Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS

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Abstract

Chemically enhanced phytoextraction is achieved by the application of chelates to soils. Using pot experiments, the effect of the combined application of EDTA and EDDS on the uptake of Cu, Pb Zn and Cd by *Zea mays* L. was studied. Among the tested application ratios of 1:1, 1:2, and 2:1 (EDTA/EDDS), 2:1 of EDTA:EDDS was the most efficient ratio for increasing the concentrations of Cu, Pb, Zn and Cd in the shoots. The combined application of 3.33 mmol kg^{-1} soil of EDTA + 1.67 mmol kg^{-1} soil of EDDS produced 650 mg kg^{-1} of Pb in the shoots, which was 2.4 and 5.9 times the concentration of Pb in the shoots treated with 5 mmol kg^{-1} of EDTA and EDDS alone, respectively. The total phytoextraction of Pb reached 1710 μg kg^{-1} soil, which was 2.1 and 6.1 times the total Pb from 5 mmol kg^{-1} EDTA and EDDS alone, respectively. The combined application of EDTA and EDDS also significantly increased the translocation of Pb from the roots to the shoots. The mechanism of enhancing the phytoextraction of Pb by the

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combined application of EDTA + EDDS did not involve a change in the pH of the soil. The increase in the phytoextraction of Pb by the shoots of *Zea mays* L. was more pronounced than the increase of Pb in the soil solution with the combined application of EDTA and EDDS. It was thought that the major role of EDDS might be to increase the uptake and translocation of Pb from the roots to the shoots of plants.

Keywords: Phytoextraction; EDTA; EDDS; Combined Application; Pb; Cu; Zn; Cd; *Zea mays* L.

1. Introduction

The contamination of soils with metals is an important environmental issue, given the rapid development of agriculture and industry throughout the world. Lead is one of the most frequently encountered heavy metals in the environment. Elevated levels of Pb in the soil are most often the result of anthropogenic activities such as mining, metallurgy, agriculture, the use of leaded gasoline, paints, and explosives, as well as the disposal of municipal sewage sludge. The severe contamination of soils with Pb may threaten the health of humans as well as plants and animals.

Of the new approaches for removing heavy metals from contaminated soils, phytoremediation has received increasing attention because of its low environmental impact and cost-effectiveness (Salt et al., 1998; Garbisu and Alkorta, 2001). The goal of phytoextraction is to reduce levels of heavy metals in soil to acceptable levels within a reasonable time frame. To achieve this, plant shoots must accumulate high levels of heavy metals and produce high amounts of biomass. Many studies have shown that hyperaccumulators, which have the capacity to concentrate high amounts of heavy metals in their shoots, could be immensely useful in phytoextraction. However, the known hyperaccumulator plants usually accumulate only a specific element, and are usually small, native plants, such as those belonging to the genus of *Thlaspi* and several others (Reeves and Brooks, 1983; Brooks et al., 1998). Moreover, Pb hyperaccumulators have been reported in the literature, but some doubts persist about their Pb uptake abilities (Baker et al., 2000).

As an alternative of using hyperaccumulating plants, it has been suggested that high-biomass crops be used, such as maize (*Zea mays* L.), peas (*Pisum sativum* L.), oats (*Avena sativa* L.), Indian mustard (*Brassica juncea* L.), and cabbage (*Brassica rapa* L. subsp. *chinensis*), along with appropriate chemical treatments to increase the solubility and uptake of Pb (Huang and Cunningham, 1996; Blaylock et al., 1997; Ebbs and Kochian, 1998; Wu et al., 1999; Kayser et al., 2000; Shen et al., 2002; Kos and Leštan, 2003a, b; Quartaccia et al., 2005). Chelates have been shown to enhance the phytoextraction of Pb from contaminated soil. Of the chelates used in studies of phytoextraction, EDTA (ethylenediaminetetraacetic acid) has been the most widely studied because it is highly effective in mobilizing metals in soils. Compared with other chelates with binding constants similar to Pb, such as DTPA (diethylenetrinitrilopentaacetic acid) and N, N′-di(2-hydroxybenzyl)ethylenediamine N, N′–diacetic acid, EDTA was the most efficient at solubilizing soil-bound Pb (Wu et al., 1999). Huang et al. (1997) found EDTA to be the most efficient at enhancing the uptake and solubility of Pb in soils among the five chelates tested. EDTA at 10 mmol kg^{-1} increased plant Pb from < 100 mg kg^{-1} in the control to 16,000 mg kg^{-1} of dry shoot weight and increased water soluble soil Pb from <10 to ~470 mg kg^{-1} (Blaylock et al., 1997). It was reported that EDTA not only increases the amount of soil Pb taken up by plants but also the transport of metals through the xylem and the translocation of Pb from roots to shoots (Huang et al., 1997; Epstein et al., 1999; Shen et al., 2002). However, the application of EDTA may increase the potential off-site migration of metals, either in surface runoff or by the leaching of metals into groundwater. In EDTA-facilitated phytoremediation, the amount of heavy metals taken up by plants is minor compared to the amount mobilized from the soil and the large quantities that are leached out of the root zone (Madrid et al., 2003, Chen et al., 2004). In laboratory columns without plants, Kedziorek et al. (1998) observed the leaching of Pb and Cd from smelter-polluted soil during the percolation of EDTA. Grčman et al. (2001, 2003) found that large quantities of Pb, Zn, and Cd in EDTA-treated soil columns growing cabbage plants had leached to drainage water. Moreover, EDTA and EDTA-heavy metal complexes can be toxic to plants and soil microorganisms and can also be persist in the environment due to their low level of biodegradability (Bucheli-Witschel and Egli, 2001; Grčman et al., 2003).

In recent years, some easily biodegradable chelates, such as NTA (nitrilotriacetate) and EDDS (S,S-ethylenediaminedisuccinic acid), have been proposed to enhance the phytoextraction of heavy metals from contaminated soils (Kulli et al., 1999; Kayser et al., 2000; Grčman et al., 2003; Kos and Leštan, 2003 a and b; Luo et al., 2005; Meers et al., 2005; Quartaccia et al., 2005). However, NTA was less effective than EDTA in increasing the phytoextraction of Pb in cabbage (Shen et al., 2002). Kos and Leštan (2003b) observed that the application of EDDS at 10 mmol kg^{-1} increased the concentration of Pb in cabbage leaves by 89 times compared to the control, to 464 mg kg^{-}

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The in situ application of EDTA may pose potential risks of water pollution through the uncontrolled solubilization and migration of metals. The mobilization of heavy metals into groundwater is not only dependent on the properties of the soil and the status of the water, but also on the balance of solubilized metals in soil solutions and the uptake of metals by the roots of plants (Shen et al., 2002). To minimize the use of EDTA and the potential risk of the migration of solubilized metals into groundwater, further research is still needed. The objectives of the present study were to (i) investigate whether amendments of biodegradable chelate EDDS or citric acid in combination with EDTA can further enhance the shoot uptake of Pb by *Zea mays* L.; and (ii) compare the solubilzation of heavy metals in soil by various combined treatments of EDTA and EDDS.

2. Materials and methods

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 $Pb_3(OH)_2(CO_3)_2$ and PbS at a Pb concentration ratio of 1:1; with Cu (500 mg kg^{-1} of soil) as $CuCO_3$; Zn (1000 mg kg^{-1} of soil) as $ZnCO_3$ and ZnS at a Zn concentration ratio of 1:1; and with Cd (15 mg kg^{-1} of soil) as $Cd(NO_3)_2{\cdot}4H_2O$ (Chen et al., 2004). The basal fertilizers applied to the soil consisted of 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 detailed measurement procedures for the soil were shown elsewhere (Luo et al., 2005). Selected physical and chemical properties of the soil are summarized in Table 1.

2.2. Effects of combined application of EDTA and EDDS on plant growth and metal uptake

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 (deionised water) on a daily basis. Eight seeds of *Zea mays* L. cv. Nongda108 were sown in each pot. After germinating, the seedlings were thinned to four plants per pot and grown for two weeks. EDTA (Na_2EDTA salt from BDH Laboratory Supplies Poole, U.K., minimum assay: 99.5%) and EDDS (Na_3EDDS salt from Fluka Chemie GmbH) were applied to the surface of the soil in five different ways: a single application of EDTA and EDDS alone (5 mmol kg^{-1} of soil), an equimolar combined application of EDTA and EDDS (2.5 mmol kg^{-1} of EDTA + 2.5 mmol kg^{-1} of EDDS), and a combined application of EDTA and EDDS at ratios of 1:2 (1.67 mmol kg^{-1} of EDTA + 3.33 mmol kg^{-1} of EDDS) and 2:1 (3.33 mmol kg^{-1} of EDTA + 1.67 mmol kg^{-1} of EDDS). A control group without chelate treatment was also used in the experiment. Each treatment was replicated four times. All experiments were conducted in the greenhouse under natural light. Air temperatures ranged from 18 to 23 ºC. Plants were harvested by cutting the shoots 0.5 cm above the surface of the soil, and removing the roots from the pots 14 d after the application of chelates. The shoots and roots were washed with tap water and rinsed with DIW, then dried at 70 ºC to constant weight. The dried plant materials were ground using an agate mill.

2.3. Effects of combined application of EDTA, EDDS and citric acid on plant growth and metal uptake

In this part, the combined application of EDTA, EDDS and citric acid was studied. *Zea mays* L. seedlings were grown for 14 d before the chelates were added. The chelates of EDTA (Na_2EDTA salt), EDDS (Na_3EDDS salt) and citric acid (from BDH Laboratory Supplies Poole, U.K., minimum assay: 99.7%) were applied as solutions to the surface of the soil as follows: a single application of EDTA, EDDS, and citric acid (5 mmol kg^{-1} soil); a combined application of EDTA and EDDS at a single dose (2.5 mmol kg^{-1} of EDTA + 2.5 mmol kg^{-1} of EDDS); a combined application of EDTA and citric acid (2.5 mmol kg^{-1} of EDTA + 2.5 mmol kg^{-1} of citric acid); and a combined application of EDDS and citric acid (2.5 mmol kg^{-1} of EDDS + 2.5 mmol kg^{-1} of citric acid). A control group without chelate treatment was also used in the experiment. Each treatment was replicated four times. The plants were harvested 14 d after the application of chelates, as mentioned above.

2.4. Extraction of metals with chelates

For the experiment on the dissolution of metals in soils, 4.0 g of soil (based on dry weight) were placed in a 50-mL polypropylene centrifuge tube. 2 ml of 10 mM chelates of EDTA, EDDS, 1EDTA:1EDDS, 1EDTA:2EDDS, and 2EDTA:1EDDS were added to the soil samples, which corresponded to the total amount of chelates added (5 mmol kg^{-1} of soil) in pot experiments. After 2 d, DIW was added to the soil (at a 1:5 soil-to-water ratio) and the suspension was shaken for 30 min. After centrifugation, the supernatant was filtered through a 0.45 µm filter paper (Whatman No 42), acidified with HNO_3, and analyzed by ICP-AES (Perkin-Elmer Optima 3300 DV) for different concentrations of metal.

Soil was extracted with different EDTA and EDDS concentrations at 0.5, 1.0, 2.5, 5.0, 5.5, 6.0 and 7.5 mmol kg^{-1} of soil, a combined application of 5 mmol kg^{-1} of soil EDTA and 0.5, 1.0, and 2.5 mmol kg^{-1} of soil EDDS. Chelates were added to a 2 ml solution. After 2 d, DIW was added to the soil (at a 1:5 soil-to-water ratio) and the suspension was shaken for 30 min. The filtration and chemical analyses were conducted as mentioned before.

2.5. Plant and soil analysis

Sub-samples of ground shoot (200 mg) and root (100 mg) dry matter were digested using a mixture of concentrated HNO_3 and $HClO_4$ (4:1, by volume), and the major and trace elements in the solutions were determined by ICP-AES (Li et al., 2001). Certified standard reference material (SRM 1515, apple leaves) from the National Institute of Standards and Technology, U.S.A. was used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery efficiencies 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 the SPSS® 11.0 statistical software package.

3. Results

3.1. Effects of the combined application of EDTA and EDDS on the growth of Zea mays L.

The dry mass yields of *Zea mays* L. are shown in Figure 1. The treatments with 5 mmol kg^{-1} of EDTA and EDDS significantly depressed the growth of the plants. The addition of EDDS appeared to be more toxic to plants than the application of EDTA, as shown by a significantly lower biomass following the addition of EDDS. Plants with the combined treatments of EDTA and EDDS exhibited a slight decrease in biomass compared to those that had received a treatment of 5 mmol kg^{-1} of EDTA alone. Among the combined treatments of EDTA and EDDS at the ratios of 1:1, 1:2 and 2:1 and the treatment of EDDS alone, there were no significant differences in dry mass yields (Fig. 1).

3.2. Effects of the combined application of EDTA and EDDS on metal concentrations and phytoextraction

Compared with the control group, the application of EDTA and EDDS at 5 mmol kg^{-1} to the soil significantly increased the concentrations of Cu, Pb, Zn, and Cd in the shoots of *Zea mays* L. (Fig. 2). When EDTA and EDDS were applied in combination at different ratios, the concentrations of Pb in shoots were significantly higher than in those where EDTA and EDDS had been applied alone. The combined application of EDTA and EDDS at the ratio of 2:1 produced the highest Pb concentration of 647 mg kg^{-1} DW in the shoots of *Zea mays* L., which was 2.4 and 5.8 times that of the 5 mmol kg^{-1} of EDTA and 5 mmol kg^{-1} of EDDS treatment alone, respectively, on the 14th day after the application of chelates. The concentration of Cd in corn shoots treated with 3.33 mmol kg^{-1} of EDTA + 1.67 mmol kg^{-1} of EDDS was similar to that in shoots treated with 5 mmol kg^{-1} of EDTA alone ($P < 0.05$), but significantly higher than that with 5 mmol kg^{-1} of EDDS alone. For Cu and Zn, there were no significant differences in concentration in shoots treated with 3.33 mmol kg^{-1} of EDTA + 1.67 mmol kg^{-1} of EDDS and those treated with 5 mmol kg^{-1} of EDDS alone ($P < 0.05$), but the concentrations were significantly higher than those treated with 5 mmol kg^{-1} of EDTA alone.

Although the dry matter yield of plants was significantly affected by the application of the chelates (Fig. 1), the total phytoextraction of Pb in shoots increased significantly with the application of chelates (Fig. 3). As expected, the maximum phytoextraction of Pb was found in the combined application of 3.33 mmol kg^{-1} of EDTA + 1.67 mmol kg^{-1} of EDDS, which reached 86, 2.1 and 6.1 times those of the control, 5 mmol kg^{-1} of EDTA alone, and 5 mmol kg^{-1} of EDDS alone, respectively. For Cu, the maximum phytoextraction was found in the EDDS and 2EDTA:1EDDS treatment, which increased the phytoextraction by up to 42 times that of the control. For Zn and Cd, the total extracted amounts were less than twice those of the control.

The distribution of metals in the plants was also significantly affected by the application of chelates (see Table 2). The application of EDTA and EDDS significantly increased the shoot-to-root ratios of the concentrations of Cu, Pb, Zn, and Cd in *Zea mays* L. EDTA was more effective than EDDS in stimulating the translocation of Pb from roots to shoots. For increasing the translocation of Cu and Zn in plants, EDDS was better than EDTA. The combined application of EDTA + EDDS was more efficient at enhancing the translocation of Pb from roots to shoots in comparison with EDTA and EDDS alone. When EDTA and EDDS were applied at ratios of 1:1 and 2:1, respectively, the mean percentage of absorbed Pb that was translocated from roots to shoots increased from 5.2% in the control group to 48% and 57%.

3.3. Effects of the combined application of EDTA, EDDS and citric acid on metal concentrations

The combined application of 2.5 mmol kg^{-1} of EDTA or EDDS + 2.5 mmol kg^{-1} of citric acid decreased the concentrations of Cu, Pb, Zn, and Cd in the shoots of *Zea mays* L. compared with those in the treatment with 5 mmol kg^{-1} of EDTA or EDDS alone, but increased the metal concentrations compared with those in the 5 mmol kg^{-1} of citric acid treatment alone (Table 3).

3.4. Effects of the combined application of chelates on the solubility of metals in soil

The concentrations of soluble metals in soil were examined to assess the relative efficiency of EDTA and EDDS in enhancing the solubilization of metals from soil (Table 4). For the dissolution of Pb and Cd, EDTA showed much higher efficiency than EDDS. The addition of EDTA and EDDS at 5 mmol kg^{-1} for 2 d produced 41 and 1.1 mg kg^{-1} of soluble Pb in soil, respectively, which were 508 and 14 times higher than the levels found in the control soil. When EDTA and EDDS were applied together at ratios of 1:1, 1:2, and 2:1 of total 5 mmol kg^{-1}, the concentration of extracted Pb remained the same as with the 5 mmol kg^{-1} of EDTA treatment alone, although the total amount of EDTA applied was reduced by 50%, 67%, and 33%, respectively. For the extraction of Cd, there was no significant difference observed among the treatments of 5 mmol kg^{-1} of EDTA alone and three different combined treatments of EDTA and EDDS during the whole experiment period. EDDS was more effective than EDTA in solubilizing Cu and Zn (see Table 4). The combined extraction of EDTA and EDDS at ratios of 1:1 and 1:2 of the total 5 mmol kg^{-1} chelate application produced the same concentration of Cu as the 5 mmol kg^{-1} of EDDS treatment alone within 2 d, which was more than 1.9-fold of the amount extracted by 5 mmol kg^{-1} of EDTA alone.

In order to evaluate the additional effects of the combined application of EDTA and EDDS on the extraction of Pb, different methods of applying EDTA and EDDS to extract Pb and Ca within 2 d were studied. When the soil was extracted by EDTA or EDDS separately, the concentrations of Pb in the soil solution increased as concentrations of EDTA and EDDS increased (Fig. 4a). In extracting Ca, the application of EDTA and EDDS produced different effects. The concentration of Ca in the soil solution increased as the concentration of EDTA increased, and decreased as the concentration of EDDS applied to the soil increased (Fig. 4b). The pH of soil increased as concentrations of EDTA and EDDS increased, and the effect of EDDS was greater than that of EDTA (Fig. 4c).

The results of extractable Pb and Ca and soil pH in the combined chelate application are shown in Figure 5. When 5 mmol kg^{-1} of EDTA was applied, the increasing levels of EDDS increased the concentration of soluble Pb and decreased the concentration of soluble Ca in the soil (Figs. 5a and 5b). The soil pH remained relatively stable with the increasing EDDS in the chelate application (Fig. 5c)

4. Discussion

The effective phytoextraction of Pb from contaminated soils depends on the uptake of metal in plant shoots. The accumulation of Pb in plants from soil relies on an adequate concentration of Pb in a soluble form in soil, and on the uptake of metals by plant roots and their translocation to the shoots. Several chelating agents, such as citric acid, EDTA, CDTA (trans -1, 2 -diaminocyclohexane -N, N, N’, N’-tetraacetic acid), DTPA, EGTA [ethyleneglycol -bis (ß -aminoethyl ether), N, N, N’, N-tetraacetic acid], EDDHA [etylenediamine-di (*o*-hydroxyphenylacetic acid)], EDDS and NTA have been tested for their ability to mobilize heavy metals and increase their accumulation (Huang and Cunningham, 1996; Blaylock et al., 1997; Ebbs and Kochian, 1998; Shen et al., 2002; Kos and Leštan, 2003a, b). In most cases, the EDTA treatment was superior in terms of solubilizing soil Pb for root uptake and translocation into the above ground biomass due to its strong chemical affinity for Pb (log K_s = 17.88). The results from this study have demonstrated that EDTA is more effective than EDDS or citric acid at increasing the concentrations of Pb in the shoots of *Zea mays* L. (Table 3).

The interesting part of the current study was that a higher efficiency (or a synergy effect) in the phytoextraction of Pb could be achieved by replacing one-third of the EDTA used with an equal amount of EDDS compared with the treatment with 5 mmol kg^{-1} of EDTA alone, although EDDS was less efficient at enhancing the phytoextraction of Pb from the soil than EDTA when EDDS was applied alone (Figs. 2 and 3). The combined application of 3.33 mmol kg^{-1} of EDTA + 1.67 mmol kg^{-1} of EDDS produced 650 mg kg^{-1} of Pb in the shoots. The value was 2.4 and 5.9 times the concentration of Pb in *Zea mays* L. shoots treated with 5 mmol kg^{-1} of EDTA and EDDS alone, respectively. The total phytoextraction of Pb reached 854 µg pot^{-1} (e.g. 1710 µg kg^{-1} soil), which was 2.1 and 6.1 times the total Pb from 5 mmol kg^{-1} of EDTA and EDDS alone, respectively. These results showed that the significantly enhanced phytoextraction of Pb by the combined application of EDTA and EDDS was not attributed to the condensing effects caused by the decrease in dry mass yields. In addition, EDDS has the advantage because it is readily biodegradable and is less toxic to fish, daphnia, and soil fungi (Jaworska et al., 1999; Grčman et al., 2003). The calculated half-life of EDDS in sludge-amended soil was 2.5 d (Jaworska et al., 1999). This implies that residual EDDS in the soil will rapidly be degraded and pose a relatively lower risk with respect to the leaching of metal into groundwater. The results suggest that the combined application of EDTA and EDDS, with less use of EDTA, can be regarded as a good alternative for the phytoextraction of Pb and other metals in soil, reducing the potential of Pb leaching into deep soil, groundwater, and surrounding sites.

Some changes in soil conditions such as pH, total ligand, or superior ion concentrations may affect the chelating 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 a partial remobilization of adsorbed metals from the metal oxides and in the dissolution of minerals (iron and aluminium oxides) and remobilization of adsorbed metal (Nowack and Sigg, 1995; Van Devivere et al., 2001). Tandy et al. (2004) reported that the extraction of Pb by EDTA seemed to depend mainly on the pH at the low chelate: metal ratio of 1, at which EDTA showed very strong ability to extract Pb up to pH 6. Above this, the efficiency of extraction declined by 50% because of the competition for EDTA from Ca. In the present study, however, Na_2EDTA salt and Na_3EDDS salt were used. A decrease in soil pH was not found in the combined treatment of EDTA + EDDS, compared to that of EDTA alone (Fig. 5). Thus, the mechanism of enhancing the phytoextraction of Pb by the combined application of EDTA + EDDS does not involve a change in the pH of the soil. Wu et al. (1999) reported that there was no simple correlation between the amount of soluble Pb extracted and the binding constant of ligand with Pb. This could be related to the presence of other cations, in particular Ca^{2+}. As shown in Figures 4 and 5, an increase in the level of EDDS led to a decrease in the concentration of soil soluble Ca with and without EDTA. Competition between heavy metals and Ca has been shown to be an important factor for the extraction of metals with EDTA (Tandy et al., 2004).

The increase in the phytoextraction of Pb by corn shoots was more pronounced than the increase of Pb in the soil solution after the partial use of EDTA was replaced with EDDS. The combined application of EDTA + EDDS at the ratio of 2:1 increased the concentration of Pb in shoots and phytoextration by 2.4 and 2.1 times. The increased concentrations of soluble Pb in soil were only 1.2 times higher than the EDTA treatment alone (Figs. 4 and 5). The shoot-to-root ratio of Pb concentration was two times higher with the application of EDTA + EDDS (2:1) than with the 5 mmol kg^{-1} of EDTA treatment alone (Table 2). The percentage of absorbed Pb translocated from roots to shoots increased from 41 % to 57 %. These results indicated that the major role of EDDS might be to increase the uptake and translocation of Pb from roots to shoots rather than to dissolve soil Pb. Vassil et al. (1998) found that the accumulation of Pb in shoots was correlated with the formation of a Pb-EDTA complex in the hydroponic solution and that Pb-EDTA was the major form of Pb taken up and translocated by the plant. A similar mechanism held true for plants grown in Pb-contaminated soils amended with EDTA (Epstein et al., 1999). To "induce" the accumulation of high EDTA or Pb-EDTA in shoots, a threshold concentration of EDTA was required in the solution (Vassil et al. 1998). At this threshold concentration, EDTA destroyed the physiological barrier(s) in roots that normally functioned to control the uptake and translocation of metals. It has been suggested that synthetic chelates may induce the uptake and accumulation of metals by chelates by removing stabilizing Zn^{2+} and Ca^{2+} from the plasma membrane, which is thought to play a major role in forming the physiological barrier(s). In the present study, the reduction in the shoot biomass was more significant in the treatment with EDDS than with EDTA (Fig. 1). It is hypothesized that the physiological barriers in roots were destroyed due to the toxic effects of EDDS, and soil Pb was solubilized by forming soluble complexes with EDTA when EDTA and EDDS were combined and applied. Ensley et al. (1999) described chemically enhanced phytoextraction as a two-step process for induced hyperaccumulation of metals in plant shoots. Plants first accumulate metals in their roots and then, through the application of an inducing agent, the enhanced transfer of the metals to the shoots occurs. It has been suggested that metal chelate complexes may enter the roots at breaks in the root endodermis and Casparian strip, and be rapidly transported to the shoots (Römheld and Marschner, 1981; Bell et al., 1991).

For environmental and cost considerations, the addition of chelates to soils should be minimized in the phytoextraction process. Some approaches that could help to minimize the amount of chelate used in soil have been explored (Wu et al., 1999; Shen et al. 2002). A significant increase in the uptake and translocation of Pb has been reported for corn transplanted into soil, then treated with EDTA, in comparison with plants that were germinated and grown in Pb-contaminated soil to which EDTA was subsequently applied (Wu et al., 1999). Shen et al. (2002) found that the application of EDTA in three separate doses was better at enhancing the accumulation of Pb in cabbage shoots than methods in which EDTA was added in one or two doses. Our current results showed that the combined application of EDTA + EDDS could significantly increase the concentrations and uptake of Cu, Zn, Pb, and Cd in the shoots of *Zea mays* L. than the application of single chelate, especially for the phytoextraction of Pb. This method may provide a more efficient approach to the phytoremediation of soils contaminated with multiple heavy metals. For soil contaminated by Cu and Zn, EDDS can be a better chelate because of its high extraction efficiency and better biodegradability (Luo et al., 2005; Meers et al., 2005).

Conclusions

The results from the present study showed that the combined application of EDTA and EDDS significantly increased the concentrations of heavy metals (especially Pb) and total metal uptake in the shoots of *Zea mays* L. The method could be as an efficient alternative for the phytoextraction of Pb and other metals in contaminated soils at reduced EDTA dosage and increased metal removal efficiency. The enhanced phytoextraction of Pb in this process was independent of pH change, and might be related to the decreased competition from Ca in soil solution.

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Table 1

The physicochemical properties of the soils used in the study (from Luo et al., 2005).

Physicochemical properties	
pH ($CaCl_2$)	7.27
Electrical conductivity at 25°C (μS 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 content (mg kg^{-1})	
Pb	44.2
Cu	26.7
Zn	131
Cd	0.45

Table 2

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

	Control	EDTA	EDDS	1EDTA:1EDDS	1EDTA:2EDDS	2EDTA:1EDDS
Shoot/root quotient for metal concentration						
Cu	0.06 ± 0.01	0.33 ± 0.05	2.1 ± 0.27	1.85 ± 0.2	1.47 ± 0.5	2.3 ± 0.35
Pb	0.03 ± 0.01	0.33 ± 0.04	0.11 ± 0.03	0.46 ± 0.05	0.56 ± 0.07	0.64 ± 0.04
Zn	0.54 ± 0.08	0.58 ± 0.07	1.1 ± 0.14	0.84 ± 0.1	0.62 ± 0.17	0.9 ± 0.09
Cd	0.34 ± 0.1	0.65 ± 0.24	0.47 ± 0.09	0.58 ± 0.03	0.52 ± 0.06	0.72 ± 0.15
Metal absorbed by shoots/metal absorbed by the entire plant (%)						
Cu	7.9 ± 1.5	36.7 ± 5.6	84.3 ± 7.8	75.1 ± 8.7	69.2 ± 7.5	82 ± 11
Pb	5.2 ± 0.8	40.9 ± 5.1	18.3 ± 1.1	48.4 ± 5.6	52 ± 6.8	56.9 ± 7.3
Zn	41 ± 0.8	58.6 ± 7.5	62.5 ± 8.9	50.7 ± 9.2	60 ± 3.6	65.5 ± 6.1
Cd	37.5 ± 5.9	63.4 ± 10	49.6 ± 6.7	57 ± 6.5	52.3 ± 4.9	67.2 ± 7.2

Values are means ± S.D. (n = 4).

Table 3

Effects of the application of chelates on the concentrations (mg kg^{-1} DW) of Cu, Pb, Zn and Cd in the shoots of corn 14 d after treatment

	Cu	Pb	Zn	Cd
control	45.6 ± 9.2	10.1 ± 4	580 ± 102	9 ± 2.6
5 mM EDTA	560 ± 69	270 ± 67	850 ± 74	29 ± 1
5 mM EDDS	2060 ± 272	91 ± 22	1310 ± 148	13.7 ± 2.1
5 mM citric acid	36.8 ± 1.3	5.8 ± 2	401 ± 58	5.2 ± 1.3
2.5 mM EDTA + 2.5 mM EDDS	2300 ± 210	569 ± 59	1460 ± 249	31.3 ± 3.2
2.5 mM EDTA + 2.5 mM citric acid	199 ± 21	54.3 ± 11	596 ± 53	17.1 ± 2
2.5 mM EDDS + 2.5 mM citric acid	770 ± 167	12.3 ± 3.4	671 ± 111	17.8 ± 2

Values are means ± S.D. (n = 4). Data for control, EDTA, EDDS and citric acid were cited from Luo et al. (2005).

Table 4

Cu, Pb, Zn and Cd concentrations (mg kg^{-1}) in soil solutions extracted with different chelates within 2 d

	Cu	Pb	Zn	Cd
Water	1.2 ± 0.2	0.08 ± 0.002	21.7 ± 3.2	0.01 ± 0.001
EDTA	73.6 ± 5.6	40.6 ± 4.1	88.8 ± 4.5	1.49 ± 0.1
EDDS	141 ± 11	1.08 ± 0.01	125 ± 15	0.03 ± 0.003
1EDTA:1EDDS	128 ± 16	48.6 ±3 .2	74 ± 3.7	1.72 ± 0.1
1EDTA:2EDDS	146 ± 12	40.2 ± 3.1	80.9 ± 6	1.41 ± 0.2
2EDTA:1EDDS	86 ± 7.2	50.1 ± 4.6	84.6 ± 7.5	1.82 ± 0.1

Values are means ± S.D. (n = 4)

Figure Captions:

Fig.1. Effects of the combined application of EDTA and EDDS at different ratios on the dry matter yields of shoots and roots in *Zea mays* L. Values are means ± S.D. (n = 4).

Fig. 2. Effects of the combined application of EDTA and EDDS at different ratios on the concentrations of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of *Zea mays* L. Values are means ± S.D. (n = 4).

Fig. 3. Effects of the combined application of EDTA and EDDS at different ratios on the uptake of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of *Zea mays* L. Values are means ± S.D. (n = 4).

Fig. 4. Changes in the soluble concentrations of Pb (a), Ca (b), and pH (c) in soil treated with EDTA and EDDS at different concentrations. Values are means ± S.D. (n = 4).

Fig. 5. Changes in the soluble concentrations of Pb (a), Ca (b), and pH (c) in soil treated with the combined application of EDTA and EDDS at different ratios. Values are means ± S.D. (n = 4).

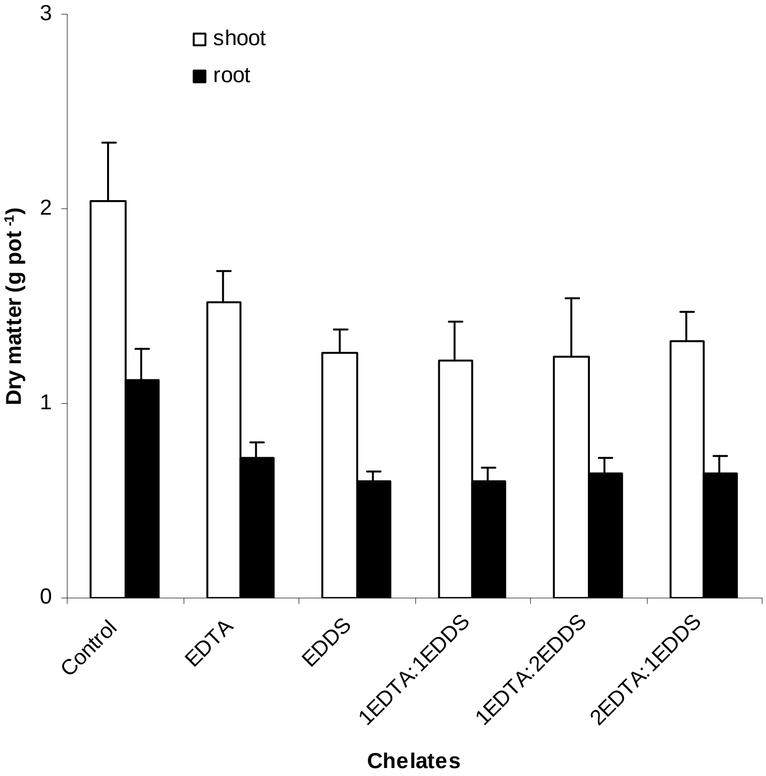

Fig. 1. Effects of the combined application of EDTA and EDDS at different ratios on the dry matter yields of shoots and roots in *Zea mays* L. Values are means ± S.D. (n = 4).

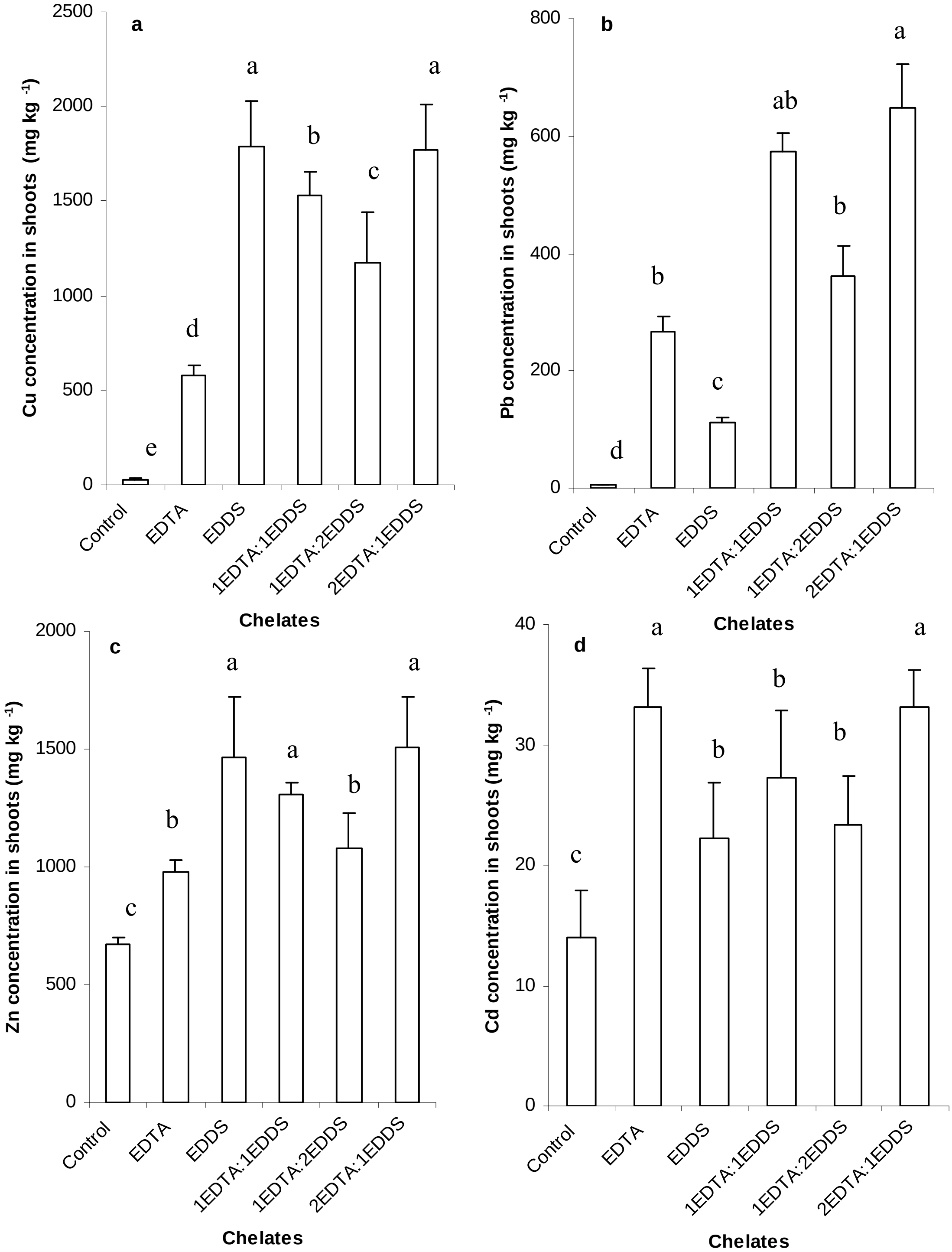

Fig. 2. Effects of the combined application of EDTA and EDDS at different ratios on the concentrations of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of *Zea mays* L. Values are means ± S.D. (n = 4). The different small letters stand for statistical significance at the 0.05 level with LSD test.

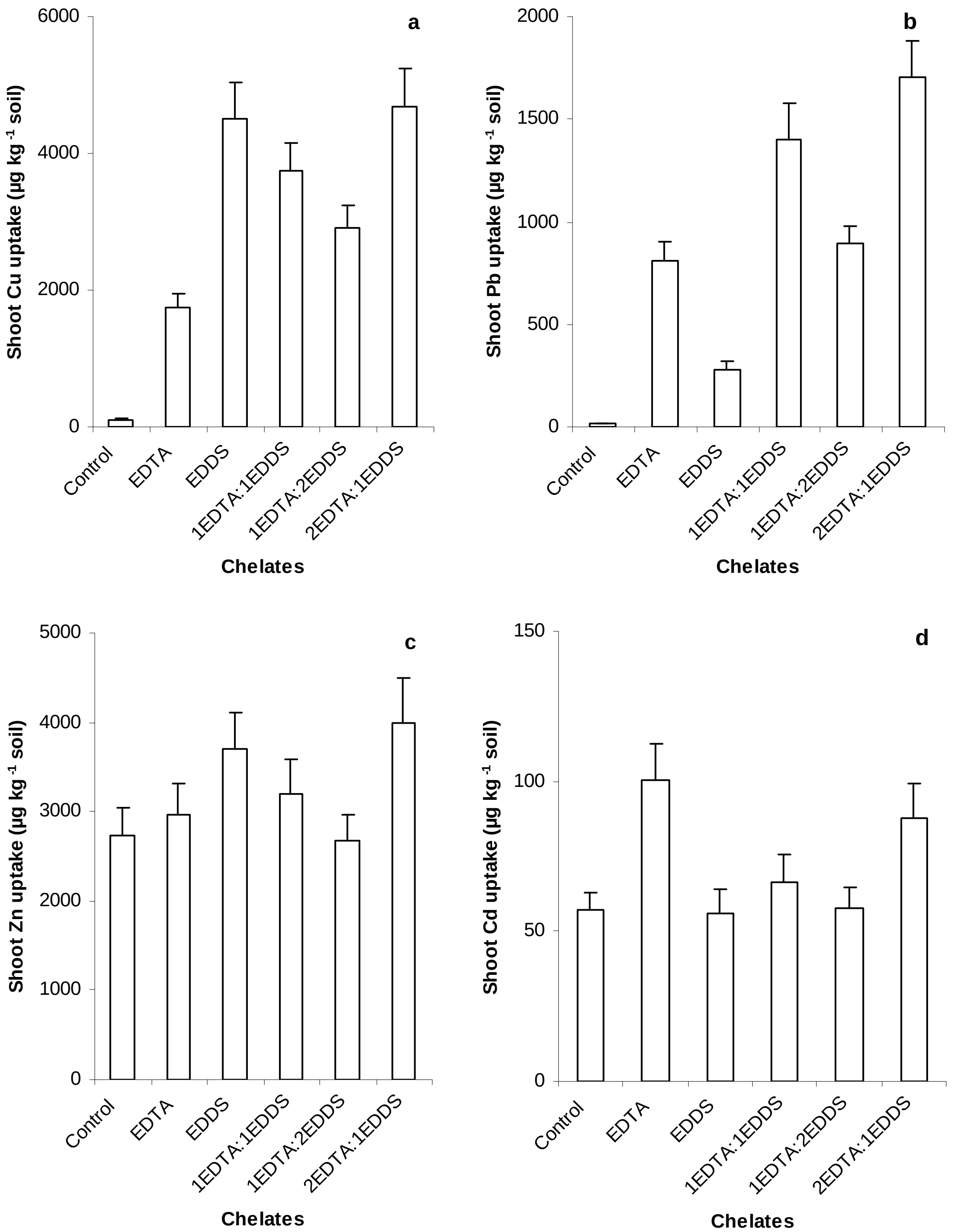

Fig. 3. Effects of the combined application of EDTA and EDDS at different ratios on the uptake of Cu (a), Pb (b), Zn (c) and Cd (d) in the shoots of *Zea mays* L. Values are means ± S.D. (n = 4).

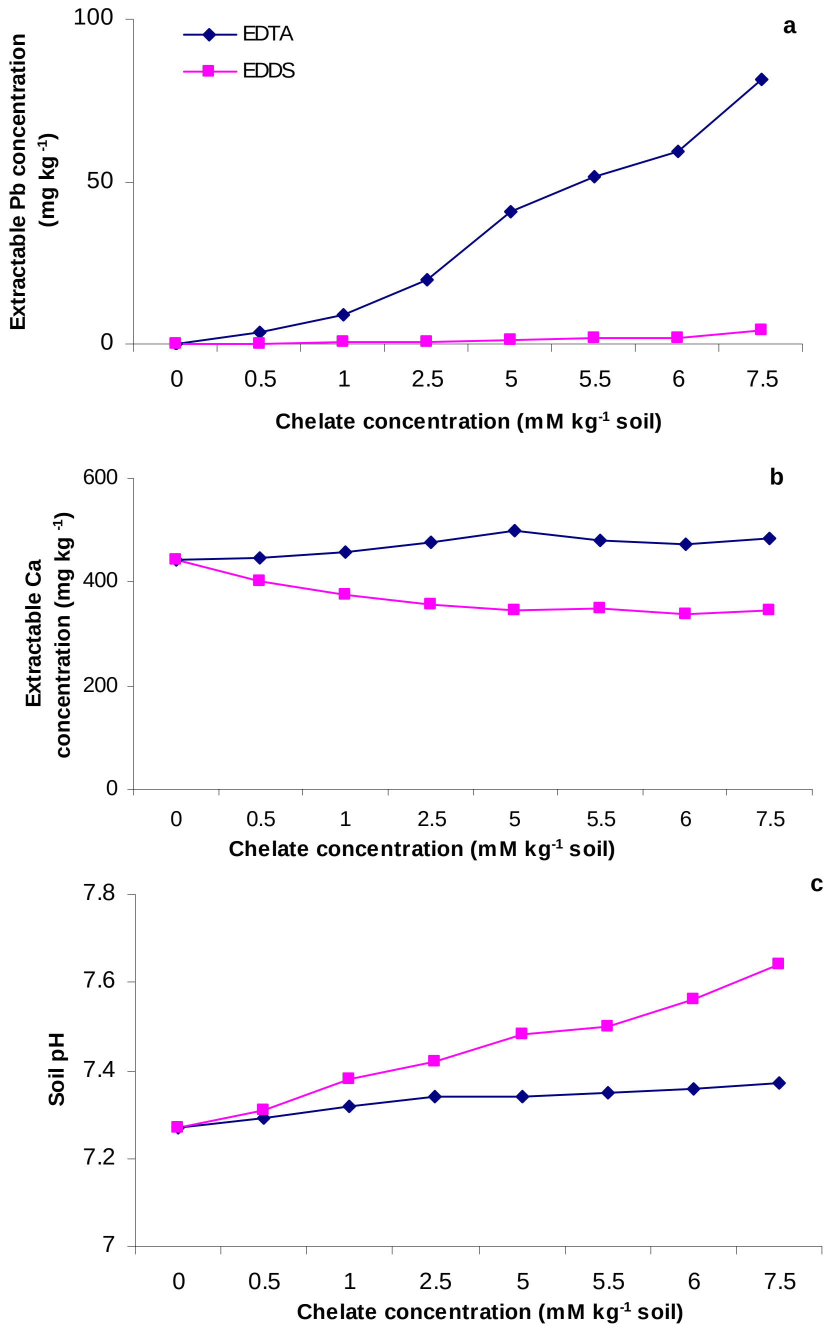

Fig. 4. Changes in the soluble concentrations of Pb (a), Ca (b), and pH (c) in soil treated with EDTA and EDDS at different concentrations. Values are means ± S.D. (n = 4).

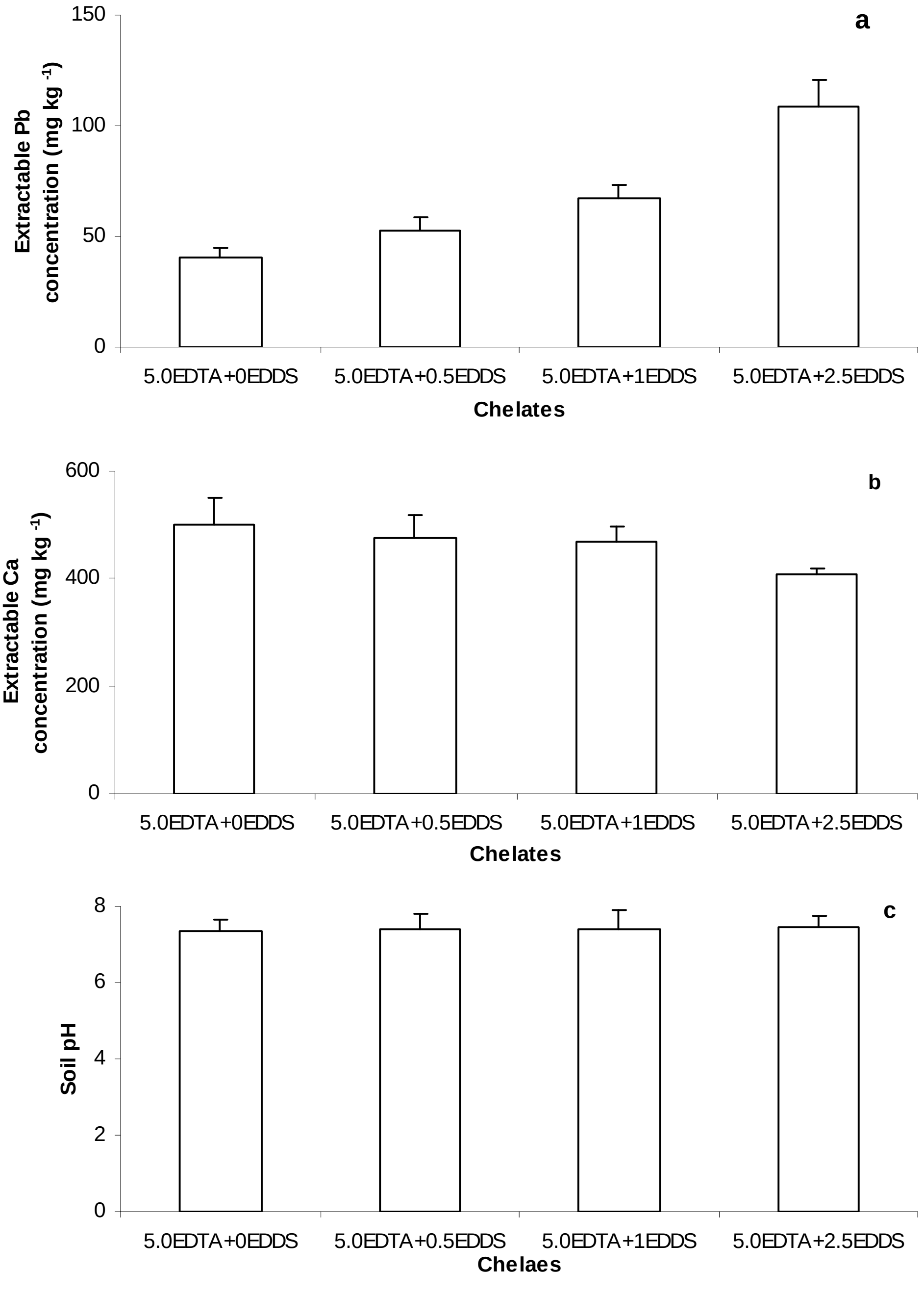

Fig. 5. Changes in the soluble concentrations of Pb (a), Ca (b), and pH (c) in soil treated with the combined application of EDTA and EDDS at different ratios. Values are means ± S.D. (n = 4).