PHYTOEXTRACTION OF PB AND CU CONTAMINATED SOIL WITH MAIZE AND MICROENCAPSULATED EDTA

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Chelate-assisted phytoextraction using agricultural crops has been widely investigated as a remediation technique for soils contaminated with low mobility potentially toxic elements. Here, we report the use of a controlled-release microencapsulated EDTA (Cap-EDTA) by emulsion solvent evaporation to phytoremediate soil contaminated with Pb and Cu. Incubation experiments were carried out to assess the effect of Cap- and non-microencapsulated EDTA (Ncap-EDTA) on the mobility of soil metals. Results showed EDTA effectively increased the mobility of Pb and Cu in the soil solution and Cap-EDTA application provided lower and more constant water-soluble concentrations of Pb and Cu in comparison with. Phytotoxicity may be alleviated and plant uptake of Pb and Cu may be increased after the incorporation of Cap-EDTA. In addition phytoextraction efficiencies of maize after Cap- and Ncap-EDTA application were tested in a pot experiment. Maize shoot concentrations of Pb and Cu were lower with Cap-EDTA application than with Ncap-EDTA. However, shoot dry weight was significantly higher with Cap-EDTA application. Consequently, the Pb and Cu phytoextraction potential of maize significantly increased with Cap-EDTA application compared with the control and Ncap-EDTA application.

KEY WORDS: soil, Pb, Cu, phytoremediation, maize, microencapsulated EDTA

INTRODUCTION

Synthetic chelating agents such as ethylene diaminetetraacetate (EDTA) have been used to artificially enhance the solubility of heavy metals from the soil solid phase to the soil solution and thus increase heavy metal phytoavailability (Blaylock *et al.* 1997; Wu *et al.* 2003; Luo *et al.* 2005; Luo *et al.* 2007; Epelde *et al.* 2008). Addition of chelating agents to soil may promote root uptake and translocation from the roots to harvestable above-ground parts of crops with high biomass production (Huang *et al.* 1997). However,

the rapid increase in the concentrations of both heavy metals and chelators in the soil solution may increase metal phytotoxicity and may pose a potential risk of groundwater pollution (Grčman *et al.* 2001; Sun *et al.* 2001; Thayalakumaran *et al.* 2003). The rate of release of heavy metals by chelating agents may therefore be important for environmental quality. To lower the speed of release of Pb by EDTA, Shen *et al.* (2002) tested split applications of EDTA and found that Pb was leached much more slowly with split application than single application. Li *et al.* (2005) developed a controlled-release EDTA by coating the EDTA with a layer of silicate. Unfortunately, the silicate-coated EDTA was released within a short period, probably due to the properties of the coating materials. Recently, Shibata *et al.* (2007) developed five types of controlled-release EDTA by coating the EDTA with a polyolefin polymer for phytoextraction of Pb and this enhanced Pb accumulation and reduced the leaching of both Pb and EDTA. However, the coating had low biodegradability and was very persistent in the environment, and the influence of coated and uncoated EDTA on the mobility of metals in the soil was not studied or compared.

Microcapsules have been widely used as controlled release products in such applications as drug delivery, textiles, petroleum, chemical industries, and agrochemicals owing to their well-defined chemical and physical characteristics (Mathiowitz *et al.* 1997; Qi *et al.* 2006; Ma *et al.* 2006; Fujiwara *et al.* 2008; Kamio *et al.* 2008). Microencapsulation technology has provided many researchers over the past decade with improved formulations in agrochemicals (e.g., pesticides, fertilizers and herbicides) and it has also enabled manufacturers to deliver microcapsules which reduce toxicity, control release, reduce evaporative losses, control environmental degradation, reduce leaching into groundwater, and consequently reduce environmentral pollution (Yeom *et al.* 2002; Ahmadi, 2007; Fernández-Pérez *et al.* 2007; Sopeña *et al.* 2007). Various microencapsulation techniques, including solvent evaporation and phase-separation, have been developed to obtain fine particles with controlled release properties (Ahmadi 2007; Constantin *et al.* 2007; Ichikawa and Fukumori, 2007; Kubota *et al.* 2007). Biodegradable polyesters such as poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) have been especially useful as carriers in controlled-release delivery systems due to their biological compatibility and degradability (Zhang *et al.* 2008). Encapsulation of EDTA in biodegradable polymeric particles may therefore provide controlled release and simultaneously protect the environment and plants and may be useful for chelate-assisted phytoextraction of soil heavy metals.

In the present study we prepared a biodegradable microcapsule containing EDTA by emulsion solvent evaporation to control the release rate of EDTA. The cumulative release of Cap- and Ncap-EDTA in deionised water, the effect of Cap- and Ncap-EDTA on the mobility of metals in the soil, and the possible risks of Cap- and Ncap-EDTA applications to groundwater quality were investigated. A pot experiment was also conducted to evaluate the effect of different management techniques (Cap- and Ncap-EDTA applications) on remediation of an agricultural soil originally contaminated with Pb and Cu from Dabaoshan Mine in Guangdong province, south China. The aim was to develop a new technique for effective phytoremediation of soils contaminated with potentially toxic elements.

MATERIALS AND METHODS

Reagents

All chemicals were commercially available and used without further purification. Analytical grade EDTA was purchased from Shanghai Chemical Reagent Factory,

Shanghai, China and analytical grade PCL (M_n = 80,000) was provided by Shenzhen Bright China Industrial Co., Ltd., Shenzhen, China. All other chemicals were purchased from Guangzhou Chemical Reagent Factory, Guangzhou, China.

Soil

The investigated soil was a fine-loamy, mixed, hyperthermic typical Fe-leachi-stagnic Anthrosol. Soil (24°28′27′′ N, 113°48′03′′ E) was collected from the top 20 cm of the soil profile in a rice field in the vicinity of Dabaoshan Mine, Guangdong Province, China. The soil was polluted by a combination of Cu, Zn, Cd, and Pb, with tailings and acid mine drainage being the main pollution sources affecting soils (Zhou *et al.* 2007). Soil used for pot experiments was air-dried, homogenized, and sieved through a 7-mm stainless sieve. Soil used for determination of soil properties and the incubation experiment was air-dried and passed through a 2-mm nylon sieve to eliminate the coarse sand fraction prior to analysis. Soil sub-samples passed through a 0.149-mm nylon sieve were digested with mixed acid [HNO_3 (guaranteed reagent, GR): $HClO_4$ (GR) at a ratio of 3: 2 (v/v)] and total concentrations of Cd and Pb were determined using graphic furnace atomic absorption spectrophotometry (GFAAS) (Analylikjena Zeenit 60, Japan). Total Cu, Fe, Mn, and Zn were determined using a flame atomic absorption spectrophotometer (FAAS) (Analylikjena Vario 6, Japan). Soil pH was measured in 1: 2.5 suspensions of soil and deionised water using a pH meter. Total organic matter was determined using a LECO CNS-2000. Cation exchange capacity (CEC) was calculated as the sum of Al, Ca, Mg, Na, and K extractable in 0.1 M $BaCl_2$ (w/v = 1:20). The contents of sand, silt and clay were determined by the hydrometer method.

The soil used was a sandy clay loam soil with the selected properties summarized in Table 1. High contents of Pb and Cu were found in the soil due to the vicinity of the local Dabaoshan mine. However, the contents of Ni and Zn were below the limit values of the Environment Quality Standard for Soils (GB 15618-1995) of the People's Republic of China. Soil contents of Fe and Mn were high, representing important sorption sites for heavy metals (Tan *et al.* 2005; Covelo *et al.* 2007).

Table 1 Selected physicochemical properties of the soil used in the pot experiment

Property	Value
pH_{H_2O}	4.10 ± 0.02
CEC (cmol(+) kg^{-1})	6.28 ± 0.78
TOC (g kg^{-1})	22.3 ± 1.6
Particle size distribution (%)	
Sand (%)	56.1 ± 6.5
Silt (%)	16.1 ± 2.1
Clay (%)	27.8 ± 3.0
Soil texture	Sandy clay loam
Total metal contents (mg kg^{-1})	
Fe	31392 ± 695
Mn	133 ± 13
Pb	302 ± 7
Cd	0.34 ± 0.01
Cu	986 ± 48
Zn	201 ± 9
Ni	38.8 ± 1.2

Microcapsules

EDTA loaded PCL microcapsules were prepared by a modified solid-in-oil-in-water (S/O/W) solvent evaporation method. First, 6.0 g of PCL polymer and 1.8 ml Span-80 were dissolved in 60 ml of methylene chloride/acetone (V/V = 1:1), and then 5.0 g EDTA granules (diameter $< 10\ \mu$m) was dispersed in 30 ml of ethanol, homogenized for 2 minutes with ultrasonic agitation, and added to the polymer solution. This S/O dispersion was ultrasonicated for a further 3 min and added through a 7-gauge needle dropwise into 500 ml of 0.5% (w/v) polyvinyl alcohol (PVA) solution to produce the double S/O/W emulsion. The resulting emulsion was stirred with an overhead paddle stirrer at 900 rpm for 60 min at room temperature and then for a further 60 min at reduced stirring rate (300 rpm) to remove the methylene chloride. The resulting EDTA-containing microcapsules were filtered out and washed three times with 20 ml alcohol each time, and after freeze-drying the final EDTA-containing microcapsules were obtained. Cap-EDTA prepared by this method was used in the following experiments.

The mean size of the microcapsules was analysed using an optical microscope connected to a computer (XTB-1 stereomicroscope, Jiangnan Photoelectric Instrument Company, China). For the analysis, the sample was placed on a slide and its particle size and size distribution were calculated using a computer program supplied by the manufacturer. Then the mean particle size was calculated by the following equation:

$$\bar{d} = \frac{\left(\sum_{i=1}^{500} n_i d_i\right)}{\sum_{i=1}^{500} n_i} \tag{1}$$

where d_i is the particle size of the microcapsules, and n_i is the number of microcapsules whose particle sizes are d_i.

40 mg of EDTA-PCL microcapsules were dissolved in triplicate in 1.0 ml of methylene chloride in a 10 ml centrifuge tube, and then 6 ml of deionised water was added and vortexed for 5 min. The resulting solution was centrifuged for 15 min at 5,000 rpm. Supernatant was diluted in deionised water to measure EDTA concentration by ion chromatography (IC) (Dionex ICS-90) (Welcher 1965). The EDTA loading was calculated as follows:

$$\text{EDTA loading}\,(\%) = \frac{\text{mass of EDTA in microcapsules}}{\text{mass of microcapsules}} \tag{2}$$

Microcapsule Release Experiments in Water

250 mg of microcapsules containing EDTA as described above were added in triplicate to 400 ml of deionised water in stoppered conical flasks. The flasks were kept in a thermostatic water bath at $30 \pm 0.1°$C. At different time intervals (in the EDTA-treated experiments after 0.5 d, 2 d, 5 d, 10 d, 20 d, 30 d, 40 d, and 60 d), 3 ml of clear solution were removed from the release medium to determine the concentration of EDTA. Then 3 ml of fresh deionised water were injected into the flasks to replace the sampled solution. The concentrations of EDTA were determined by ion chromatography (IC) (Dionex ICS-90) (Welcher 1965).

A preliminary study was carried out to establish the method for determining EDTA using IC (Dionex ICS-90, equipped with a gradient pump, a suppresser, and conductivity detector). A guard column (Dionex IonPac AG14A) (50 mm × 4 mm) and a hydrophilic

anion column (Dionex IonPac AS14A) (250 mm × 4 mm) were used. The eluant, consisting of 8 mM Na_2CO_3 and 1 mM $NaHCO_3$, was pumped through the columns isocratically at a flow rate of 1 ml min^{-1}. The eluant solutions were filtered through a 0.22-μm membrane and de-gassed by purging with helium. The EDTA solution was also filtered through a 0.22-μm membrane prior to injection. This method resulted in satisfactory separation of EDTA and major inorganic anions.

Effects of Cap- and Ncap-EDTA on the Mobility of Metals in Soil

Experiments on the release of the microcapsules were carried out to evaluate the effects of Cap- and Ncap-EDTA on the mobility of metals in the soil. 400 g of air-dried, sieved (≤2 mm) and homogenized Cap- or Ncap-EDTA soil were placed in acid-washed 500-ml polyethylene bottles. Five treatments were prepared in triplicate: control (no EDTA), two Ncap-EDTA rate doses (3 and 6 mmol EDTA/ kg soil) and two Cap-EDTA rate doses (3 and 6 mmol EDTA/ kg soil). The soil was then adjusted to 60% of water holding capacity (WHC) with 240 ml water. At appropriate time intervals (in the EDTA-treated experiments after 0.5 d, 2 d, 5 d, 10 d, 20 d, 30 d, 40 d, and 60 d), 20 ml of soil solution were removed from the incubated soils using 'Rhizon' suction samplers, and immediately 20 ml of fresh deionised water were injected back into the bottles. Soil solution pH was measured immediately using a pH meter. The concentrations of water soluble Cd and Pb in the solutions were determined using GFAAS, and Cu, Fe, Mn, and Zn were determined using FAAS as described above.

Pot Experiment

Samples of air-dried, homogenized, and sieved (≤7 mm) soil (5 kg) were fertilised with 1 g N as $CO(NH_2)_2$, 0.16 g P and 0.4 g K as K_2HPO_4 and transferred to pots. Eight seeds of maize (*Zea mays* L. cv. LvBa Chaotian No. 15) were sown in each pot and thinned to two plants after one week in the glasshouse at Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou, China. Soil moisture was maintained at 30% of WHC by watering daily with deionised water. The same EDTA treatments were prepared as described above for the batch incubation experiments. Cap- and Ncap-EDTA (3 and 6 mmol EDTA/ kg soil) were prepared 25 days after sowing. Treatments were set up in triplicate. Soil solution was collected using 'Rhizon' samplers one day after EDTA application and then at appropriate time intervals: in the EDTA-treated pots after 0.5 d, 2 d, 5 d, 10 d, 20 d, 30 d, 40 d, and 60 d. Pots were watered with deionised water to 60% of WHC and left for one day to reach equilibrium before sampling. The same elements were determined as in the batch incubation experiment described above. Plants were harvested at the booting stage 33 days after the EDTA addition.

Plant Sample Collection and Metal Determination

Plant shoots were harvested by cutting the stems 0.5 cm above the soil surface. The roots were gently washed using a brush pen to remove adhering soil particles. The harvested shoots and roots were then gently washed with deionised water. After drying at 70°C to constant weight, they were finely ground with a medicinal herb grinder. Plant samples were digested with 3:2 (V/V) nitric:perchloric acid. Pb was determined using GFAAS and Cu by FAAS. Data are presented as the means of three replicates together with standard errors.

Calculation of Phytoextraction Potential

The phytoextraction potential (PP) represents the total amount of heavy metals extracted per pot of soil in one single phytoextraction cycle and can be calculated as follows (Kos *et al.* 2003):

$$PP = M_{plant} \times B_{plant} \tag{3}$$

where M_{plant} is the content of heavy metal in the dry above-ground biomass (mg kg^{-1}) and B_{plant} is the above-ground dry matter yield (g pot^{-1}).

Statistical Analysis

Statistical analysis was performed using the SPSS 16.0 for Windows software package. All the values reported here are means of three replicates. Analysis of variance (ANOVA) was performed and subsequently the mean values were compared using Duncan's new multiple range test at the 5% level.

RESULTS AND DISCUSSION

Characterization of Biodegradable Microcapsules Containing EDTA

The biodegradable microcapsules were spherical with a mean diameter of approximately 65 ± 15 μm and contained about $(33 \pm 1)\%$ EDTA. The time course of changing EDTA concentration is presented in Figure 1 for two types of solution: the mixture of Cap-EDTA dispersed in distilled water and the reference mixture of Ncap-EDTA dispersed
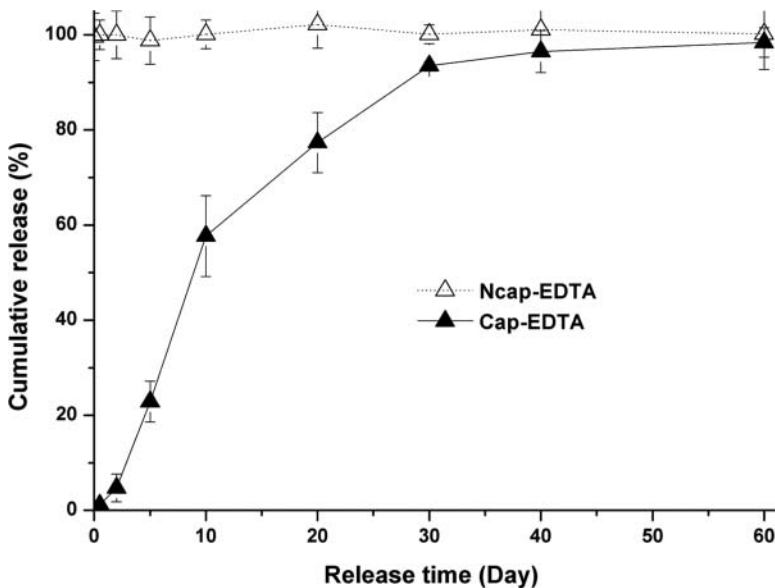

Figure 1 Cumulative release percentages of microencapsulated EDTA (Cap-EDTA) and non-microencapsulated EDTA (Ncap-EDTA).

in distilled water. The release of EDTA from Cap-EDTA increased with time and the cumulative release of EDTA reached 93% after 30 days. However, the cumulative release of Ncap-EDTA remained approximately constant at 100% throughout the observation period of 60 days. This indicates that the microcapsules possessed excellent slow-release properties as carriers of EDTA and the rate of release may be favourable for phytoremediation. Lower concentrations of EDTA in the soil might reduce the leaching of heavy metals which could lead to environmental contamination. Synchronous slow-release of EDTA and heavy metals in the soil solution may alleviate phytotoxicity.

Effect of EDTA on Heavy Metal Solubility in Soil

The time course of release of water-soluble Pb and Cu in the batch incubation experiment is presented in Figure 2. As reported previously (Huang *et al.* 1997; Wu *et al.* 2004), EDTA is an efficient chelator of Pb and Cu. EDTA application significantly increased the concentrations of water-soluble Pb and Cu whereas water-soluble concentrations in the control remained low over time. The higher dose of Ncap-EDTA (6 mmol EDTA/kg soil) resulted in a 53-fold increase in initial water-soluble Pb concentration and 134-fold increase in initial water-soluble Cu compared to the control. The initial water-soluble fractions of Pb and Cu were 91.6 and 195.9 mg kg^{-1}, respectively. Such high Pb and Cu concentrations with Ncap-EDTA may be phytotoxic and may also lead to secondary pollution by leaching into groundwater. Water-soluble Pb and Cu concentrations with Cap-EDTA applications were about 9 and 5 times lower than those with Ncap-EDTA after 12 h. By using Cap-EDTA, water-soluble Pb and Cu concentrations increased slowly within about 10 days (Figure 2). At the same time, water-soluble Pb and Cu concentrations sharply decreased treated with Ncap-EDTA. These patterns may be explained by the cumulative release of Cap- and Ncap-EDTA in water (Figure 1). The rapid increase in water-soluble Pb and Cu in the soil solution may be controlled by Cap-EDTA application. Consequently the phytotoxicity and the potential risk to groundwater pollution by potential uncontrolled solubilisation and leaching would decrease.

Applications of Ncap-EDTA led to decreased water-soluble Pb and Cu concentrations over time and applications of Cap-EDTA also resulted in decreased water-soluble Pb and Cu concentrations 10 days later as shown in Figure 2. These results are consistent with observation of Wu *et al.* (2004) who suggested that the decrease in water-soluble Pb was due to an increase in the pH of soil solution which resulted from soil organic matter, EDTA degradation and the appearance of a CO_2 emission peak (Wu *et al.* 2004). In contrast, Neugschwandtner *et al.* (2008) ascribed the decrease in water-soluble Pb to a continuous increase in Fe in the water extract which originated from the slow dissolution of Fe oxides and hydroxides. In our study, in the first 10 days the concentrations of water-soluble Fe in the soil solutions increased after application of EDTA and decreased slowly 10 days later (Figure 3a). At the same time the pH of the soil solutions decreased in the first 5 days and increased rapidly from the 5th to the 20th day, and 20 days later the soil solution pH increased slowly (Figure 3b). From the above results we confirm that the decrease in water-soluble Pb and Cu seems to be due to an increase in Fe in the water extracts (Figure 3a) during the first 10 days, and the subsequent decrease in water-soluble Pb and Cu after 10 days was associated with an increase in soil solution pH resulted from dissolution of Fe-oxides and hydroxides, soil organic matter, EDTA degradation, and the appearance of a CO_2 emission peak (Figure 3b). Fe was the main competitor due to the higher stability of Fe-EDTA complexes (log $K_{Fe(III)\text{-}EDTA}$ = 26.5) compared to other metal complexes (log
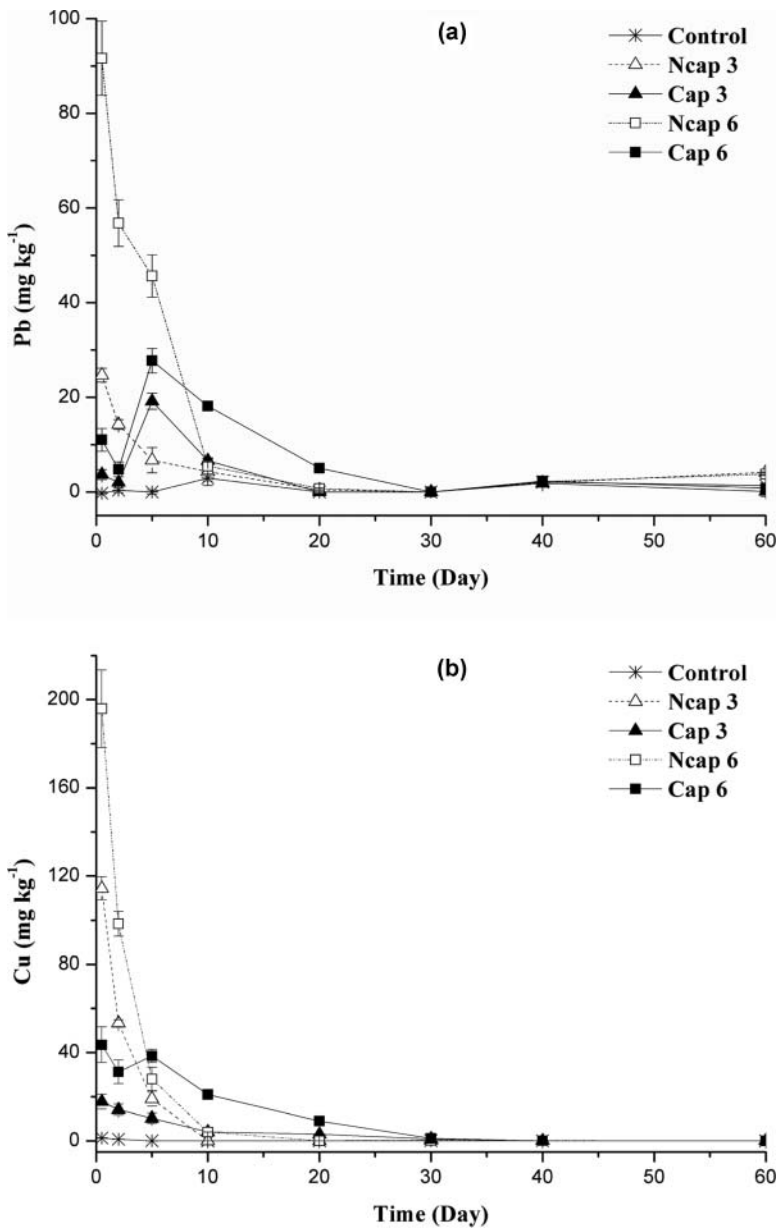
Figure 2 Dynamics of water-soluble (a) Pb and (b) Cu concentrations following Cap- and Ncap-EDTA application. Values are means $\pm$ SE, n = 3.

$K_{Pb\text{-}EDTA} = 19.0$ and log $K_{Cu\text{-}EDTA} = 19.7$) (Nowack and Sigg 1997; Sun *et al.* 2001; Nowack *et al.* 2006). However, Cap-EDTA can resist Fe competition. Applications of Cap-EDTA therefore resulted in lower initial mobilization of heavy metals but provided more constant amounts of water-soluble Pb and Cu compared to Ncap-EDTA.
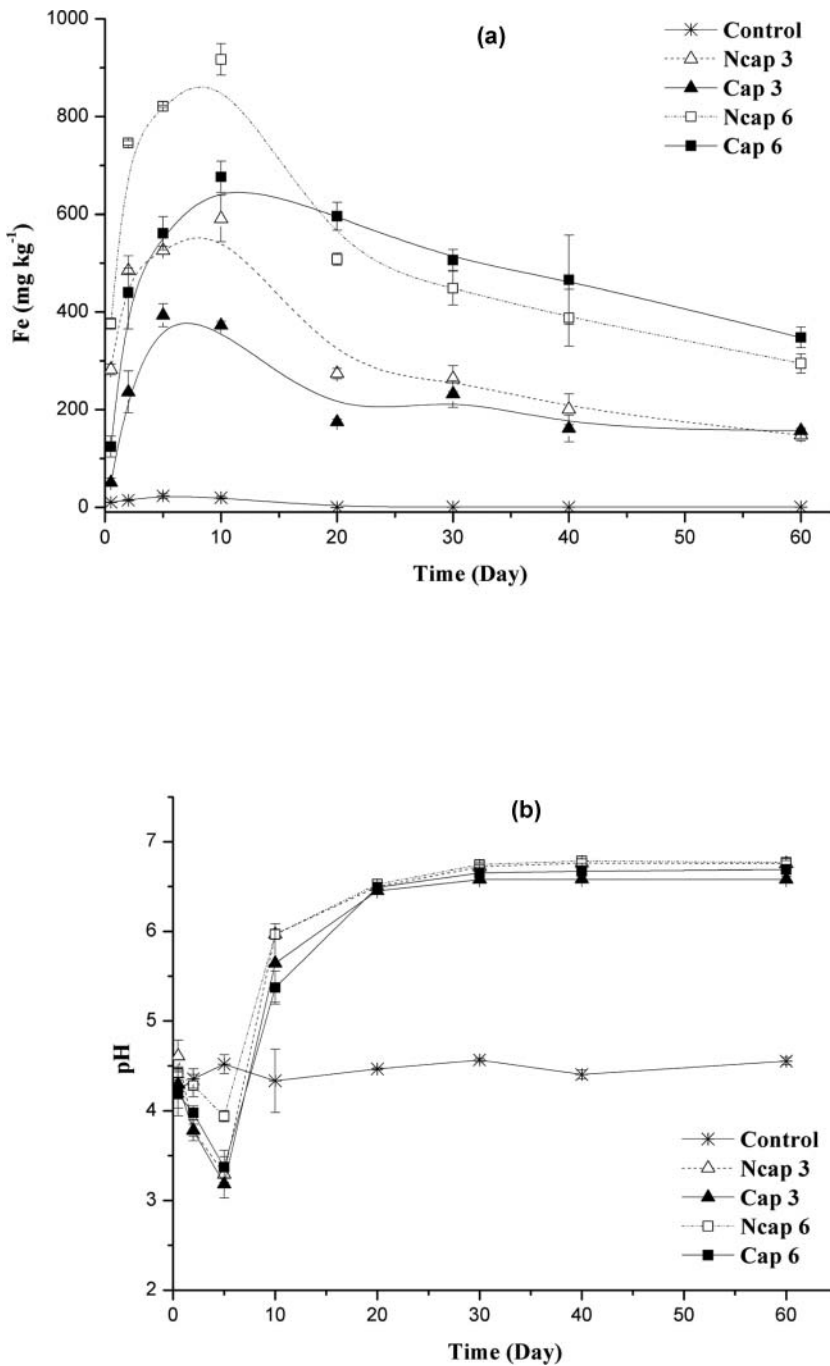
Figure 3 Dynamics of water-soluble (a) Fe concentrations and (b) pH following Cap- and Ncap-EDTA application. Values are means ± SE, n = 3.

Effect of EDTA on Maize Biomass

Maize exhibited visible phytotoxicity symptoms (wilting and plant necrosis) after all EDTA treatments. Plants treated with the higher Ncap dose of EDTA (6 mmol/kg soil) showed visible signs of phytotoxicity within four days which led to plant death. The plants with applications of higher dose of Cap-EDTA (6 mmol EDTA/kg soil) and lower dose of Ncap-EDTA (3 mmol EDTA/kg soil) were only slightly damaged and survived till the end (60 days) of the experiment. However, plants treated with control and lower dose of Cap-EDTA (3 mmol EDTA/kg soil) were hardly damaged. Application of Cap-EDTA provided continuous lower doses of dissolved metals compared to the equal Ncap-EDTA addition (Figure 1). Barocsi *et al.* (2003) suggested that addition of lower EDTA doses provides time for plants to initiate their adaptation mechanisms and also to raise their damage threshold (Barocsi *et al.* 2003). Toxicity to plants is mainly attributed to free EDTA and the resulting uptake of heavy metals after addition of EDTA.

Phytotoxicity was associated with a significant decrease in biomass production (shoot and root dry matter) of maize with the exception of plants treated with 3 mmol/kg soil Cap-EDTA as shown in Figure 4. This is in accordance with the results reported by Neugschwandtner *et al.* (2008) and Li *et al.* (2005). The decrease in plant biomass was greater with increasing EDTA dose. The decline in biomass production with Ncap-EDTA applications was greater than with the corresponding Cap-EDTA applications, especially at the higher Ncap-EDTA dose (6 mmol/kg soil). Lower rates of EDTA application (including continuous applications) resulted in lower phytotoxicity. These results indicate that Cap-EDTA applications can decrease phytotoxicity and thereby increase plant dry matter production compared with Ncap-EDTA applications.
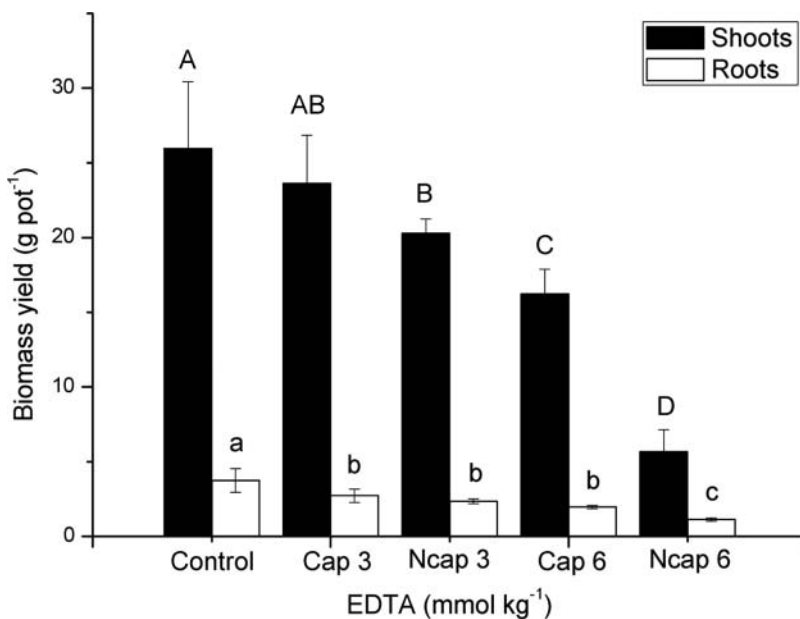

Figure 4 Shoot and root dry matter biomass of maize following the addition of different rates of EDTA (0, Cap 3, Ncap 3, Cap 6, Ncap 6 mmol kg^{-1}). Values are means ± SE, n = 3. Values followed by different letters in the same column are significantly different at $P < 0.05$.

Accumulated Concentrations of Pb and Cu in Maize

As reported previously, EDTA significantly increased the uptake of heavy metals (Sims and Kline 1991; Grčman *et al.* 2001; Wu *et al.* 2004). Table 2 presents the Pb and Cu concentrations in the shoot dry matter of maize after different application rates, with EDTA (Ncap- and Cap-EDTA) significantly increasing the concentrations of Pb and Cu in the shoots compared to the control. Shoot Pb and Cu contents increased with increasing rate of applied NCap- and Cap-EDTA. The differences in shoot Pb and Cu contents between lower application rates of NCap- and Cap-EDTA (3 mmol/kg soil) were not significant. However, higher doses of Ncap-EDTA (6 mmol/kg soil) resulted in significantly higher Pb and Cu contents compared with the corresponding Cap-EDTA applications. This may be explained by the importance of physiological stress for the increased unrestricted uptake of Me-chelate complexes (Thayalakumaran *et al.* 2003). When EDTA was present, Pb accumulation in plant shoots was correlated with the formation of Pb-EDTA complex which was absorbed and translocated by the plant (Vassil *et al.* 1998). Shoot accumulation of Me-EDTA is strongly dependent on physiological stress (Neugschwandtner *et al.* 2008). Therefore, higher Me-EDTA concentrations (Figure 3) may result in higher physiological stress for shoot uptake of Me-EDTA. However, the maximal accumulated concentrations of Pb in maize in our study is about twice less than values reported by Chunling Luo *et al.* (2005) for the concentrations of Pb in maize (257–270 mg kg^{-1}). The results indicate that our data do not suggest an efficient phytoextaction in the pot with the current system used. It may change with a different corn variety or different plant species or different protocol in our next experiments.

Table 2 also shows the phytoextraction potential of maize with applications of NCap- and Cap-EDTA in the pot experiment. For example, Cap-EDTA (6 mmol/kg soil) addition significantly increased the Pb phytoextraction potential of maize to 1.18 mg pot^{-1} of Pb in one growing season, almost a 1.9-fold increase compared with the control and a 2.4-fold increase compared with corresponding Ncap-EDTA (6 mmol/kg soil) application. This may be explained by the high concentrations of Me-EDTA in the soil solutions reducing plant yield and also little free Pb^{2+} or Pb-EDTA resulting in low Pb concentrations in the plants. Cap-EDTA application was therefore an effective regime for increasing the Pb phytoextraction potential of maize. However, the Cu phytoextraction was less efficient compared to Pb phytoextraction. For example, phytoextraction potential (PP) for Cu at Cap 3, Ncap 3 and Ncap 6 was not significantly different from that of the control and this is in

Table 2 Concentrations of Pb and Cu in the shoots of maize, calculated dry shoots yields per hectare and phytoextraction potential (PP) per pot by application of Ncap and Cap-EDTA

Treatment	Concentration (mg kg^{-1})		Shoot DM yield (g pot^{-1})		PP (mg pot^{-1})	
	Pb	Cu	Pb	Cu	Pb	Cu
Control	25.7 ± 1.7 d	22.1 ± 1.2 d	25.9 ± 2.1	25.9 ± 2.1	0.67 ± 0.05 c	0.57 ± 0.04 b
MC 3	53.2 ± 7.2 c	30.2 ± 0.8 c	23.6 ± 1.8	23.6 ± 1.8	1.26 ± 0.17 a	0.71 ± 0.06 ab
NMC 3	44.6 ± 3.4 c	32.0 ± 3.7 c	20.3 ± 0.5	20.3 ± 0.5	0.90 ± 0.06 b	0.65 ± 0.07 b
MC 6	72.9 ± 5.6 b	49.7 ± 2.4 b	16.9 ± 1.0	16.9 ± 1.0	1.18 ± 0.09 a	0.81 ± 0.05 a
NMC 6	91.3 ± 5.1 a	119 ± 8 a	5.67 ± 0.68	5.67 ± 0.68	0.52 ± 0.07 c	0.67 ± 0.08 b

*: Values followed by different letters in the same column are significantly different at the 5% level, n = 3.

contrast to PP for Pb (Table 2). The reason may be that the order of the dissolution rates was PbEDTA > CuEDTA from natural iron oxides (Nowack and Sigg 1997). Interestingly, the PP for Cu was significantly enhanced by prolonging the exposure time of the plants in appropriate water-soluble Cu concentrations solution or in other words by application of 6 mmol/kg soil Cap-EDTA.

CONCLUSION

A comparison of applications of Ncap and Cap-EDTA and the evaluation of phytoextraction from the pot scale are presented. Applications of Cap-EDTA resulted in lower initial mobilization of heavy metals but provided generally a more constant amount of water-soluble Pb and Cu concentrations compared to applications of Ncap-EDTA. Moreover, applications of Cap-EDTA proved to be lower phytotoxic and enhance plant dry above-ground biomass compared with Ncap-EDTA addition. Furthermore, addition of Cap-EDTA significantly increased the Pb PP of maize compared with the control and the corresponding Ncap-EDTA application. The Cu phytoextraction was less efficient compared to Pb phytoextraction. Last, the risk of groundwater contamination may be alleviated due to a lower maximal solubility of Pb and Cu after applications of Cap-EDTA in comparison with Ncap-EDTA additions. However, the concentrations of Pb and Cu in the plants are very low in the pot with the current system used. It may change with a different corn variety or different plant species or different protocol in our next experiments.

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