and shoots, cadmium translocation factor (TF), cadmium uptake index (UI) and soil available (diethylene-triamine-penta-acetic acid extractable) cadmium were measured. Results indicated that EDTA reduced roots dry weight while none of the roots and shoots was affected by other chelating agents and by electrical treatment as well. Highest concentration of cadmium in shoots was measured in 10 V-control with no significant differences with 30 V-PME and 30 V-EDTA. Utilization of chelating agents did not increase the cadmium TF and cadmium UI while highest values for cadmium TF and cadmium UI were observed in 10 and 30 V treatments, respectively. Available cadmium in the soil near root system treated with 10 and 30 V was relatively lower compared with the soil far from root system. Results of this experiment indicated that charging the plant with direct current electricity ameliorated the efficiency of cadmium phytoremediation.

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Keywords Contaminated soil · Electrokinetic · *Helianthus annuus* · Phytoremediation

Introduction

Due to the toxicity and carcinogenic effects on human, animals, and plants, heavy metal pollution is one of the major anxieties nowadays (Giannis et al. 2010). The widespread use of sewage sludge, waste waters, pesticide in agriculture and the emissions of vehicle exhausts, mining, and smelting has resulted in large accumulation of heavy metals in soils (Shi et al. 2009; Dede et al. 2012). While it is believed that soil contamination with cadmium (Cd) is the most serious health risk, Cd is likely to be accumulated into the soil surface under the influence of human activities (Kabata-pendias 2011). It is reported that the Cd concentration in top soil can be very high near Pb and Zn mines and especially smelting operations (Kabata-pendias 2011).

There are several reviews explaining that the phosphate fertilizers and sewage sludge are also accepted as important sources of Cd in soil (Fleischer et al. 1974; Williams and David 1976, 1981; Street et al. 1977; Smolders and Degryse 2006). The polluted soils with large quantities of contaminants, in turn, can be a long-term source of pollution for groundwater and the ecosystems (Kabata-pendias 2011); therefore, remediation of these contaminants for decreasing subsurface contamination is very important (Li et al. 2009). In situ technologies are noninvasive techniques used to remove pollutants from soils (Pazos et al. 2010). Phytoremediation is one of the in situ procedures, which has been applied by many scientists (Huang et al. 1997; Kulli et al. 1999; Cooper et al. 1999; Safari Sinegani and Khalilikhah 2011; Safari Sinegani and Ahmadi 2012) and has received increasing attention as a cost-effective alternative to usual engineering-based remediation approaches (Salt et al. 1998). Only plants with high biomass and high metal uptake capacity are suitable for phytoextraction of contaminants from soils. Because most hyperaccumulators have low annual biomass, some researchers aimed to increase the availability of heavy metals in soil for plants (Wong et al. 2004). Ethylene-diamine-tetraacetic acid (EDTA) was found to be the most effective chelating agent to enhance heavy metal bioaccumulation (Ebbs et al. 1997; Huang et al. 1997; Wu et al. 1999; Safari Sinegani and Khalilikhah 2011). Application of EDTA in soil causes a large fraction of total metals to become available for phytoextraction (Elliott and Brown 1989; Haag-Kerwer et al. 1999) and also enhances the root metal flux through the apoplast and then subsequently increase metal translocation factor (TF) (Hernandez-Allicac et al. 2007).

On the other hand, electrokinetic decontamination of soil is one of the promising remediation techniques that has been utilized to extract various contaminants from the soil, sediment, or sludge (Kim et al. 2009; Chen et al. 2011; Alcantara et al. 2012; Gomes et al. 2012).

The principle of operation of electrokinetic is to use a direct electrical field in soil to run pollutants within the soil pores toward the electrode (Wang et al. 2009). The major mechanisms of electrokinetic remediation are electromigration and electroosmosis (Kim et al. 2002). Electromigration is defined as a directional movement in which ions move toward the electrode with opposite charge (Zhou et al. 2006). Hence, metal ions and complexes can move under electrokinetic treatments and absorb easily by plants cultured near electrodes (Cang et al. 2011). The other process caused by applied electrical potential is known as electroosmosis, i.e., the movement of an ionic liquid under the action of an applied electric field which relates to a charged surface (Giannis et al. 2007). Due to the hydrolysis reaction of water during the electrokinetic process, a hydrogen ion is produced at the anode. While moving toward the cathode, the H+ exchanges with the soil exchangeable cations (Kim et al. 2009). The released metal ions move toward the cathode in electromigration. An acidic solution can be used to improve the removal efficiency of heavy metals from soils (Baek et al. 2009).

In recent years, combination of electrokinetic remediation and phytoremediation to decontaminate metal-polluted soil has attracted many researches (O'Connor et al. 2003; Lim et al. 2004; Cang et al. 2011; Bi et al. 2011). However, there was no study on the effects of choosing a plant as cathode in electrokinetic phytoremediation. The objective of this study was to evaluate the effects of application of some chelating agents and electrical fields on Cd uptake by *Helianthus annuus* from a calcareous mine soil to achieve more remediation efficiency. Here, the sunflower was negatively charged by inserting a stainless steel needle in the lowest part of the stem directly. The current study was conducted in the spring of 2012 in Hamadan Province, Iran.

Materials and methods

Primary analysis of soil

The experimented soil was gathered from a pasture situated around a Pb and Zn mine in Hamadan Province, Iran. Selected soil properties were determined according to the standard methods. Particle size was measured using the hydrometer method. Equivalent calcium carbonate (ECC) was measured by back titration procedure so that 1 g of the experimental soil was weighted and put into a 250 cm^3 beaker; 100 cm^3 of pure water was added followed by 25 cm^3 0.5 M sulfuric acid. When effervescence has finished, the solution was heated for 3 min. The mixture was filtered, and soil was washed with distilled water 3 times. All filtered waters titrated with standard 0.25 M sodium hydroxide solution until the appearance of a pink color of phenolphthalein indicator. Soil pH and electrical conductivity (EC) were measured in a 1:5 soil:water extract after shaking for 30 min. Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate. Cation-exchange capacity (CEC) was measured by the method of sodium saturation. It was done by addition of 33 ml Na acetate (1 M) on 5 g soil in 50 ml centrifuge tube for three times, and extraction was done to remove the released cations from soil solution (Bower et al. 1952). Excess Na acetate was removed with 3 washing (33 ml each) of 95 % ethanol. The exchangeable Na was extracted with 3 washing (33 ml each) of 1 N ammonium acetate and then measured by a flame photometer instrument. These soil characteristics were measured according to methods of soil analysis published by Soil Science Society of America (SSSA) (Klute 1986; Page et al. 1992). Texture of the experimented soil was loamy. The soil pH, EC, OC, ECC, and CEC were 7.7, 580.65 µs cm^{-1}, 0.59, 22 %, and 14.24 cmolc (+) kg^{-1}, respectively. Soil total-Cd was extracted by HNO_3–H_2O_2–HCl (USEPA 2000), and soil available Cd was analyzed by diethylene-triamine-penta-acetic acid (DTPA) so that 10 g of air-dry soil was shaken with 20 ml of extractant (0.005 M DTPA, 0.1 M triethanolamine, and 0.01 M $CaCl_2$, with a pH of 7.3.) for 2 h. The leachate was filtered, and Cd was measured in the filtrate by atomic absorption spectrophotometry (Lindsay and Norvell 1978). Soil Cd active fractions were extracted by the modified Sposito et al. (1982) method and analyzed by atomic absorption spectrometry (Varian 220). The soil total-Cd, carbonate-bound, organic-bound, soluble and available Cd were 10.20, 2.11, 1.54, 1.31, and 0.03 mg kg^{-1}, respectively. Total amount of Cd in manure extracts was too low and not detectable by the atomic absorption instrument.

Experimental setup

A seven and half kilograms of Cd-polluted (air-dried) soil was fertilized with 150 mg kg^{-1} N (about 321.4 mg kg^{-1} of urea), 100 mg kg^{-1} K (about 233.1 mg kg^{-1} of K_2SO_4), and 70 mg kg^{-1} P (about 307.1 mg kg^{-1} of KH_2PO_4) and put into plastic pot (diameter 22 cm and height 20 cm). Each pot was divided into two parts (Fig. 1a) with a cylindrical vertical galvanized-size mesh (25 µm pore size, 11 cm diameter, and 20 cm height) to separate rhizospheric and non-rhizospheric soil. *H. annuus* seeds were germinated on filter paper and transferred to the center of cylindrical galvanized-iron mesh (center of the pots), and the pots were daily irrigated. The pots were arranged in a completely randomized experimental design with 3 replicates.

Four graphite electrodes (15 × 1 × 1 cm) were placed around the pots as anodes (Fig. 1a, b). The plant was used as a cathode instead of a central graphite electrode. The sunflower was negatively charged by inserting a stainless steel needle in the lowest part of the stem directly (Fig. 1c). Electrical fields were produced by DC electricity (0, 10 and 30 V) with a TNI-U power supply instrument model TU305D.

The experiment was set out in a glasshouse as a completely randomized design. Because of favorable conditions for plant growth in spring, all of optimizing systems of the glasshouse were switched off. After 53 days from culturing the sunflowers in the pots, at the beginning of flowering stage, the soils were treated by 2 g kg^{-1} soil of EDTA as a chemical chelator, cow manure extract (CME), and poultry manure extract (PME) as organic chelators. The chelating agents were dissolved in irrigation water and applied. Seven days later (60 days from the beginning), DC electricity was applied (10 and 30 V). Electrical fields were applied for an hour each day for 14 days (Lim et al. 2004). The EDTA was purchased in reagent grade form Sigma Chemical. The CME and PME were prepared after extracting of 1:5 manure/distilled water suspension by shaking (120 rounds per min for 20 min), centrifuging, and filtering. The pH of CME and PME were 7.72 and 8.2, respectively. Total solid of CME and PME was measured for calculating the required amounts of amendment for soil treatment (2 g kg^{-1} soil). Two weeks after the application of electricity (74 days after starting the experiment), the plants were carefully harvested for shoot and root analysis at the flowering stage.

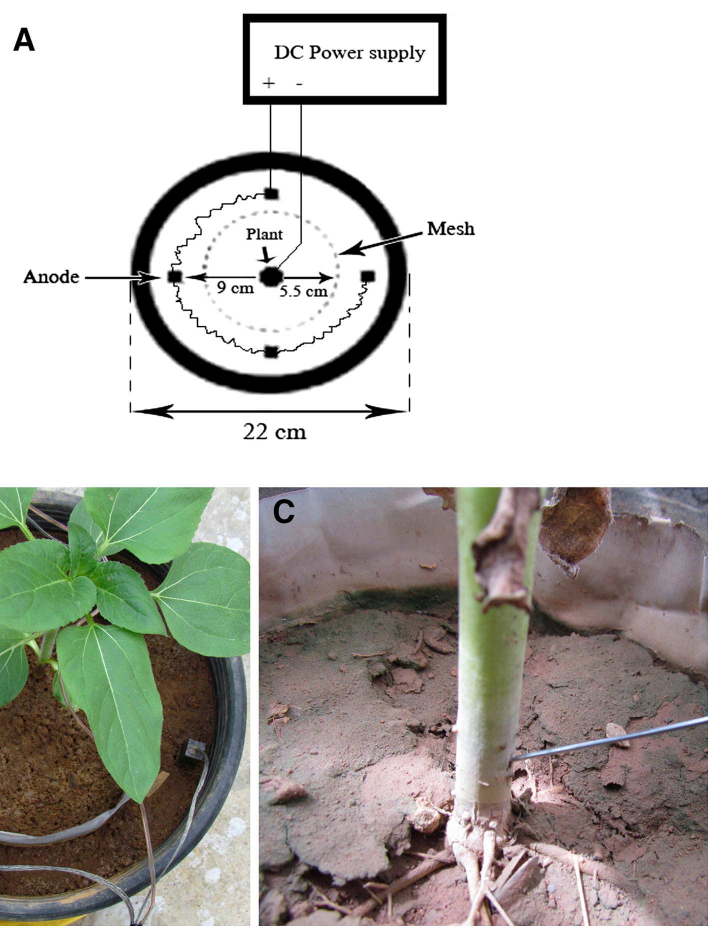

Fig. 1 **a** Diagram of the soil remediation system, **b** graphite electrodes placed around the pots as anodes, **c** stainless steel needle inserted in the lowest part of sunflower stem for charging the plant with negative charge

Plant analysis

The plants were harvested by cutting the stem 1 cm above the soil surface carefully. The harvested plants (shoot and root) were washed with 0.1 M HCl, rinsed with de-ionized water, and then dried in the oven at 75 °C for 48 h (Lim et al. 2004; Bi et al. 2011). Then the dried shoots and roots were weighted and milled into fine powder using a grinding machine and stored in glass containers until analysis. 0.5 g of shoots and roots were placed in a glass tube and 2 ml of concentrated nitric acid was added. The tubes were heated on an electrical heater at 65 °C, 60 min and then 120 °C, 60 min. Once cooled to room temperature, 0.2 ml of hydrogen peroxide was added to each tube and the mixture was left for 30 min to complete reaction. The final volume was brought to 25 ml (Figueroa et al. 2008). After appropriate dilution, the determination of Cd was carried out by atomic absorption spectrometry (Varian model 220).

The metal TF was calculated from the shoot and root Cd concentrations. It describes the ability of the plant to translocate Cd from root to shoot (Li et al. 2006). The Cd TF was calculated by Eq. 1:

$$\begin{aligned} \mathrm{TF} = {} & \text{Cd concentration in shoot } \left(\mathrm{mg\ kg^{-1}}\right) \\ & \div \text{ Cd concentration in root } \left(\mathrm{mg\ kg^{-1}}\right) \end{aligned} \tag{1}$$

Cadmium uptake index (UI) was calculated by Eq. 2 (Bi et al. 2011):

$$\begin{aligned} \mathrm{UI}\ \left(\mathrm{mg\ pot^{-1}}\right) = {} & \text{Cd concentration in shoot } \left(\mathrm{mg\ kg^{-1}}\right) \\ & \times \text{ Shoot dry weight of plant } \left(\mathrm{kg\ pot^{-1}}\right) \end{aligned} \tag{2}$$

Soil chemical analysis

The soil out of the galvanized-iron mesh (5.5 cm between iron mesh and edge of pot) was collected and labeled as anodic soil or soil far from root system (FFRS). The soil within the galvanized-iron mesh (5.5 cm radius from pot center) was collected and labeled as cathodic soil or soil near root system (NRS). The soil available Cd was extracted by DTPA and analyzed by atomic absorption spectrometry on a Varian 220 instrument (Lindsay and Norvell 1978).

Statistical analysis

This experiment was considered a completely randomized design as factorial in three replicates. Applied factors were chelating agents (no chelating agent, EDTA, CME, and PME) and electrical fields produced by DC electricity (0, 10 and 30 V). Analysis of variance was used to determine the significance of the effects of chelating agents and negatively charging of plant on Cd phytoremediation indices and soil available Cd in comparison with the control. Data were statistically analyzed for standard deviation, means were calculated, and Duncan's new multiple range tests were performed to assess the effects. Statistical analyses and graphical works were performed on the SAS 9.1 software and Microsoft Excel 2010.

Results and discussion

Shoot and root dry weight

Application of electrical fields had no significant effect on the plant root and shoot dry weights (Table 1). It can be attributed to the late and short time of application of electricity. Table 2 represented values of shoots dry weight of *H. annuus*. Bi et al. (2011), by the application of DC electrical fields in soil, have also reported that the application of electrical field did not affect the plant biomass significantly; However, the application of chelating agents had significant effect on the root dry weight of sunflower ($p < 0.05$). EDTA compared with CME and PME decreased root dry weight of plant significantly. Root biomass of sunflower which was treated with EDTA (2.41 ± 0.80 g pot^{-1}) was significantly lower than its root biomass in CME (4.33 ± 1.02 g pot^{-1}) and PME (4.29 ± 2.04 g pot^{-1}) treatments. However, the roots and shoots dry weights of sunflower treated with different chelating agents had no significant difference with those in control soil (3.69 ± 1.39 g pot^{-1}). Many scientists have reported that the application of EDTA in soil reduces plant's root and shoot biomass (Safari Sinegani and Khalilikhah 2011). This different result may be related to the time of application of chelating agents in soil. Safari Sinegani and Khalilikhah (2011) reported that when EDTA was applied late, negative effect of this treatment on plant growth and plant dry weight was markedly low.

Cadmium concentration in plant organs

Although it is considered that Cd is a nonessential element for metabolic processes, it can be effectively absorbed by both root and leaf systems of plants and also highly accumulate in soil organisms (Kabata-pendias 2011). The results showed that there is a significant ($p < 0.01$) interaction between the electrical fields and chelating agents' treatment on Cd concentrations in plant's root and shoot (Table 1).

As depicted in Table 3, the highest amount of Cd in shoots (8.1 mg kg^{-1}) was found in the plants treated with 10 V-control. The lowest amount of Cd concentration in shoot was observed in no voltage-control and no voltage-PME treatments. On the other hand, highest amount of Cd concentration in roots (6.68 mg kg^{-1}) was also measured in 10 V-control treatment. It seems that the application of a

Table 1 Analysis of variance (mean squares) of analyzed factors affected by chelating agents and electrical fields

	DF	SDW	RDW	Sh conc.	R conc.	TF	UI	A. Cd	
								NRS	FFRS
Electricity	2	73.56 ns	0.19 ns	12.91**	1.39 ns	1.56**	0.006*	10^{-3}**	4×10^{-4} ns
Chelating agents	3	30.20 ns	6.47*	2.10 ns	2.08 ns	0.12 ns	3×10^{-4} ns	10^{-3}**	4×10^{-4} ns
Interaction	6	45.95 ns	2.24 ns	5.57**	4.44**	0.42 ns	0.003 ns	0.01**	7×10^{-3}**
Error	24	30.41	1.99	0.71	0.85	0.19	0.001	10^{-4}	3×10^{-4}

SDW shoot dry weight, *RDW* root dry weight, *Sh conc.* shoot Cd concentration, *R conc.* root Cd concentration, *TF* Cd translocation factor, *UI* Cd uptake index, *A. Cd* available (DTPA extractable) Cd, *NRS* soil near the root system, *FFRS* soil far from root system, *ns* nonsignificant at 0.05

* Significant impacts at the 0.05; ** significant impacts at the 0.01

Table 2 Shoots dry weight (g pot^{-1}) of *Helianthus annuus* in chelating agents and electrical field treatments

Electrical treatment	Shoots dry weight	Chelating agents treatment	Shoots dry weight
30 V	28.00^{a} (±6.06)	CME	28.04^{a} (±4.79)
10 V	23.38^{a} (±5.57)	PME	26.04^{a} (±4.68)
Control	25.87^{a} (±5.57)	EDTA	24.06^{a} (±5.45)
		Control	25.07^{a} (±8.43)

Values with different letters show significant differences at the 0.05 probability level for each treatment

CME cow manure extract, *PME* poultry manure extract

Table 3 Cadmium concentration in shoot and root (mg kg^{-1}) of *Helianthus annuus* in different chelating agent and electrical field treatments

	30 V	10 V	No voltage
Shoot			
CME	3.90^{ed} (±1.00)	5.82^{bc} (±0.37)	5.23^{dc} (±0.40)
PME	7.35^{ab} (±1.63)	5.98^{bc} (±0.55)	3.37^{e} (±0.32)
EDTA	6.58^{abc} (±0.88)	6.37^{bc} (±0.80)	5.67^{bc} (±1.17)
Control	5.80^{bc} (±0.55)	8.10^{a} (±0.92)	3.15^{e} (±0.55)
Root			
CME	3.18^{c} (± 0.43)	2.48^{c} (± 0.41)	5.63^{ab} (±0.63)
PME	3.98^{bc} (± 0.66)	3.43^{c} (± 1.28)	3.47^{c} (±0.03)
EDTA	3.82^{bc} (± 0.74)	4.25^{bc} (± 1.47)	3.80^{bc} (<0.01)
Control	3.48^{c} (± 1.38)	6.68^{a} (± 1.02)	4.18^{bc} (±0.71)

CME cow manure extract, *PME* poultry manure extract

Values with different letters show significant differences at the 0.05 probability level for each organ

medium voltage of DC electrical field between plant and soil can increase the accumulation of Cd in both root and shoot of sunflower. Application of higher voltage possibly increases insoluble form of Cd as a result of generating hydroxyl ions and increasing the soil pH during the hydrolysis of water. Treating the sunflower with 30 V-CME causes the plant to reduce the accumulation of Cd in its root to the minimum level (3.18 mg kg^{-1}). This may be related to higher plant growth and root dry weight in manure treatments and dilution of absorbed Cd in root tissue of plant (Safari Sinegani and Ahmadi 2012).

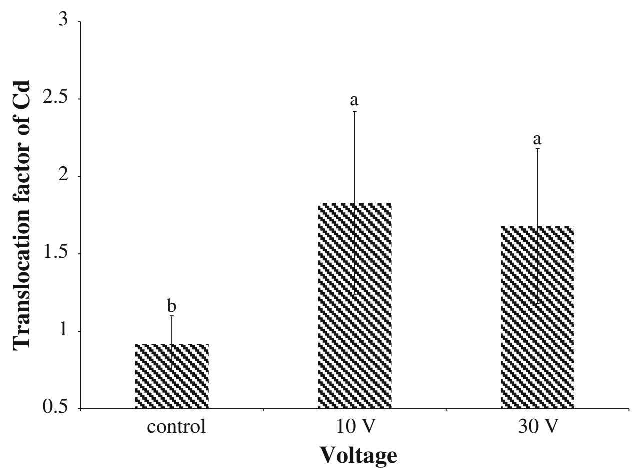

Fig. 2 Cadmium translocation factor (TF) in the electrical field treatments. *Values with different letters* show significant difference at the 0.05 probability level. *Thin bars* show standard deviation

Translocation factor (TF)

Translocation of the Cd from the root to the shoot of sunflower is the second step of phytoextraction after the Cd absorption by root. It has been revealed that the application of chelating agents like EDTA can enhance the translocation of metals from the root to shoot (Barber and Lee 1974; Hamon et al. 1995; Vassil et al. 1998; Gleba et al. 1999; Safari Sinegani and Khalilikhah 2011). Result of this experiment showed that the translocation of Cd from the root to shoot was not affected by chelating agents maybe because of the late time of application of the chelating agents or equal changing in concentration of Cd in shoots and roots; however, it was affected by application of the electrical fields (Table 1). Studying the TF of Cd showed an appreciable increase by application of the electrical treatment (Fig. 2). The values of TF increased more than 98 and 82 % in 10 and 30 V treated plant compared with

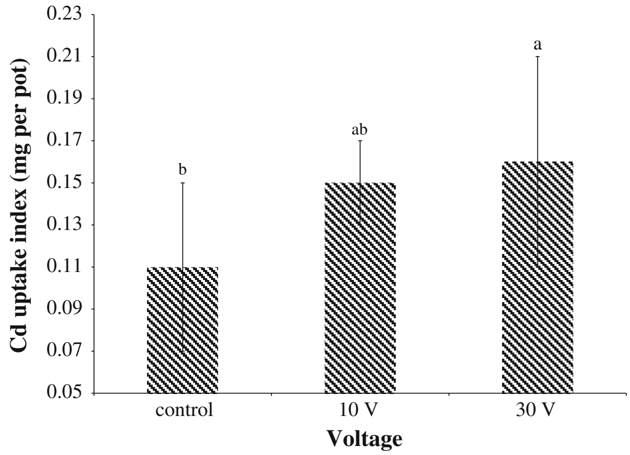

Fig. 3 Cadmium uptake index (UI) by *Helianthus annuus* (mg pot^{-1}) in the electrical field treatment. *Values with different letters* show significant differences at the 0.05 probability level. *Thin bars* show standard deviation

the control, respectively. Maximum TF was calculated in 10 V (1.83), and minimum TF was obtained in control (0.92); however, no significant difference was observed between 10 and 30 V (1.68).

Metal uptake index (UI)

Production of plant biomass as well as the concentration of the Cd in shoot is an important factor affecting the efficiency of Cd phytoremediation of soil. In this experiment, treating the soil with the chelating agents seems to have not had significant impacts on Cd UI (Table 1). This finding is in contrast to the other reports. EDTA is found to be the most effective chelating agent to enhance heavy metal bioaccumulation (Ebbs et al. 1997; Huang et al. 1997; Wu et al. 1999; Safari Sinegani and Khalilikhah 2011). This difference may be related to the time of application of the chelating agents. Here, we use chelating agents very late for lowering its possible toxicity to plant. Nevertheless, application of the electrical field and plant charging had a significant effect on Cd uptake by plant (Table 1). It resulted in higher Cd uptake by *H. annuus* (Fig. 3). The amount of UI increased from 0.11 in control to 0.16 in 30 V treated plants. There was no significant difference between the 10 V and control and also 10 and 30 V while control was significantly lower than 30 V. It was reported that a certain electrical field is proven to make biomembranes more permeable without damaging membrane structures (Neumann and Rosenheck 1972, 1973; Kinosita and Tsong 1997). The induced permeability leading to a transient exchange of matter across the perturbed membrane structure (Rosenheck et al. 1975; Lindner et al. 1977) may in turn result in more Cd uptake by plant. Also, role of the electrical gradient from soil toward the plant shoot should not be ignored in this study. Negatively charged plant can absorb and translocate Cd from soil to upper parts effectively.

Obtained correlation coefficients between Cd UI and the shoot dry weight, Cd concentration in shoot and Cd TF were positive and significant (Table 4). Here, the negative correlation between TF and root Cd concentration was predictable because the value of TF is increased by increasing Cd concentration in shoot and decreasing Cd concentration in root. These findings may be related to the applied electrical fields as discussed earlier.

Soil available Cd

One of the common methods used to determine plant bioavailable heavy metals in soil is DTPA test “a non-equilibrium extraction” developed by Lindsay and Novell in 1987 (Kirkham 2006). In this experiment, a significant ($p < 0.01$) interaction was observed between the electricity and chelating agent treatments on the available (DTPA extracted) Cd in soil (Table 1). Table 5 shows the means of soil available Cd in different treatments of chelating agents and electrical fields in soils near the root system (NRS) and FFRS.

Test of the soil available Cd at the end of the experiment illuminated that the highest amount of available Cd in NRS soil was measured in no voltage-PME (0.14 mg kg^{-1}) without any significant difference with 30 V-control (0.12 mg kg^{-1}). But the soil NRS in 30 V-EDTA treatment had the lowest available Cd (0.003 mg kg^{-1}). The changes of available Cd in the FFRS soil were almost similar to that in the NRS soil. The maximum (0.13 mg kg^{-1}) and the minimum (0.005 mg kg^{-1}) concentration of available Cd in FFRS soil were detected in the 30 V-control and 30 V-EDTA treatments, respectively. High amount of Cd uptake by plant may be responsible for the low available Cd concentration in the 30 V-EDTA treatment although there is no significant correlation between the DTPA extractable Cd and concentration of Cd in plant organs (Table 4).

In general, cathodic soil that was NRS in 10 and 30 V treatments compared with anodic soil (FFRS) had relatively lower available Cd, which maybe because of better Cd absorption and acquisition in negatively charged plants or increasing in insoluble form of Cd. This difference was reverse in soils treated with chelating agents with no application of electrical fields (Table 4). In the no voltage treatment, Cd availability was markedly higher in soil NRS especially in PME and CME treatments, which maybe because of higher soil biological activity. Safari Sinegani and Ahmadi (2012) found that soil Pb and Cd in soluble and exchangeable fractions were increased by manure applications in cannabis cultivation.

Table 4 Linear correlation coefficients between analyzed factors in soil near the root system of sunflower

	pH	SDW	RDW	Sh conc.	R conc.	TF	UI	A. Cd
pH	1							
SDW	0.04	1						
RDW	0.03	0.42*	1					
Sh conc.	−0.17	−0.25	−0.3	1				
R conc.	−0.26	−0.35*	−0.24	0.32	1			
TF	0.13	0.15	0.08	0.46**	−0.46**	1		
UI	−0.11	0.52**	0.05	0.64**	−0.02	0.53**	1	
A. Cd	0.16	−0.13	−0.17	−0.12	<0.01	−0.16	−0.20	1

SDW shoot dry weight, *RDW* root dry weight, *Sh conc*. shoot Cd concentration, *R conc*. root Cd concentration, *TF* Cd translocation factor, *UI* Cd uptake index, *A. Cd* available (DTPA extractable) Cd

* Correlation is significant at the 0.05; ** correlation is significant at the 0.01

Table 5 Available (DTPA extractable) Cd (mg kg^{-1}) in NRS and FFRS of *Helianthus annuus* in application of different electrical fields and chelating agents

	30 V	10 V	No voltage
NRS			
CME	0.04^{d} (±0.007)	0.009^{e} (±0.007)	0.10^{c} (±0.01)
PME	0.04^{d} (±0.002)	0.05^{d} (±0.01)	0.14^{a} (±0.01)
EDTA	0.003^{e} (±0.003)	0.11^{bc} (±0.01)	0.04^{d} (±0.02)
Control	0.12^{ab} (±0.01)	0.04^{d} (±0.01)	0.005^{e} (±0.009)
FFRS			
CME	0.04^{cd} (±0.01)	0.10^{ab} (±0.006)	0.08^{bc} (±0.02)
PME	0.06^{cd} (±0.01)	0.03^{de} (±0.01)	0.09^{b} (±0.02)
EDTA	0.005^{e} (±0.005)	0.11^{ab} (±0.02)	0.07^{bc} (±0.03)
Control	0.13^{a} (±0.01)	0.06^{cd} (±0.02)	0.03^{de} (±0.03)

Values with different letters show significant differences at the 0.05 probability level for each soil

NRS soil near the root system, *FFRS* soil far from root system, *CME* cow manure extract, *PME* poultry manure extract

Conclusion

In this experiment, the effects of EDTA, CME, and PME as chelating agents and using the plant as a cathode on the phytoremediation indices were investigated. Application of chelating agents and electrical fields had no significant impacts on shoots and roots dry weights of *H. annuus*, except that EDTA reduced roots dry weight. Treating the plants with EDTA along with increasing the voltage of electrical fields increased the concentration of Cd in plants' shoots. None of the chelating agents had significant impacts on Cd uptake and acquisition by *H. annuus*. Highest Cd TF was measured in 10 V treatment, while Cd UI increased by increasing the voltages so that the highest UI was obtained in the 30 V with no significant difference with 10 V. Based on the obtained results of the current experiment, charging the plant with DC electricity, which could be recognized and improved as a new method of phytoextraction, enhanced the efficiency of Cd phytoremediation.

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