A novel strategy using biodegradable EDDS for the chemically enhanced phytoextraction of soils contaminated with heavy metals

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Abstract

For the sake of cost and potential environmental risk, it is necessary to minimize the amount of chelants used in chemically-enhanced phytoextraction. In the present study, a biodegradable chelating agent, EDDS was added in a hot solution at 90°C to the soil in which garland chrysanthemum (*Chrysanthemum coronarium* L.) and beans (*Phaseolus vulgaris* L., white bean) were growing. The application of hot chelant solutions was much more efficient than the application of normal chelant solutions (25°C) in improving the uptake of heavy metals by plants. When 1 mmol kg^{-1} of EDDS as a hot solution was applied to soil, the concentrations of Cu, Zn and Cd and

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the total phytoextraction by the shoots of the two plant species exceeded or approximated those in the shoots of plants treated with 5 mmol kg^{-1} of normal EDTA solution. The concentrations of metals in the shoots of beans were significantly correlated with the relative electrolyte leakage rate of root cells, indicating that the root damage resulting from the hot solution might play an important role in the process of chelant-enhanced metal uptake. The soil leaching study demonstrated that decreasing the dosage of chelant resulted in decreased concentrations of soluble metals in soils. On the 28th day following the application of chelant, the concentrations of soluble metals in the EDDS treated soil were not significantly different from the concentrations in the control soil to which chelants had not been applied. The application of biodegradable EDDS in hot solutions to soil may be an efficient alternative in chemical-enhanced phytoextraction to increase metal removal and to reduce possible leaching.

Introduction

The clean-up of soils contaminated with heavy metals is one of difficult tasks faced by environmental engineers. A number of techniques have been developed to remove heavy metals from contaminated soil, including *ex situ* washing with physical-chemical methods, and *in situ* phytoextraction. Recently, phytoextraction techniques, using plants to extract heavy metals from contaminated soil, have become more attractive because they cost less and are more environmentally friendly than conventional *ex situ* clean-up technologies (Salt et al., 1998; Garbisu and Alkorta, 2001).

In order to obtain higher efficiency in accumulating heavy metals in the shoots of target plants, many chelants such as EDTA (ethylenediaminetetraacetic acid), CDTA (trans -1, 2 -diaminocyclohexane -N, N, N', N'-tetraacetic acid), and EDDHA [etylenediamine-di (*o*-hydroxyphenylacetic acid)]) have been applied in chemical-enhanced technology (Blaylock et al., 1997; Wu et al., 1999; Shen et al., 2002). Among all of the chelants, EDTA is one of the most widely used and can produce the highest metal extraction efficiency, especially for the phytoremediation of Pb. However, EDTA and EDTA-heavy metal complexes can be toxic to plants and soil microorganisms and they can also persist in the environment due to their low biodegradability (Bucheli-Witschel and Egli, 2001; Grčman et al., 2003). This may increase the potential off-site migration of metals, either in surface runoff or by the leaching of metals into groundwater (Nowack, 2002; Römkens et al., 2002; Madrid et al., 2003; Chen et al. 2004a). Therefore, in addition to the use of appropriate plants and suitable techniques for applying chelating agents, the addition of chelants to soil should be minimized for environmental and cost reasons.

In the last few years, the use of some easily biodegradable chelating agents, such as NTA (nitrilotriacetate) and EDDS (S,S-ethylenediaminedisuccinic acid) has been proposed to enhance the uptake of heavy metals in soil phytoextraction (Kulli et al., 1999; Kayser et al., 2000; Grčman et al., 2003; Kos and Leštan, 2003a, b; Meers et al., 2005). However, NTA and EDDS have generally been less effective than EDTA in increasing the phytoextraction of Pb and other metals in plant shoots (Shen et al., 2002; Kos and Leštan, 2003a; Luo et al., 2005a). Kos and Leštan (2003a) observed that the application of EDDS at 10 mmol kg^{-1} increased the concentration of Pb in cabbage leaves by 89 times compared to the control, to 464 mg kg^{-1}. But the effects were still considered insufficient for practical application in field, even at the highest concentrations of heavy metals achieved in the harvestable plant tissues (Grčman et al., 2003).

Several studies on the accumulation of Pb in plants showed that both Pb and EDTA were present in the shoots, suggesting that the metal was absorbed and transferred as a Pb-EDTA complex (Vassil et al., 1998; Epstein et al., 1999). Bell et al. (1991) suggested that the plant uptake of metal chelant complexes occurs at the breaks in the root endodermis and Casparian strip. Our previous study (Luo et al., 2006) has shown that some physiological damage to the roots, such as hot water pretreatment would be useful in enhancing the uptake of metal-chelants, such as metal-EDTA, by plants, which in turn can minimize the amounts of chelants that need to be applied in the practical operation of chelant-assisted phytoremediation, and the associated environmental risks of mobilized metals in soils.

The objectives of the present study were: (i) to investigate whether soil amendments with biodegradable EDDS, in comparison to EDTA, in hot solutions can further enhance the uptake of heavy metals by plants from artificially metal contaminated soils; (ii) to evaluate using soil dissolution experiments the potential leaching of solubilized metals after the application of chelants; and (iii) to further study the mechanisms involved in chelant-induced metal accumulation in plants using hydroponic experiments.

Materials and methods

Soil properties

Soil samples (gray fluvo-aquic soil) were collected from a disused agricultural field in the Yuen Long area of Hong Kong. The samples passed through a 2 mm sieve and air-dried for one week. The soils were artificially contaminated with Cu (400 mg kg^{-1} of soil) as $CuCO_3$ (copper carbonate); Pb (500 mg kg^{-1} of soil) as $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (lead sulfide – galena, a common lead mineral in mining areas) at a Pb concentration ratio of 1:1; Zn (500 mg kg^{-1} of soil) as $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulphide) at a Zn concentration ratio of 1:1; and Cd (15 mg kg^{-1} of soil) with $Cd(NO_3)_2{\cdot}4H_2O$ (cadmium nitrate). The basal fertilizers applied to the soil were 80 mg P kg^{-1} of dry soil, and 100 mg K kg^{-1} of dry soil as KH_2PO_4 (Shen et al., 2002). After the addition of heavy metals, the soils were equilibrated for two months, undergoing seven cycles of saturation with de-ionized water and air-drying processes. The electrical conductivity (EC) of the soil was measured using a conductivity meter on the soil extract, obtained by shaking soil with double-distilled water at a water-to-soil ration of 1:2 (w/v). The soil pH was measured by 0.01 M $CaCl_2$ at a 1:5 ratio (w/v) using a pH meter. The cation exchange capacity (CEC) of the soil was determined using the ammonium acetate saturation method. The soil texture, organic matter content, total N, and field capacity were measured by the procedures described by Avery and Bascomb (1982). The total metal concentrations were determined by ICP-AES (Perkin-Elmer Optima 3300 DV) after strong acid digestion (1:4 concentrated HNO_3 and $HClO_4$ (v/v)) (Li et al., 2001). The selected physical and chemical properties of the soil are presented in Table 1.

Hot EDDS and EDTA treatments

Air-dried soils (500 g) were placed in plastic pots (12 cm i.d. x 12 cm height). Soil moisture was maintained to near field water capacity by adding deionized water (DIW) on a daily basis. Seeds of garland chrysanthemum (*Chrysanthemum coronarium* L.) and beans (*Phaseolus vulgaris* L., white bean) were sown directly in the soils. In order to acquire uniform seedlings, beans were sown 14 d after that of the garland chrysanthemum seeds. After germination, seedlings were thinned to four plants per pot. On the 35th day after the sowing of garland chrysanthemum, EDTA (BDH Laboratory Supplies Poole, UK, minimum assay: 99.5%) and EDDS (Fluka Chemie GmbH, UK) were applied to the surface of the soils in two different ways (heated and not heated) at rates of 0 (control), 1.0, 3.0, and 5.0 mmol kg^{-1} of soil as 100 ml Na_2EDTA and Na_3EDDS solutions. To make up the different amounts of chelant treatments, EDTA and EDDS were diluted from 50 mM Na_2EDTA (pH 4.8) and Na_3EDDS (pH 10.1) salt solutions. The hot chelant solution treatments were conducted by adding boiled solution to soil in the pots, which resulted in the final temperature of the soils being about 40 ºC at the 2/3 depth of the pot. Three replicates were conducted for each treatment. All experiments were conducted in a glasshouse under natural light. Air temperatures ranged from 16 to 21 ºC. All plants were harvested by cutting the shoots 0.5 cm above the surface of the soil, and removing the roots from the pots 7 d after the application of chelants. The shoots and roots were washed with tap water and rinsed with DIW (deionzed water), and dried at 70 ºC in a drying oven to a constant weight for dry weight measurements. The dried plant materials were ground using an agate mill.

Metal leaching study

After harvesting the plants, soils in pots were brought to 2/3 field capacity. On Day 0, 7, 14 and 21 (i. e. on Day 7, 14, 21 and 28 after the application of chelants), the soil in every pot was mixed thoroughly and 4.0 g of soil (based on dry weight) were placed in a 50 mL polypropylene centrifuge tube. DIW was added to the soil (at a soil:water ratio of 1:5) and the suspension shaken for 30 min. After centrifugation, the supernatants were filtered through a 0.45 μm filter paper (Whatman UK] No 42), acidified with $HNO_{3,}$ and analyzed for metal concentrations by ICP-AES (Perkin Elmer 3000DV).

Root pretreatment with hot water

Seeds of beans were sterilized in 0.1% (w/v) $HgCl_2$ for 10 min, and rinsed four times in deionized water before being placed on filter paper for germination. After germination, plants of the same size were selected and transferred to 2 L polyethylene vessels containing a modified 0.2-strength Rorison's nutrient solution (Hewitt, 1966) with the following composition (in μmol L^{-1}): 400 $Ca(NO_3)_2$, 200 $Mg(SO_4)_2$, 50

K_2HPO_4, 300 KCl, 9.2 H_3BO_3, 1.8 $MnSO_4 \cdot 4H_2O$, 0.21 $Na_2MoO_4 \cdot 2H_2O$, 0.31 $CuSO_4 \cdot 5H_2O$, 10 $ZnSO_4 \cdot 7H_2O$, and 10.8 Fe-EDTA at pH 6.0. Nutrient solutions were aerated continuously and renewed every two days. The plants were grown in a glasshouse where the temperature ranged from 17 ºC to 22 ºC.

After seven days of transplanting, different pretreatments were conducted to assess the effects of root damage by hot water on the accumulation of Pb in shoots. Nine pretreatments were included: the roots were exposed in hot water at 30 ºC, 40 ºC, 50 ºC, 60 ºC, and 80 ºC for 15 min. For the pretreatment at 40 ºC, the roots were exposed in hot water for 15, 30, 45, and 60 min. The plants without hot pretreatment (where the room temperature was about 25 ºC) were used as the control. After pretreatment, 15 plants from each treatment were used to measure the relative electrolytic leakage rate of root cells by electrical conductivity (Zhu et al., 1990; Zhou and Leul, 1998). The root samples (0.5 g) were placed in a test tube containing 15 ml of deionized water and the root tissue was immersed and vibrated at room temperature for 2 h. The conductivity of the solution was measured using a conductivity meter (DDS - 11A). After boiling the samples for 10 min, the conductivity was measured again when the solution had cooled to room temperature. The relative electrical conductivity (REC) was calculated as follows: $REC = C_1 / C_2 \times 100$, where C_1 and C_2 were the electrolyte conductivities measured before and after boiling, respectively. Half of the remaining 30 plants from each treatment were treated with 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDTA and another half were treated with 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDDS for 2 d, respectively (pH 6.0). Pb, EDTA, and EDDS were applied in the forms of $Pb(NO_3)_2$, Na_2EDTA, and Na_3EDDS solutions, respectively. Each treatment was replicated three times. At the end of these experiments, the shoots and roots were harvested for further chemical analysis. The effects of root damage on the accumulations of Cu, Zn, and Cd were studied in the same way, whereby Cu, Zn, Cd were applied in the forms of $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, and $CdNO_3 \cdot 4H_2O$ solutions, respectively.

Plant analysis

Sub-samples of ground shoot dry matter (200 mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1 v/v), and the major and trace elements in the solutions were determined with ICP-AES (Chen et al., 2004b). A certified standard reference material (SRM 1515, apple leaves) from the National Institute of Standards and Technology, U.S.A., was used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery rates were around 93 ± 9% for all of the metals in the plant reference material. The data reported in this paper are the mean values based on the three replicated experiment results. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software.

Results

Plant growth

Application of EDTA and EDDS had a significant effect on the growth of plants and shoot biomass yield. The dry weights of the shoots of garland chrysanthemum and beans decreased as the level of the chelant applied to the soil increased (Fig. 1). The results also showed that the decrease was more pronounced when EDTA and EDDS were applied as hot solutions to the surface of the soil than was the case with the treatments without heating. Compared to shoot dry matter yields in the treatments with corresponding chelant solutions without heating, such yields on the 7^{th} day after the application of the chelants decreased 13% and 15% for garland chrysanthemum, and 21% and 24% for beans as a result of the treatments with hot solutions of EDTA and EDDS, respectively.

Metal concentrations and phytoextraction in hot EDDS and EDTA treatments

Compared to the control group, the addition of EDDS and EDTA significantly increased the concentrations of Cu, Pb, Zn, and Cd in the shoots of both plant species (Figs. 2 and 3). EDDS was more effective at increasing the concentration of Cu in the shoots of the two species than EDTA, but less effective for Pb and Cd. In all treatments, the uptake of the metals in the shoots of garland chrysanthemum was greater than in beans.

At the same application dosage, application of hot chelant solutions produced higher concentrations of Cu, Pb, Zn, and Cd in the shoots of both plant species than the application of chelant solutions without heating (Figs. 2 and 3). The concentrations of Cu ranged from 3850 to 5850, and 2710 to 3710 mg kg^{-1} in the shoots of garland chrysanthemum treated with hot EDDS and EDTA, respectively, which were 4 - 21 and 6.8 - 16 times those with the normal chelants treatments without heating, and 136 - 207 and 96 - 131 times that in the control group, respectively. The highest Pb concentration of 2330 mg kg^{-1} was found in the shoots of garland chrysanthemums treated with hot EDTA at the rate of 5 mmol kg^{-1}, followed by 2080 mg kg^{-1} in the treatment with hot EDDS of 5 mmol kg^{-1}. The average enhanced effects of hot EDTA and EDDS on the Pb shoot uptake were 10.4 and 6.7 times that in the corresponding chelant treatment without heating. Chelants were found to have a less significant stimulatory effect on the uptake of Zn and Cd in these two plants. When EDTA and EDDS were applied at rates of 1 - 5 mmol kg^{-1}, the concentrations of Zn and Cd in the shoots of both plant species did not exceed 3.2 and 5.9 times those of the controls. The applications of hot EDTA and EDDS increased the concentration of metals in shoots by 3.8-13.1 and 2.6-11 times for Zn, and by 5.5 - 67 and 1.4 - 23 times for Cd, compared with the controls, respectively. The concentrations of Cd were much higher in the shoots of both plant species treated with hot EDTA than in those treated with hot EDDS.

Total metal phytoextraction by the shoots of garland chrysanthemum and beans is shown in Table 2. Of the two plant species tested, garland chrysanthemum was superior at the phytoextraction of metals than beans. Similar to the effects of chelants on the concentration of metals in the shoots, the maximum phytoextraction of Cu was found in the heated EDDS treatments at the rate of 1 and 3 mmol kg^{-1} of soil, which increased 82- and 35-fold in garland chrysanthemum and beans, respectively, compared with the control group (adding hot water). For Pb, the plants treated with 5 mmol kg^{-1} of hot EDTA attained the maximum level of phytoextraction of approximately 118- and 101-fold that in the corresponding control garland chrysanthemum and bean plants. The total amounts of Zn that were extracted did not exceed 8.8 times that of the controls, but were significantly higher in the plants treated with hot EDTA and EDDS than in those treated with chelants without heating. The maximum Cd phytoextraction was observed in the heated EDTA treatment at the rate of 3 mmol kg^{-1} of soil, which was 6 and 40 times the level seen in the control group of garland chrysanthemum and beans.

Metal leaching study after the treatment with EDDS and EDTA

In order to examine the potential of metal leaching in pots, the soil solution was extracted within 28 days after the application of chelants. For the same metal, the concentrations of water-soluble metals in soil were mainly dependent upon the chelant type and application rate (Table 3 and Fig. 4). No significant differences were observed in the concentrations of soluble metals in the soils between the treatments with hot chelant solutions and those with normal chelant solutions at the same application dosage (Table 3). The concentrations of soluble Cu were higher in the soil treated with EDDS than those with EDTA. However, EDTA was more effective in solubilizing soil Pb and Cd than EDDS. In all treatments, the concentrations of water-soluble metals increased as increasing levels of EDTA and EDDS were applied to soils, and decreased as time progressed (Fig. 4). This decrease was more pronounced in soil treated with EDDS than in soil treated with EDTA. For example, average concentrations of soluble Cu, Pb, Zn and Cd decreased by 97, 44, 81, and 82%, respectively, from the 7^{th} to 28^{th} day after the application of EDDS. On the 28^{th} day after application of chelant, no significant differences were found in the concentrations of soluble metals between the EDDS treatments and the controls (without the application of chelant). In the soil treated with EDTA, the concentrations of soluble Cu, Pb, Zn, and Cd decreased only by 26, 36, 39 and 40%, respectively, from the 7^{th} to the 28^{th} day after application of chelant, and were still significantly higher than those in the control group.

Effects of pretreatment with hot water on the accumulation of Pb in beans

The roots of beans were pretreated with hot water at different temperatures before they were exposed in solutions containing 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDTA and 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDDS, respectively. Two days after Pb + EDTA or EDDS exposure, Pb concentrations in shoots were measured. The results showed that there was a significantly positive correlation between the water temperature and root cell electrolyte leakage rate (R^2 = 0.92, n = 18) (see Fig. 5). A significantly positive correlation was also shown between the Pb concentration in shoots and the relative electrolyte leakage rate of root cells (R^2 = 0.91, n = 27 for EDTA treatment; and R^2 = 0.90, n = 27 for EDDS treatment) (see Fig. 6). Similar significantly positive correlation results were also obtained for Cu, Zn and Cd (see Table 4).

Discussion

The chemically-enhanced phytoextraction of soils contaminated with heavy metals has been shown to be a potential way of removing heavy metals from soils with high biomass plants (Huang et al., 1997; Liphadzi et al., 2003). In the present study, the results demonstrated that the application of chelants to soils led to a rapid and significant increase in the concentrations of heavy metals in the shoots of garland chrysanthemum and beans. Our results also showed that the accumulation of heavy metals in plant shoots improved substantially when chelants, including non-degradable EDTA and biodegradable EDDS, were added as hot solutions to soil. For all heavy metals that were studied when chelants were applied as hot solutions at the rate of 1 mmol kg^{-1}, metal concentrations and total phytoextraction of Cu, Zn and Cd by plant shoots exceeded or at least approximated those in the shoots of plants treated with normal chelants at a rate of 5 mmol kg^{-1}. The enhanced effect was most significantly for Cu. For Pb, the concentration and total phytoextraction observed at the treatment of 1 mmol kg^{-1} of hot EDDS were lower than that achieved by the application of 5 mmol kg^{-1} of normal EDTA. However, they were still higher than that of 1 mmol kg^{-1} normal EDTA, with an average 13 and 9.5–fold improvements compared with the control group (with the application of normal water) in the two plant species, respectively. This result implies that the amount of chelant applied could be greatly decreased, for the given effectiveness of chelants in enhancing phytoextraction of heavy metals from contaminated soils.

The *in situ* application of chelants may pose the potential risk of causing groundwater pollution through uncontrolled metal solubilization and migration (Nowack, 2002; Römkens et al., 2002; Shen et al., 2002; Madrid et al., 2003; Chen et al. 2004a). Concentrations of soluble metals in soil significantly increased with the level of chelant applied to the soil (Table 3). A reduction in the amount of chelant applied could result in a marked decrease in the concentrations of water-soluble metals in the soil. Therefore, the application of hot chelant solution could not only help to reduce the cost of the operation but also alleviate the potential risk of the migration of chelant and heavy metals to groundwater and to the surrounding environment.

Previous studies indicated that EDDS was more effective at increasing the concentration of Cu in shoots than EDTA (Luo et al., 2005a, b; Meers et al., 2005). It was suggested that EDDS-assisted phytoextraction could be an acceptable approach for the remediation of Cu-contaminated soils (Luo et al., 2005a). The results of the current study show that EDDS is superior to EDTA in the extraction of Cu by plant shoots from contaminated soil. The increased uptake of Cu by the application of hot EDDS was much higher than that of EDTA (Lombi et al., 2001; Meers et al., 2005), EDDS (Kos and Leštan, 2003a, b; Meers et al., 2005) and NTA (Kulli et al., 1999; Kayser et al., 2000). The percentage of Cu extracted was 3.4-6% of the total Cu in the soil by the shoots of garland chrysanthemum during a 42-d period of plant growth and 1-1.3% by beans for 28 days. These values were higher than the data reported by Kos and Leštan (2003b) and comparable with the results of Blaylock et al. (1997) for Pb extraction with EDTA.

Of the chelants tested for solubilizing soil Pb and enhancing the accumulation of the metal in plant shoots, EDTA has been found to be the most effective due to its strong chemical affinity for Pb (log K_s = 17.88) (Huang et al., 1997; Tandy et al., 2004; Shen et al., 2002; Luo et al., 2005a, b). In the present study, the concentrations of Pb in the shoots of garland chrysanthemums and beans reached 2080 and 1320 mg kg^{-1} on the 7^{th} day after the addition of 5 mmol kg^{-1} of hot EDDS solutions to the soil (Figs. 2 and 3), respectively, which represented a 365- and 176-fold increase compared to that in the corresponding controls; and increased 7.2- and 11.5-fold compared with that in the plants treated with 5 mmol kg^{-1} of normal EDTA. For the extraction of Pb in the shoots of garland chrysanthemum and beans, increases of up to 94- and 74-fold were also found with 5 mmol kg^{-1} of hot EDDS compared with those in the control (Table 2). The increased uptake of Pb was much higher by the application of hot EDDS than that of normal EDTA at the same rates of application, as reported previously (Grčman et al., 2003; Luo et al., 2005a, b). This indicated that hot EDDS solutions might also be effective in the phytoremediation of Pb-contaminated soils. In the pot experiments described in the literature, the concentrations of Pb in plant shoots were generally lower than 2000 mg kg^{-1} DW after the application of EDTA (Wu et al., 1999; Bricker et al., 2001; Grčman et al., 2001; Lombi et al., 2001; Barocsi et al., 2003; Grčman et al., 2003; Kos and Lestan, 2003a; Kos et al., 2003; Walker et al., 2003; Wenzel et al. 2003; Chen et al., 2004a; Lim et al., 2004; Meers et al., 2004), except for the results in a few experiments (Blaylock et al., 1997; Huang et al., 1997; Epstein et al., 1999; Shen et al., 2002). Blaylock et al. (1997) reported that the concentrations of Pb in the shoots of Indian mustard increased from less than 100 to 15 000 mg kg^{-1} when the plants were grown in soil containing 600 mg kg^{-1} of Pb amended with 10 mmol kg^{-1} of EDTA. Huang et al. (1997) measured more than 10 000 mg kg^{-1} of Pb in the shoots of corn grown in soil containing 2 500 mg kg^{-1} of Pb with the addition of 5.5 mmol kg^{-1} of EDTA. The different Pb phytoextraction efficiencies of the EDTA treatment might be attributed to different experimental conditions, for example, soil properties, plant status and methods of applying chelant.

EDTA and its complexes with metals were usually toxic and poorly photo-, chemo-, and biodegradable in soil environments, which can persist in soil for several months after harvest of the phytoextraction crops (Bucheli-Witschel and Egli, 2001; Nowack, 2002; Grčman *et al.*, 2003). In comparison to EDTA, EDDS has a clear advantage because it is readily biodegradable and is less toxic to fish, *Daphnia*, and soil fungi (Jaworska et al., 1999; Grčman et al., 2003).The calculated half-life of EDDS in sludge-amended soil is 2.5 days (Jaworska et al., 1999). The results from the leaching study showed that, at the end of the experiment of 28 d, after the harvesting of the plants, metal solubility in the soil treated with EDDS was not significantly different from that in the control group. This implied that residual EDDS in the soil had been degraded and that the risk of metal leaching to the surrounding environments was relatively low.

Several studies on the accumulation of Pb in plants have shown that this metal was absorbed and transferred as a Pb-EDTA complex in the presence of high concentrations of EDTA (Vassil et al., 1998; Epstein et al., 1999). Sarret et al. (2001)

reported that both Pb and EDTA could be absorbed by plants, and that some of the Pb present in the leaves of *P. vulgaris* was complexed by EDTA. If plant uptake of metal chelating complexes occurs at breaks in the endodermis of the root and in the Casparian strip as suggested by Bell et al. (1991), in the chemically-enhanced phytoextraction process uptake of metal would be strongly dependent on the concentration of the metal-chelant complex in the solution and on the breakdown of the root exclusion mechanism. In our pot experiment, it was presumed that high temperatures caused the breakdown of the root exclusion mechanism, and that the chelant increased the concentrations of the metal-chelant complex in soil solution, especially when the chelants were applied in hot solutions, which led to the rapid equilibration of metal-chelant between the external solution and the sap of the xylem. After entering the xylem, metal-chelant would be translocated from the roots to shoots in the transpiration stream, leading to high concentrations and the accumulation of metals in shoots. It was found that in the temperature range of 8 - 48 ºC each 10 ºC increment resulted in a 6% increase in the metal extracted from soil for Zn, Pb and Cd (Vandevivere et al., 2001). Enhanced concentrations of metals in plant tissues with increasing temperature were observed in other experiments (Antoniadis and Alloway, 2000; Fritioff et al., 2005). This hypothesis was also confirmed by the data obtained from the hydroponic experiment presented here. Figure 6 shows a significantly positive correlation between the Pb concentration in the shoots of beans and the relative electrolyte leakage rate of root cells (root damage by hot water). Therefore, the root damage treatment can play an important role in increasing metal uptake in chemically-enhanced phytoextraction. The application of hot EDDS solutions could be a good alternative approach in this direction.

Conclusions

The biodegradable chelant EDDS added in hot solutions to soil greatly enhanced the phytoextraction of metals by shoots of garland chrysanthemum and beans, and did not promote further leaching of metals compared to normal application of EDDS without heating. The significantly enhanced uptake of metals by plants might be attributed to an increased metal solubilization in the short term, and the root damage to the further breakdown of a root exclusion mechanism. The application of hot EDDS solution may be a more efficient alternative in chemical-enhanced phytoextraction.

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*Table 1.*The physicochemical properties of the soils used in the study

pH ($CaCl_2$)	7.12
Electrical conductivity at 25°C (μS cm^{-1})	262
Sand (%) > 0.05 mm	79.5
Silt (%) 0.05 - 0.001 mm	13
Clay (%) < 0.001 mm	7.5
N_{Total} (%)	0.15
Organic matter (%)	2.7
Cation exchange capacity (cmol kg^{-1})	4.2
Field water capacity (%)	39.7
Total metal concentration after amendment (mg kg^{-1})	
Cu	480
Pb	575
Zn	700
Cd	17

Table 2. Total phytoextraction (μg pot^{-1}) of Cu, Pb, Zn, and Cd in the shoots of garland chrysanthemum and beans 7 d after the application of EDTA and EDDS at different concentrations (mmol kg^{-1} soil)

	Garland chrysanthemum				**Beans**			
Treatments	Cu	Pb	Zn	Cd	Cu	Pb	Zn	Cd
Water	80.4 ± 9.5 a	16 ± 3 a	1330 ± 210 a	52.9 ± 6.5 a	36.2 ± 4 a	11.6 ± 5 a	228 ± 35 a	2.6 ± 1 a
Hot-water	146 ± 24 a	36.5 ± 4.8 a	1190 ± 150 a	47.6 ± 5.9 a	72.8 ± 9 a	14.5 ± 3 a	202 ± 21 a	2.7 ± 0.9 a
1mM EDTA	448 ± 69 a	98.4 ± 11 a	1360 ± 150 a	87.9 ± 14 a	190 ± 29 a	34.6 ± 6 a	312 ± 54 a	9.7 ± 3 a
Hot-1mM EDTA	5800 ± 672 c	1040 ± 185 b	3830 ± 450 b	218 ± 35 c	1560 ± 250 c	335 ± 40 b	1180 ± 210 b	69.6 ± 8.9 c
3mMEDTA	833 ± 53 b	333 ± 47 a	1540 ± 250 a	82.9 ± 9 a	201 ± 35 a	63 ± 12 a	317 ± 54 a	9.6 ± 1.9 a
Hot-3mM EDTA	7090 ± 912 c	3220 ± 410 d	5180 ± 680 c	288 ± 40 c	2110 ± 350 c	987 ± 75 c	1740 ± 210 c	108 ± 17 c
5mM EDTA	1170 ± 190 b	622 ± 75 b	1730 ± 248 a	79.1 ± 13 a	284 ± 39 a	133 ± 25 a	370 ± 19 a	11.5 ± 3 a
Hot-5mM EDTA	6900 ± 824 c	4330 ± 450 d	4860 ± 610 c	263 ± 35 c	2270 ± 489 c	1470 ± 210 c	1770 ± 279 c	104 ± 15 c
1mM EDDS	656 ± 59 a	23.3 ± 3.8 a	1550 ± 360 a	52.5 ± 6 a	313 ± 47 a	9.2 ± 2 a	239 ± 21 a	2.3 ± 1 a
Hot-1mM EDDS	12100 ± 980 d	258 ± 35 a	2520 ± 340 a	54 ± 6 a	2560 ± 390 d	32.9 ± 4 a	490 ± 35 a	3.55 ± 2 a
3mM EDDS	1130 ± 235 b	516 ± 80 b	1650 ± 240 a	66.1 ± 8.9 a	352 ± 42 a	47 ± 5 a	289 ± 10 a	2.6 ± 0.5 a
Hot-3mM EDDS	7310 ± 800 c	2180 ± 250 c	4120 ± 500 b	99.8 ± 15 a	2590 ± 360 d	752 ± 68 b	1310 ± 153 b	27.3 ± 3.8 b
5mM EDDS	2060 ± 310 b	1840 ± 280 c	2130 ± 380 a	88 ± 15 a	689 ± 78 b	346 ± 45 b	492 ± 29 a	7.8 ± 2 a
Hot-5mM EDDS	6810 ± 782 c	3460 ± 490 d	3760 ± 485 b	127 ± 21 b	2080 ± 115 c	1080 ± 190 c	1160 ± 174 b	31.2 ± 2.6 b

The values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 3. Effects of the application of EDTA and EDDS at different rates (mmol kg^{-1} soil) on metal solubility (mg kg^{-1} soil) 7 d after the application

Treatments	**Cu**	**Pb**	**Zn**	**Cd**
Water	2.6 ± 0.1 a	2.22 ± 0.1 a	4.37 ± 0.2 a	0.07 ± 0.01a
Hot-water	2.9 ± 0.3 a	2.49 ± 0.2 a	4.8 ± 0.3 a	0.08 ± 0.01 a
1mM EDTA	36.7 ± 2.5 b	3.39 ± 0.2 a	18.1 ± 1.2 b	1.06 ± 0.2 b
Hot-1mM EDTA	31.6 ± 3 b	3.47 ± 0.3 a	16.6 ± 0.6 b	0.85 ± 0.1 b
3mMEDTA	90.1±5.5 c	14.1 ± 0.9 b	65.6 ± 2.9 bc	3.8 ± 0.2 c
Hot-3mM EDTA	88.8 ± 6 c	12.8 ± 1.1 b	57.5 ± 3.7 bc	3.2 ± 0.3 c
5mM EDTA	131 ± 9.7 cd	43.9 ± 3.3 c	90 ± 5.9 c	5.72 ± 0.4 c
Hot-5mM EDTA	132 ± 6.5 cd	48 ± 2.5 c	85 ± 7.2 c	5.78 ± 0.2 c
1mM EDDS	87 ± 4.7 c	2.52 ± 0.1 a	8.34 ± 0.5 a	0.04 ± 0.01 a
Hot-1mM EDDS	85 ± 3.5 c	2.56 ± 0.2 a	9.37 ± 0.3 a	0.07 ± 0.01 a
3mM EDDS	176 ± 17 d	3.44 ± 0.3 a	62.3 ± 2.1 b	0.11 ± 0.02 a
Hot-3mM EDDS	169 ± 15 d	2.96 ± 0.2 a	65.2 ± 3.6 b	0.1 ± 0.01 a
5mM EDDS	203 ± 12 d	5.04 ± 0.4 a	97 ± 4 c	0.36 ± 0.03 a
Hot-5mM EDDS	198 ± 18 d	4.1 ± 0.1 a	95 ± 6.8 c	0.22 ± 0.04 a

The values are means ± S.D. (n = 3); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 4. The correlation between the relative electrolyte leakage rate of roots and the concentrations of Cu, Zn, and Cd in the shoots of beans (R^2 was shown in the Table). Plants were pretreated with hot water at different temperatures, then exposed in solutions containing 500 μmol L^{-1} of Cu, Zn, or Cd + 500 μmol L^{-1} of EDTA or EDDS for 2 d, respectively. The root cell electrolytic leakage was measured immediately after the pretreatment with hot water

Treatments	R^2
500 μmol L^{-1} of Cu + 500 μmol L^{-1} of EDTA	0.88
500 μmol L^{-1} of Cu + 500 μmol L^{-1} of EDDS	0.95
500 μmol L^{-1} of Zn + 500 μmol L^{-1} of EDTA	0.90
500 μmol L^{-1} of Zn + 500 μmol L^{-1} of EDDS	0.94
500 μmol L^{-1} of Cd + 500 μmol L^{-1} of EDTA	0.86
500 μmol L^{-1} of Cd + 500 μmol L^{-1} of EDDS	0.87

Figure captions:

Fig. 1. Effects of the application of EDTA and EDDS on the dry matter yields of garland chrysanthemums (a) and beans (b). The values are means ± S.D. (n = 3).

Fig. 2. Effects of the application of EDTA and EDDS on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of garland chrysanthemums. The values are means ± S.D. (n = 3).

Fig. 3. Effects of the application of EDTA and EDDS on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of beans. The values are means ± S.D. (n = 3).

Fig. 4. Effects of the application of hot EDTA and EDDS at different concentrations on the solubility of Cu (a), Pb (b), Zn (c), and Cd (d). The values are means ± S.D. (n = 3).

Fig. 5 The correlation between the relative electrolyte leakage rate of roots and water temperature in the pretreatment.

Fig. 6. The correlation between the relative electrolyte leakage rate of roots and the concentration of Pb in the shoots of beans. Plants were pretreated with hot water at different temperatures, then exposed in solutions containing 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDTA or 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDDS for 2 d. The root cell electrolytic leakage was measured immediately after the pretreatment with hot water.

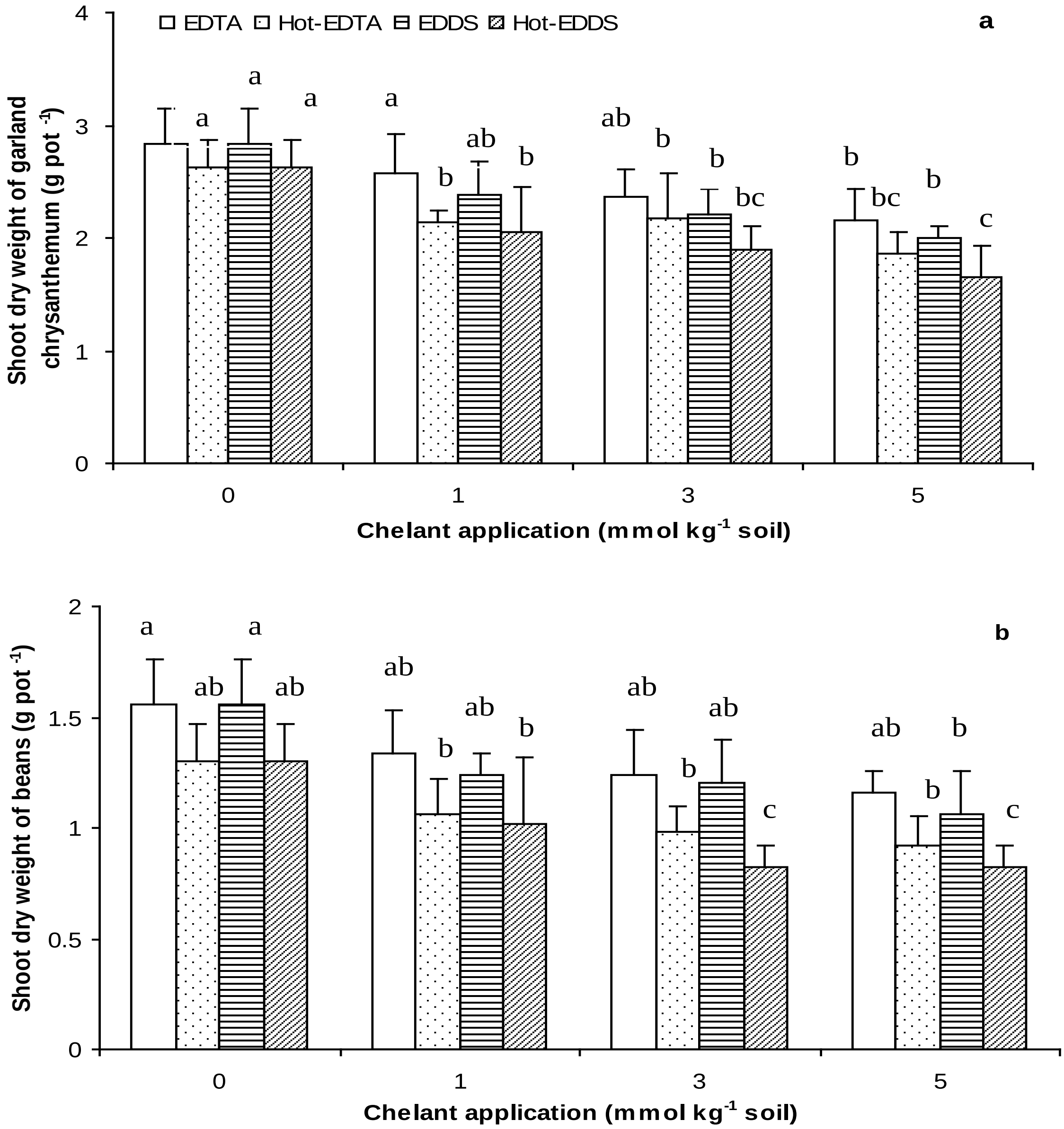

Fig. 1. Effects of the application of EDTA and EDDS on the dry matter yields of garland chrysanthemums (a) and beans (b). The values are means ± S.D. (n = 3). The different small letters stand for statistical significance at the 0.05 level with the LSD test.

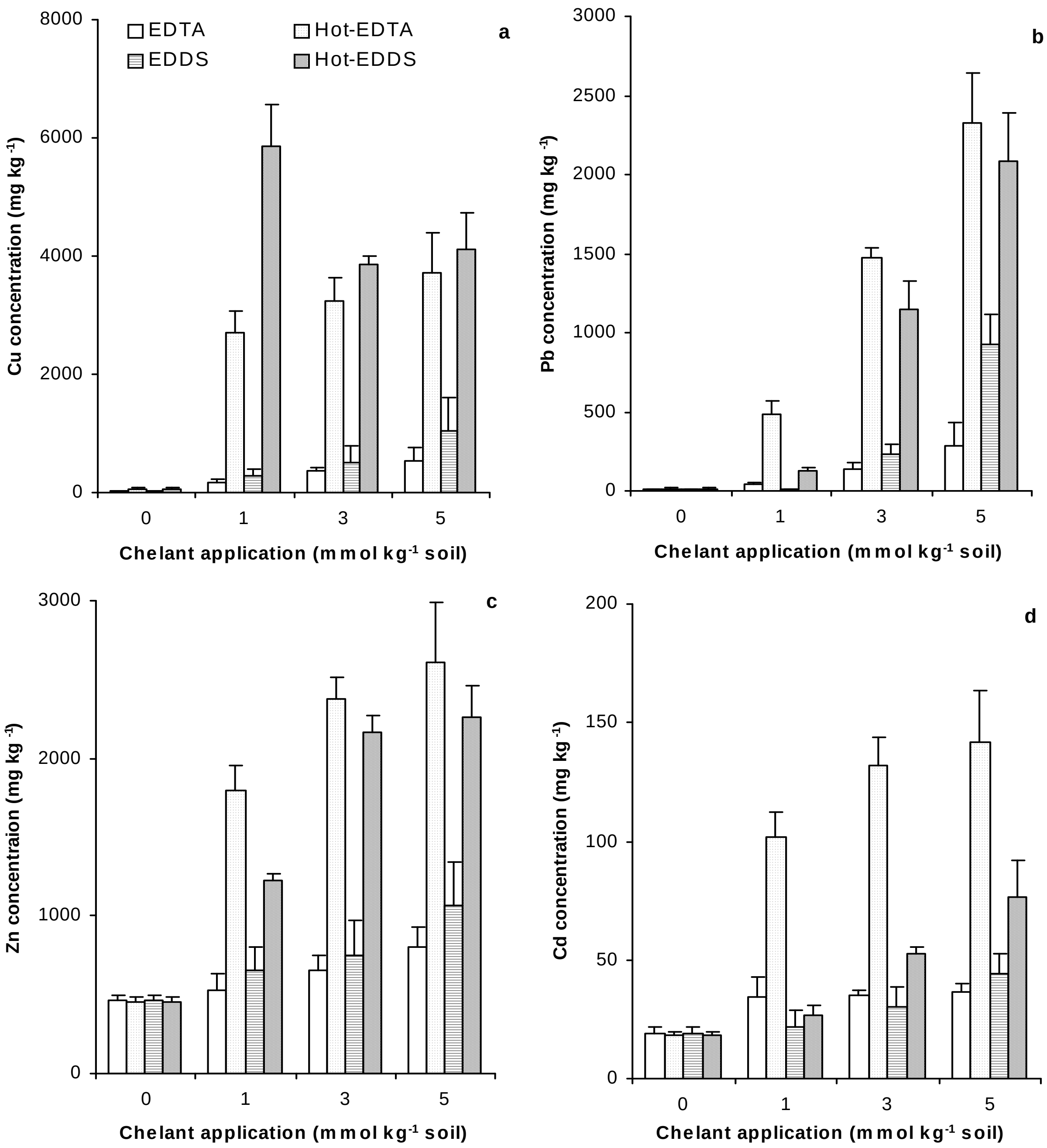

Fig. 2. Effects of the application of EDTA and EDDS on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of garland chrysanthemums. The values are means ± S.D. (n = 3).

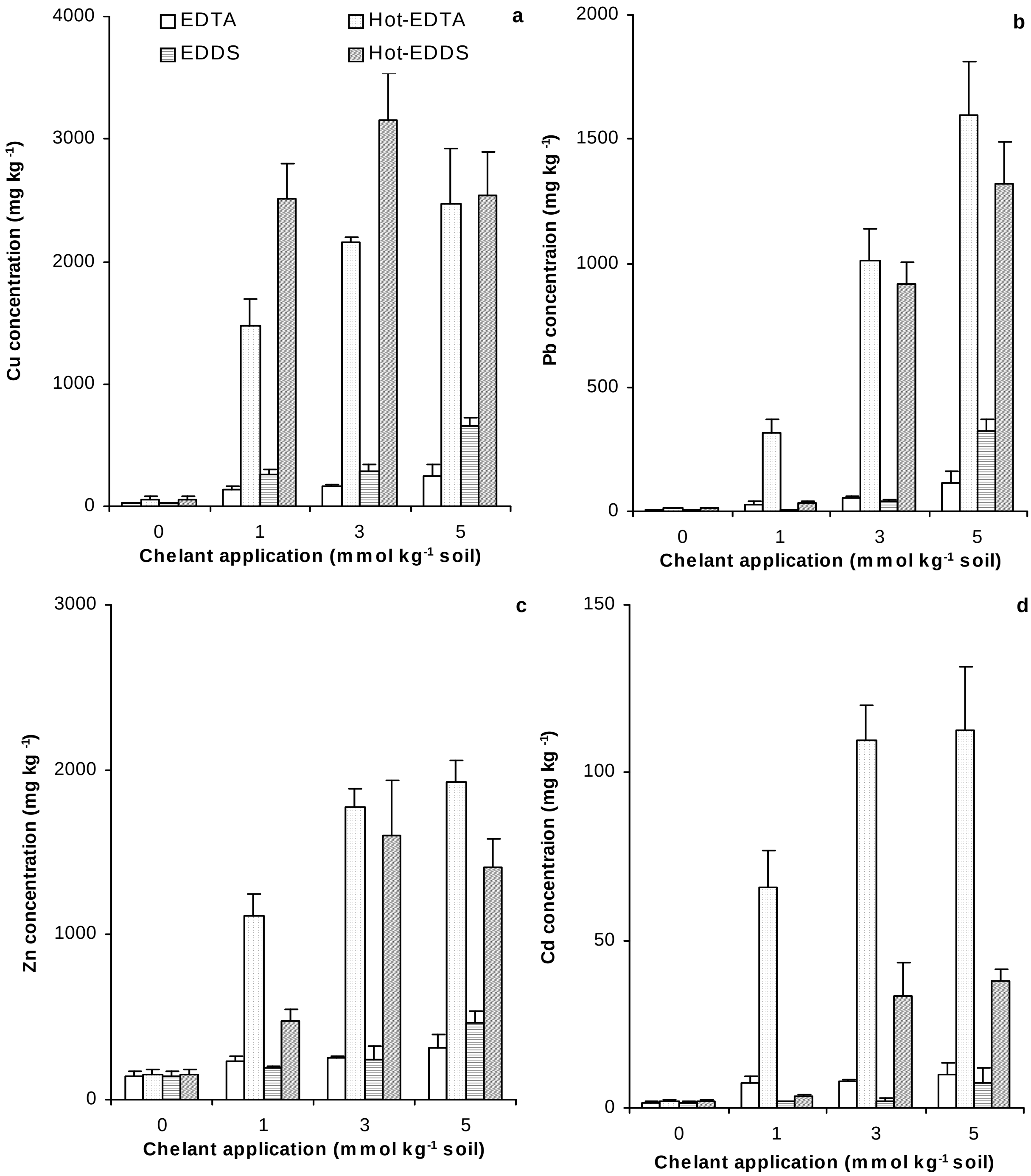

Fig. 3. Effects of the application of EDTA and EDDS on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of beans. The values are means ± S.D. (n = 3).

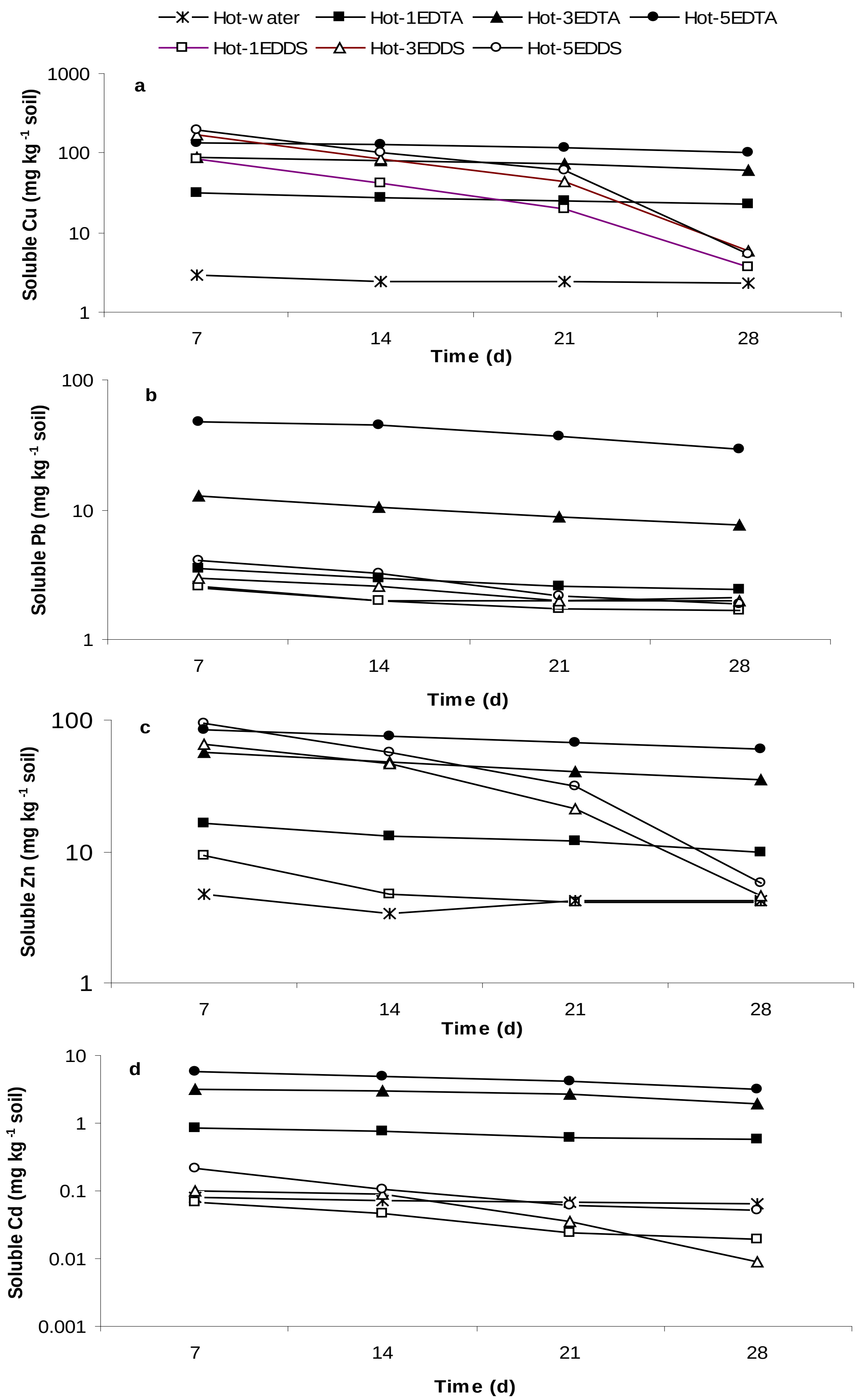

Fig. 4. Effects of the application of hot EDTA and EDDS at different concentrations on the solubility of Cu (a), Pb (b), Zn (c), and Cd (d). The values are means ± S.D. (n = 3).

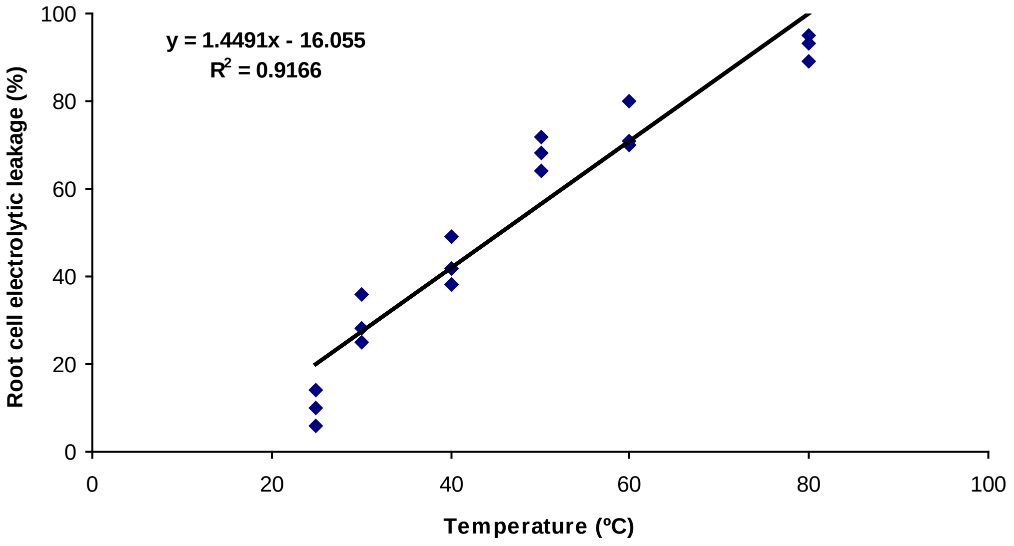

Fig. 5. The correlation between the relative electrolyte leakage rate of roots and water temperature in the pretreatment.

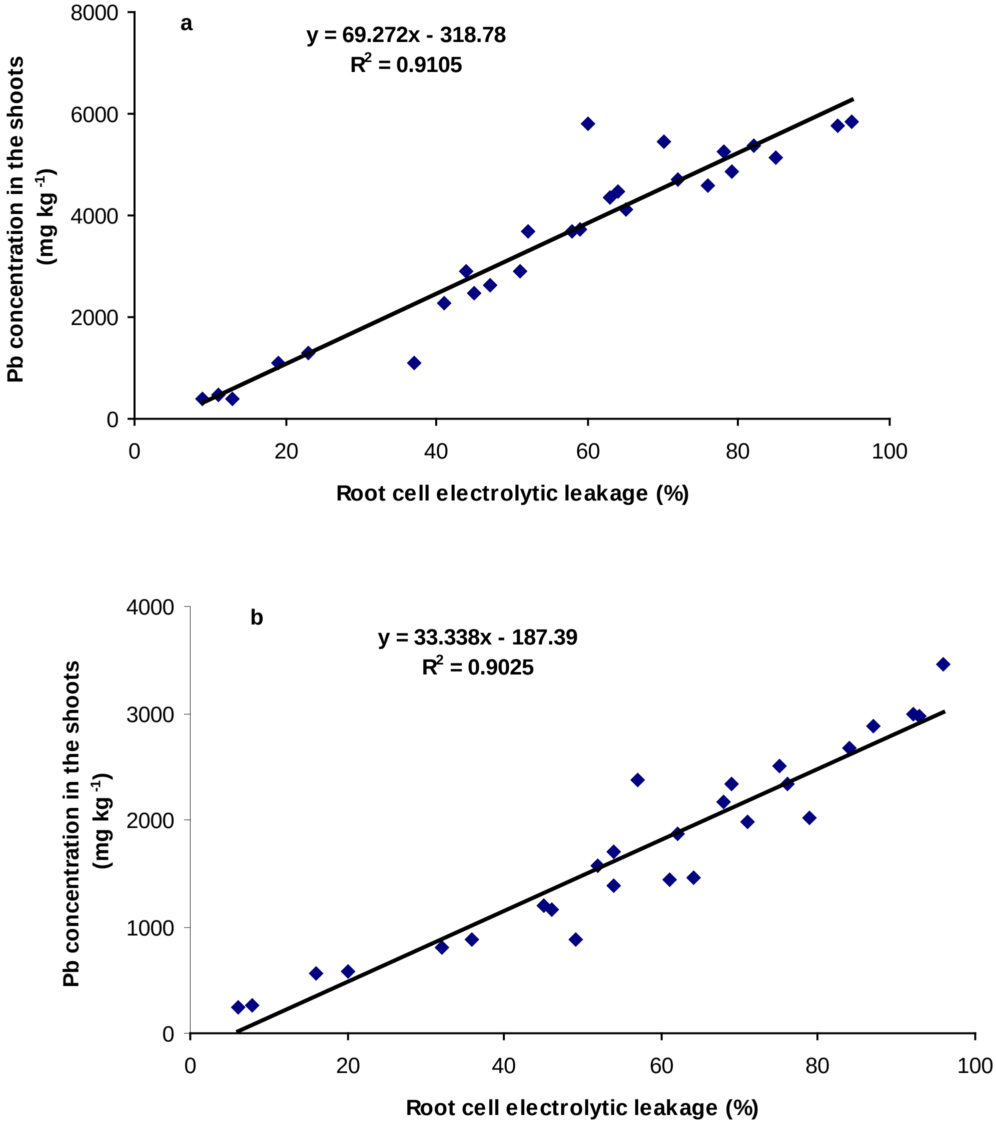

Fig. 6. The correlation between the relative electrolyte leakage rate of roots and the concentration of Pb in the shoots of beans. Plants were pretreated with hot water at different temperatures, then exposed in solutions containing 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDTA (a), or 500 μmol L^{-1} of Pb + 500 μmol L^{-1} of EDDS (b) for 2 d. The root cell electrolytic leakage was measured immediately after the pretreatment with hot water.