

**EDDS and EDTA-enhanced phytoextraction of metals from artificially
contaminated soil and residual effects of chelant compounds**

Chunling Luo^a, Zhenguo Shen^{a,b}, Laiqing Lou^b, Xiangdong Li^{a*}

^a *Department of Civil and Structural Engineering, The Hong Kong Polytechnic
University, Hung Hom, Kowloon, Hong Kong*

^b *College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China*

“Capsule” - Chrysanthemum coronarium L. was the most sensitive species to the application of chelants, and EDDS biodegrades much more rapidly than EDTA in soil.

Abstract

The potential of 18 different plants to be used in the chemically enhanced phytoextraction of Cu, Pb, Zn and Cd was assessed using pot experiments. The results showed that, of all of the plants that were tested, *Chrysanthemum coronarium* L. was the species most sensitive to the application of EDTA (ethylenediaminetetraacetic acid), and had the highest enhancement of Cu and Pb concentrations in its shoots. For Cu and Pb, 9.5- and 69-fold increases in metal concentrations were achieved 7 d after the application of 3 mmol kg⁻¹ of EDTA, respectively. Compared with EDTA, EDDS

* Corresponding author (X. D. Li). *E-mail address:* cexdli@polyu.edu.hk; Fax: +852-2334-6389;

Tel.: +852-2766-6041.

(S,S-ethylenediaminedisuccinic acid) was more effective in enhancing the concentration of Cu in the shoots of *Chrysanthemum coronarium* L. and *Zea mays* L. grown on multi-metal contaminated soils. With regard to Pb, when the chelant application rate was higher than 5 mmol kg⁻¹, EDDS was more effective than EDTA in increasing the concentration of Pb in the shoots of the two plants. The study of the residual effects of various chelant treatments in soils indicated that the EDTA-treated soil still had a significant ability to enhance the concentrations of Cu and Pb in the shoots of *Zea mays* L. six months after the chelant treatment. However, the EDDS-treated soil did not have any effect in enhancing the concentrations of metals in the shoots of *Zea mays* L. in the second crop test. The results may indicate that EDDS biodegrades more rapidly than EDTA in soil, and has better chance in limiting the potential metal leaching from soil.

Keywords: Phytoextraction; EDTA; EDDS; *Chrysanthemum coronarium* L.; *Zea mays* L.; Residual effects

1. Introduction

Soil contamination by heavy metals is a global environmental issue due to the rapid development of intensive agriculture and industry in many parts of the world. Elevated concentrations of heavy metal not only lead to reductions in the microbial activity and fertility of the soil, and in crop production (McGrath et al., 1995), but also threaten human health through the food chain. The remediation of soil and water

contaminated with heavy metals has become a challenging task facing regulators and scientific communities. Recently, phytoextraction, the use of plants to extract heavy metals from contaminated soils, has been receiving an increasing amount of attention (Salt et al., 1998).

The success of phytoextraction is dependent on large biomass production and high concentrations of heavy metals in the shoots of plants. Hyperaccumulators were able to accumulate unusually high levels of heavy metals in their aboveground harvestable parts. But, their low biomass production limits the overall amount of heavy metals extracted by plant shoots per harvest. In addition to hyperaccumulators, some fast-growing high-biomass plant species have been also evaluated for their potential use in chemically enhanced phytoextraction (Kumar et al., 1995; Ebbs et al., 1997; Stoltz and Greger, 2002). Synthetic chelants, such as CDTA (trans -1, 2 -diaminocyclohexane -N, N, N', N'-tetraacetic acid), EDDHA [ethylenediamine-di (*o*-hydroxyphenylacetic acid)], EDDS (S,S-ethylenediaminedisuccinic acid), EDTA (ethylenediaminetetraacetic acid), EGTA [ethyleneglycol -bis (β -aminoethyl ether), N, N, N', N'-tetraacetic acid], and NTA (nitrilotriacetate) have been used to enhance the solubility of metals in soils and their subsequent uptake and translocation in plant shoots (Blaylock et al., 1997; Huang et al., 1997; Cooper et al., 1999; Wu et al., 1999; Shen et al., 2002; Kos and Leštan, 2003a). Despite the high efficiency of EDTA for inducing the extraction of metals, some concerns have been expressed regarding the enhanced mobility of metals in soil and their potential risks of spreading metal contaminants to groundwater and the surrounding environment due to its high affinity

with heavy metals and its poor biodegradability in the environment. EDDS is an easily biodegradable, low-toxic chelant with a strong chemical affinity to Pb, Cu, and other metals. The use of this chelant in the phytoremediation process has received much attention in the past few years (Vandevivere et al., 2001a; Grčman et al., 2003; Kos and Leštan, 2003b; Tandy et al., 2004; Luo et al., 2005a and b). Large differences have been reported in the stimulating effects of chelants on the accumulation of metals in the shoots of different species of plants (Huang et al., 1997; Shen et al., 2002; Chen et al., 2004). Therefore, efforts have been focused on identifying high biomass plant species that can accumulate significant amounts of heavy metals in response to chelant treatments to soil. Screening plant species that are more sensitive to chelant treatments will not only help minimize the amount of chelants applied in the field, but also decrease the environmental risk of mobilised metals.

Copper can be particularly toxic to many species of plants (Pahlsson, 1989). The threshold concentration of Cu toxicity for crops is about $30 \text{ mg kg}^{-1} \text{ DM}$ (Marschner, 1995). Copper toxicity might be a limiting factor in the phytoremediation of multi-metal contaminated soils (Lombi et al., 2001). It would be difficult to produce enough biomass before the application of chelants if the soil were heavily contaminated with metals such as Cu. A few Cu hyperaccumulators were reported in the literature, but there are still some doubts about their Cu uptake abilities (Baker et al., 2000). The chelant-enhanced uptake of Cu in plant shoots has generally been minimal (Kulli et al., 1999; Kayser et al., 2000; Lombi et al., 2001; Römkens et al., 2002; Shen et al., 2002; Thayalakumaran et al., 2003; Wenzel et al., 2003), except for the results

reported by Blaylock et al. (1997). In their study, the concentration of Cu in *Brassica juncea* shoots grown in soil containing 200 mg kg⁻¹ of Cu reached 1000 mg kg⁻¹ DM one week after 2.5 mmol kg⁻¹ of EDTA were applied.

The objectives of the present study were: (i) to study the potential use of the 18 different species and cultivars of plants in the chemically enhanced phytoextraction of heavy metals (Cu, Pb, Zn, and Cd) from artificially contaminated soils; (ii) to evaluate the relative effectiveness of biodegradable chelant EDDS in enhancing the accumulation of heavy metals in selected species of plants grown in multi-metal contaminated soil, particularly in soil with high concentrations of Cu; and (iii) to compare the residual effects of EDDS and EDTA in soil after the first cropping with the application of these two chelants.

2. Materials and methods

2.1. Phytoextraction of heavy metals from artificially contaminated soil by 18 different plants with EDTA application

The experiment was conducted in Nanjing Agricultural University, Nanjing, China. Soil samples were collected from a farm in Nanjing. After being air-dried, the samples were crushed to pass through a 1-cm diameter sieve. The soils were artificially amended with multi-metals: Cu (500 mg kg⁻¹ of soil) as CuSO₄·5H₂O (copper sulfate); Pb (500 mg kg⁻¹ of soil) as Pb(NO₃)₂ (lead nitrate); Zn (400 mg kg⁻¹

of soil) as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (zinc sulfate); and Cd (1 mg kg^{-1} of soil) with $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (cadmium nitrate). The basal fertilizers applied to the soil were 250 mg N kg^{-1} of dry soil (as NH_4NO_3), 100 mg P kg^{-1} of dry soil, 250 mg K kg^{-1} of dry soil as KH_2PO_4 , and 60 mg S kg^{-1} of dry soil (as MgSO_4). The soil was treated to five cycles of saturation with de-ionized water and air-dried before being used for pot experiments. The selected physical and chemical properties of the soils are presented in Table 1.

Eighteen different species/cultivars of plants, including thirteen dicotyledons and five graminaceous monocotyledons, were used in this study: *Zea mays* L. cv. Nongda No. 108, *Triticum aestivum* L. cv. Weimai No. 8, *Triticum aestivum* L. cv. Shangnong No. 93-52, *Triticum aestivum* L. cv. Zimai No. 12, *Sorghum bicolor* L. cv. Moench-S. vulgare Pers, *Brassica juncea* L. Czern. Et Coss. cv. Liyangkucai, *Brassica chinensis* L. cv. Xiaoairen, *Brassica chinensis* L. cv. Xiaoza No. 56, *Brassica pekinensis* Rupr L., *Brassica campestris* L. cv. Suyou No. 1, *Brassica campestris* L. cv. Qinyou No. 8, *Lactuca sativa* L., *Glycine max* L. Merrill, *Chrysanthemum coronarium* L., *Coriandrum sativum* L., *Daucus carota* L. var. sativa DC., *Raphanus sativus* L., *Spinacia oleracea* L.. The seeds were sown directly in the soil. After germination, the plants were thinned to 6 plants per pot (2.5 kg dried soils, 20 cm i.d. x 20 cm height) for all species and varieties.

On the 56th day after sowing, EDTA (from BDH Laboratory Supplies Poole, minimum assay: 99.5%) was added in a 250 ml 30mM Na_2EDTA salt solution with the pH of 4.8 to the surface of the soil to make up the amount of EDTA to 3 mmol

EDTA kg^{-1} of soil. All of the experiments were conducted in a glasshouse under natural light. The air temperature ranged from 27 to 35 °C. Each treatment was replicated three times, and was in a completely randomized block design. Three plants were harvested by cutting the shoots 0.5 cm above the surface of the soil, and the roots were removed from the pots 7 d after the application of EDTA. The shoots were washed with tap water and rinsed with DIW, dried at 70 °C in an oven to a constant weight for dry weight measurement.

2.2. Comparison of EDDS and EDTA for enhanced phytoextraction of heavy metals by Chrysanthemum coronarium L. and Zea mays L., and residual effects of chelants in soils

The experiment was conducted in the Hong Kong Polytechnic University, Hong Kong. Soil samples were collected from a disused agricultural field in the Yuen Long area of Hong Kong. The samples were sieved to pass through a 2 mm sieve and air-dried for one week. The soils were artificially contaminated with multi-metals: Cu (400 mg kg^{-1} of soil) as CuCO_3 (copper carbonate); Pb (500 mg kg^{-1} of soil) as $\text{Pb}_3(\text{OH})_2(\text{CO}_3)_2$ (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 sulfide) at a Zn concentration ratio of 1:1; and Cd (15 mg kg^{-1} of soil) with $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (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 air-dried soils (500 g) were placed in plastic pots (12 cm i.d. x 12 cm height). The moisture of the soil was maintained at near field water capacity by adding DIW daily. Seeds of *Chrysanthemum coronarium* L. and *Zea mays* L. cv. Nongda No. 108 were sown directly in the soils. In order to acquire uniform seedlings, *Chrysanthemum coronarium* L. was sown three weeks before *Zea mays* L. After germination, the seedlings were thinned to four plants per pot. On the 35th day after the sowing of *Chrysanthemum coronarium* L., EDTA (the same as the mentioned above) and EDDS (from Fluka Chemie GmbH) were applied to the surface of the soils at rates of 0 (control), 0.5, 1.0, 1.5, 3.0, and 5.0 mmol kg⁻¹ soil in 50 ml Na₂EDTA and EDDS-Na₃ salt solutions. To make up the different amounts of chelant treatments, EDTA and EDDS were diluted from 50 mM Na₂EDTA (pH 4.8) and EDDS-Na₃ (pH 10.1) salt solutions. Three replications were used for each treatment. All of the experiments were conducted in a glasshouse under natural light. The air temperature ranged from 18 to 23 °C. The plants were harvested 7 d after the application of chelants. Their shoots and roots were separated for drying and further analysis.

In order to determine the residual effects of the applied chelants on the second cropping, the pots were kept at two-thirds of the field moisture capacity after the harvest of the first crop of corn. Six months after the harvest, the remaining soils were mixed thoroughly, and the *Zea mays* L. seeds were again sown directly in the soils.

Zea mays L. was selected in this experiment due to its low sensitivity to chelants treatment in soils than *Chrysanthemum coronarium* L. The seedlings were thinned to four plants per pot after germination. The experiment was conducted in the same greenhouse under natural light. The air temperature ranged from 22 to 28 °C. The shoots of the *Zea mays* L. were harvested 14 d after growing in the pots and dried for dry biomass measurements and an analysis of metal concentrations.

2.3. Soil and plant analysis

The electrical conductivity (EC) of the soils was measured using a conductivity meter on an extract of soil obtained by shaking soil with DIW at a 1:2 (w/v) soil: water ratio. The pH of the soil was measured in a 0.01 M CaCl₂ solution at a 1:5 ratio of soil:solution (w/v) using a pH meter. The cation exchangeable capacity (CEC) of the soil was determined using the ammonium acetate saturation method. The texture, organic matter content, total N and field water capacity of the soil were measured by the procedures described by Avery and Bascomb (1982). The total concentrations of metal were determined by ICP-AES (Perkin-Elmer Optima 3300 DV) after strong acid digestion (1:4 concentrated HNO₃ and HClO₄ (v/v)) (Li et al., 2001).

For the analysis of plant samples, ground shoot samples (200 mg) were digested in a mixture of concentrated HNO₃ and HClO₄ (4:1, by volume) and the major and trace elements in the solutions were determined by ICP-AES (Shen et al., 2002). A certified standard reference material NIST (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 test the accuracy and precision of the analysis. The recovery rates were around $90 \pm 6\%$ for all of the metals in the plant reference material (NIST SRM 1515). The data reported in this paper were the mean values based on the three replicates. A statistical analysis of the experimental data, such as the correlation coefficient and significant difference calculation, was performed using SPSS® 11.0 statistical software.

3. Results

3.1. Effects of EDTA on the growth of different plants

Before the treatment of EDTA, all the plants showed normal development without visible toxic symptoms of heavy metals. The application of 3 mmol kg^{-1} of EDTA significantly decreased the yields of shoot dry matter (DM) in most of the plants tested (see Table 2). Compared with the dicotyledon species, the five graminaceous monocotyledon plants showed relatively less response to the application of EDTA, with less chlorosis and smaller reductions in shoot biomass.

3.2. Effects of EDTA on the uptake of heavy metals by different plants

In the control group, there were large variations in the concentration of metals in the shoots of different plant species/cultivars (Table 2). The concentrations of Cu, Pb, Zn, and Cd in the plants were in the range of 13 to 62, 2.1 to 7.5, 56 to 211, and 0.1 to 2.7 mg kg⁻¹, respectively. The application of EDTA at a rate of 3 mmol kg⁻¹ dramatically increased the concentrations of Cu, Pb, and Zn in the shoots of the plants. The enhancement was more pronounced for the dicotyledon plants than for the graminaceous monocotyledon plants (Table 2). Of all the plants tested, *Chrysanthemum coronarium* L. showed the greatest sensitivity to the application of EDTA, with the highest enhancement of the Cu, Pb, Zn concentrations in the shoots. It was noted that the EDTA application reduced the concentrations of Cd in some plant species tested in the present study (Table 2).

With regard to the total amount of metals phytoextracted from the soil, the application of EDTA also produced significant enhancing effects in the plants, although these effects were smaller than that on the metal concentrations in the shoots due to the reduction in shoot biomass as a result of the toxic effects of EDTA on the plants. The highest amounts of Cu and Pb extracted were achieved by *Chrysanthemum coronarium* L., with 1.42 mg Cu kg⁻¹ soil and 0.93 mg Pb kg⁻¹ soil, respectively. For Zn, the highest extraction (2.0 mg Zn kg⁻¹ soil) was achieved by *Brassica chinensis* L. cv. Xiaoza No. 56. The application of 3 mmol kg⁻¹ of EDTA did not significantly improve the phytoextraction of Cd by all species of plants. Noticeably, the total phytoextraction of Cu, Pb, and Zn in the *Chrysanthemum coronarium* L. shoots 7 days after chelant application reached 590, 390, and 590 µg

plant⁻¹, which were 5.2, 38, and 3.5 times the levels seen in the control group, respectively, although the biomass of the shoots decreased by about 45% in comparison with that in the control group.

3.3. Effects of EDDS and EDTA on the growth of *Chrysanthemum coronarium* L. and *Zea mays* L.

In this experiment, *Chrysanthemum coronarium* L. and *Zea mays* L. were chosen for a further study of their potential use in the chelant-enhanced phytoextraction of heavy metals from contaminated soil. The treatments with 0.5 mmol kg⁻¹ soil EDDS and EDTA significantly affected the growth of *Chrysanthemum coronarium* L., leading to a respective 23% and 18% decrease in shoot dry matter yields compared with the control group (Fig. 1). The effects of EDDS and EDTA on the growth of *Zea mays* L. were less significant than on the *Chrysanthemum coronarium* L. (see Fig. 1).

3.4. Effects of EDDS and EDTA on the uptake of metals by *Chrysanthemum coronarium* L. and *Zea mays* L.

The concentrations of Cu in the shoots of *Chrysanthemum coronarium* L. and *Zea mays* L. significantly increased with the increasing level of EDDS and EDTA applied to the soil (Fig. 2). Compared with EDTA, EDDS was more effective at increasing the concentration of Cu in the shoots of the two species. Between the two species, a larger

increase was observed in *Chrysanthemum coronarium* L. than in *Zea mays* L. On Day 7 after the application of 5 mmol kg⁻¹ of EDDS, the concentration of Cu in the shoots reached 2340 mg kg⁻¹ DW in *Chrysanthemum coronarium* L., which was 69-fold the amount found in the control (without the application of chelants). The maximum concentration of Cu in the shoots of *Zea mays* L. was 330 mg kg⁻¹ DW, representing an 8.2-fold increase over that seen in the control group.

The total amount of Cu accumulated in shoots also increased with the rate of application of EDDS and EDTA to soil (Fig. 3). A higher level of Cu phytoextraction was always found in the EDDS treatment, in which with the application of 5 mmol kg⁻¹ of EDDS, the phytoextraction of Cu was 38 and 5 times that of the control for *Chrysanthemum coronarium* L. and *Zea mays* L., respectively.

The results from the current study showed that the concentrations of Pb in the shoots of *Chrysanthemum coronarium* L. and *Zea mays* L. increased with the level of EDTA applied to the soil (Fig. 4). The application of EDDS at rates of 0.5-1.5 mmol kg⁻¹ had no significant effect on the Pb concentration in the shoots of the two plant species. However, at the application rate of 5 mmol kg⁻¹, EDDS was much more effective than EDTA at increasing the uptake of Pb by the plants, particularly in *Chrysanthemum coronarium* L. The concentration of Pb in the shoots of *Chrysanthemum coronarium* L. treated with 5 mmol kg⁻¹ of EDDS reached 628 mg kg⁻¹, which was 4.7- and 126- fold the level in the plants treated with 5 mmol kg⁻¹ of EDTA and the control group, respectively.

The enhancing efficiencies of total Pb phytoextraction by the two plant species

through EDDS and EDTA application (Fig. 5) were consistent with the increased concentrations of Pb in the shoots (Fig. 4), although the treatments with the chelants had a significant effect on the shoot biomass production of *Chrysanthemum coronarium* L. and *Zea mays* L. (Fig. 1). The maximum amount of Pb that was phytoextracted was found in *Chrysanthemum coronarium* L. treated with 5 mmol kg⁻¹ of EDDS, which reached 1.95 mg Pb kg⁻¹ soil. This represents an increase of 70 times over the control groups.

The concentrations of Zn and Cd were enhanced through the application of chelating agents to soil (Table 3). However, the concentrations of Zn and Cd in the shoots treated with the chelants never exceeded those of the controls by more than 1.85 times, except for Zn in the 5 mmol kg⁻¹ of EDDS treatment. The total amount of Zn extracted did not exceed that of the control groups by more than 1.5 times. No significant stimulating effect from the chelants was found on the phytoextraction of Cd in these plants.

In the present study, the sum of the phytoextraction of Cu, Pb, Zn, and Cd by *Chrysanthemum coronarium* L. reached 14.7 mg kg⁻¹ soil with the application of 5 mmol kg⁻¹ of EDDS, which accounted for 1.04% of the total amount of Cu, Pb, Zn, and Cd in the soil. Those values were 2.9-fold those of the EDTA treatments. These results indicated that EDDS was superior to EDTA in the phytoremediation of contaminated soils with multiple heavy metals.

3.5. The residual effects of EDDS and EDTA on the growth and uptake of metals by

Zea mays L.

To evaluate the residual and time effects of the application of EDDS and EDTA in soil, a second cropping with *Zea mays L.* was conducted six months after the first crops were harvested. The growth of *Zea mays L.* in the second cropping was strongly dependent on the chelant treatment in the first cropping experiment (Fig. 6). The yields of shoot DM showed a strong negative effect from the amount of the original EDTA application. The biomass of *Zea mays L.* at the second harvest decreased significantly as the level of EDTA applied in the first cropping increased due to the toxicity of the residual chelant in soil. The concentration of Cu in the shoots of *Zea mays L.* increased as the rate of application of EDTA increased in the first cropping (see Table 4). The highest concentration of Cu ($200 \text{ mg kg}^{-1} \text{ DW}$) in the shoots of the second *Zea mays L.* cropping appeared in the 5 mmol kg^{-1} of EDTA treatment, which was 6.8-fold that of the control group. For Pb, a significant increase was found only in the treatments with 3 and 5 mmol kg^{-1} of EDTA compared with the control (Table 4).

For the plants grown on the EDDS-treated soils, no significant residual effects of the EDDS were found in the *Zea mays L.* shoot DM and the concentrations of the four metals, regardless of the different rates of EDDS application (Table 4).

4. Discussion

In chemically enhanced phytoextraction processes, the increased uptake of Pb induced by the application of EDTA can be explained by the effect of improved solubility of Pb, and the uptake of the Pb-EDTA complex by plants (Huang et al., 1997; Vassil et al., 1998; Epstein et al., 1999; Shen et al., 2002). It is thought that EDTA can destroy the physiological barrier(s) of plant roots that normally function to control the uptake and translocation of solutes, which would lead to the rapid equilibration of the hydroponic or soil solution with the sap of the xylem (Vassil et al., 1998). For different plant species, even different cultivars within the same species, there are significant differences in the metal tolerance and uptake ability. Monocotyledon species are usually more tolerant to metals than dicotyledon species (Marschner, 1995). Our results showed that the dicotyledon species suffered from more severe phytotoxicity than the graminaceous monocotyledon species after the application of EDTA (Table 2). Therefore, it is possible that the roots of dicotyledon species would experience from heavier physiological damages, which could lead to a breakdown of the root exclusion mechanism, and in turn to the indiscriminate uptake of solutes by plants, than the roots of monocotyledon species. This assumption was consistent with the fact that the enhancement of metal concentrations in the shoots of the plants was more pronounced for the dicotyledon plants than for the graminaceous monocotyledon plants (see Table 2). Of all the plants tested, *Chrysanthemum coronarium* L. showed the greatest sensitivity of growth to the application of EDTA and the highest enhancement of the concentrations of Cu, Pb, Zn in the shoots. Chen et al. (2004) also reported that the growth of and metal accumulation by mung bean

and buckwheat had higher sensitivity to the EDTA treatment in soils than the growth of and metal accumulation by other monocotyledon plants species.

It has been suggested that a threshold concentration of EDTA is required to induce the accumulation of metals in plant shoots (Vassil et al., 1998). Blaylock et al. (1997) observed that the application of a threshold concentration of chelant of between 1 and 5 mmol kg⁻¹ of EDTA induced a dramatic accumulation of Pb in the shoots of Indian mustard grown in soil containing 600 mg Pb kg⁻¹. At this threshold concentration (5 mmol kg⁻¹ of EDTA) and above, synthetic chelants including EDTA could damage the membrane of root cells, which normally function to control the uptake and translocation of solutes (Vassil et al., 1998). In the present study, a dramatic increase in the concentrations of Cu, Pb, and Zn in plant shoots occurred between the 3 and 5 mmol kg⁻¹ EDDS treatments. However, this increase in the accumulation of the metals in shoots was not found in the EDTA treatments ranging from 0.5 to 5 mmol kg⁻¹ soil. It was speculated that less than 5 mmol kg⁻¹ of EDTA application was insufficient to break down plant uptake barriers under the conditions of the present experiment. This observation was consistent with the observation that EDTA was less toxic to *Chrysanthemum coronarium* L. than EDDS (Fig. 1). It could partially explain why EDDS had a higher efficiency than EDTA in increasing the accumulation of metals in shoots at the application rate of 5 mmol chelant kg⁻¹ to soil. Grčman et al. (2003) observed that single addition of 10 mmol kg⁻¹ EDDS was similarly effective as EDTA for the phytoextraction of Pb from soil.

Several chelating agents, such as EDTA, CDTA, EGTA, EDDS, and NTA have been tested for their ability to mobilize and increase the accumulation of heavy metals by plants. EDDS was more effective in solubilizing soil Cu for root uptake and translocation into aboveground biomass (Luo et al., 2005a and b; Meers et al., 2005). In the present study, the percentages of Cu phytoextracted by *Chrysanthemum coronarium* L. for 42 days and *Zea mays* L. for 21 days with the EDDS treatment in one phytoextraction cycle were 0.11-1.81 % and 0.06-0.11% of the total Cu in the soil (400 mg kg^{-1}), respectively. The values by *Chrysanthemum coronarium* L. in this study were higher than the data on the phytoextraction of Cu with NTA (Kulli et al., 1999; Kayser et al., 2000), with EDDS (Kos and Leštan, 2004), and with EDTA (Thayalakumaran et al., 2003; Wenzel et al., 2003). The results suggest that EDDS can be regarded as a better candidate chelant for the phytoextraction of Cu in soils. EDDS and EDTA have an almost equal chemical affinity for Cu ($\log K_s = 18.4$ and 18.8 , respectively) (Martell et al. 2001). But for Fe, Pb, Zn, and Cd, EDDS has a much lower chemical affinity than EDTA. Thus, EDDS would be more effective at solubilizing soil Cu than EDTA (Tandy et al., 2004; Luo et al., 2005a). Tandy et al. (2004) reported that a very small fraction of EDTA and about 20-35% of EDDS were predicted to be in a free and non-complex form when soil was extracted with EDTA and EDDS at a chelate:metal ratio of 1:1. It is possible that there is more free EDDS than free EDTA in soil when EDDS or EDTA is applied at the same rate. A uncoordinated chelant would be available to bind various essential divalent cations in root cells, including Fe^{2+} , Zn^{2+} , and Cu^{2+} , causing the breakdown of exclusion

mechanisms in plants. Metal chelant complexes may enter the root through breaks in the root endodermis and Casparian strips, and be rapidly transported to the shoots (Romheld and Marschner, 1981; Bell et al., 1991). Also, it is likely that Cu may enter the roots of plants and be transported to their shoots as a Cu-EDDS complex.

In most cases, the EDTA treatment was superior in terms of solubilizing soil Pb for root uptake and translocation into the aboveground biomass due to its strong chemical affinity for Pb ($\log K_s = 17.88$) (Martell et al., 2001). The accumulation of Pb in plant shoots was correlated with the formation of the Pb-EDTA complex, and Pb-EDTA was the major form of Pb absorbed and translocated by plants (Vassil et al., 1998; Epstein et al., 1999). The highest percentages of Pb extracted by *Chrysanthemum coronarium* L. and *Zea mays* L. in one phytoextraction cycle were calculated to be 0.39% and 0.01% that of the total Pb (500 mg kg^{-1}) present in the soil, respectively. These values by *Chrysanthemum coronarium* L. were higher than the data reported by Kos and Leštan (2003a) and Grčman et al. (2003) for the extraction of Pb by corn with EDDS and EDTA.

Phytoremediation processes of contaminated soils usually require several successive croppings of selected plants. For an efficient phytoextraction with the application of chelants, the application dosage in the first cropping should not produce significant negative effects on the following plants to ensure substantial amounts of plant biomass established prior to the next round addition of chelants. Results from the residual effects study indicated that the EDTA-treated soil still had a significant ability to depress plant growth and enhance the concentrations of Cu and Pb in the

shoots of *Zea mays* L. six months after the chelant treatment to soil. Grčman et al. (2001) also found that residual EDTA had a strong inhibitory effect on red clover. However, there were relatively low concentrations of residual EDDS and metal-EDDS complexes in the soil. EDDS and metal-EDDS complexes could have been degraded before the seeds of *Zea mays* L. were sown in the second cropping six months later. Vandevivere et al. (2001b) reported that the Ca-, Mg-, Cd-, Fe(III)-, Al-, Pb-, and Cr(III)-EDDS complexes biodegraded readily at an average rate of 0.3 mmol d⁻¹. The calculated half-life of EDDS in sludge-amended soil was 2.5 days (Jaworska et al., 1999). Meers et al. (2005) observed that the half-life of EDDS varied between 3.8 and 7.5 days when the dose that was applied ranged from 0.8 to 4 mmol kg⁻¹ soil. However, the minimum observed effective half-life of EDTA was 36 days. When EDTA was applied at the rate of 4 mmol kg⁻¹ to soil, no decrease on the mobilization of metals was observed 40 days after the application. Thus, compared with EDTA, EDDS not only has the advantage of being readily biodegradable and less toxic to fish, daphnia, and soil fungi (Jaworska et al., 1999; Grčman et al., 2003), it also poses less risk with respect to the leaching of metals to the groundwater and other surrounding environmental media.

5. Conclusions

The present study demonstrated that, compared with 17 other plant species/cultivars that were tested, *Chrysanthemum coronarium* L. was the most

efficient at accumulating Cu and Pb in its shoots with the application of EDTA. EDDS was superior to EDTA in the phytoremediation of contaminated soils with multiple heavy metals. The treatment of 5 mmol EDDS kg⁻¹ soil produced a total phytoextraction of 14.7 mg kg⁻¹ soil of Cu, Pb, Zn, and Cd by *Chrysanthemum coronarium* L., which was 2.9-fold that of the corresponding EDTA treatments. The results from the post-harvest effect study indicated that EDDS in soil degraded rapidly, which could reduce the risks associated with the leaching of metals to the groundwater and surrounding environment in comparison with the long half-life of EDTA in soil.

Acknowledgments

The project was supported by the Research Grants Council of the Hong Kong SAR Government (PolyU5046/02E), the Natural Science Foundation of China (No: 30300211) and a Postdoctoral Research Fellowship from the Hong Kong Polytechnic University (G-YX07). We thank Professor William J. Manning and four reviewers' constructive comments and suggestions which have significantly improved the quality of this manuscript.

References

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

- Baker, A.J.M., McGrath, S.P., Reeves, R.D., Smith, J.A.C., 2000. Metal hyperaccumulator plants: A review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: Terry, N., Bañuelos, G. (Eds.), *Phytoremediation of Contaminated Soil and Water*. Lewis Publishers, Boca Raton, FL., pp. 85-107.
- Bell, P.F., Chaney, R.L., Angle, J.S., 1991. Free metal activity and total metal concentrations as indices of metal availability to barley (*Hordeum vulgare* cv 'Klages'). *Plant and Soil* 130, 51-62.
- Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in Indian Mustard by soil-applied chelating agents. *Environmental Science and Technology* 31, 860-865.
- Chen, Y.H., Li, X.D., Shen, Z.G., 2004. Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. *Chemosphere* 57, 187-196.
- Cooper, E.M., Sims, J.T., Cunningham, S.D., Huang, J.W., Berti, W.R., 1999. Chelat-assisted phytoextraction of lead from contaminated soils. *Journal of Environmental Quality* 28, 1709-1719.
- Ebbs, S.D., Lasat, M.M., Brady, D.J., Cornish, J., Gordon, R., Kochian, L.V., 1997. Phytoextraction of cadmium and zinc from a contaminated soil. *Journal of Environmental Quality* 26, 1424-1430.
- Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., Orser, C.S., 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant and Soil* 208, 87-94.
- Grčman, H., Velikonja-Bolta, Š., Vodnik, D., Kos, B., Leštan, D., 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant and Soil* 235, 105-114.
- Grčman, H., Vodnik, D., Velikonja-Bolta, Š., Leštan, D., 2003. Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *Journal of Environmental Quality* 32, 500-506.
- Huang, J.W., Chen, J.J., Berti, W.R., Cunningham, S.D., 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology* 31, 800-805.
- Jaworska, J.S., Schowanek, D., 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.
- Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H.R., Gupta, S.K., Schulin, R., 2000. Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environmental Science and Technology* 34, 1778-1783.
- Kos, B., Leštan, D., 2003a. Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. *Plant and Soil* 253, 403-411.
- Kos, B., Leštan, D., 2003b. Induced phytoextraction /soil washing of lead using biodegradable chelate and permeable barriers. *Environmental Science and*

- Technology 37, 624-629.
- Kos, B., Leštan, D., 2004. Chelator induced phytoextraction and in situ soil washing of Cu. *Environmental Pollution* 132, 333-339.
- Kulli, B., Balmer, M., Krebs, R., Lothenbach, B., Geiger, G., Schulin, R., 1999. The influence of nitrilotriacetate on heavy metal uptake of lettuce and ryegrass. *Journal of Environmental Quality* 28, 1699-1705.
- Kumar, P.B.A.N., Dushenkov, V., Motto, H., Raskin, I., 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology* 29, 1232-1238.
- Li, X.D., Poon, C.S., Liu, P.S., 2001. Concentration and chemical partitioning of road dusts and urban soils in Hong Kong. *Applied Geochemistry* 16, 1361-1368.
- Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P., 2001. Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. *Journal of Environmental Quality* 30, 1919-1926.
- Luo, C.L., Shen, Z.G., Li, X.D., 2005a. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* 59, 1-11.
- Luo, C.L., Shen, Z.G., Li, X.D., Baker, A.J.M., 2005b. Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS. *Chemosphere* (in press).
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London.
- Martell, A.E., Smith, R.M., Motekaitis, R.J., 2001. NIST critically selected stability constants of metal complexes, version 6.0; NIST: Gaithersburg, MD.
- Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., Tack, F.M.G., 2004. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere* 58, 1011-1022.
- McGrath, S.P., Chaudri, A.M., Giller, K.E., 1995. Long-term effects of metals in sewage sludge on soils, microorganisms and plants. *Journal of Indian Microbiology* 14, 94-104.
- Pahlsson, A.M.B., 1989. Toxicity of heavy - metals (Zn, Cu, Cd, Pb) to vascular plants – a literature – review. *Water, Air, and Soil Pollution* 47, 287-319.
- Piechalak, A., Tomaszewska, B., Baralkiewicz, D., 2003. Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. *Phytochemistry* 64, 1239-1251.
- Römheld, V., Marschner, H., 1981. Effect of Fe stress on utilization of Fe chelates by efficient and inefficient plant-species. *Journal of Plant Nutrition* 3, 551-556.
- Römken, P., Bouwman, L., Japenga, J., Draaisma, C. 2002. Potentials of drawbacks of chelate-enhanced phytoremediation of soils. *Environmental Pollution*. 116, 109-121.
- Salt, D.E., Smith, R.D., Raskin I., 1998. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* 49, 643-648.
- Shen, Z.G., Li, X.D., Wang, C.C., Chen, H.M., Chua, H., 2002. Lead phytoextraction from contaminated soil with high-biomass plant species. *Journal of Environmental Quality* 31, 1893-1900.

- Stoltz, E., Greger, M., 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. *Environmental and Experimental Botany* 47, 271-280.
- Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., Nowack, B., 2004. Extraction of heavy metals from soils using biodegradable chelating agents. *Environmental Science and Technology* 38, 937-944.
- Thayalakumaran, T., Robinson, B.H., Vogeler, I., Scotter, D.R., Clothier, B.E., Percival, H.J., 2003. Plant uptake and leaching of copper during EDTA-enhanced phytoremediation of repacked and undisturbed soil. *Plant and Soil* 254, 415-423.
- Vassil, A.D., Kapulnik, Y., Raskin, I., Salt, D.E., 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiology* 117, 447-453.
- Vandevivere, P., Hammes, F., Verstraete, W., Feijtel, T., Schowanek, D., 2001a. Metal decontamination of soil, sediment, and sewage, sludge by means of transition metal chelant [S, S]-EDDS. *Journal of Environmental Engineering – ASCE*. 127, 802-811.
- Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C.J., Schowanek, D.R., 2001b. Biodegradation of metal-[S,S]-EDDS complexes. *Environmental Science and Technology* 35, 1765-1770.
- Wenzel, W.W., Unterbrunner, R., Sommer, P. Sacco, P., 2003. Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. *Plant and Soil* 249, 83-96.
- Wu, J., Hsu, F.C., Cunningham, S.D., 1999. Chelate-Assisted Pb phytoextraction: Pb availability, uptake and translocation constraints. *Environmental Science and Technology* 33, 1898-1904.

Table 1

The physicochemical properties of the soils used in the study

	Experiment 1	Experiment 2
pH (CaCl ₂)	6.57	7.12
Electrical conductivity at 25°C ($\mu\text{S cm}^{-1}$)	287	262
Sand (%) > 0.05 mm	57	79.5
Silt (%) 0.05-0.001 mm	28	13
Clay (%) < 0.001 mm	15	7.5
N _{Total} (%)	0.10	0.15
Organic matter (%)	1.5	2.7
Cation exchange capacity (cmol kg^{-1})	3	4.2
Field water capacity (%)	42.3	39.7
Total metal concentration (mg kg^{-1})		
Pb	530	480
Cu	517	575
Zn	475	700
Cd	3	17

Table 2

Dry biomass yields (g plant⁻¹) and the concentrations of Cu, Pb, Zn and Cd (mg kg⁻¹ DW) in the shoots of 18 different plants 7 days after the application of EDTA

EDTA (mmol kg ⁻¹ soil)	Biomass		Cu		Pb		Zn		Cd	
	0	3	0	3	0	3	0	3	0	3
Crop plants										
Monocotyledon										
<i>Zea mays</i> L. cv. Nongda No. 108	3.8 ± 1	3.4 ± 0.25	19.6 ± 0.1	43.7 ± 12	4.2 ± 0.5	29.3 ± 3	147 ± 16	173 ± 12	1.0 ± 0.2	0.5 ± 0.1
<i>Triticum aestivum</i> L. cv. Weimai No. 8	2.7 ± 0.33	2.6 ± 0.24	26.4 ± 3.1	40.2 ± 5.5	2.4 ± 0.4	10.4 ± 1.5	138 ± 19	186 ± 21	0.2 ± 0.01	0.3 ± 0.02
<i>Triticum aestivum</i> L. cv. Shangnong No. 93-52	2.0 ± 0.69	2.0 ± 0.03	15.8 ± 2	72.1 ± 3.7	3.3 ± 0.2	32.5 ± 4.5	96.1 ± 7.8	157 ± 14	0.6 ± 0.1	0.4 ± 0.05
<i>Triticum aestivum</i> L. cv. Zimai No. 12	2.6 ± 0.84	2.7 ± 0.2	17.5 ± 2.1	40.1 ± 5.2	2.6 ± 0.3	12.3 ± 1.5	148 ± 16	191 ± 23	1.2 ± 0.1	0.2 ± 0.02
<i>Sorghum bicolor</i> L. cv. Moench-S. vulgare Pers	1.2 ± 0.15	1.0 ± 0.05	35.9 ± 2.7	68.3 ± 5.5	2.2 ± 0.3	37.9 ± 0.9	211 ± 35	255 ± 47	1.5 ± 0.2	0.8 ± 0.1
Dicotyledon										
<i>Brassica juncea</i> L. Czern. Et Coss. cv. Liyangkucai	3.5 ± 0.2	2.5 ± 0.45	30.7 ± 3	112 ± 18	3.8 ± 0.7	49.7 ± 5.2	141 ± 42	210 ± 19	1.9 ± 0.3	0.9 ± 0.1
<i>Brassica chinensis</i> L. cv. Xiaoairen	3.8 ± 0.46	2.9 ± 0.67	41.2 ± 5.3	125 ± 15	4.5 ± 0.3	62.4 ± 0.7	209 ± 17	188 ± 29	2.7 ± 0.3	1.1 ± 0.2
<i>Brassica chinensis</i> L. cv. Xiaozha No. 56	5.0 ± 0.5	3.5 ± 0.81	27.2 ± 3.8	116 ± 25	3.6 ± 0.4	53.5 ± 4.5	130 ± 19	241 ± 39	1.4 ± 0.2	1.4 ± 0.2
<i>Brassica pekinensis</i> Rupr. L.	3.4 ± 0.4	2.3 ± 0.52	22.2 ± 3	100 ± 8.5	3.2 ± 0.3	46.4 ± 6.5	147 ± 18	246 ± 31	1.5 ± 0.1	1.1 ± 0.2
<i>Brassica campestris</i> L. cv. Suyou No. 1	2.8 ± 1.68	2.6 ± 1.04	25.7 ± 3	98.6 ± 11	3.1 ± 0.6	50.9 ± 6	123 ± 14	214 ± 22	1.6 ± 0.2	0.8 ± 0.1
<i>Brassica campestris</i> L. cv. Qinyou No. 8	2.4 ± 0.97	1.9 ± 0.29	26.6 ± 1	108 ± 7.5	3.3 ± 0.4	42.6 ± 3.9	129 ± 15	201 ± 27	1.4 ± 0.1	0.7 ± 0.1
<i>Lactuca sativa</i> L.	1.5 ± 0.6	1.1 ± 0.57	22.8 ± 1.7	107 ± 8	7.5 ± 0.9	65.5 ± 7.3	99 ± 12	187 ± 19	1.8 ± 0.2	0.9 ± 0.1
<i>Glycine max</i> L. Merrill	3.9 ± 0.37	3.7 ± 0.58	13 ± 0.8	42 ± 3.5	2.1 ± 0.1	16.3 ± 0.2	79 ± 9.5	91 ± 11	0.4 ± 0.1	0.1 ± 0.01
<i>Chrysanthemum coronarium</i> L.	1.8 ± 0.51	1.0 ± 0.37	61.9 ± 7.2	585 ± 43	5.6 ± 0.7	385 ± 41	92 ± 16	586 ± 78	0.2 ± 0.03	1.3 ± 0.2
<i>Coriandrum sativum</i> L.	0.8 ± 0.16	1.0 ± 0.13	34.8 ± 4.9	96.8 ± 7.9	7.1 ± 0.9	72.4 ± 6.3	105 ± 13	159 ± 21	2.2 ± 0.2	1.2 ± 0.1
<i>Daucus carota</i> L. var. sativa DC.	0.9 ± 0.27	0.7 ± 0.24	28.6 ± 3.2	83.2 ± 7	3.2 ± 0.2	31.1 ± 0.5	56 ± 7.5	91 ± 11	0.1 ± 0.01	0.6 ± 0.04
<i>Raphanus sativus</i> L.	4.4 ± 1.11	3.6 ± 0.93	24.3 ± 2.5	50.3 ± 4.6	6.0 ± 0.8	14.9 ± 2.5	132 ± 17	177 ± 25	1.9 ± 0.2	1.1 ± 0.1
<i>Spinacia oleracea</i> L.	2.0 ± 0.43	1.2 ± 0.05	29.9 ± 2.9	187 ± 21	3.5 ± 0.5	146 ± 16	190 ± 41	341 ± 27	1.8 ± 0.2	1.9 ± 0.2
ANOVA										
Plant Species	***		***		**		***		*	
EDTA Treatment	***		***		**		**		NS	
Species × treatment	*		**		*		*		NS	

Values are means ± S.D. (n = 3); ***, P < 0.001; **, P < 0.01; *, P < 0.05, NS, not significant.

Table 3

The concentration and phytoextraction of metals in the shoots of *Chrysanthemum coronarium* L. and *Zea mays* L. with the application of EDTA and EDDS

	<i>Chrysanthemum coronarium</i> L.				<i>Zea mays</i> L.			
	EDTA		EDDS		EDTA		EDDS	
	<u>Concentration (mg kg⁻¹ DW)</u>							
	Zn	Cd	Zn	Cd	Zn	Cd	Zn	Cd
0	624 ± 70a	34.8 ± 4a	624 ± 70a	34.8 ± 4a	212 ± 20a	24.5 ± 2a	212 ± 20a	24.5 ± 2a
0.5	726 ± 22a	47 ± 1.3a	685 ± 96a	36.6 ± 5a	183 ± 28a	17.5 ± 1.6a	202 ± 33a	22.5 ± 3.3a
1	619 ± 61a	42.6 ± 5.9a	900 ± 200b	46.6 ± 8a	205 ± 17a	24.3 ± 3.7a	208 ± 3a	24.3 ± 2.1a
1.5	778 ± 120a	55 ± 8.8b	703 ± 76b	39.4 ± 7.8a	225 ± 25a	24.2 ± 2.4a	263 ± 17a	20.8 ± 2.3a
3	924 ± 75b	63.2 ± 8b	964 ± 68b	44.7 ± 3.8a	221 ± 41a	29.4 ± 1b	325 ± 67b	35.6 ± 4.7b
5	1050 ± 117b	52.6 ± 8.2b	1700 ± 83c	55.4 ± 2.3b	297 ± 20b	26.3 ± 1.6b	383 ± 32b	38.9 ± 7.9b
	<u>Phytoextraction (µg kg⁻¹ soil)</u>							
0	3470 ± 420b	193 ± 20a	3470 ± 420a	193 ± 20b	432 ± 56b	50 ± 6b	432 ± 56a	50 ± 6a
0.5	3300 ± 246b	214 ± 26a	2930 ± 314a	156 ± 16b	282 ± 34a	27 ± 4a	471 ± 68a	52.6 ± 8a
1	2800 ± 242a	193 ± 24a	3260 ± 372a	169 ± 8b	399 ± 48a	47 ± 3.6b	535 ± 64a	62.6 ± 8a
1.5	2830 ± 220a	200 ± 30a	2330 ± 278a	131 ± 18a	475 ± 76b	51 ± 5.2b	636 ± 92a	50.5 ± 6a
3	2570 ± 134a	176 ± 18a	2590 ± 280a	120 ± 14a	321 ± 40a	42.8 ± 6b	533 ± 72a	58.3 ± 10a
5	2820 ± 242a	142 ± 12a	5280 ± 692b	172 ± 22b	574 ± 62b	50.9 ± 12b	490 ± 54a	49.8 ± 7.4a

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

Table 4

The concentration of metals in the shoots of *Zea mays* L. grown in the second cropping

Chelates (mmol kg ⁻¹)	Metal concentrations treated by EDTA (mg kg ⁻¹ DW)				Metal concentrations treated by EDDS (mg kg ⁻¹ DW)			
	Cu	Pb	Zn	Cd	Cu	Pb	Zn	Cd
0	29.2 ± 1.44d	4.7 ± 0.55b	186 ± 35.1a	10.6 ± 0.65a	29.2 ± 1.44a	4.7 ± 0.55a	186 ± 35.1a	10.6 ± 0.65a
0.5	44.3 ± 5.6d	2.3 ± 0.65c	203 ± 11.3a	9.48 ± 1a	30 ± 1.06a	1.12 ± 0.1c	190 ± 19.6a	10.7 ± 1.85a
1.0	66.5 ± 8.9c	3.43 ± 1.09b	191 ± 25.7b	7.96 ± 1.59b	32.9 ± 2.19a	1.13 ± 0.18c	192 ± 34.7a	9.05 ± 0.58a
1.5	76.1 ± 14.2c	3.94 ± 0.59b	133 ± 17.1b	5.85 ± 0.94b	32.3 ± 5.67a	1.72 ± 0.57b	182 ± 18.4a	8.20 ± 1.35b
3.0	127 ± 35.1b	14.8 ± 2.76a	139 ± 49.2b	7.29 ± 2.28b	35.8 ± 4.72a	1.27 ± 0.26b	186 ± 31.7a	7.75 ± 0.79b
5.0	200 ± 29.5a	16.8 ± 3a	133 ± 32.6b	7.45 ± 1.85b	39 ± 5a	0.99 ± 0.49c	150 ± 19.8b	7.69 ± 0.59b

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

Figure Captions:

Fig. 1 Effects of the application of chelant on the dry matter yields of shoots in *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

Fig. 2 Effects of the application of chelant on the concentration of Cu in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

Fig. 3 Effects of the application of chelant on the uptake of Cu in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

Fig. 4 Effects of the application of chelant on the concentration of Pb in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

Fig. 5 Effects of the application of chelant on the uptake of Pb in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

Fig. 6 The dry matter yields of shoots in *Zea mays* L. grown in the second cropping. Values are means \pm S.D. (n = 3).

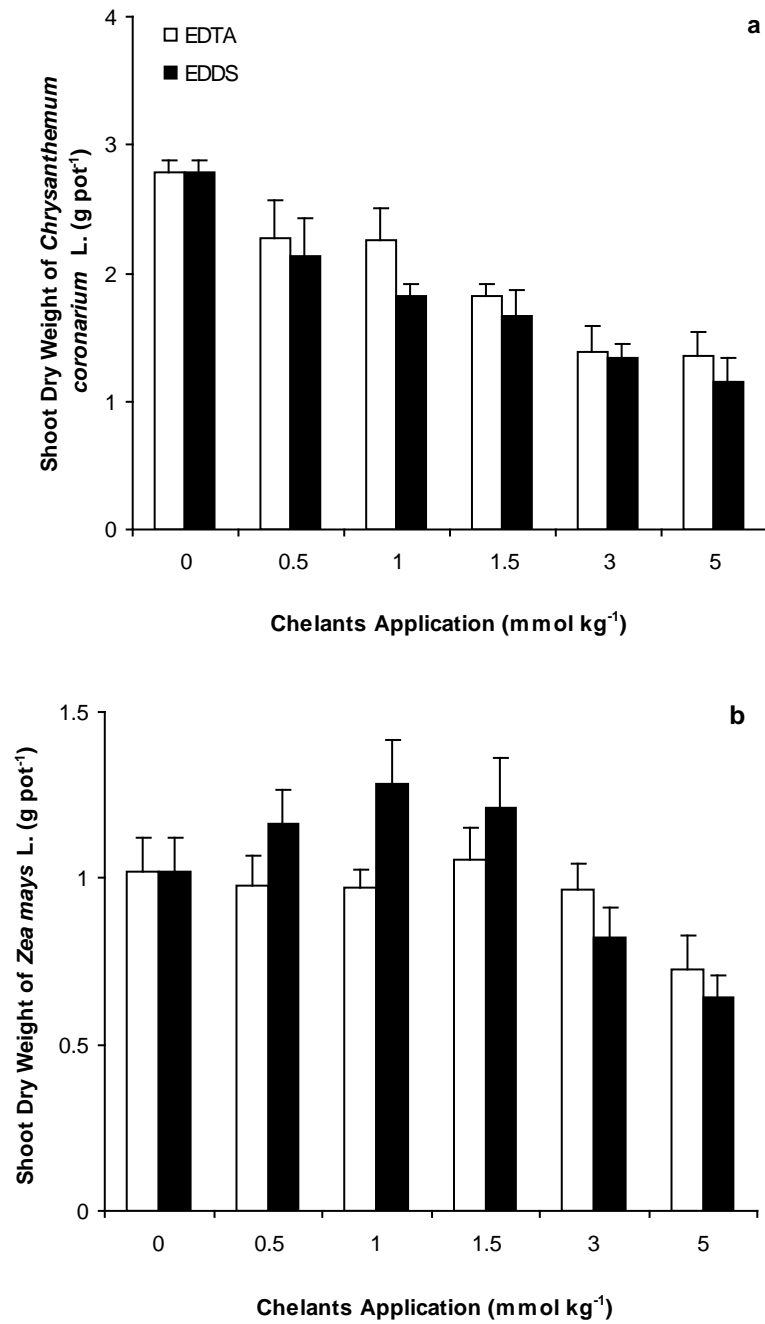


Fig. 1. Effects of the application of chelant on the dry matter yields of shoots in *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

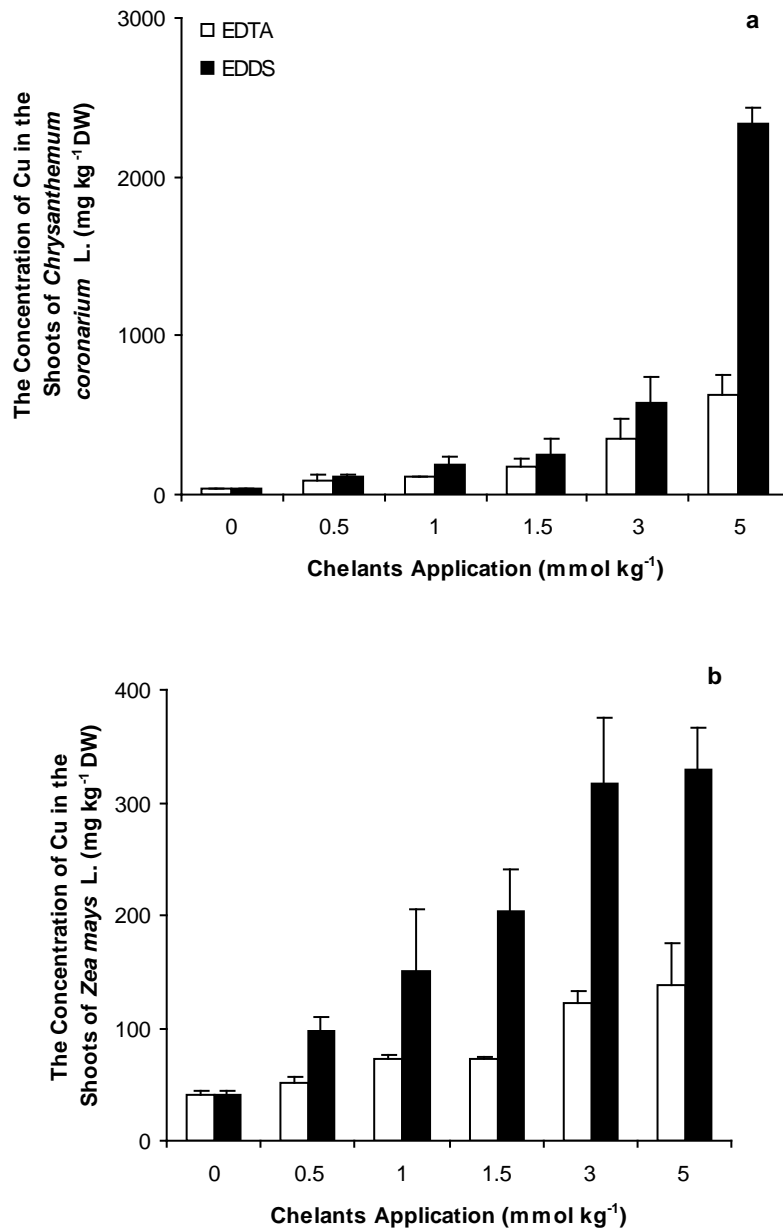


Fig. 2. Effects of the application of chelant on the concentration of Cu in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

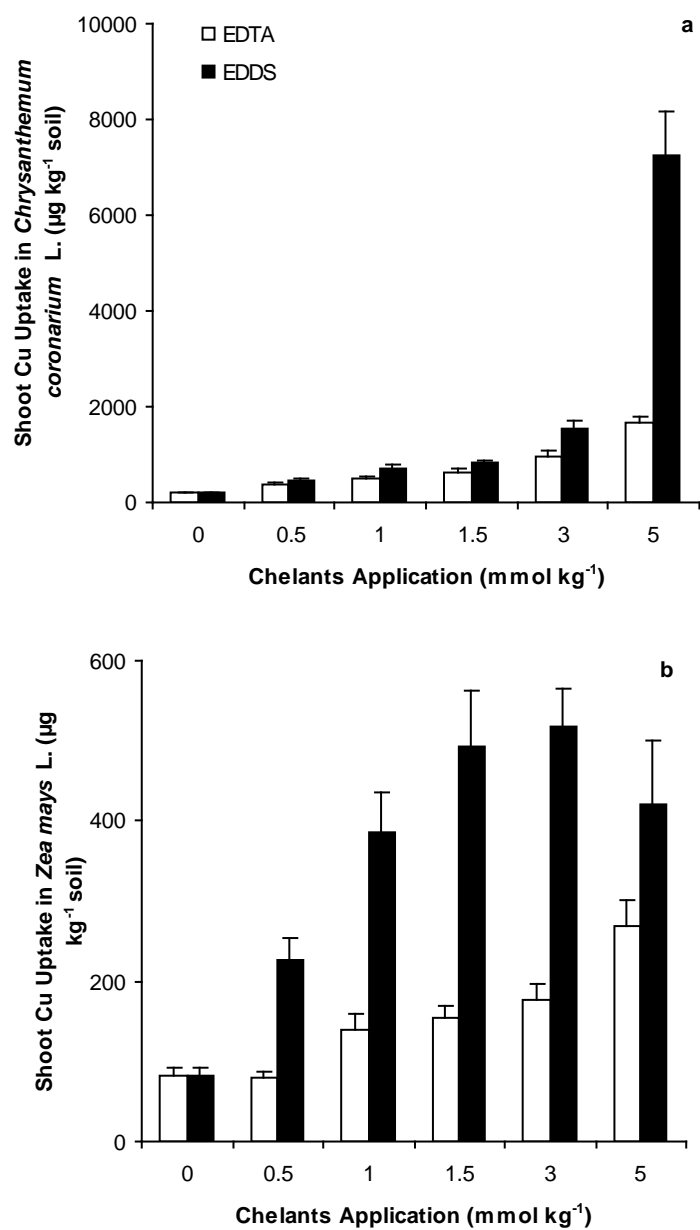


Fig. 3. Effects of the application of chelant on the uptake of Cu in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

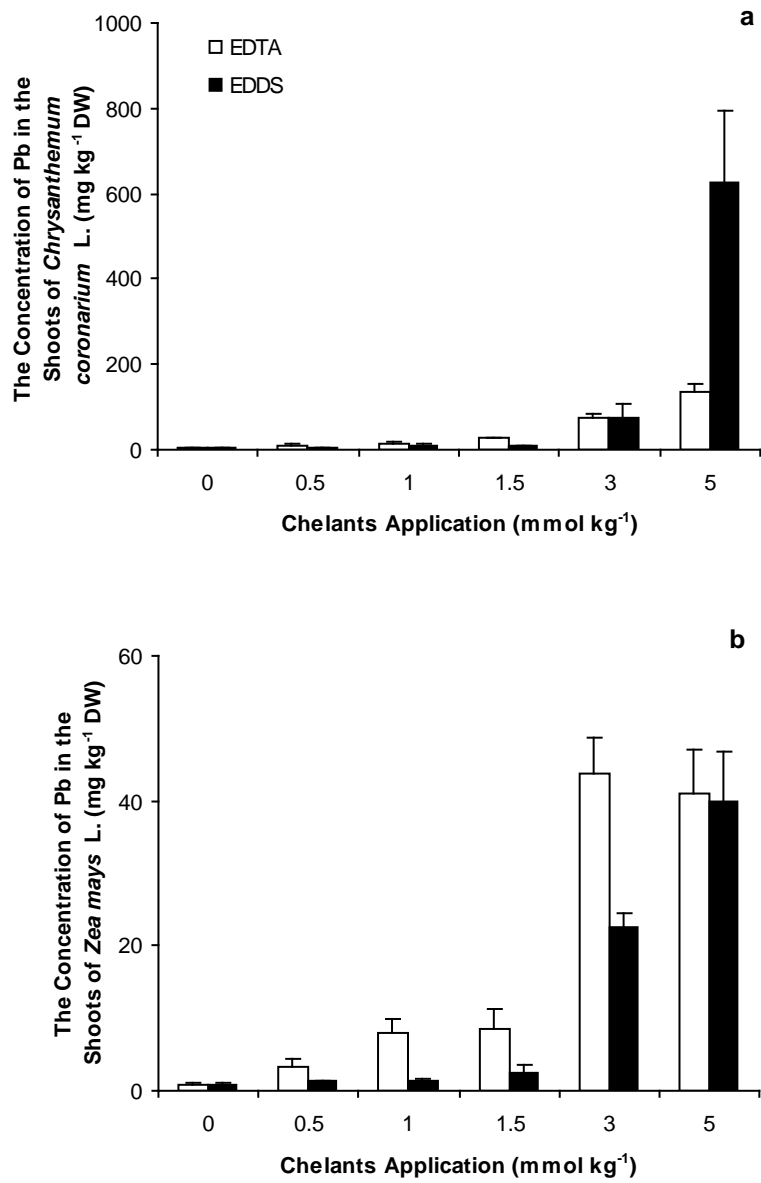


Fig. 4. Effects of the application of chelant on the concentration of Pb in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

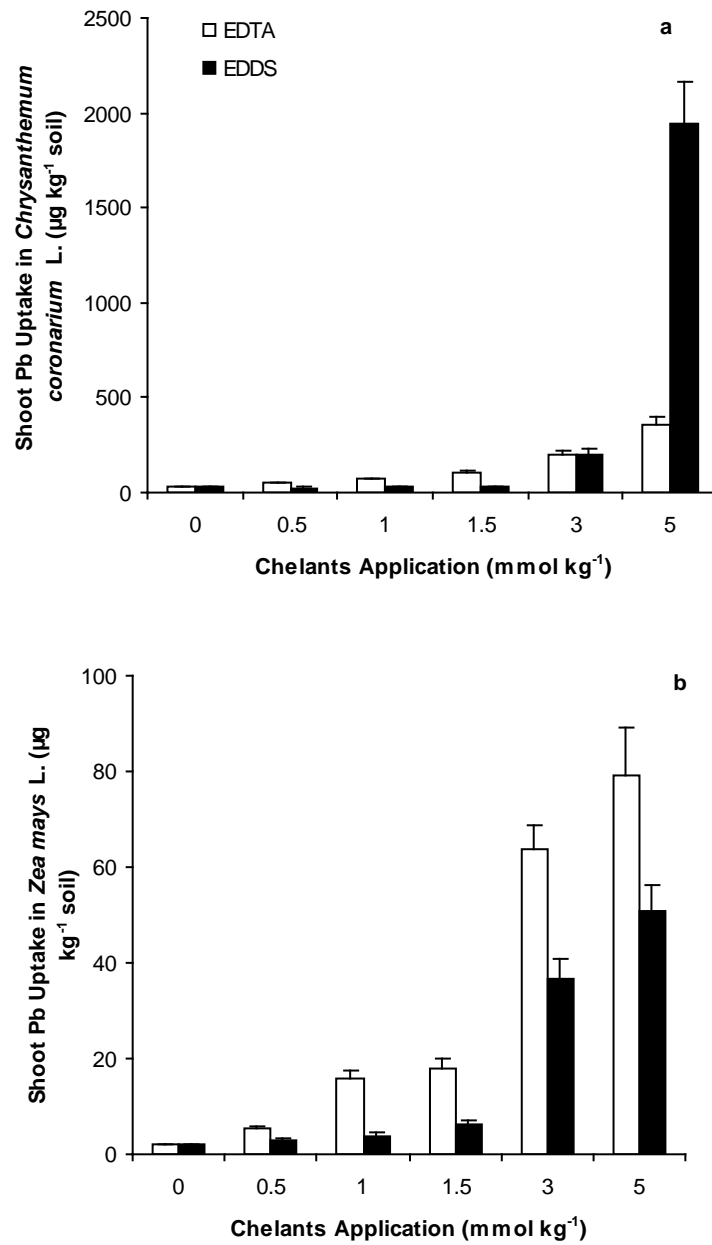


Fig. 5. Effects of the application of chelant on the uptake of Pb in the shoots of *Chrysanthemum coronarium* L. (a) and *Zea mays* L. (b). Values are means \pm S.D. (n = 3).

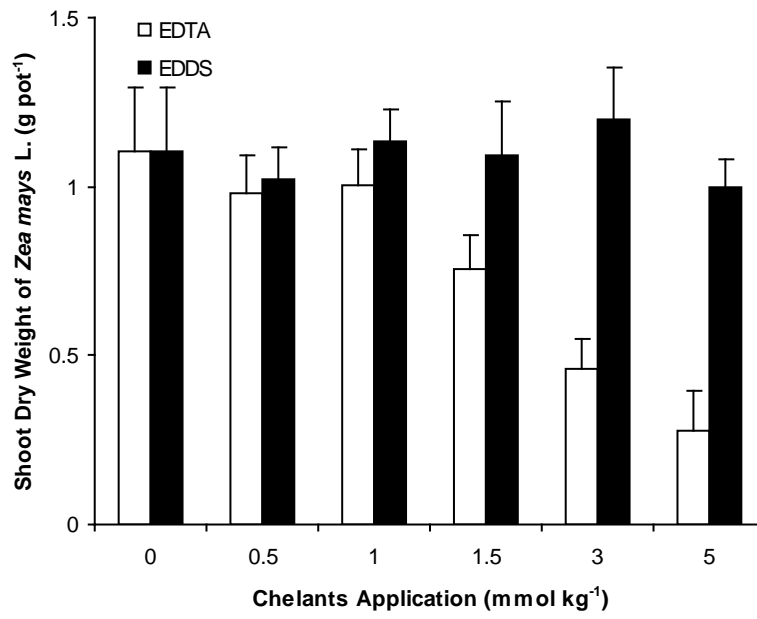


Fig. 6. The dry mass yields of *Zea mays* L. grown in the second cropping. Values are means \pm S.D. (n = 3).