PLANT UPTAKE AND LEACHING OF METALS DURING THE HOT EDDS-ENHANCED PHYTOEXTRACTION PROCESS

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ABSTRACT

Using pot experiments, the effect of the application of the biodegradable chelating agent EDDS in hot solutions at 90°C on the uptake of Cu, Pb, Zn, and Cd by corn (*Zea mays* L. cv. Nongda No. 108) and beans (*P vulgaris* L. white bean), and the potential leaching of metals from soil were studied. When EDDS was applied as a hot solution at the rate of 1 mmol kg^{-1}, the concentrations and total phytoextraction of metals in plant shoots exceeded or approximated those in the shoots of plants treated with normal EDDS at the rate of 5 mmol kg^{-1}. On the other hand, the leaching of Cu, Pb, Zn, and Cd after the application of the hot EDDS solution at the rate of 1 mmol kg^{-1} was reduced by 46%, 21%, 57%, and 35% in comparison with that from the application of normal EDDS at 5 mmol kg^{-1}, respectively. For the treatment with 1 mmol kg^{-1} of EDDS, the leached metals decreased to the levels of the control group (without EDDS amendment) 14 days after the application of EDDS. The soil amendment with biodegradable EDDS in hot solutions may provide a good alternative

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to chelate-enhanced phytoextraction in enhancing metal uptake by plants and limiting metals from leaching out from soil.

KEY WORDS: phytoextraction, metals, EDDS, hot solution, leaching

INTRODUCTION

The efficient phytoremediation of metal-contaminated soils in practical applications should satisfy two basic requirements. First, it should be possible to remove the metals in the soil to concentration levels below relevant regulation standards by harvesting the plants within a reasonable period of time. Second, the technology should not pose a second threat of contamination to the surrounding environment. The widely tested chelate – EDTA, and its complexes with metals are usually toxic and poorly photo-, chemo-, and biodegradable in soil environments, persisting in soil for several weeks or months after the harvesting of the phytoextraction crops (Bucheli-Witschel and Egli, 2001; Nowack, 2002; Grčman *et al.*, 2003). The combination of its widespread use in detergents and its slow decomposition in surface environments has led to background concentrations of EDTA in surface waters in Europe ranging from 10 to 50 mg L^{-1} (Kari *et al.*, 1995). The EDTA-enhanced leaching of heavy metals in soils has been extensively documented using batch and column-leaching experiments (Papassiopi *et al.*, 1999; Römkens *et al.*, 2002; Kos and Leštan, 2003a, b; Madrid *et al.*, 2003; Chen *et al.*, 2004a, b). In soil columns where cabbage plants were grown, Grěman *et al.* (2001) observed that 38%, 10%, and 56% of the initial total Pb, Zn, and Cu, respectively, in soil were leached down the soil profiles during the 10 mmol kg^{-1} EDTA treatments. Chen *et al.* (2004a) found that the application of 5 mmol kg^{-1} of EDTA caused the leaching of a large portion of the total amounts of Pb, Cu, Zn, and Cd initially present in the soil. All of the potential risks of using chelates for the phytoextraction of metals from contaminated soils should therefore be thoroughly evaluated before this remediation technology is applied in the field.

As a biodegradable chelate, EDDS (S,S-ethylenediaminedisuccinic acid) can strongly complex with transitional metals and radionuclides (Jones and Williams, 2001). EDDS mineralizes rapidly in sludge-amended soil, with the process being completed in 28 d (Jaworska *et al.*, 1999). The application of EDDS to metal-contaminated soils should dramatically improve the phytoextraction of metals, especially Cu (Luo *et al.*, 2005, 2006a; Meers *et al.*, 2005). In a soil column treated with weekly additions of 10 mmol kg^{-1} of EDDS, about 0.8% and 1.5% of initial total Pb and Cd in soil were leached through the soil profile. However, the same amount of EDTA caused 22.7% and 39.8% of the initial total Pb and Cd to leach out (Grěman *et al.*, 2003). A biotest with red clover indicated that EDTA has a greater phytotoxic effect than EDDS on soil. EDDS is also less toxic to soil fungi and causes less stress to microorganisms in the soil (Grěman *et al.*, 2003). In addition, the combination of EDDS and permeable barriers has been found to possibly lead to a more environmentally safe method of phytoextracting Pb and to the *in situ* washing of Pb (Kos and Leštan, 2003a, b).

There are two hypotheses for the process of metal-chelate uptake by plants. One is the split-uptake mechanism, by which only free metal ions are absorbed by plant roots (Chaney *et al.*, 1972; Marschner *et al.*, 1986). Another possible mechanism theory that has been suggested is that some of the purportedly intact metal chelates are taken up by plants (Wallace, 1983; Bell *et al.*, 1991; Laurie *et al.*, 1991; Salt *et al.*, 1995). 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), the breakdown of the root exclusion mechanism will play an important role in increasing metal uptake after the application of chelates. Reducing metal leaching associated with the application of chelates can be accomplished by decreasing the application dosage and using easily biodegradable chelate. Our previous results showed that some physiological damage to plant roots, such as the pretreatments with MC (methanol : trichloromethane = 2 : 1,v / v), HCl, and hot water dramatically increased the concentrations of Pb in plant shoots treated with EDTA (Luo *et al.*, 2006b). Therefore, pretreating plant roots would be helpful in reducing the dose of chelates applied in a practical operation. In the present study, two plants, namely corn (*Zea mays* L. cv. Nongda No. 108) and beans (*P vulgaris* L. white bean), were used. Corn is a typical monocotyledon plant and has been extensively studied in research on phytoremediation (Huang *et al.*, 1997; Wu *et al.*, 1999). The bean plant is a dicotyledon plant that has shown high efficiency in metal uptake with the application of chelates (Luo *et al.*, 2005; Luo *et al.*, 2006c). The biodegradable EDDS was added in hot solutions to soil in order to study the effects of this technique on the phytoextraction of metals. Emphasis was also given to the assessment of metal leaching behaviors arising from different methods of applying EDDS to soil.

MATERIALS AND METHODS

Soil Preparation

Soil samples were collected from a disused agricultural field in the Yuen Long area of Hong Kong. The samples were 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 the soil with double-distilled water at a water-to-soil ratio 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). For the extractable metal in the soil, 4.0 g of soil (based on dry weight) were placed in a 50-mL polypropylene centrifuge tube. About 20 ml 1 mM of EDDS solutions were added to the soil samples, which corresponded to the total amount of chelate added at a rate of 5 mmol kg^{-1} of soil. The soil extracted with 20 ml of DIW was used as the control. The suspension was shaken for 30 min. After centrifugation, the supernatant was filtered through a 0.45 µm paper filter (Whatman [Maidstone, UK] 42), acidified with HNO_3, and analyzed for metal concentrations by ICP-AES (Luo *et al.*, 2005). The selected physical and chemical properties of the soil are presented in Table 1.

EDDS Treatment on Plant Growth and Metal Uptake

Air-dried soils (500 g) were placed in plastic pots (12 cm i.d. x 12 cm height). A glass fiber filter GC-50 (D = 1.2 μm) was placed at the bottom of the pot to retain the soils. The moisture of the soil was maintained to near field water capacity by adding deionized water (DIW) on a daily basis. Seeds of corn (*Zea mays* L. cv. Nongda No. 108) and beans (*P vulgaris* L. white bean) were sown directly in the soils. After germination, the seedlings were thinned to four plants per pot. On the 14^{th} day after the plants were sown, EDDS (from Fluka Chemie GmbH) was applied to the surface of the soils in two different ways (heated and not heated) at rates of 0 (control), 1.0, and 5.0 mmol kg^{-1} of soil as 100 ml of Na_3EDDS solutions. To make up the different chelate treatments, EDDS was diluted from 50 mM of Na_3EDDS (pH 10.1) salt solutions. The hot chelate solution treatments were conducted by adding boiled solution to soil in the pots, with the final temperature of the soils being about 40 ºC at a depth of two-thirds into the pot. Twelve replicates were used for each treatment so that there would be two replicates for the following leaching experiments within different time spans. All of the experiments were operated in the glasshouse under natural light. Air temperatures ranged from 25 to 33 ºC. All of the plants were harvested by cutting the shoots 0.5 cm above the surface of the soil 7 d after the application of chelates. The shoots were washed with tap water, rinsed with DIW, and dried at 70 ºC in an oven to a constant weight so that dry weight measurements could be made and a metal analysis carried out. During the treatment and after the plants had been harvested, the moisture of the soil was also maintained at field water capacity by the addition of DIW.

Pot Leaching Study with Artificial Rainwater

A leaching study was carried out with artificial rainwater on Day 0, 7, 14, 21, 28 and 35 after the application of EDDS. The composition of the artificial rainwater (mg l^{-1}) used in the present leaching experiment was NO_3^-: 1.94; NH_4^+: 0.49; Na^+: 1.87; Mg^{2+}: 0.25; Ca^{2+}: 0.29; Cl^-: 3.41; SO_4^{2+}: 2.65; pH = 4.40 (Hodson *et al.*, 2001). In the leaching experiment, 800 ml of artificial rainwater (equivalent to about 71 mm of rainwater precipitation) were first added to two pots from each EDDS treatment (12 replicates for every EDDS treatment). One 800 ml plastic beaker was placed under the bottom of the pots to collect the leachate. The same artificial rainwater was then added to another two pots from each EDDS treatment. For the leaching study on Day 0 of the EDDS application, 700 ml of artificial rainwater were added because the leaching was carried out immediately after the addition of 100 ml of EDDS solution.

Chemical Analysis

The leachate solutions were filtered through Whatman No. 42 filter paper. The volume and pH of the leachate were measured immediately after being collected from the bottom of the pots. The dissolved total organic carbon (DOC) was measured with a Shimadzu TOC-5000A analyzer. A sub-sample was digested with concentrated HNO_3, then diluted with 5% HNO_3 and analyzed for heavy metals using ICP-AES (Perkin-Elmer Optima 3300 DV).

Subsamples of ground shoot samples (200 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). Certified standard reference materials of SRM 1515 (apple leaves) of the National Institute of Standards and Technology, U.S.A., were used in the analysis as part of the QA/QC protocol for analyzing metals. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery rates were around 90 ± 7% for all of the metals in the plant reference material. The data reported in this paper were the mean values based on the twelve replicates for plant samples and the two replicates for leachate solutions. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software.

RESULTS

Plant Growth

The dry mass yields of corn and beans are shown in Fig. 1. Before the application of EDDS, all of the plants grew very well and no visual symptoms of toxicity were observed. The application of EDDS had a significant effect on the growth of the plants and on shoot biomass yields. The dry weights of the shoots of corn and beans decreased as the level of the chelates applied to the soil increased (Fig. 1). In addition, applying the same dosage of EDDS, the decrease in the dry biomass yields of the plants was more pronounced when EDDS was applied as hot solutions to the surface of the soil than when a normal EDDS solution was applied. The shoot biomass yields of corn and beans decreased by 18% and 24%, respectively, when EDDS was added in hot solutions compared to when EDDS was added in a normal solution at the same dosage (Fig. 1).

Concentrations and Phytoextraction of Metals

Compared with the control group, the addition of EDDS significantly increased the concentrations of Cu, Pb, Zn, and Cd in the shoots of both plant species (Figs. 2 and 3). In all of the treatments, a larger increase in metal concentrations was always observed in beans than in corn. The concentrations of metals in the shoots of corn and beans increased significantly as increasing levels of EDDS were applied to the soil (Figs. 2 and 3). At the same dosage, the application of hot EDDS solutions produced much higher concentrations of Cu, Pb, Zn, and Cd in the shoots of both plant species than did the application of EDDS solutions without heating (Figs. 2 and 3). Compared with the normal application of EDDS, when EDDS was applied to the soil in hot solutions (P = 0.05) the concentrations of Cu, Pb, Zn, and Cd in the shoots increased to about 6.6, 12.3, 2.1, and 3.8 times for corn; and 8.1, 11, 3.2, and 12.2 times for beans, respectively. The highest concentrations of Cu, Pb, Zn, and Cd at 3420, 1980, 1430, and 82 mg kg^{-1} were found in the shoots of beans with the application of hot EDDS at a rate of 5 mmol kg^{-1}. These concentrations were 61, 198, 11, and 37 times those found in the control group (without the application of EDDS).

Total metal phytoextraction by the shoots of corn and beans is shown in Table 2. Of the two plant species tested, beans were superior to corn at the phytoextraction of metals. Similar to the effects of chelates on the concentration of metals in the shoots, significantly higher levels of metal phytoextraction were achieved in the treatment with the application of hot EDDS than with the application of normal EDDS at equal dosages. The maximum phytoextraction of Cu (8940 μg kg^{-1} of soil) was found in beans with the heated EDDS application at a rate of 1 mmol kg^{-1} of soil. Here, the increase was 9- and 100-fold compared with beans that had received an equal dose of normal EDDS and the control group (adding normal water), respectively. The highest levels of phytoextraction of Pb, Zn, and Cd were 4000, 2880, and 166 μg kg^{-1} of soil, respectively, observed in beans at the 5 mmol kg^{-1} hot EDDS application. The levels were 3.7, 2.5, and 13.8 times that of the normal EDDS treatment at the same dose and 200, 6.3, and 18 times the levels of the control group (adding normal water), respectively.

The Leaching of Metals from the Pots

In order to examine the potential leaching of metals in pots and the effects of root growth on the leaching of metals, the soil in the pots was leached with artificial rainwater within 35 d after the application of EDDS. The volume of the leachate solutions ranged from 600 to 690 ml (equivalent to about 53-61 mm of rainfall), and the pH ranged from 6.95 to 7.26. Slightly higher pHs were observed in the treatments with the 5 mmol kg^{-1} EDDS application on the day of the application, which might be related to the higher pH of the EDDS solutions applied to the soil. But, no significant difference was found between different treatments (data not shown).

The amounts of DOC and metals leached from the pots were also measured (see Table 3 and Fig. 4). In the leachates from the control group (without the application of EDDS), the amounts of DOC ranged from 42 to 69 mg kg^{-1} of soil. The average amounts of Cu, Pb, Zn, and Cd in the leachates were 2.1, 1.8, 3.1, and 0.056 mg kg^{-1} of soil, respectively, which accounted for only 0.53%, 0.36%, 0.62%, and 0.37% of the total initial metals in the soil, respectively. The application of EDDS dramatically enhanced the amounts of DOC in the leachate solutions. The DOC in the leachates increased as the levels of EDDS applied to the soils increased (Fig. 4). The highest DOC amounts reached 2120, 1960, and 1900 mg kg^{-1} of soil in the leachates without plants, and with corn and bean growth, respectively, just after the application of 5 mmol kg^{-1} of EDDS. Thereafter, the amounts of DOC decreased over the period of the test, and the decrease was more pronounced in the soil with plant growth than in the soil without plants. No significant differences were observed in the amounts of DOC in the leachates between the pots grown with corn and beans, or between the treatments with hot EDDS solutions and those with normal EDDS solutions at the same application dosage (see Table 3).

In all treatments, the amounts of Cu, Pb, Zn, and Cd in the leachate solutions closely followed the pattern of DOC. Total metal amounts in the leachate solutions increased with the increasing levels of EDDS applied, and decreased with the leaching time (Fig. 4). On the day that the chelates were applied, the application of 1 mmol kg^{-1} of EDDS as normal solutions led to an average 140, 4, 15.7, and 0.144 mg kg^{-1} of soil of Cu, Pb, Zn, and Cd leached from the pots. This was a respective 73.3, 2.2, 4.8, and 2.6 times the leaching that was seen in the control group (without the EDDS application). When the application of EDDS was increased to 5 mmol kg^{-1}, the average amounts of Cu, Pb, Zn, and Cd in the solutions increased to 246, 7, 163, and 0.5 mg kg^{-1} of soil, respectively, which accounted for 61.5%, 1.4%, 40.1%, and 3.4% of the initial metals, respectively. At the same application dosage, the hot EDDS treatment only resulted in slightly higher amounts of metals in the leachate solutions. On average, about 10%, 12%, 14%, and 13% enhancement of leaching was observed for Cu, Pb, Zn, and Cd, respectively, compared with the normal EDDS treatment.

In contrast to the results observed on the day of the EDDS application, from 7 d to 35 d (the end of the leaching study), at the same EDDS application dosage, the amounts of metals in the leachate solutions from the pots treated with hot EDDS solutions were slightly lower than that from the pots treated with normal EDDS solutions. The amounts of metals in the leachate solutions from the pots with plants were significantly lower than that from the pots without plants (see Fig. 4). For example, on Day 7 after the application of 5 mmol kg^{-1} of normal EDDS, the amounts of Cu, Pb, Zn, and Cd in the leachate solution from the pots without plants were 204, 5.38, 133, and 0.4 mg kg^{-1} of soil, respectively, which were 1.3, 1.3, 1.4, and 1.25

times that from the pots grown with plants, respectively. For the soils with corn and bean growth, the average amounts of Cu, Pb, Zn, and Cd in the leachate solutions decreased by 58%, 30%, 50%, and 39%, respectively, from Day 7 to Day 14 after the application of EDDS. But in the soil without the plants, the amounts of leached Cu, Pb, Zn, and Cd decreased only by 40%, 24%, 42%, and 26%, respectively. The result indicated that the leached metals decreased more quickly in the soil with plant growth than in the soil without plants. There were no significant differences in the amounts of leached metals from soil between the pots grown with corn and beans.

DISCUSSION

The main concerns about chemically enhanced phytoextraction have been the efficiency of removing metals from soil, the cost of the operation, and the potential negative effects on the surrounding environment resulting from mobilized metals. Compared with the traditionally used chelate EDTA, the biodegradable chelate EDDS is more promising for the chemically enhanced phytoextraction of heavy metals in contaminated soils because of the latter’s higher biodegradability and lower leachability (Nowack, 2002; Grěman *et al.*, 2003). The present study demonstrated that when the EDDS was applied as a hot solution, the accumulation of heavy metals in plant shoots could be greatly enhanced compared with a normal solution of EDDS applied at the same dose (Table 2). For all heavy metals that were studied when EDDS was applied as a hot solution at the rate of 1 mmol kg^{-1}, the concentrations and total phytoextraction of metals by plant shoots exceeded or approximated those in the shoots of plants treated with normal EDDS at a rate of 5 mmol kg^{-1} (Figs. 2 and 3, and Table 2). It is hypothesized that the hot solution first destroyed the physiological barrier(s) of plant roots that normally function to control the uptake and translocation of solutes. The rapid equilibration of the soil solution with the sap of the xylem was then achieved. On the other hand, the leaching study showed that when the EDDS application was decreased, there was a significant decrease in the metals that leached out from the pots (Fig. 4). On average, compared with the treatment of 5 mmol kg^{-1} of EDDS, the total leached amounts of Cu, Pb, Zn, and Cd were reduced by 46%, 21%, 57%, and 35%, respectively, after the application of EDDS at the rate of 1 mmol kg^{-1}. From this respect, for a given phytoextraction efficiency, the application of hot EDDS would be very useful in reducing operating costs and the potential risk of metals leaching to the surrounding environment.

Increasing temperatures could have a positive effect on the extraction of metals (Vandeviere *et al.*, 2001). It was found that in the temperature range of 8 to 48 ºC, each 10 ºC increment resulted in a 6% increase in metal extraction, with the exception of Cu (Vandeviere *et al.*, 2001). In the present leaching study, at the same dosage, the hot EDDS application only resulted in slightly higher amounts of metals in the leachate solutions. On average, about 12%, 14%, and 13% enhancement of leaching was observed for Pb, Zn, and Cd, respectively. In contrast to the results of Vandeviere *et al.* (2001), the amount of Cu also increased by 10%. This might be due to the different soil properties, such as different soil textures, metal concentrations, and chemical forms. The higher amounts of metal in the leachate solutions from the pots with hot EDDS application than in those with the normal EDDS application probably indicates a higher degree of metal solubility in the soils, which contributed to the higher metal concentrations in the shoots of corn and beans (see Figs. 2 and 3), although none of the enhancements reached statistically significant levels (P = 0.05) under the current experimental conditions.

The amounts of metals in the leachate solutions from the pots with plants were significantly lower than that from the pots without plants on the seventh day at the same EDDS application dosage. This difference could not be attributed to the metal uptake by the two plants. The data in Table 2 show that when the EDDS was applied at the same dosage, significantly more metals were phytoextracted by the plants treated with hot EDDS solutions than by those that had received the normal EDDS. However, the maximum metal uptake by plants did not surpass 8.9, 4, 2.9, and 0.17 mg kg^{-1} of soil for Cu, Pb, Zn, and Cd, respectively, which accounted for only 1.5%, 0.8%, 0.4%, and 1% of the total metals in the soil, respectively (see Tables 1 and 2). Therefore, it is more reasonable to attribute the difference to the biodegradability of EDDS. EDDS is the only commercially available chelate that is readily decomposed into degradation products when it is present in soil (Witschel and Egli, 1998). The calculated half-life of EDDS in sludge-amended soil was 2.5 days, and it would be completely mineralized within 28 d (Jaworska *et al.*, 1999). The smaller amounts of metal that were leached from the pots with plants may be due to the presence of plant roots, which would facilitate more microbial population growth and thus accelerate the degradation of EDDS in soil. It was also reported that the application of EDDS did not have toxic effects on the microorganisms in the soil, which was capable of chelate biodegradation, even at the higher concentration of 20 mmol kg^{-1} (Kos and Lestan, 2003a). Consistent with the results of Day 7, the following leaching analysis on Days 14, 21, and 28 also showed that significantly lower amounts of Cu, Pb, Zn, and Cd were found in the leachate solutions from pots with plants when the EDDS was applied at the same dosage. On Day 28, no significant differences could be found in the amounts of Cu, Pb, Zn, and Cd in the leachate solutions between the pots grown with plants and the control group (without EDDS application). For the pots with no plants, the differences in the amounts of metal in the leachate solutions disappeared by the 35th day following the application of EDDS (Fig. 4).

In the present study, the metals leached from the 1 mmol kg^{-1} EDDS treatment decreased to the control group levels 14 days after the application of EDDS. Therefore, if there is no rainfall within the first 14 d after the application of EDDS, the amount of metals leached to the surrounding environment would be minimal. Moreover, if some plants with deep root systems, such as vetiver grass, can be introduced to intercrop with the target plants, heavy metals can be efficiently removed by harvesting the shoots of high biomass plants; at the same time, the leaching of metals may be prevented by their retention and re-adsorption in soil due to the deep root systems of the introduced plant species.

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REFERENCES

Avery, B.W., and Bascomb, C.L. 1982. Soil Survey Laboratory Methods. Harpenden, Soil Survey Technical Monograph No. 6. Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.

Bell, P.F., Chaney, R.L., and Angle, J.S. 1991. Free metal activity and total metal concentrations as indexes of micronutrient availability to barley (*Hordeum vulgare* cv 'Klages'). *Plant Soil* **130,** 51-62.

Bucheli-Witschel, M. and Egli, T. 2001. Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiol. Rev.* **25,** 69-106.

Chaney, R.L., Brown, J.C., and Tiffin, L.O. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. *Plant Physiol.* **50,** 208-213.

Chen, Y.H., Li, X.D. and Shen, Z.G. 2004a. Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process, *Chemosphere* **57,** 187-196.

Chen, Y.H., Shen, Z.G., and Li, X.D. 2004b. The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. *Appl. Geochem.* **19,** 1553-1565.

Grčman, H., Velikonja-Bolta, Š., Vodnik, D., Kos, B., and Leštan, D. 2001. EDTA enhanced heavy metal phytoextraction : metal accumulation, leaching and toxicity. *Plant Soil* **235,** 105-114.

Grčman, H., Vodnik, D., Velikonja-Bolta, Š., and Leštan, D. 2003. Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *J. Environ. Qual.* **32,** 500-506.

Hodson, M.E., Valsami-Jones, E., Cotter-Howells, J.D., Dubbin, W.E., Kemp, A.J., Thornton, I., and Warren, A. 2001. Effect of bone meal (calcium phosphate) amendments on metal release from contaminated soils – a leaching column study. *Environ. Pollut.* **112,** 233-243.

Huang, J.W., Chen, J.J., Berti, W.R., and Cunningham, S.D. 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* **31,** 800-805.

Jaworska, J.S., Schowanek, D., and Feijtel, T.C.J. 1999. Environmental risk assessment for trisodium [S,S]-ethylene diamine disuccinate, a biodegradable chelator used in detergent applications. *Chemosphere* **38,** 3597-3625.

Jones, P.W., and Williams, D.R. 2001. Chemical speciation used to assess [S,S']-ethylenediaminedissuccinic acid (EDDS) as a readily-biodegradable replacement for EDTA in radiochemical decontamination formulations. *Appl. Radiat. Isot.* **54,** 587-593.

Kari, F.G., Hilger, S., and Canonica, S. 1995. Determination of the reaction quantum yield for the photochemical degradation of Fe(III)-EDTA: Implication for the environmental fate of EDTA in surface waters. *Environ. Sci. Technol.* **29,** 1008-1017.

Kos, B. and Leštan, D. 2003b. Induced phytoextraction /soil washing of lead using biodegradable chelate and permeable barriers. *Environ. Sci. Technol.* **37,** 624-629.

Kos, B., and Leštan, D. 2003a. Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogen modified soil water sorption capacity on Pb phytoextraction and leaching. *Plant Soil* **253,** 403-411.

Laurie, S.H., Tancock, N.P., McGrath, S.P., and Sanders, J.R. 1991. Influence of complexation on the uptake by plants of iron, manganese, copper and zinc. I. Effect of EDTA in a multi-metal and computer-simulation study. *J. Experi. Bot.* **42,** 509-513.

Li, X.D., Poon, C.S., and Liu, P.S. 2001. Concentration and chemical partitioning of road dusts and urban soils in Hong Kong. *Appl. Geochem.* **16,** 1361-1368.

Luo, C.L., Shen, Z.G., and Li, X.D. 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* **59,** 1-11.

Luo, C.L., Shen, Z.G., Baker, A.J.M., and Li, X.D. 2006b. The role of root damage in the chelate-enhanced accumulation of lead by Indian mustard plants. *Internat. J. Phytorem.* (in press)

Luo, C.L., Shen, Z.G., Li, X.D. and Baker, A.J.M. 2006a. Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS. *Chemosphere* **63,** 1773-1784.

Luo, C.L., Shen, Z.G., Lou, L.Q., and Li, X.D. 2006c. EDDS and EDTA-enhanced phytoextraction of metals from artificially contaminated soil and residual effects of chelant compounds. *Environ. Pollut*. (in press)

Madrid, F., Liphadzi, M.S., and Kirkham, M.B. 2003. Heavy metal displacement in chelate-irrigated soil during phytoremediation. *J. Hydrol.* **272,** 107-119.

Marschner, H., Romheld, V., and Kissel, M. 1986. Different strategies in higher-plants in mobilization and uptake of iron. *J. Plant Nutr.* **9,** 695-713.

Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., and Tack, F.M.G. 2005. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere* **58,** 1011-1022.

Nowack, B. 2002. Environmental chemistry of aminopolycarboxylate chelating agents. *Environ. Sci. Technol.* **36,** 4009-4016.

Papassiopi, N., Tambouris, S., and Kontopoulos, A. 1999. Removal of heavy metals from calcareous contaminated soils by EDTA leaching. *Water Air Soil Pollut.* **109,** 1-15.

Römkens, P., Bouwman, L., Japenga, J., and Draaisma, C. 2002. Potentials of drawbacks of chelate-enhanced phytoremediation of soils. *Environ. Pollut.* **116,** 109-121.

Salt, D.E., Prince, R.C., Pickering, I.J., and Raskin, I. 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109,** 1427-1433.

Shen, Z.G., Li, X.D., Wang, C.C., Chen, H.M., and Chua, H. 2002. Lead phytoextraction from contaminated soil with high-biomass plant species. *J. Environ. Qual.* **31,** 1893-1900.

Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C.J., and Schowanek, D.R. 2001. Biodegradation of metal-[S,S]-EDDS complexes. *Environ. Sci. Technol.* **35,** 1765-1770.

Wallace, A. 1983. A one-decade update on chelated metals for supplying micronutrients to crops. *J. Plant Nutr.* **6,** 429-438.

Witschel, M., and Egli, T. 1998. Purification and characterization of a lyase from the EDTA-degrading bacterial strain DSM 9103 that catalyses the splitting of [S,S']-ethylenediaminedissuccinate, a structural isomer of EDTA. *Biodegradation* **8,** 419-428.

Wu, J., Hsu, F.C., and Cunningham, S.D. 1999. Chelate-Assisted Pb phytoextraction: Pb availability, uptake and translocation constraints. *Environ. Sci. Technol.* **33,** 1898-1904.

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
Extractable metal with water (mg kg^{-1})	
Cu	2.6
Pb	2.2
Zn	4.4
Cd	0.07
Extractable metal after addition of 5 mM EDDS (mg kg^{-1})	
Cu	235
Pb	7.5
Zn	120
Cd	0.5

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

	Corn				Beans			
Treatments	Cu	Pb	Zn	Cd	Cu	Pb	Zn	Cd
Water	70 ± 5.2 a	8 ± 1.6 a	360 ± 28 a	9 ± 2.2 a	89 ± 1.2 a	20 ± 0.6 a	454 ± 27 a	9 ± 0.9 a
Hot-water	85 ± 8.8 a	9.6 ± 2.2 a	410 ± 50 a	5.7 ± 1.9 a	254 ± 26 a	43 ± 6 a	394 ± 66 a	5.4 ± 0.8 a
1mM EDDS	176 ± 34 a	9 ± 0.2 a	408 ±36 a	13.7 ± 1.8 a	992 ± 114 a	38 ± 10 a	600 ± 112 a	7.8 ± 2 a
Hot-1mM EDDS	1390 ± 160 c	73 ± 7 b	884 ± 95 b	39.5 ± 4.2 a	8940 ± 532 d	532 ± 56 b	1500 ± 150 b	40 ± 8 ab
5mM EDDS	630 ± 75 b	63 ± 5.6 b	712 ± 20 b	18.5 ± 5 b	1800 ± 74 b	1090 ± 30 b	1140 ± 69 ab	12 ± 0.4 a
Hot-5mM EDDS	1920 ± 210 c	762 ± 100 c	934 ± 134 b	62 ± 8.8 b	6920 ± 700 c	4000 ± 420 c	2880 ± 320 c	166 ± 18 b

The values are means ± S.D. (n = 12); the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Table 3 Total amounts of DOC and metals in the leachate solutions 7 d after the application of EDDS

Plants	**Treatments**	**DOC (mg kg^{-1} of soil)**	**Metals (mg kg^{-1} of soil)**			
			Cu	Pb	Zn	Cd
No plant	0 EDDS	50.8 ± 11.4	2.18 ± 0.6	1.86 ± 0.2	3 ± 0.2	0.068 ± 0.002
	Hot-0 EDDS	49.4 ± 7.8	2.26 ± 0.4	1.84 ± 0.6	3.24 ± 0.4	0.054 ± 0.008
	1 EDDS	484 ± 84	107 ± 13	3.3 ± 0.4	12.2 ± 1.4	0.112 ± 0.02
	Hot-1 EDDS	418 ± 74	100 ± 9.6	3.1 ± 0.8	12.5 ± 0.8	0.13 ± 0.02
	5 EDDS	1400 ± 212	204 ± 24	5.4 ± 0.4	135 ± 16	0.4 ± 0.06
	Hot-5EDDS	1600 ± 112	190 ± 13	5.2 ± 1	132 ± 11	0.384 ± 0.04
Corn	0 EDDS	46 ± 10	2 ± 1	1.82 ± 0.2	2.9 ± 0.4	0.058 ± 0.008
	Hot-0 EDDS	54.6 ± 8.8	2.2 ± 0.2	1.79 ± 0.2	2.4 ± 0.6	0.052 ± 0.008
	1 EDDS	340 ± 50	75 ± 8.6	2.44 ± 0.4	10.8 ± 0.8	0.09 ± 0.002
	Hot-1 EDDS	376 ± 62	80 ± 10	2.2 ± 0.6	10 ± 1.4	0.08 ± 0.004
	5 EDDS	1020 ± 54	160 ± 12	4.18 ± 0.4	97 ± 16	0.316 ± 0.04
	Hot-5EDDS	1000 ± 92	162 ± 18	4.38 ± 0.4	95 ± 11	0.29 ± 0.04
Beans	0 EDDS	60 ± 12	1.8 ± 0.2	1.84 ± 0.2	2.62 ± 0.4	0.064 ± 0.006
	Hot-0 EDDS	70 ± 9.6	1.6 ± 0.3	1.74 ± 0.2	2.42 ± 0.2	0.060 ± 0.008
	1 EDDS	360 ± 50	73 ± 8.9	2.6 ± 0.4	10.4 ± 1	0.084 ± 0.01
	Hot-1 EDDS	332 ± 34	75 ± 14	2.8 ± 0.8	10.3 ± 1.6	0.1 ± 0.01
	5 EDDS	1090 ± 112	161 ± 19	4.34 ± 1	91 ± 10	0.33 ± 0.04
	Hot-5EDDS	1200 ± 100	164 ± 22	4.64 ± 0.6	83 ± 10	0.3 ± 0.02

Values are means ± S.D. (n = 2).

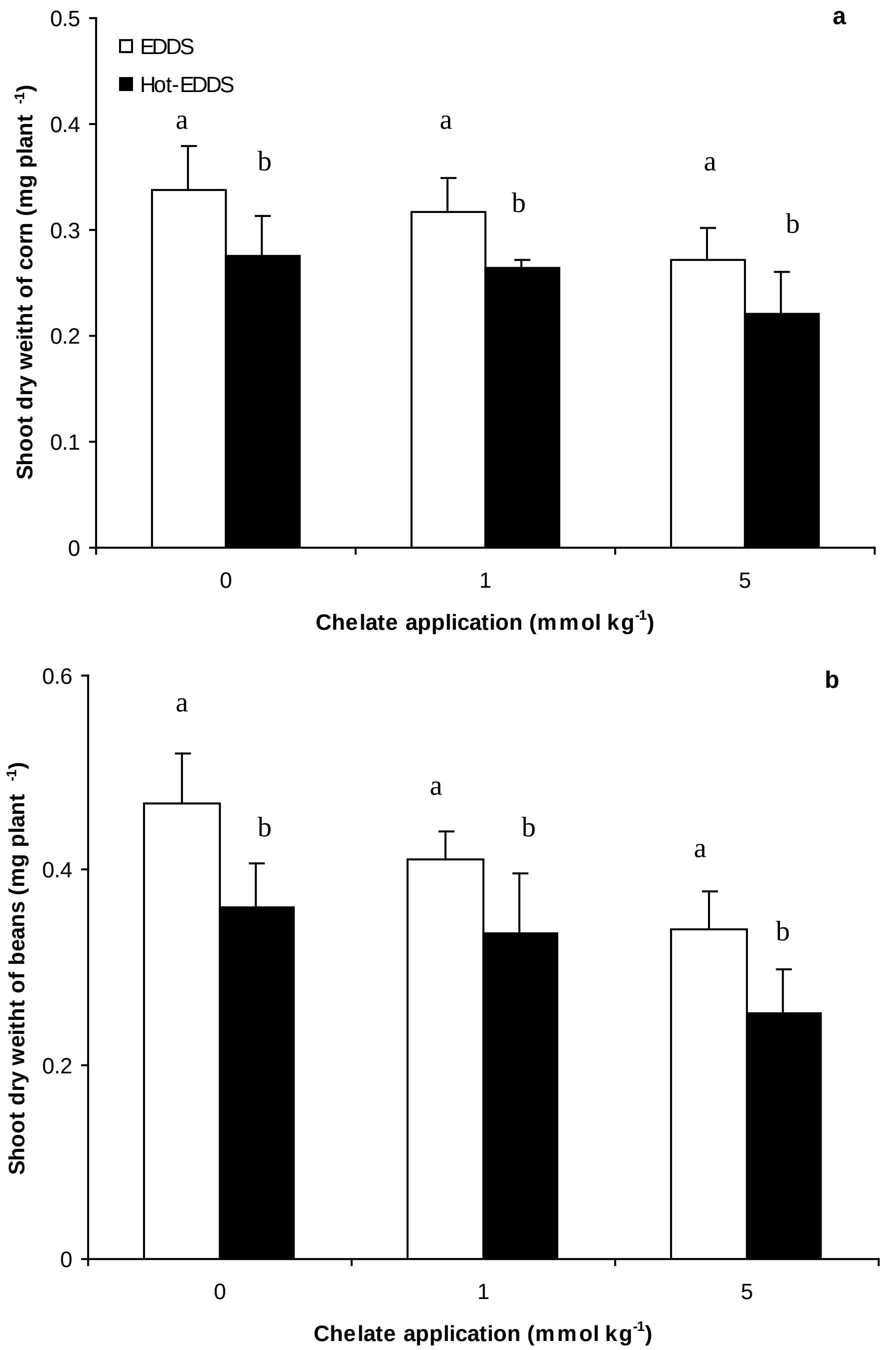

Fig. 1 The effects of the normal and hot EDDS solutions at different application dosages on the shoot dry matter yields of corn (a) and beans (b). The values are means ± S.D. (n = 12). The different small letters stand for statistical significance at the 0.05 level with the LSD test.

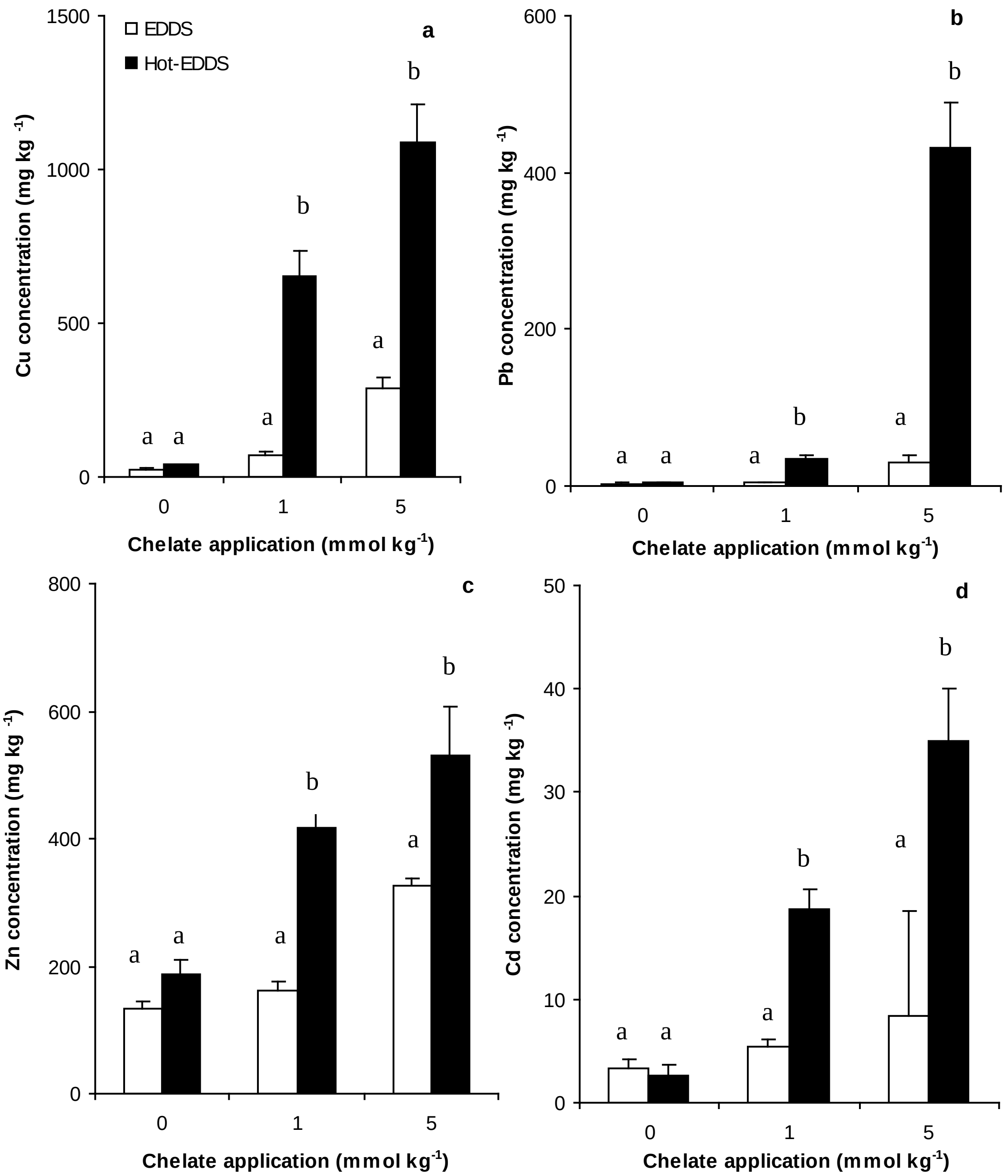

Fig. 2 The effects of normal and hot EDDS solutions at different application dosages on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of corn. The values are means ± S.D. (n = 12). The different small letters stand for statistical significance at the 0.05 level with the LSD test.

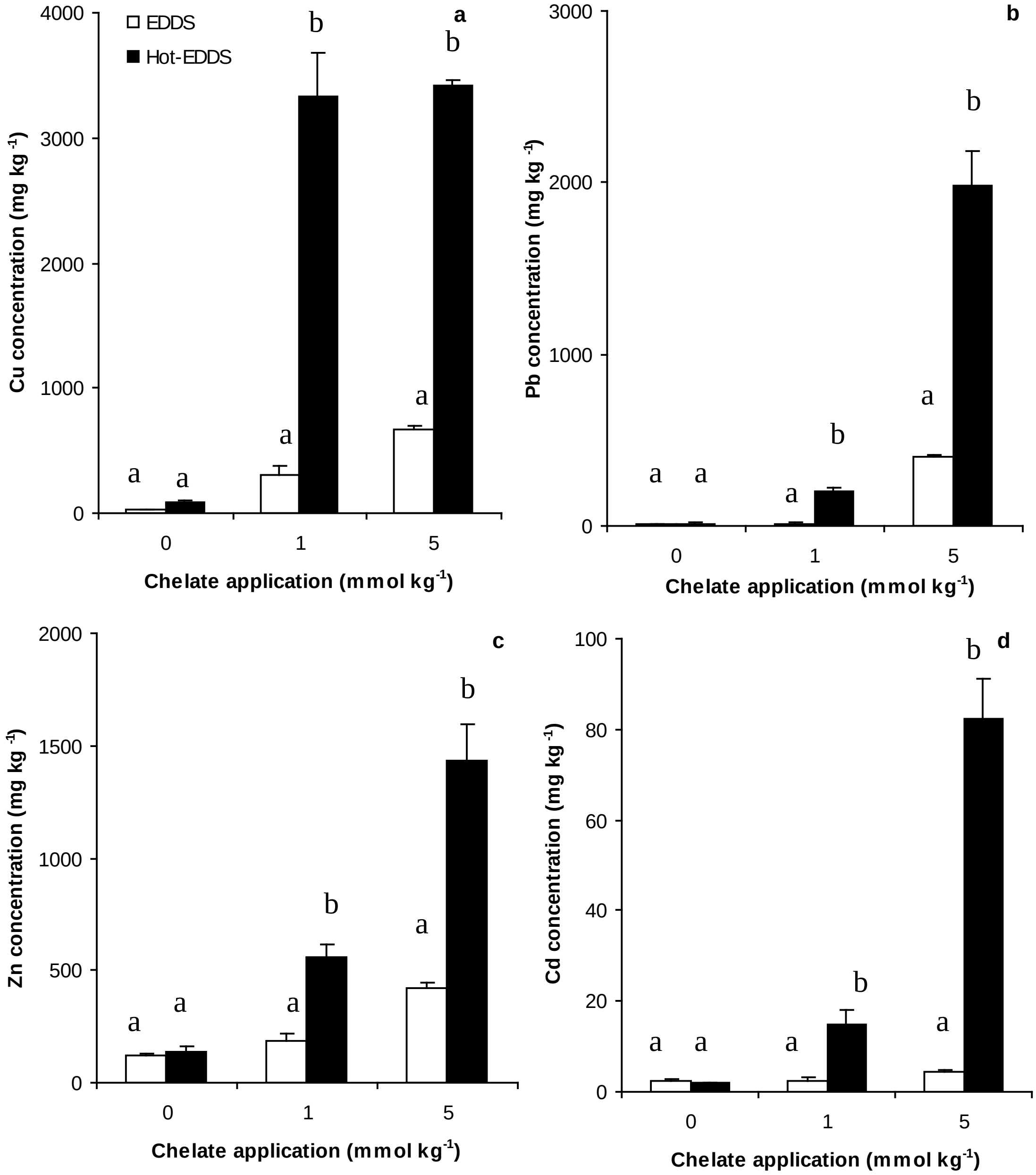

Fig. 3 The effects of normal and hot EDDS solutions at different application dosages on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of beans. The values are means ± S.D. (n = 12). The different small letters stand for statistical significance at the 0.05 level with the LSD test.

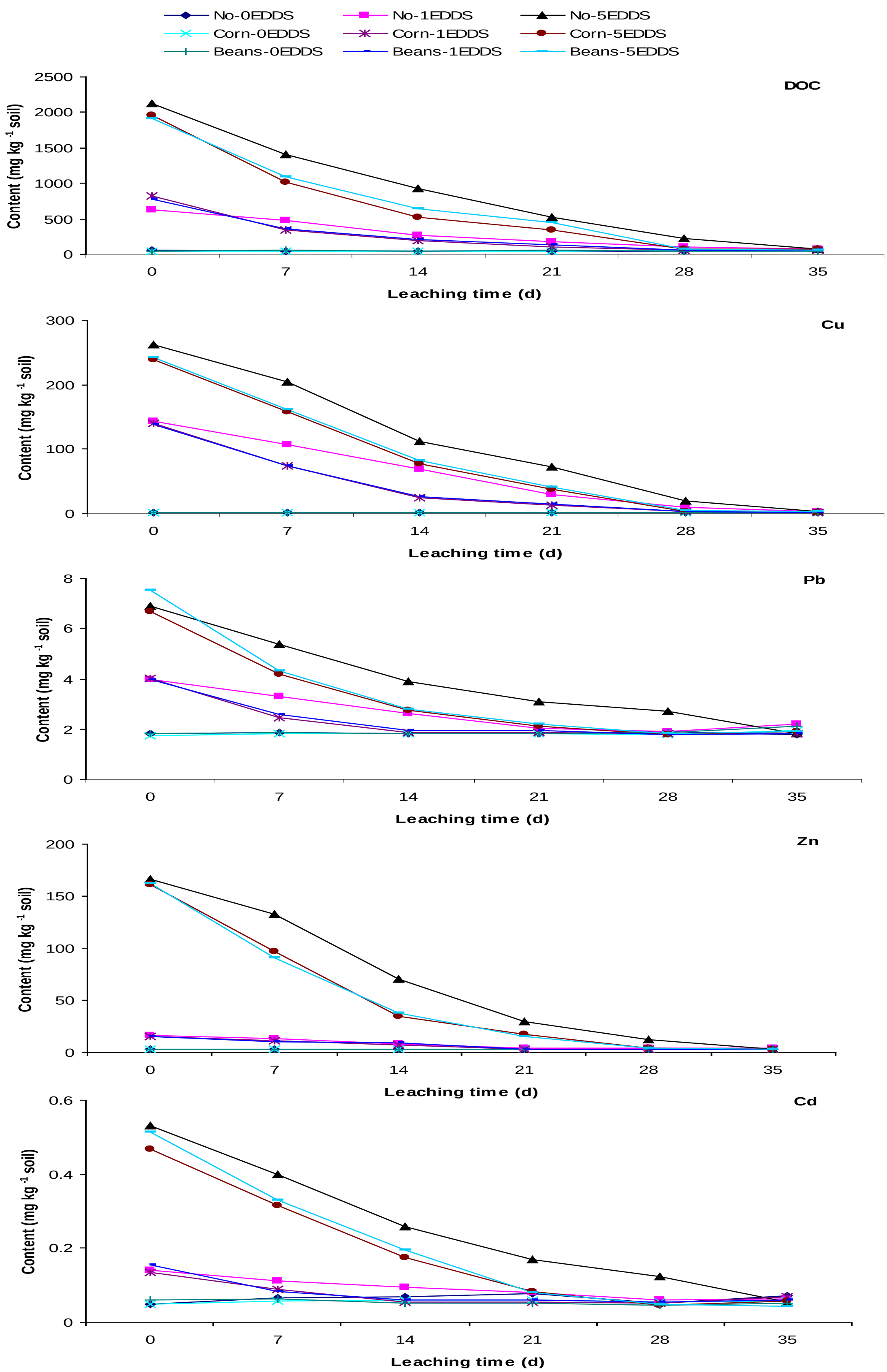

Fig. 4 The amounts of DOC, Cu, Pb, Zn, and Cd in the leachates from the soil without plants and the soil grown with corn and beans after the application of normal EDDS at different dosages during the whole leaching study of 35 days.