The role of Fe- and Mn-oxides during EDTA-enhanced phytoextraction of heavy metals

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ABSTRACT

In several cases ethylenediaminetetraacetic acid (EDTA) proved to be an efficient mobilising amendment during chemically enhanced phytoextraction of heavy metals. The presence of Fe-(hydr)oxides and their dissolution after the addition of EDTA can limit the phytoextraction of the targeted heavy metals due to the high stability of the formed Fe(III)EDTA complexes. This study has focused on the influence of Fe- and Mn-oxides and hydroxides dissolution on heavy metal uptake by *Zea mays* in a two-year EDTA-enhanced phytoextraction process. Incubation experiments and speciation modelling proved the increased concentrations of Mn and Fe through the dissolution of Mn-and Fe-(hydr)oxides. Furthermore, increased Fe and Mn accumulation was observed in maize plants after the second year of the phytoextraction process. Therefore, the presence of Mn- and especially Fe-(hydr)oxides proved to be a limiting factor during EDTA-enhanced phytoextraction of heavy metals from contaminated soils.

Keywords: EDTA; phytoextraction; Fe oxides; Mn oxides; heavy metals; *Zea mays*

Phytoextraction of heavy metals, i.e. the use of plants for accumulating contaminating metals in the aboveground parts, has received much attention in the past decade as a cost-effective and environmentally safe method of soil remediation (Macková et al. 2006). Ensley (2000) calculated the approximate costs of different remediation technologies; for example, the excavation-landfill procedure, commonly used in the USA, costs between $150 and $350 per ton; remediation of such contaminated soils using phytoextraction methods would cost only $20–$80 per ton. On the other hand, due to its lower efficiency compared to conventional methods, phytoextraction is a long-term process that can take several years/decades (Robinson et al. 2003).

Most of the recent heavy metal phytoextraction studies have focused on the use of agricultural crops with high biomass yields (e.g., *Brassica juncea* L., *Zea mays* L., *Helianthus annuus* L.) combined with a chemical alteration of heavy metal mobility (Chen and Cutright 2001, Wu et al. 2004, Luo et al. 2005, Meers et al. 2005, Tandy et al. 2006). A successful phytoextraction process depends strictly on adequate biomass yields and high heavy metal contents in the harvestable parts of the plants (Macek et al. 2004). The harvested biomass containing high concentrations of heavy metals can be removed from the site and stored at a landfill or burned (Keller et al. 2005).

The low mobility and bioavailability of some heavy metals (especially Pb) is one of the main drawbacks of the phytoextraction process. Besides soil organic matter, Fe- and Mn-oxides and hydroxides play an important role in heavy metal sorption due to their high specific surface and reactivity. Their strong affinity to heavy metals makes them important sorbents of these risk ele-

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ments in soils and sediments (Carty et al. 1998, Chrastný et al. 2006). It was found that Mn-oxides were much more efficient in adsorbing Pb compared to Fe-oxides (Dong et al. 2000). An increase of heavy metal mobility can be achieved by adding synthetic chelating agents capable of solubilising and complexing heavy metals into the soil solution as well as to promote heavy metal translocation from roots to the harvestable parts of the plants (Blaylock et al. 1997, Huang et al. 1997). Among many others, EDTA (ethylenediaminetetraacetic acid) has proved to be the most efficient chelant used for Pb phytoextraction from contaminated soils in several cases, mainly because of its high affinity to form soluble PbEDTA complexes ($\log K_{\mathrm{PbEDTA}} = 18.0$) (Huang et al. 1997, Shen et al. 2002, Kos and Leštan 2003, Wu et al. 2004, Do Nascimento et al. 2006, Komárek et al. 2007).

Other elements present in soils (e.g. Ca, Fe) are likely to compete with the target metal during the extraction due to their high concentrations in soils and stability of their complexes (Martell et al. 2001, Nowack et al. 2006). The extraction efficiency of the chelating agent is influenced not only by the stability constant (log K) of the formed chelate complex, but to a great extent by the concentrations of the metals and the ligand (chelant). Compared to Ca, formed Fe(III)EDTA complexes are much more stable ($\log K_{\mathrm{Fe(III)EDTA}} = 27.2$; $\log K_{\mathrm{CaEDTA}} = 10.7$) than most heavy metal complexes (Martell et al. 2001). Although low concentrations of Fe(III) are present in soil solution under common conditions, the addition of EDTA leads to a slow dissolution of Fe-(hydr)oxides (HFO) and subsequent preferential formation of Fe(III)EDTA complexes. This is, however, a slow process depending on the amount of Fe-oxides, their crystallinity and the concentration of the present chelating agent. Dissolution of these mineral phases is mainly attributed to ligand exchange reactions (Nowack and Sigg 1997, Komárek et al. 2007). Therefore, the dissolution of amorphous HFO controls to a great extent the solubility of Fe and its subsequent competition for the chelating agent, especially at pH values below 6. With a pH above 6, the abundance of EDTA complexes with other metals (e.g., Zn, Ca, Pb) will increase (Nowack 2002).

The aim of this study was (i) to evaluate the influence of the partial dissolution of Fe- and Mn-oxides and the subsequent competition of Fe and Mn for EDTA during the EDTA-extraction of heavy metals from a soil; (ii) to assess the phytoextraction potential of *Zea mays* L. after the addition of EDTA in a two-year phytoextraction process.

MATERIAL AND METHODS

Soil sampling and sample preparation. Samples were taken from the arable layer (0–20 cm) of an agricultural soil originating from the mining and smelting area of Příbram (N49°40.350'; E13°58.440'). The soil profile (classified as a Gleyic Cambisol) has developed on a Proterozoic volcano-sedimentary rock complex (basalt veins in clastic sediments) belonging to the Příbram ore district. The contamination of soils from the area has been studied by several authors (Šichorová et al. 2004, Ettler et al. 2005, Vaněk et al. 2005). Pb isotopic studies proved the predominant influence of the smelter on the contamination in the area (Komárek et al. 2006). Samples used for the soil characteristics determination, heavy metal contents, heavy metal chemical fractionation and incubation experiments were air-dried, homogenised and sieved through a 2-mm stainless sieve prior to analyses. Soil samples used for pot experiments were air-dried, homogenised and passed through a 10-mm stainless sieve.

Basic soil characteristics. Soil pH was measured in suspension using a 1:2.5 (w/v) ratio of soil and deionised water or 0.2M KCl. Particle size distribution was determined by the hydrometer method. Cation exchange capacity (CEC) was determined as a sum of basic cations and Al extracted with 0.1M $BaCl_2$ solution. Basic cations and Al were determined using FAAS (SpectrAA 300, Varian, Australia) and ICP-OES (Vista Pro, Varian, Australia) under standard analytical conditions. In order to determine the amount of amorphous and poorly crystalline Fe- and Mn-oxides and hydroxides, the oxalate extraction (0.2M ammonium oxalate/oxalate acid at pH 3) was performed (Carty et al. 1998). The oxalate extracts obtained were analysed for Fe and Mn using ICP-OES. Total organic carbon (TOC) was determined by catalytic oxidation (1250°C) using the ELTRA (Neuss, Germany) Metalyt CS1000S elemental analyser. Available forms of nutrients (Ca, K, Mg, P) were determined using the Mehlich 3 soil extraction procedure (Zbíral 2000).

Total heavy metal contents and chemical fractionation. Total heavy metal contents were determined as follows: (i) soil samples were decomposed using the dry ashing procedure in a mixture of oxidising gases ($O_2 + O_3 + NO_x$) at 400°C for 10 h in the Dry Mode Mineraliser Apion (Tessek, Czech Republic); (ii) the ash was then decomposed using HNO_3 and HF (Analytika Ltd., Czech Rep.), evaporated to dryness at 160°C and dissolved in diluted *aqua regia*. In order to determine heavy metal fractionation in the studied soils, the sequential extraction technique by Quevauviller (1998) was used. Extraction solutions were prepared using chemicals of analytical grade (Lachema, Czech Republic and Merck, Germany) and deionised water. The fractions determined in this work were as follows: (i) Fraction A – exchangeable and weak acid-extractable; (ii) Fraction B – reducible; (iii) Fraction C – oxidisable; (iv) Fraction D – residual, computed as the sum of fractions A, B, C subtracted from the total content. A detailed experimental scheme of the sequential extraction is given elsewhere (Quevauviller 1998). Heavy metal contents in digests were analysed using ICP-OES. For the evaluation of measurement precision and accuracy, the certified reference material CRM Light Sandy Soil 7001 (Analytika Prague, Czech Republic) was used.

Incubation experiments. Incubation experiments for a period of 0.5–28 days were carried out in order to evaluate the changes in heavy metal solubility and the possible competition of Fe and Mn in the studied soil after the addition of EDTA (pure acid of analytical grade, Fluka, Germany). An aliquot part of 50 g of air-dried soil was placed into acid-cleaned 250-ml polyethylene bottles. An amount of 15 ml (~ 60% water field capacity) of the EDTA solution (3, 6, and 9 mmol/kg soil) was added to the soil. The pH of the solution was set to 5.5 and no significant change of soil pH after the incubation experiment was observed. The control variant was treated with 15 ml of deionised water. Samples were extracted with 125 ml of deionised water for 1 h on an end-over-end shaker at 30 rpm. Water-soluble contents of heavy metals (Pb, Cd, Cu, Zn) and other elements (Fe, Mn, Ca, Mg, K) in solutions were analysed using ICP-OES and FAAS. The PHREEQC-2 speciation model (Parkhurst and Appelo 1999) version 2.13.00 for Windows, was used to determine the metal speciation in extracts. Metal concentrations (Pb, Cd, Zn, Cu, Fe, Mn, Ca, Mg, K) obtained from the incubation experiment and pH values of the water extracts were used for the calculation. The calculations were carried out for 1 day and 28 days in order to evaluate the changes of metal speciation during the incubation experiment and the effect of possible competition of other metals (in particular Fe).

Pot experiments and plant analyses. The phytoextraction efficiency of maize was assessed during a two-year pot experiment. Maize (*Z. mays*) was chosen as the tested plant species because of its high biomass yields and heavy metal tolerance and it represents a commonly cultivated crop in Central Europe. Each pot contained 5 kg of air-dried and sieved (10-mm sieve) soil. Ten seeds of maize were sown in each pot. Maize plants were fertilised with 2 g of N (NH_4NO_3); 0.16 g of P and 0.4 g of K (K_2HPO_4) per pot. Pots were kept in a controlled outdoor vegetation hall and were watered twice per day using only deionised water in order to maintain ~ 60% of the water holding capacity. Maize plants were thinned to four plants per pot after two weeks of growth. EDTA was applied to plants in three 100-ml doses (3 × 1, 3 × 2, 3 × 3 mmol EDTA/kg soil) in order to achieve higher phytoextraction efficiency by reducing the phytotoxicity effects together with minimising the risks associated with leaching of the mobilised metals (Shen et al. 2002). Each EDTA treatment as well as the control treatment was conducted in quadruplicates. The first application of the mobilising agents was added to plants after 100 days of growth. The following applications were added after 10 and 20 days. The control variants were treated with deionised water. The aboveground biomass was harvested after 10 days from the last EDTA addition (i.e., 130 days after planting). The applications of fertilisers followed the same pattern during the second vegetation period. EDTA was not further applied during the second year. The harvested aboveground biomass was washed carefully using deionised water and dried at 60°C until constant weight and finely ground prior to decomposition. Plant samples were decomposed using the dry ashing procedure (Miholová et al. 1993). Heavy metal contents in digests were analysed using ICP-OES. In order to evaluate measurement precision and accuracy, the certified reference material DC73350 Leaves of Poplar (China National Analysis Centre for Iron and Steel, China) was used.

Statistical analyses. All statistical analyses were performed using analyses of variance (ANOVA) with consequent Duncan test and linear regression analyses (software Statistica 6.0, StatSoft). The results were evaluated on the basis of homogenous groups at a given significance level ($P < 0.05$).

RESULTS AND DISCUSSION

Soil characteristics

The basic physico-chemical properties of the studied soil are summarised in Table 1. The near-neutral pH of the soil extract prevented leaching

Table 1. Basic physico-chemical soil characteristics and total heavy metal contents in the studied soil

pH_{H_2O}	6.6
pH_{KCl}	5.8
CEC (meq/kg)	134
TOC (%)	2.6
Particle size distribution (%)	
Sand	43.6
Silt	47.5
Clay	8.9
Oxalate-extractable (g/kg) (n = 3)	
Fe	3.04 ± 0.19
Mn	0.69 ± 0.02
Al	0.59 ± 0.15
Available nutrients after Mehlich 3 (mg/kg) (n = 3)	
Ca	1058 ± 30
K	392 ± 1
Mg	121 ± 5
P	162 ± 0.1
Total heavy metal contents (mg/kg) (n = 3)	
Pb	200 ± 2
Cd	1.61 ± 0.18
Cu	61.9 ± 9.2
Zn	169 ± 3

of the nutrients and bases (e.g., Mg, Ca) from the soil as it was observed in agricultural acidic soils located in the vicinity of the local smelter (Komárek et al. 2007). Amorphous and poorly crystalline Fe-(hydr)oxides are the predominant oxide/hydroxide phase in the soil (Table 1). The chemical fractionation of heavy metals in the soils is given in Figure 1. Only a small portion of Pb (0.6%) and Cu (2.9%) is readily available (present in fraction A). Pb is predominantly bound to the reducible and oxidisable fractions of the soil complex. Because of high contents of anthropogenic Pb, only a small portion (5.3%) is present in the residual fraction of the soil. A high portion of Cd in the soil (26%) was present in the mobile phase. The majority of Zn (49%) and Cu (81%) were present in the residual fraction D (Figure 1).

Incubation experiments

The evolution of soluble heavy metals after the addition of EDTA is given in Figure 2. EDTA was successful at mobilising the target heavy metals (Pb, Cd, Cu, Zn) from the soil. Without any EDTA present, water-soluble contents of heavy metals remained low and relatively constant throughout the 28-day incubation period. As expected, the addition of 9 mmol EDTA/kg most significantly increased heavy metal solubility [by a factor of 214 (Pb); 281 (Cd); 40 (Cu) and 24 (Zn) compared to the control]. The high efficiency of EDTA to desorb Pb and Cd from the soil is due to the relatively high stability constants of PbEDTA (log K = 18.0) and CdEDTA (log K = 18.2) (Martell et al. 2001). Additionally, the high concentration of Pb present in the soil influences greatly EDTA complexation. A decrease of soluble Pb, Cd, Cu and Zn contents was observed after 3 days and was more evident at lower EDTA concentrations (3–6 mmol/kg). It is attributed to the slow dissolution of Fe-(hydr)oxides after the EDTA addition and the subsequent exchange reactions between the complexed heavy metals and mobilised Fe(III). This fact was proved by the increasing Fe concentration in time after the addition of EDTA, leading to a strong competition between the target heavy metals and Fe(III) for the EDTA ligand in the end of the incubation period (Figure 2). Besides the dissolution of Fe-(hydr)oxides, Mn-oxides could play an important role during EDTA complexation as well. Although the addition of EDTA solubilised Mn in the soil, the

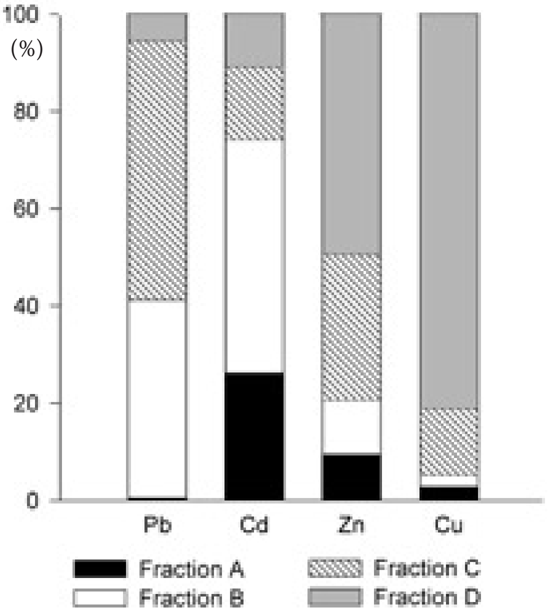

Figure 1. Chemical fractionation of Pb, Cd, Cu and Zn in the studied soil: Fraction A – exchangeable and weak acid-extractable; Fraction B – reducible; Fraction C – oxidisable; Fraction D – residual

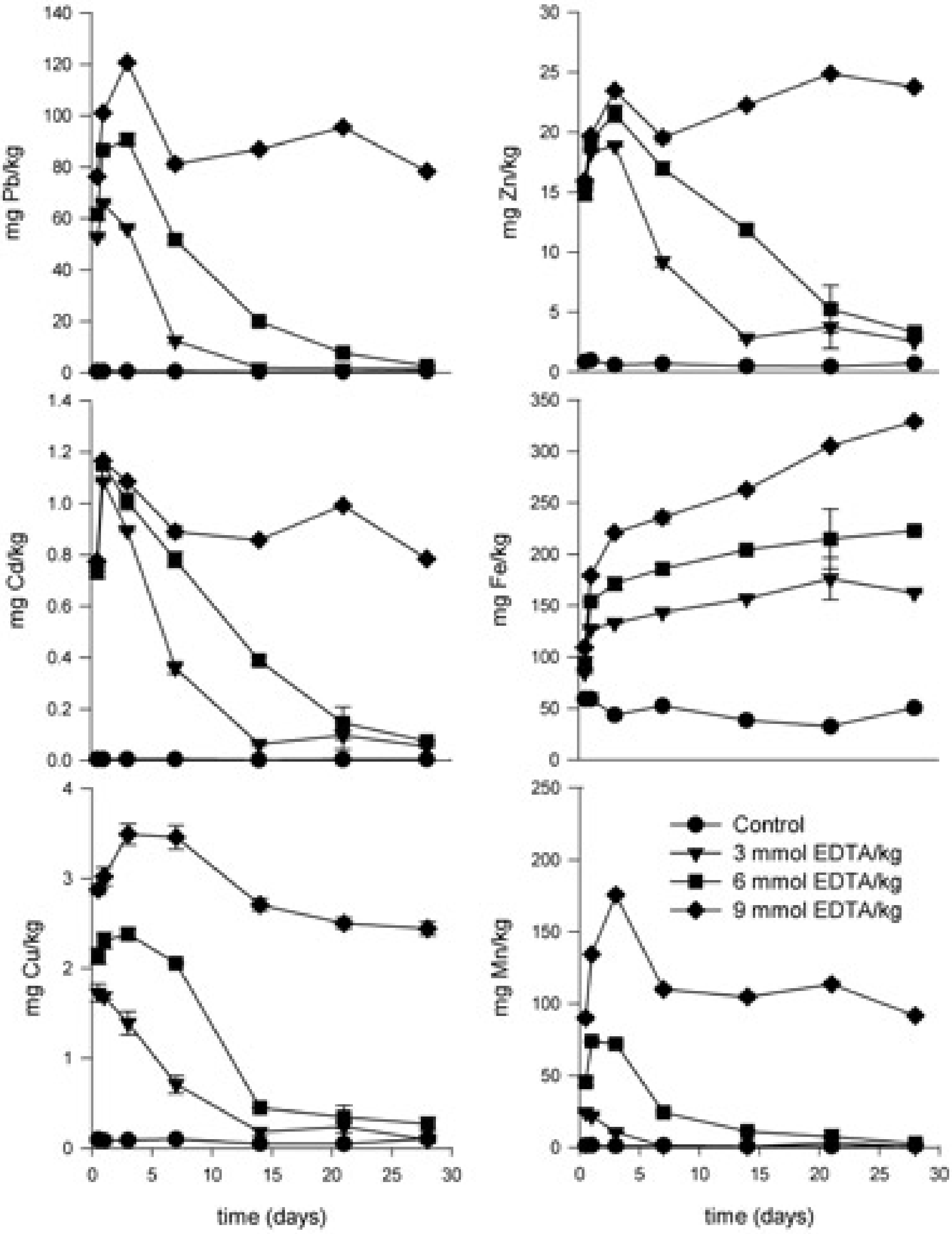

Figure 2. Time evolution of soluble Pb, Cd, Cu, Zn, Fe and Mn after the addition of EDTA. Data shown are means ± SD ($n = 3$)

role of Fe(III) is more emphasised due to the higher content of Fe-(hydr)oxides in the soil compared to Mn-oxides (Table 1) and the higher stability constant of Fe(III)EDTA ($\log K_{Fe(III)EDTA} = 27.2$; $\log K_{MnEDTA} = 15.6$) (Martell et al. 2001). The Mn concentration decreasing in time is associated with increased solubilised contents of Fe(III). Heavy metal extraction is therefore reduced in time mainly due to the co-extraction of Fe(III), which outcompetes other metals (Nowack et al. 2006). Because of its high concentration, the addition of 9 mmol EDTA/kg helps to maintain relatively high soluble heavy metal contents during the 28-day incubation period (Figure 2).

The PHREEQC-2 speciation modelling showed that the vast majority of divalent metals are mainly present as $[MeEDTA]^{2-}$ complexes after the application of EDTA. Fe mainly forms $[FeEDTA]^{-}$ and $[FeOHEDTA]^{2-}$ species. Complexes of Pb, Fe and Cu with EDTA are the predominant species, other competing cations (Zn, Cd) form complexes accounting for up to 1% of the total EDTA speciation. The abundance of MnEDTA complexes is negligible, proving that the dissolution of Fe-(hydr)oxides, rather than Mn-oxides, controls the EDTA extraction efficiency. The relative abundance of $[PbEDTA]^{2-}$ complexes increased after 28 days. This increase was associated with a decrease in the abundance of $[FeEDTA]^{-}$ and $[FeOHEDTA]^{2-}$ complexes. This fact can be explained by a relatively high pH of the soil (Table 1). While lower pH support the formation of Fe(III)EDTA complexes, higher pH favours the formation of other metal-EDTA complexes (such as ZnEDTA or PbEDTA) (Nowack 2002).

Pot experiments

Biomass yields of *Z. mays* during the two-year phytoextraction experiment are summarised in Figure 3. During the first year, the addition of 3 × 1 and 3 × 2 mmol EDTA/kg did not significantly influence maize biomass yields compared to the control (with only deionised water added). On the other hand, during the second year, already 3 × 2 mmol EDTA/kg decreased the biomass production of maize. In addition, maize plants treated with 9 mmol EDTA/kg exhibited visual chlorosis and necrosis symptoms and died. The growth reductions of plants treated with higher EDTA concentrations are probably due to the presence of heavy metals mobilised to the soil solution and possibly due to the toxicity of free EDTA, if present (Vassil et al. 1998). This phenomenon was even more emphasised during the second year (Figure 3).

Heavy metal contents in maize shoots are given in Figure 4. An increase of the EDTA concentration led to a linear increase of Pb contents in the aboveground biomass of maize in both years ($R^2 = 0.99$) (Figure 4a). Pb content in the control plants reached 3.98 ± 0.71 mg/kg and increased to 10.2 ± 2.0 mg/kg after the addition of 9 mmol EDTA/kg. During the first year, Cu and Zn concentrations in maize biomass decreased in the presence of EDTA (especially after the addition of the lowest EDTA concentration). This can be explained by the fact that the accumulation of Cu and Zn, as micronutrients, is governed by uptake of free metal ions through the symplastic pathway. The uptake of nonessential elements, such as Pb, will be thus limited in the absence of chelants. The presence of EDTA leads to the complexation of Cu and Zn and reduction of their uptake (Nowack et al. 2006). Pb uptake will be increased as all Pb is taken up in the form of PbEDTA complexes in the presence of EDTA (Sarret et al. 2001). When EDTA is present in higher concentrations, the passive uptake through the apoplastic pathway controls the uptake of all heavy metals and leads to an increase of heavy metal contents in plant shoots (Figure 4) (Nowack et al. 2006). During the second year, a vast majority of metals was taken up as chelated species and no decrease in accumulation after the addition of EDTA was observed (as it was during the first year) (Figure 4). A significant increase of

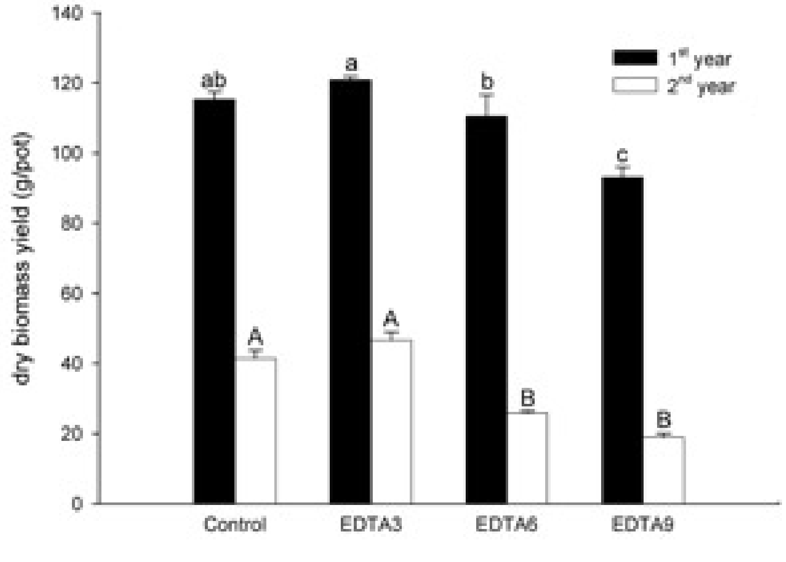

Figure 3. Biomass yields of maize during the two-year phytoextraction process after the addition of 0 (Control), 3 × 1 (EDTA3), 3 × 2 (EDTA6), and 3 × 3 (EDTA9) mmol EDTA/kg. Data shown are means ± SD ($n = 4$). Data with the same letter represent statistically identical values ($P < 0.05$)

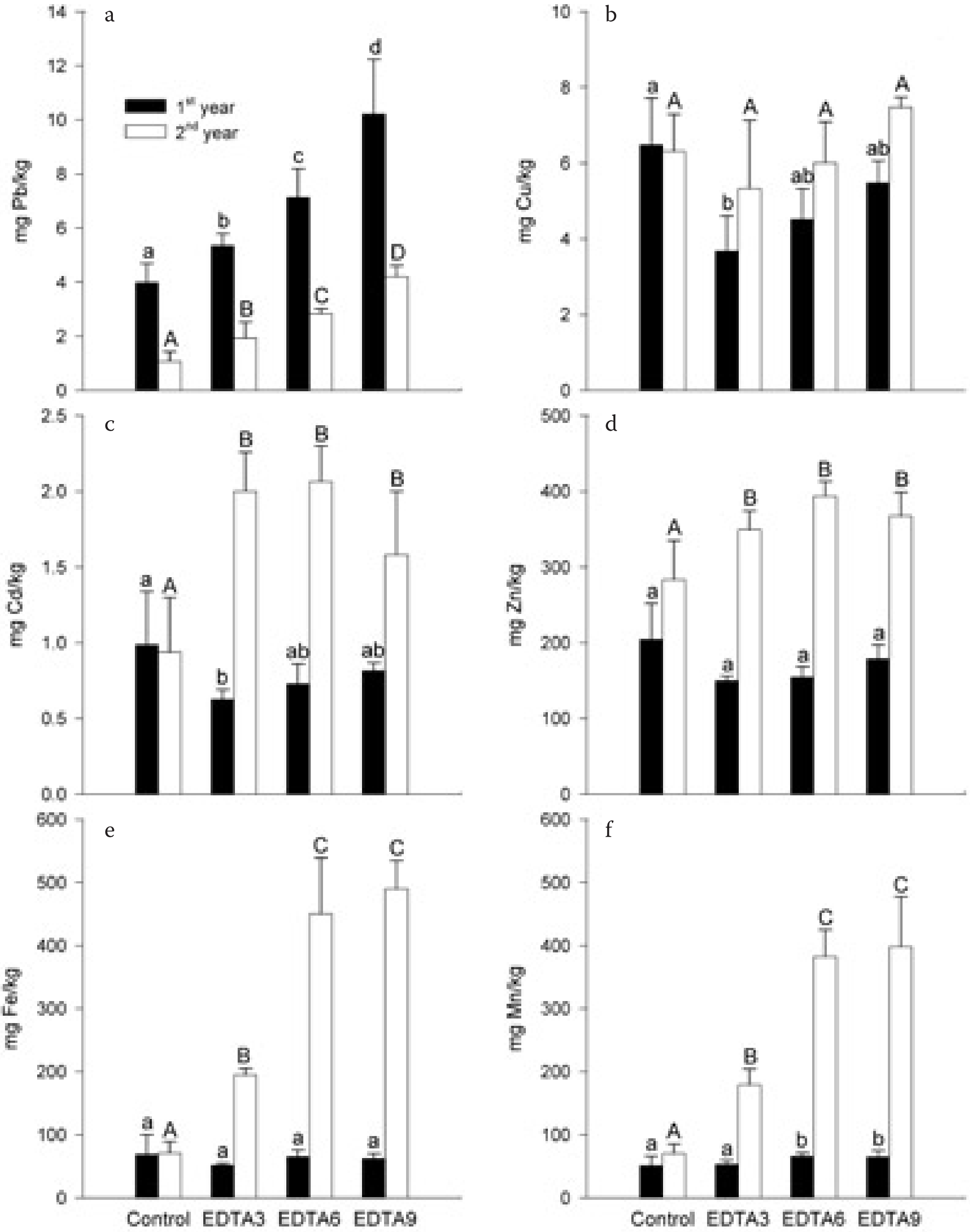

Figure 4. Contents of Pb, Cd, Cu, Zn, Fe and Mn in maize plants after the addition of 0 (Control), 3 × 1 (EDTA3), 3 × 2 (EDTA6) and 3 × 3 (EDTA9) mmol EDTA/kg. Data shown are means ± SD ($n = 4$). Data with the same letter represent statistically identical values ($P < 0.05$)

Fe and Mn accumulation was observed after the second year. This suggests that the dissolution of Fe and Mn oxides has played an important role during the two-year phytoextraction process and thus has limited the phytoextraction efficiency of *Z. mays*, especially for Pb (Figures 2 and 4a, e, f). Additionally, not only free EDTA, but even metal-chelant complexes (e.g., PbEDTA) present in the soil are able to dissolve Fe-(hydr)oxides (Nowack and Sigg 1997).

The dissolution of Mn- and especially Fe-(hydr)oxides controls, to a great extent, the phytoextraction efficiency and could present a limiting factor when using EDTA as the mobilising agent during chemically enhanced phytoextraction of heavy metals. Nevertheless, chelant-enhanced phytoextraction of heavy metals remains a viable option for remediation of low to moderately contaminated soils. EDTA proved to be, in most cases, the most successful chelating agent used for enhancing the phytoextraction of Pb. However, the toxicity of free EDTA and the persistence of the metal-EDTA complexes are the main drawbacks associated with the use of this chelating agent in chemically enhanced phytoextraction of heavy metals. Therefore, more research needs to be focused on the use of environmentally friendlier and less toxic chelating agents, such as biodegradable EDDS (ethylenediaminedisuccinic acid).

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