Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS

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Abstract

Chemically enhanced phytoextraction has been proposed as an effective approach to removing heavy metals from contaminated soil through the use of high biomass plants. Using pot experiments, the effects of the application of EDTA, EDDS and citric acid on the uptake of Cu, Pb, Zn and Cd by corn (*Zea mays* L. cv. Nongda 108) and bean (*Phaseolus vulgaris* L. white bean) plants were studied. The results showed that EDDS was more effective than EDTA at increasing the concentration of Cu in corn and beans. The application of 5 mmol kg^{-1} soil EDDS application to soil significantly increased concentrations of Cu in shoots , with maximum levels of 2060 and 5130 mg kg^{-1} DW in corn and beans, respectively, which were 45-fold and 135-fold higher than that in the corresponding control plants to which chelate had not been applied. Concentrations of Zn in shoots were also higher in the plants treated with EDDS than in those treated with EDTA. For Pb and Cd, EDDS was less effective than EDTA. The maximum Cu

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phytoextraction was found with the EDDS treatment. The application of EDTA and EDDS also significantly increased the shoot-to-root ratios of the concentrations of Cu, Pb, Zn and Cd in both plant species. The results of metal extraction with chelates showed that EDDS was more efficient at solubilizing Cu and Zn than EDTA, and that EDTA was better at solubilizing Pb and Cd than EDDS.

Keywords: Phytoextraction; EDTA; EDDS; Metals; Corn; Beans

1. Introduction

The contamination of soils with metals is a major environmental problem throughout the world. Soils polluted with metals may threaten ecosystems and human health. The remediation of soils contaminated with toxic metals is a challenging task because metals do not degrade and the danger they pose is aggravated by their almost indefinite persistence in the environment. Conventional cleanup technologies are generally too costly to be used to restore contaminated sites, and are often harmful to the normal properties of the soil (i.e., texture and organic matter) (Holden, 1989; Smith et al., 1995). The emerging phytoremediation techniques, with their lower cost and environmental friendly nature, have received increasing attention in the last decades (Salt et al., 1998; Garbisu and Alkorta, 2001).

The success of the phytoremediation process, whereby metals are effectively removed from soil, is dependent on an adequate yield of plants and on the efficient transfer of metals from the roots of the plants into their shoots. Most hyperaccumulators, such as *Thlaspi, Urtica, Chenopodium, Polygonum sachalase and Alyssim* are characterized by slow growth and low-biomass production, which make these plants impractical for use in phytoextraction in the field (Mulligan et al., 2001; Puschenreiter et al., 2001). For this reason, more recent research projects on phytoextraction have focused on high biomass crop species, such as maize (*Zea mays*), peas (*Pisum sativum*), oats (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*), and on relevant plant husbandry and soil management practices to enhance the metal uptake of these high biomass species (Blaylock et al., 1997; Huang et al., 1997; Ebbs and Kochian, 1998; Shen et al., 2002; Chen et al., 2004a). Although several conditions must be met in order for phytoremediation to be effective, the bioavailability of metals to plant roots is considered to be a critical requirement for plant uptake to occur (Kayser et al., 2000). Soil factors such as pH, cation exchange capacity, or organic matter content play an important role in successful soil remediation processes. To increase metal availability, a number of chelates such as EDTA (ethylenediaminetetraacetic acid), CDTA (trans -1, 2 -diaminocyclohexane -N, N, N', N'-tetraacetic acid), EGTA [ethyleneglycol -bis (ß -aminoethyl ether), N, N, N', N-tetraacetic acid], and EDDHA [etylenediamine-di (*o*-hydroxyphenylacetic acid)]) have been used to desorb metals from the soil matrix into soil solution to facilitate the transport of metals into xylem, and increase the translocation of metals from the roots to shoots of some fast-growing, high-biomass-producing plants (Blaylock et al., 1997; Huang et al., 1997; Cooper et al., 1999; Wu et al., 1999; Shen et al., 2002).

EDTA has been the most widely used chelating agent in studies of phytoremediation because of its high efficiency in extracting many metals. Although EDTA is very effective in mobilizing metals in soils, EDTA and EDTA-heavy metal complexes can be toxic to plants and soil microorganisms and they can be also persistent in the environment due to their low biodegradability (Bucheli-Witschel and Egli, 2001; Grčman et al., 2003). This nature may increase the potential off-site migration of metals, either in surface runoff or by the leaching of metals into groundwater (Nowack, 2002).

In recent 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 phytoremediation (Kulli et al., 1999; Kayser et al., 2000; Grčman et al., 2003; Kos and Leštan, 2003 a, b). However, the accumulation of Cu, Zn and Cd, in maize, Indian mustard and other plants only increased by a factor of 2 to 3, although the solubility of these metals in soil increased by a factor of 9 to 21 (Kayser et al., 2000). Kulli et al. (1999) reported that at the highest NTA application rate (200 mmol pot^{-1} containing 7.5 kg soil), the concentrations of Cu, Zn and Cd in the aboveground plant biomass were 4- to 24-fold greater than in the control plants. But the total extraction of heavy metals was never more than 2.5 time more than that of the control due to the reduction in yields resulting from the application of NTA. EDDS at an application of 10 mmol kg^{-1} increased the concentrations of Pb, Zn and Cd in cabbage leaves 102-, 4.7- and 3.5-fold, respectively (Grčman et al., 2003). It was concluded that this procedure was far from effective, even at the highest concentrations of heavy metals achieved in the harvestable plant tissues.

Copper is usually much more bioavailable in soils, and thus particularly toxic to many plant species (Pahlsson, 1989). However, few studies have been performed with biodegradable chelates as the ligand to enhance the phytoextraction of Cu from contaminated soils (Kulli et al., 1999; Kayser et al., 2000). In the present study, the effects of three chelating agents (EDTA, EDDS and citric acid) on the uptake of Cu, Pb, Zn and Cd by corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.) were investigated. The solubilization processes of heavy metals in soil with the application of EDTA and EDDS were also studied.

2. Materials and methods

2.1 Soil characterization

Soil samples were collected from a disused agricultural field in the Yuen Long area of Hong Kong. The samples were sieved through a 2 mm sieve and air-dried for 3 d. The soils were artificially contaminated with Pb (2500 mg kg^{-1} of soil) as $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (a common lead sulfide in mining areas – galena) at a Pb concentration ratio of 1:1; Cu (500 mg kg^{-1} of soil) as $CuCO_3$ (copper carbonate); Zn (1000 mg kg^{-1} of soil) as $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulfide) 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) (Chen et al., 2004b). 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 adding heavy metals and fertilizers, the soils were equilibrated for 15 d, undergoing five cycles of saturation with de-ionized water and air-drying. 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 mol $CaCl_2$ at a 1:5 ratio (w/v) using a pH meter. The cation exchangeable 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.

2.2 Effects of different chelate treatments on plant growth and metal uptake

Air-dried soils (500 g) were placed in plastic pots. Soil moisture was maintained to near field water capacity by adding deionised water (DIW) on a daily basis. Eight seeds of corn (*Z mays* L.cv. Nongda108) and beans (*P vulgaris* L. white bean) were sown in each pot. After germination, the seedlings were thinned to four plants per pot and grown for two weeks. The subsets of pots for each species were treated with 50 mL of 50 mM EDTA (as Na_2EDTA salt), EDDS (as Na_3EDDS salt) and citric acid in a single application to the surface of the soil at 5.0 mmol chelate per kg soil. Each treatment was replicated four times. All of the experiments were conducted in the greenhouse under natural light. Air temperatures ranged from 18 to 23 ºC. Two plants were harvested by cutting the shoots 0.5 cm above the surface of the soil, removing the roots from the pots 7 and 14 d after the application of chelates. The shoots and roots were washed with tap water and rinsed with DIW, and dried at 70 ºC in a drying oven to a constant weight for dry weight measurements. The dried plant materials were ground using agate mill.

2.3 Extracting metals with chelates

For the soil metal dissolution experiment, about 4.0 g of soil (based on dry weight) were placed in a 50-mL polypropylene centrifuge tube. About 2 ml 10 mM chelate of EDTA and EDDS were added to the soil samples, which corresponded to the total amount of chelate added (5 mmol kg^{-1} soil) in the pot experiments. After 0.5 d, 1 d, 2 d, 4 d, 6 d, 8 d, 10 d, 12 d and 14 d, DIW was added to the soil (at a soil-to-water ratio of 1:5) and the suspension was shaken for 30 min. After centrifugation, the supernatant will be filtered through a 0.45 μm paper filter (Whatman [Maidstone, UK] 42), acidified with HNO_3 and analyzed for different metal concentrations by ICP-AES.

EDTA and EDDS with different concentrations of 0.5, 1.0, 2.5 and 5.0 mmol kg^{-1} soil in a 2 ml solution were added to 4 g of soil. After 2 d, DIW was added to the soil (at a soil-to-water ratio of 1:5) and the suspension was shaken for 30 min. The filtration and chemical analysis were conducted as mentioned before.

2.4 Plant and soil analysis

Subsamples of ground shoot samples (200 mg) and root samples (100 mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, by volume) 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) of 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 90 ± 6% for all of the metals in the plant reference material. The data reported in this paper were the mean values based on the four replicated experiment results. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software.

3. Results

3.1 Effects of EDTA, EDDS and citric acid on plant growth

The dry mass yields of corn and beans are shown in Figure 1. When no chelates were added to the soil, all of the plants showed normal development without visual symptoms of metal toxicity. The treatments with 5 mmol kg^{-1} soil EDTA and EDDS significantly affected plant growth (Fig. 1). The plants were strongly chlorotic and necrotic at the end of the experiment (14 d after the application of chelates), and root growth was severely impaired. The addition of EDTA appeared to be less toxic to both species of plants compared to EDDS. On the 14th day after the application of EDTA and EDDS, shoot dry matter yields decreased to 60% and 52% of the control plants for corn, and 76% and 61% for beans, respectively. There were no significant effects from the application of citric acid on the dry-yield production of the two plant species.

3.2 Effects of EDTA, EDDS and citric acid on shoot metal concentrations and phytoextraction

Compared with the control group, the addition of citric acid did not significantly increase the concentrations of heavy metals in the shoots of corn and beans except for Cu in beans (Table 2). The application of EDTA and EDDS to the soil significantly increased the concentrations of Cu, Pb, Zn and Cd in the shoots of both plant species. EDDS was more effective at increasing the concentration of Cu in shoots than EDTA. On Day 14 after the application of EDDS, the concentrations of Cu in the shoots of corn and beans reached 2060 and 5130 mg kg^{-1} DW, respectively, which were 45-fold and 135-fold that of the controls (without the application of chelates). The concentration of Zn in shoots was also higher in the plants treated with EDDS than in those treated with EDTA. For Pb and Cd, EDDS was significantly less effective than EDTA, although enhanced uptake was also observed. Concentrations of Pb and Cd in the shoots of corn increased 9- and 1.5-fold, and in beans 43- and 1.5-fold, respectively, in comparison with those in the control group. Comparing the harvest time between 7 d and 14 d, only Cu concentrations in corn and beans increased significantly over time with the treatment of EDDS.

Total metal phytoextraction by the shoots of corn and beans is shown in Figure 2. Of the two plant species tested, beans were better at the phytoextraction of metals than corn. As expected, the maximum phytoextraction of Cu was found in the EDDS treatment, which increased 22- and 81-fold in corn and beans, respectively, relative to the control. For Pb, the plants treated with EDTA attained a maximum level of phytoextraction of approximately 16- and 53-fold that in the corresponding control corn and bean plants, respectively. The total amount of Zn extracted did not exceed 2.7 times that of the controls, but was always higher than the total extraction of Pb in all treatments of both plant species. Chelates were found to have a less significant stimulating effect on the Cd phytoextraction of these two plants.

The distribution of metals in the shoots and roots of corn and beans was also significantly affected by the application of chelates (see Table 3). In the control group, the concentrations of Cu, Pb, Zn and Cd in the roots of the two plant species were significantly higher than those in the shoots. Most of the Cu and Pb absorbed by the plants were concentrated in the roots. The application of EDTA and EDDS significantly increased the shoot-to-root ratios of the concentrations of Cu, Pb, Zn and Cd in both plant species. EDTA was more effective than EDDS in stimulating the translocation of Pb from roots to shoots. But for the translocation of Cu and Zn, the most effective agent was EDDS. When 5 mmol kg^{-1} of EDTA and EDDS were applied, the mean percentage of absorbed Pb translocated from the roots to the shoots of both species of plants increased from 3.5% in the control samples to 48% and 18%, respectively; and from 9.4% to 45% and 88% for Cu. Citric acid was less effective in increasing the translocation of metals from the roots to the shoots of the plants.

3.3 Effects of EDTA and EDDS on the solubility of metals in soil

The concentrations of water-soluble metals in soil were examined to assess the relative efficiency of EDTA and EDDS in enhancing metal solubilization from the soil. In all treatments, the concentrations of soluble Cu, Pb, Zn and Cd increased as increasing concentrations of EDTA and EDDS were applied (see Fig. 3). The addition of EDTA at 5 mmol kg^{-1} for 2 d significantly increased the concentrations of soluble Cu, Pb, Zn and Cd in soil, which were 102-, 496-, 5- and 114-fold higher than those in the control soil (see Fig. 4). After 2 d, the concentrations of soluble metals remained relatively constant over the period of the experiment, for up to 14 d. However, in the soil treated with EDDS, considerably different rates of solubilization for different metals were shown. EDDS was more effective in solubilizing soil Cu than Zn. Compared with the control, EDDS increased soluble Cu and Zn concentrations by a factor of 192 and 8, respectively. Within the first 8 d, concentrations of soluble Cu tended to increase as the time spent on the EDDS treatment increased, indicating that soil Cu could be solubilized by EDDS at a slower rate than by EDTA. However, the soluble Zn concentration reached a peak 2 d after the addition of EDDS, and then tended to decrease with time. For solubilizing soil Pb and Cd, EDDS was less effective than EDTA.

4. Discussion

Chemically enhanced phytoextraction has been proposed as an effective approach for removing heavy metals from soils using plants (Huang et al., 1997; Blaylock et al., 1997; Liphadzi et al., 2003). Several chelating agents, such as citric acid, EDTA, CDTA, DTPA, EGTA, EDDHA, EDDS, HEDTA and NTA have been tested for their ability to mobilize and increase the accumulation of heavy metals, particularly Pb. In most cases, the EDTA treatment was superior in terms of solubilizing soil Pb for root uptake and translocation into above-ground biomass due to its strong chemical affinity for Pb ($\log K_s = 17.88$). The results of this study demonstrated that EDTA was most effective in increasing shoot Pb concentrations in corn and beans (Table 2). In the EDTA treatments, the concentrations of Pb in the shoots of corn and beans reached up to 270 and 487 mg kg^{-1} DW, which was 3 and 1.6 times higher than the levels achieved with the EDDS treatments. EDDS has a relatively lower chemical affinity for Pb ($\log K_s = 12.7$) (Tandy et al., 2004). Citric acid has a low chemical affinity for Pb ($\log K_s = 6.5$) and is easily biodegradable in soil (Römkens et al., 2002), leading to lower effectiveness in increasing concentrations of Pb in shoots.

The addition of some chelating agents to the soil dramatically increased the solubility of Cu. These chelating agents included EDTA (Wu et al., 1999; Lombi et al., 2001), EDDS (log Ks = 18.4, Tandy et al., 2004), EGTA (Römkens et al., 2002), HBED (Wu et al., 1999), and NTA (Kulli et al., 1999; Kayser et al., 2000). However, the chelate-enhanced Cu uptake by plant shoots was generally minimal (Kulli et al., 1999; Wu et al., 1999; Kayser et al., 2000; Lombi et al., 2001; Römkens et al., 2002) except for the result reported by Blaylock et al. (1997). In that study, a 2.5 mmol kg^{-1} EDTA treatment to the soil increased the concentration of Cu to 1000 mg kg^{-1} DW in *B. juncea* shoots. In the present study, the highest concentration of Cu reached 5130 mg kg^{-1} DW in bean shoots on the 14th day after the application of 5 mmol kg^{-1} of EDDS to soil. The effectiveness of EDDS in enhancing the accumulation of Cu was significantly higher than that of EDTA and citric acid (see Fig. 2). Compared with the control group, up to 22 and 81-fold increases in the extraction of Cu were found in the shoots of corn and beans on the 14th day after the addition of 5 mmol kg^{-1} EDDS. Similar results were observed in several of our other experiments. The increased uptake of Cu by the application of EDDS in the present study was much higher than that of EDTA, reported previously (Lombi et al., 2001), and of NTA (Kulli et al., 1999; Kayser et al., 2000). The current results showed that the percentage of Cu phytoextracted in one phytoextraction cycle was 0.6-1.0% of the total Cu in the soil by corn and 1.9-5.3% by beans during a 30-day period of plant growth. These values were higher than the data reported by Kos and Leštan (2003b) for Pb extraction with EDDS and EDTA, and comparable with the results of Blaylock et al. (1997). Blaylock et al. (1997) reported that EDTA could enhance Pb uptake in *B. juncea* shoots (plant Pb = 15 000 mg kg^{-1}), and remove 60 kg Pb ha^{-1} in one harvest from soil containing 600 mg kg^{-1} of Pb (assuming 6 000 kg ha^{-1} dry weight per crop). Based on their study, percentage of Pb extracted in one phytoextraction cycle was calculated to be 4.4% of the total Pb present in the soil (assuming 2250 tons ha^{-1} soils). For efficient soil remediation within a reasonable time span, shoot Pb concentrations exceeding 1% of dry biomass would be required to reduced soil Pb concentrations by 500 mg kg^{-1} over 20-25 years using plants with a high biomass yield (20 000 kg ha^{-1} of dry matter). Unlike Pb, Cu tends to have much higher bioavailability, and it is also more phytotoxic. Copper hyperaccumulators were reported in the literature, but there are still some doubts about their Cu uptake abilities (Baker et al., 2000). In the current study, assuming a constant efficiency of Cu removal, approximately 16 crops of beans with an EDDS application in a concentration of 5 mmol kg^{-1} would be required to reduce the total Cu in the soil from 527 to 100 mg kg^{-1} (P.R. China guidelines for agricultural soil). It appears that chemically enhanced phytoextraction could be an acceptable approach for the remediation of Cu-contaminated soils. The phytoextraction sum of four toxic elements (Cu, Pb, Zn and Cd) reached 8.92 and 40.2 mg metal kg^{-1} soil by corn and beans with the application of EDDS, which were 1.8- and 3.3-fold of the EDTA treatment, respectively

(Fig. 2). This indicated that EDDS was superior to EDTA in the phytoremediation of contaminated soils with multiple heavy metals. In addition, EDDS has the advantage of being readily biodegradable and 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 was 2.5 days (Jaworska et al., 1999). This implies that residual EDDS in the soil will rapidly be degraded and pose little risk from the leaching of metals to groundwater. The results suggest that EDDS can be regarded as a good chelate candidate for the environmentally safe phytoextraction of Cu and other metals in soils. Further research is needed to determine the most appropriate plant species and best methods of application before the chelate-assisted phytoextraction technique can be tested in the field.

The effectiveness of EDDS to stimulating the accumulation of Zn in plants was greater than that of EDTA and citric acid (see Fig. 2). The effectiveness of chelate-enhanced metal accumulation was consistent with the ability of EDDS to solubilize soil metals (Fig. 3 and 4). Some changes in soil conditions such as pH, total ligand or superior ion concentrations may affect the chelation power of chelates (Jones and Williams, 2001). It was reported that the addition of free EDTA or the existence of other metal-EDTA complexes could result in the partial remobilization of adsorbed metals from metal oxides and in the simultaneous dissolution of minerals (iron and aluminum oxides) and remobilization of adsorbed metals (Vandeviere et al., 2001). Tandy et al. (2004) suggested that EDDS is a better extractant for Cu and Zn than EDTA at pH values of above 6 with low chelate-metal ratios because it forms only a weak Ca complex. The comparatively low extraction efficiency of EDTA for Cu resulted from competition between the heavy metals and co-extracted Ca. The extraction of Pb at a low chelate-metal ratio seems to depend mainly on the stability constants of the Pb complexes, apart from the competition of Ca in the case of EDTA at high pHs. For Pb and Cd, the lower extraction efficiency of EDDS compared to EDTA may be attributed to the possible rapid biodegradability of EDDS. The biodegradation of chelate-metal complexes strongly depends on the type of metal involved and is not related to the stability constant of the chelate complex (Vandevivere et al., 2001). For example, the biodegradability of the metal-EDDS complex decreased in the following order: Cd- > Pb- > Zn- > Cu-EDDS. Pb- and Cd-EDDS complexes biodegraded much more readily than Zn- and Cu-EDDS, although Pb- and Zn-EDDS complexes have practically the same stability constant. Cd-EDDS complexes were readily biodegraded as Ca-EDDS, although Ca-EDDS has a much greater stability constant.

In their patent on the induced hyperaccumulation of metals in plant shoots, Ensley et al. (1999) described chemically enhanced phytoextraction as a two-step process. Plants first accumulate metals in their roots. An inducing agent is then applied, which enhances the transfer of the metals to the shoots. This transfer is attributed to a disruption of the plant's metabolism, which regulates the transport of metal to shoots. Large amounts of Cu absorbed by plants accumulated in roots (Lou et al, 2004). The results of this study indicate that the application of EDDS can rapidly and dramatically increase Cu concentrations in soil solutions as well as translocate Cu from the roots to the shoots of the two plant species tested. With 5 mmol kg^{-1} of EDDS treatments, up to 46- and 108-fold increases in the shoot-to-root ratios of Cu concentrations were found in corn and beans, respectively (Table 3). When 5 mmol kg^{-1} of EDDS was applied, the percentage of absorbed Cu translocated from roots to shoots increased from 8.5% to 83% and from

10.2% to 93% in corn and beans, respectively. Most of the increased uptake of Pb after the chelate treatments could be explained as an effect of enhanced Pb solubility (Wu et al., 1999; Kayser et al., 2000). It was reported that the accumulation of Pb in plant shoots correlated with the formation of the Pb-EDTA complex, and that Pb-EDTA was the major form of Pb absorbed and translocated by the plant (Vassil et al., 1998; Epstein et al., 1999). Metal chelate complexes may enter the root through breaks in the endodermis of the root and the Casparian strip, and be rapidly transported to the shoots (Romheld and Marschner, 1981; Bell et al., 1991). Also, it is likely that the physiological barriers in the roots might be destroyed due to the toxic effects of EDDS. Copper may enter the roots of the plant and be transported to the shoots as a Cu-EDDS complex. Kulli et al. (1999) found that a rather abrupt increase in Cu concentrations in the shoots of lettuce and ryegrass when the rate at which NTA, another biodegradable chelate, was applied increased from 70 to 200 mmol pot^{-1} (7.5 kg soil). Plant growth was badly damaged at the 200 mmol pot^{-1} NTA application level. It appears that only at very high Cu concentrations can the breakdown of exclusion mechanisms result in a greatly enhanced Cu uptake (Baker and Brooks, 1989). Wenger et al. (2003) reported that NTA increased the uptake and translocation of Cu into the shoots of tobacco. Neither growth reduction nor any other visible sign of Cu toxicity was found in the presence of 126 µM Cu and 500 µM NTA with a Cu concentration of 190 mg kg^{-1} in the shoots. It was hypothesized that the uptake of Cu-NTA complex by tobacco occurs via an apoplastic pathway (a passive extracellular transport into the xylem).

Conclusions

This study demonstrated that EDDS could be regarded as a good candidate chelate for the environmentally safe phytoextraction of Cu and other metals in contaminated soils. EDDS rapidly and dramatically increased Cu concentrations in the shoots of two species of plants. It also enhanced the translocation of Cu from the roots to the shoots of those two plant species. The percentage of Cu that was phytoextracted in one phytoextraction cycle was 0.6-1.0% of the total Cu (5.26 mg /kg soil) in the soil by corn and 1.9-5.3% (27.9 mg kg^{-1} soil) by beans. Assuming a constantly high efficiency of Cu removal, approximately 16 crops of beans would be required to reduce the total Cu in the soil from 527 to 100 mg kg^{-1}. For Pb, the plants treated with EDTA reached maximum phytoextraction levels, approximately 16- and 53-fold higher than that in the corresponding control plants in corn and beans, respectively. The effectiveness of chelate-enhanced metal accumulation in plants was consistent with the ability of EDDS or EDTA to solubilize metals in soils.

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Table 1

The physicochemical properties of the soils used in the study

Physicochemical Properties	Soils used in the study
pH ($CaCl_2$)	7.27
Electrical conductivity at 25°C ($\mu S\ cm^{-1}$)	282
Sand (%) > 0.05 mm	54.3
Silt (%) 0.05-0.001 mm	31.1
Clay (%) < 0.001 mm	14.6
N_{Total} (%)	0.10
Organic matter (%)	1.67
Cation exchange capacity (cmol kg^{-1})	3.29
Field water capacity (%)	27.4
Background total metal concentration (mg kg^{-1})	
Pb	44.2
Cu	26.7
Zn	131
Cd	0.45

Table 2

Shoot metal concentrations in corn and beans grown in soil with and without the application of chelates (mg kg^{-1})

	Corn				Beans			
	Control	EDTA	EDDS	Citric acid	Control	EDTA	EDDS	Citric acid
				7 d after treatment				
Cu	56.8 ± 2.5	428 ± 32	1220 ± 129	38.1 ± 6.2	37 ± 3.1	625 ± 36.4	2230 ± 89	50.2 ± 9.7
Pb	8.9 ± 1.9	257 ± 65	67 ± 22	13.7 ± 1.8	8.2 ± 1.4	411 ± 13.3	293 ± 77	28.4 ± 13
Zn	395 ± 51	778 ± 52	1200 ± 93	374 ± 84	399 ± 36	857 ± 62.3	1440 ± 161	459 ± 100
Cd	7.8 ± 0.5	19.8 ± 1.2	14.9 ± 2.9	5.4 ± 0.9	3.7 ± 0.1	8.8 ± 2.2	4.7 ± 0.9	3.2 ± 0.7
				14 d after treatment				
Cu	45.6 ± 9.2	560 ± 69	2060 ± 272	36.8 ± 1.3	38.1 ± 1.8	551 ± 4.5	5130 ± 349	51.5 ± 3.4
Pb	10.1 ± 4.1	270 ± 19.1	90.9 ± 7.5	5.8 ± 2	7 ± 2.5	487 ± 23	298 ± 19	6.4 ± 0.6
Zn	580 ± 10.2	851 ± 174	1310 ± 148	401 ± 58	434 ± 41	761 ± 74.8	1950 ± 66	437 ± 8.9
Cd	9 ± 2.6	29 ± 4	13.7 ± 2.1	5.2 ± 1.3	3.1 ± 0.6	10.1 ± 1.5	4.6 ± 0.7	2.8 ± 0.3

Values are means ± S.D. (n = 4)

Table 3

Effects of chelate treatments on the translocation of metals from the roots to the shoots of corn and beans 14 d after treatment

	Corn				Beans			
	Control	EDTA	EDDS	Citric acid	Control	EDTA	EDDS	Citric acid
	Shoot-to-root ratio of metal concentration							
Cu	0.05 ± 0.01	0.3 ± 0.02	2.3 ± 0.05	0.06 ± 0.01	0.06 ± 0.01	0.49 ± 0.02	6.5 ± 0.3	0.07 ± 0.01
Pb	0.03 ± 0.01	0.41 ± 0.02	0.08 ± 0.01	0.03 ± 0.01	0.007 ± 0.001	0.45 ± 0.02	0.12 ± 0.01	0.008 ± 0.001
Zn	0.5 ± 0.03	0.6 ± 0.05	0.97 ± 0.08	0.55 ± 0.06	0.12 ± 0.01	0.3 ± 0.02	0.81 ± 0.06	0.13 ± 0.02
Cd	0.39 ± 0.03	0.74 ± 0.08	0.54 ± 0.06	0.33 ± 0.02	0.02 ± 0.01	0.1 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
	Metal absorbed by shoots/metal absorbed by the entire plant (%)							
Cu	8.5 ± 0.6	39.8 ± 3.2	82.8 ± 7.8	10.6 ± 1.7	10.2 ± 1.6	50.9 ± 1.9	93.2 ± 8.3	12.3 ± 2.6
Pb	5.5 ± 0.5	47.4 ± 5.9	14.6 ± 2.6	4.9 ± 3.9	1.5 ± 0.8	48.7 ± 2.8	20.8 ± 3.2	1.7 ± 0.8
Zn	46.9 ± 0.3	56.7 ± 6.1	67.4 ± 8.6	50.7 ± 6.1	19.5 ± 0.9	39.0 ± 4.8	63.4 ± 7.8	20.4 ± 2.9
Cd	41.2 ± 0.5	61.8 ± 5.2	53.3 ± 5.6	37.7 ± 4.8	3.3 ± 0.2	18 ± 2.7	7.9 ± 0.5	4.9 ± 0.2

Values are means ± S.D. (n = 4)

Figure Captions:

Fig. 1 Effects of the application of chelate on the dry matter yields of shoots and roots in corn (a) and beans (b). Values are means ± S.D. (n = 4).

Fig. 2 Effects of the application of chelate on the uptake of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of corn and beans. Values are means ± S.D. (n = 4).

Fig. 3 Effects of the application of chelate at different concentrations on the solubilization of Cu (a), Pb (b), Zn (c) and Cd (d). Values are means ± S.D. (n = 4).

Fig. 4 Changes in soluble Cu (a), Pb (b), Zn (c) and Cd (d) concentrations in soil treated with chelates with time. Values are means ± S.D. (n = 4).

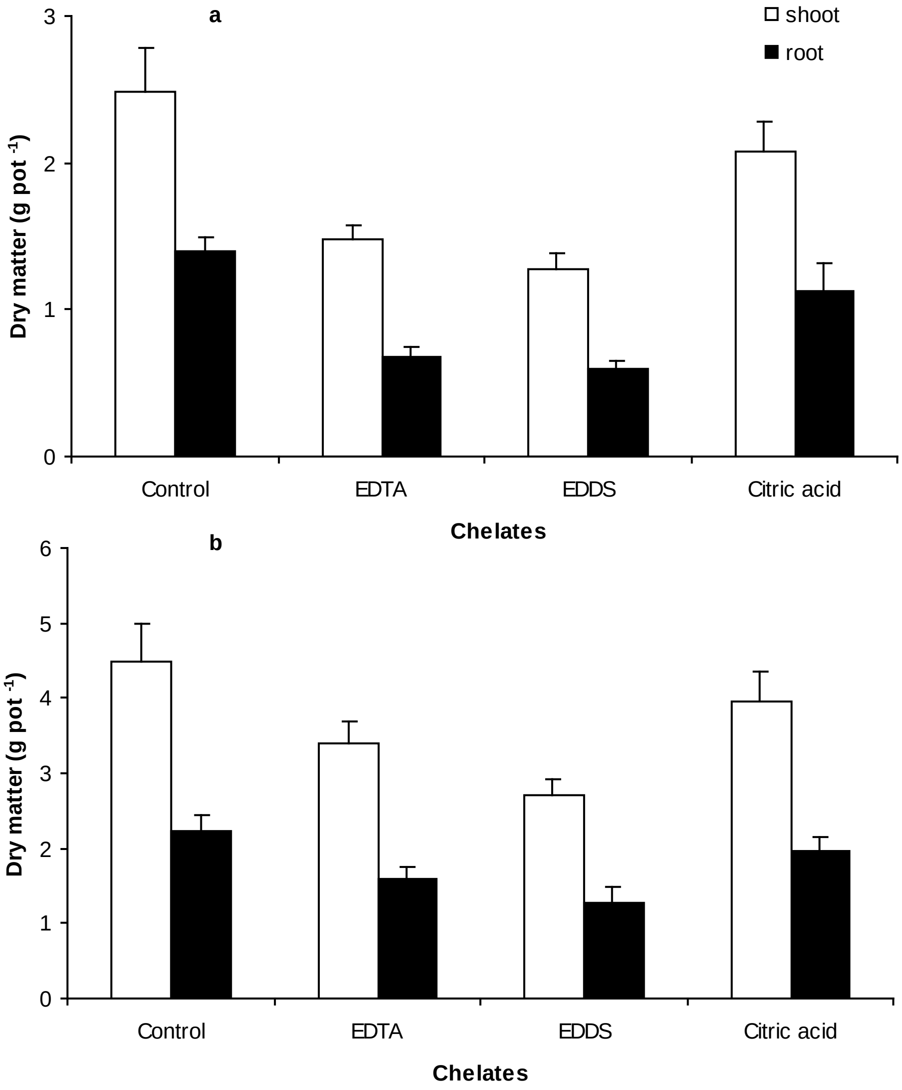

Fig. 1 Effects of the application of chelates on the dry matter yields of shoots and roots in corn (a) and beans (b). Values are means ± S.D. (n = 4).

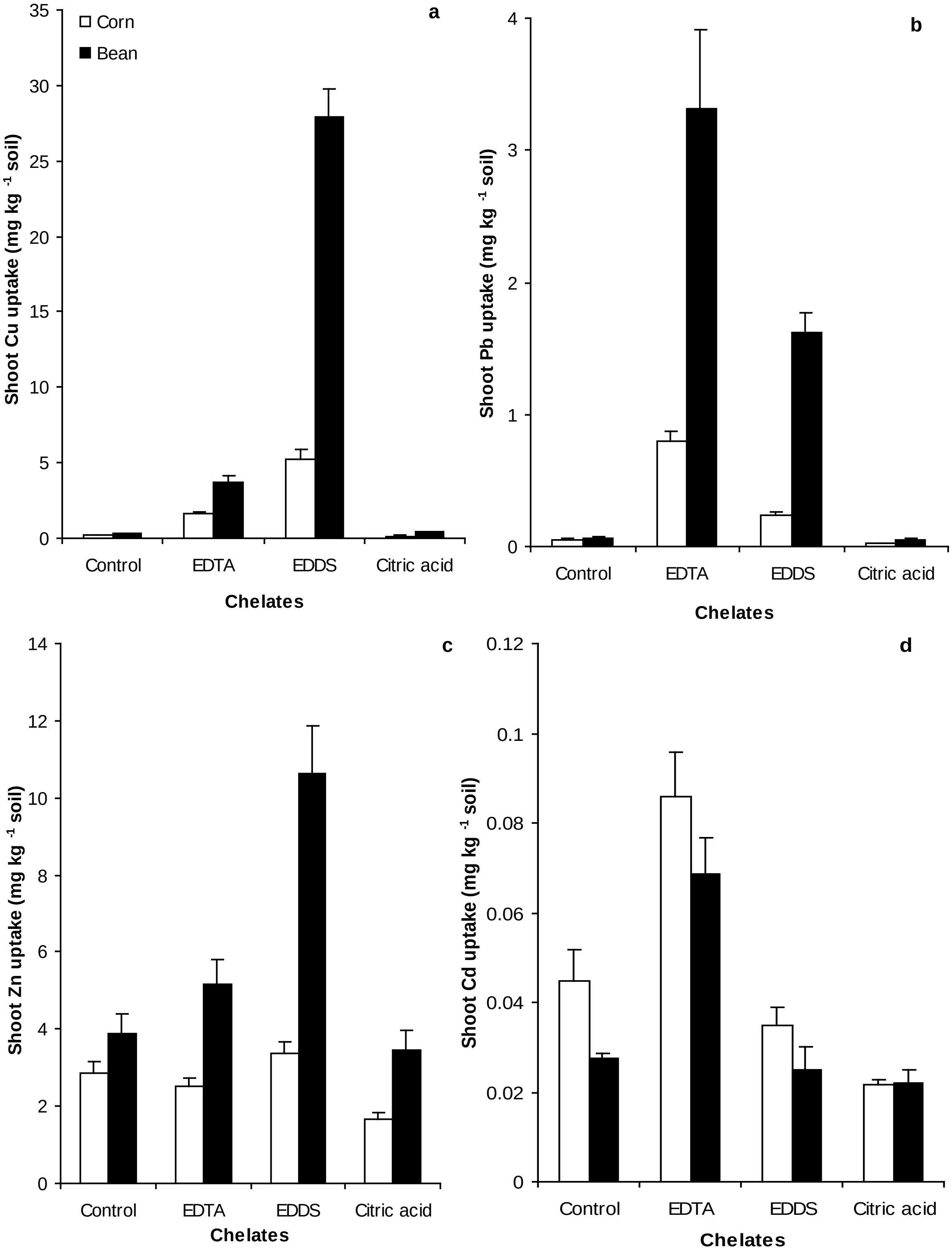

Fig. 2 Effects of the application of chelate on the uptake of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of corn and beans. Values are means ± S.D. (n = 4).

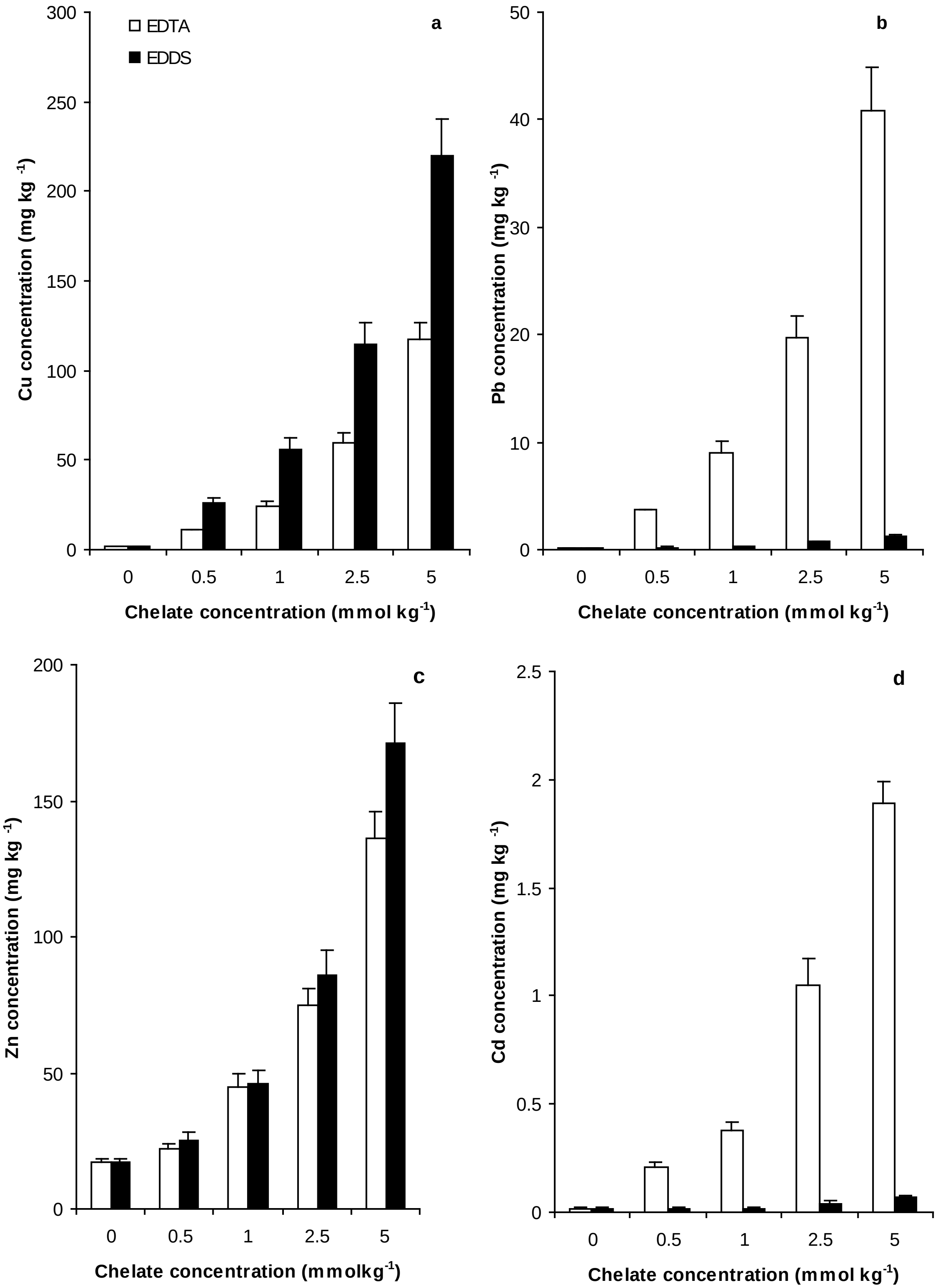

Fig. 3 Effects of the application of chelate at different concentrations on the solubilization of Cu (a), Pb (b), Zn (c) and Cd (d). Values are means ± S.D. (n = 4).

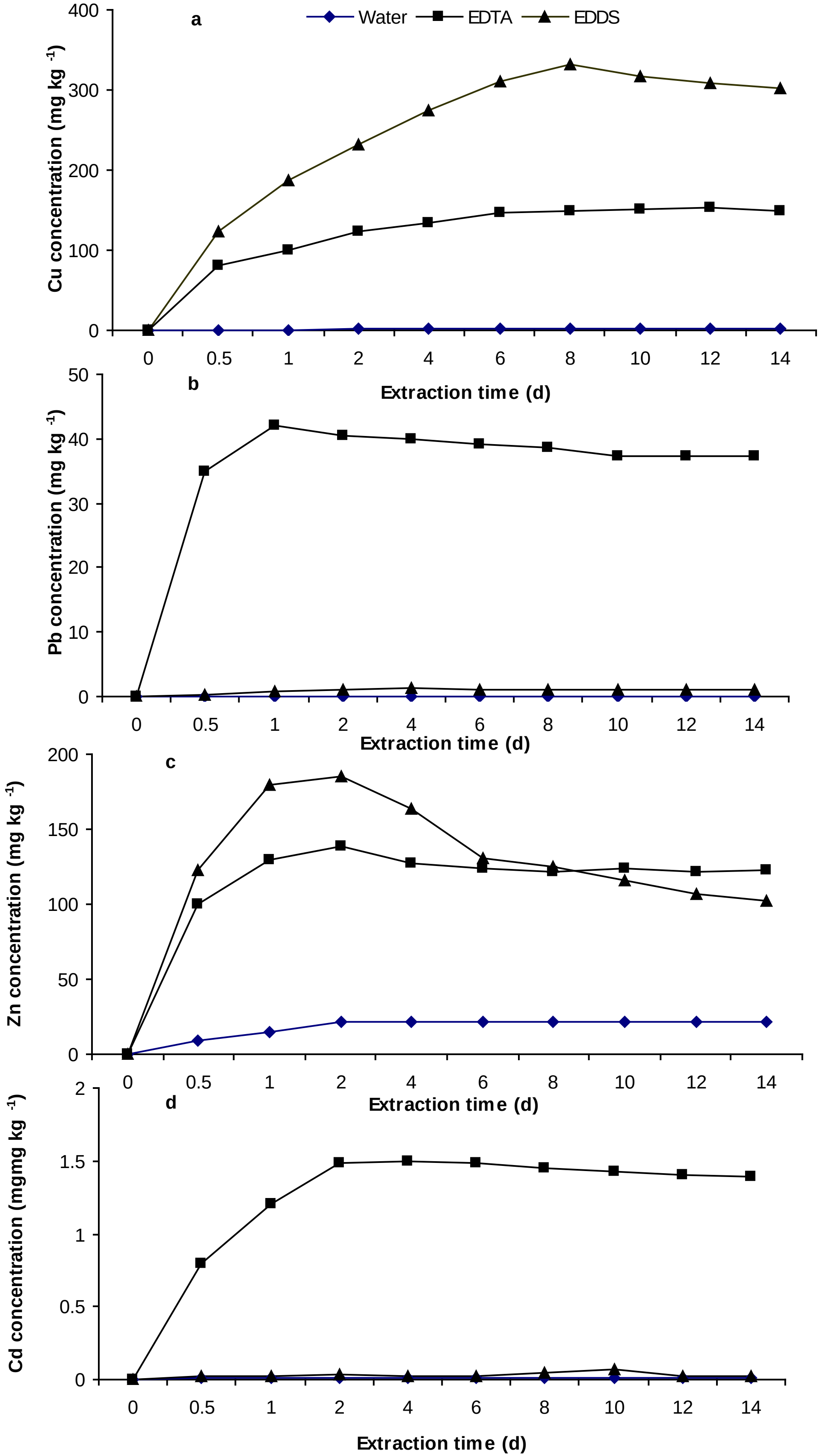

Fig. 4 Changes in soluble Cu (a), Pb (b), Zn (c) and Cd (d) concentrations in soil treated with chelates with time. Values are means ± S.D. (n = 4).