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Uptake of Metals during Chelant-Assisted Phytoextraction with EDDS Related to the Solubilized Metal Concentration

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The use of chelants to enhance phytoextraction is one method being tested to make phytoextraction efficient enough to be used as a remediation technique for heavy metal pollution in the field. We performed pot experiments with sunflowers in order to investigate the use of the biodegradable chelating agent SS-EDDS for this purpose. We used singly and combined contaminated soils (Cu, Zn) and multimetal contaminated field soils (Cu, Zn, Cd, Pb). EDDS (10 mmol kg^{-1} soil) increased soil solution metals greatly for Cu (factor 840−4260) and Pb (factor 100−315), and to a lesser extent for Zn (factor 23−50). It was found that Zn (when present as the sole metal), Cu, and Pb uptake by sunflowers was increased by EDDS, but in multimetal contaminated soil Zn and Cd were not. EDDS was observed in the sunflower roots and shoots at concentrations equal to metal uptake. The different metal uptake in the various soils can be related to a linear relationship between Cu and Zn in soil solution in the presence of EDDS and plant uptake, indicating the great importance of measuring and reporting soil solution metal concentrations in phytoextraction studies.

Introduction

Phytoextraction is seen as a cost-effective, environmentally friendly in-situ remediation technique, which strives to maintain soil fertility and structure (*1*). Because many plants are lacking in the ability to extract Cu and Pb, a lot of attention has been focused on the induced accumulation of metals using chelants by high biomass plants (*2*−*4*). EDTA has been the most commonly used chelating agent for this purpose (*5*−*11*). Because EDTA is rather recalcitrant in the environment (*12*) and is able to increase metal mobility, it is not suitable for chelant-assisted phytoremediation (*13*).

(*S*,*S*)-*N*,*N*′-ethylenediamine disuccinic acid (EDDS) is a structural isomer of EDTA but is easily biodegradable (*14*, *15*). A few studies have recently been carried out using EDDS for chelant-assisted phytoextraction in pot or column experiments, mainly for Pb (*16*−*20*), but also for Cu, Zn, and Cd (*19*−*22*). We have shown previously that EDDS increases the uptake of Pb from hydroponic solution but this was not the case for the essential metals Cu and Zn (*23*). EDDS was also found to be better than EDTA at solubilizing Cu and Zn from soils at pH 7 at equimolar ratios of chelating agent to metals (*24*).

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EDTA and EDDS have been measured in plant roots, shoots, and xylem sap either as individual complexes (*5*, *25*, *26*) or as total compound (*6*, *23*, *27*). It has been proposed that chelates pass through the roots to the xylem via a fully apoplastic pathway (*5*, *28*, *29*) through the root free space (*30*). Chelants can cross the barrier of the Casparian strip where it is not fully formed at the root tip, damaged by lateral roots or through passage cells, to reach the xylem and from there to be transported to the shoots (*28*, *31*, *32*).

If chelants are taken up by this mechanism then, as it is passive in nature and driven by transpiration, the relationship between soil solution metal (complexed to chelants) and shoot metal uptake should be linear. This has indeed been found for Pb (*7*, *9*), whereas Lai and Chen (*33*) observed a nonlinear relationship for Pb and Zn.

The soil solution concentration of complexed metals is therefore an important parameter to be measured and reported in chelant-assisted phytoextraction studies. However, this has only occasionally been done (*5*, *7*, *9*, *33*). Although some work has been carried out with EDDS previously, either no measurement of soil solution metals has been taken (*16*−*20*, *22*), or when taken, they were not linked to the shoot metal concentrations (*21*).

The aim of this work was to investigate if EDDS could be used for chelant-assisted phytoextraction and to investigate the link between soil solution and shoot metal concentration. Sunflowers were used because they are a high biomass plant with reported metal tolerance and accumulation potential in the field (*34*). Given the literature reviewed it was expected that EDDS would be taken up by the plants and that the metals would be increased in plant shoots by the addition of EDDS to soil as a linear function of the solubilized metal concentration.

Materials and Methods

Soils. A moist (84% dry weight (DW)) loamy topsoil from an agricultural field (Soil 1) was 2 mm sieved, and for each treatment 20 kg (DW) of soil was contaminated with either Cu, Zn, or both. For the Cu treatment 9.02 g Cu(II)O powder was added to the soil and thoroughly mixed to give an addition of 360 mg kg^{-1} Cu. The Zn treatment had 13.02 g Zn(II)O powder added (530 mg kg^{-1} Zn) and the ZnCu had both Cu(II)O and Zn(II)O added together in the quantities mentioned before. The soils were then stored in closed plastic containers at 15 °C in a dry place for 7 months. Periodically the soils were mixed and samples were taken to assess the 0.1 M $NaNO_3$ extractable metals. During the whole time period the extractable Cu hovered around the detection limit (0.25 mg kg^{-1}). The extractable Zn decreased over time. After day 137 it remained constant at 2.8−3.2 mg kg^{-1} (about 40% of the original value). The characteristics for Soil 1 and the different treatments are shown in Table 1S in the Supporting Information.

Three field contaminated soils were taken from contaminated sites in northwest Switzerland. The soil characteristics are shown in Table 1S. Soils 2 and 3 were cultivated soils which had been contaminated with Cu, Zn, and Cd from an adjacent brass smelter. Soil 4 was a topsoil taken from an agricultural field contaminated with Zn, Pb, and Cd from sewage sludge applications. All soils were dried at 40 °C and sieved to <2 mm prior to use.

Experimental Setup. A disk of fine nylon mesh (60 μm) was placed in the bottom of each pot and 1 kg (DW) of soil was added to each pot. A Rhizon Flex soil moisture sampler (Rhizosphere Research Products, Wageningen, Netherlands) was placed through the soil at a 45° angle. The soil was

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fertilized with 130 mg N kg^{-1} soil (NH_4NO_3), 164 mg kg^{-1} K (KH_2PO_4), 130 mg kg^{-1} P (KH_2PO_4), and 42 mg kg^{-1} Mg ($MgSO_4 \cdot H_2O$) given in the form of 100 mL of nutrient solution per pot. Each pot was moistened to about 60% of the water holding capacity (WHC) by adding ultrapure water (Millipore, Bedford, MA). The pots were kept at about this water content throughout the experiment using ultrapure water. Any drainage water was added back to the pots. The experiment was carried out in a growth chamber on a 16 h (21 °C)/8 h (16 °C) day/night cycle with a light intensity at leaf height of 10900 Lux.

For each treatment and soil type, 6 replicate pots were planted with sunflowers *Helianthus annuus* (cv Iregi) and 6 replicates were left bare. In each planted pot 5 seeds were planted initially. One week after germination they were thinned to 1 seedling per pot. After 3 weeks of growth (4 weeks after planting) 200 mL of either ultrapure water (3 replicates) or EDDS (pH 7.12, 50 mM, to give 10 mmol kg^{-1}) (3 replicates) was added to each pot (bare and planted). After 24 h a 20 mL sample of soil solution was extracted from the bare pots via the Rhizon Flex samplers. After 3 more days (total 4 days) 100 mL water was added to the bare pots and after 2 h the soil solution was again extracted. Planted pots were watered according to the needs of the plants, i.e., as much as necessary to keep the soil moist. Samples were refrigerated until analysis.

The plants were harvested 5 days after the addition of EDDS by cutting the stem 1 cm above the soil.The shoots were washed with deionized water and dried at 40 °C. The soil from the planted pots was sieved (2 mm) in order to collect the roots. The roots were well-washed with deionized water and dried in the same manner as the shoot samples. The oven-dried plant material was ground in a titanium mill.

Metal and EDDS Analysis. Plant samples were microwave digested in 5 mL of HNO_3 (65%), 2 mL of H_2O_2 (30%), and 2 mL of H_2O, and diluted to 25 mL. Digests and soil solution samples were analyzed for metals by Flame-AAS (Varian, SpectraAA 220FS) and GF-AAS (Varian, SpectraAA 300 with GTA96) (Soil 1) or by ICP-OES (Varian, Vista-MPX CCS simultaneous) (Soil 2−4). Soil solution results were normalized to a gravimetric soil water content of 30%. This was done to be able to compare soil solution results from the different soils which had different water holding capacities and because in the planted pots it was difficult to control soil water content due to plant water consumption. The water content of the planted pots was calculated using the fresh weight of the plants at harvest on day 5 (water content = total weight of soil − dry weight of soil − plants).

$NaNO_3$ extractable soil metal concentrations were determined by extraction of the soil for 2 h by 0.1 M $NaNO_3$ followed by vacuum filtration (cellulose acetate filter, 0.45 μm) (*35*). Total metal analysis of the soil was carried out by X-ray fluorescence spectroscopy (Spectro X-lab 2000, Germany).

For EDDS analysis the dried plant material was extracted with pure water (10 mg/10 mL) by sonication with a microtip sonic probe for one minute. The samples were then centrifuged and filtered (nylon syringe filter, 0.45 μm). EDDS derivatization and analysis was carried out as described by Tandy et al. (*36*). This method involves the derivatization of EDDS by FMOC (fluorenylmethyl chloroformate, puriss, Fluka) followed by separation by HPLC (Jasco PU-980) and fluorescence detection (Jasco 821-FP).

We have determined the speciation of EDDS for day 4 of the experiments with Soil 1. For speciation of EDDS we assumed that all Cu, Zn, and Fe in solution was complexed with EDDS. This is justified because speciation calculations have shown that in the presence of excess EDDS at the pH value found in our solution the free metal ion concentration will be very low.

Calculation of Shoot Metal Uptake after EDDS Addition. For comparing shoot metal and EDDS uptake after the addition of EDDS, the metal uptake during exposure to EDDS was calculated by subtracting the pre EDDS addition metal uptake from the total metal content. To do this we summed up the concentrations of Cu, Zn, and Fe (Soil 1) and Cu, Zn, Pb, Cd, Ni, and Fe (Soils 2−4) in the plant shoots for each treatment. Eighty % of the value from the treatments in which only water was added (H_2O treatments) was then subtracted from the corresponding EDDS treatment. Only 80% was subtracted because the plants were grown for 26 days in total, 21 days prior to the addition of EDDS and 5 days afterward. We have therefore assumed a linear increase in Cu and Zn concentration with time which has been found to be the case in sunflower shoots and leaves grown in polluted soils (*37*).

For investigating the mechanism of metal uptake in the presence and absence of EDDS, the shoot metal uptake for the 5 days after EDDS addition was used. For EDDS treatments it was calculated as above but using individual metal values rather than the sum of the different metals. For the H_2O treatments 20% of the summed metal value was considered as uptake in the last part of the experiment.

Chemicals. All chemicals were obtained from Merck unless stated otherwise and were analytical grade or HPLC grade for the solvents. SS-EDDS (Octaquest E30) was obtained from Octel, Cheshire for the experiments and from Procter and Gamble (Belgium) as the Na_3EDDS salt for the EDDS analysis. All solutions were made with high-purity water (Millipore, Bedford, MA).

Statistical Analysis. All statistical analyses were carried out with Systat 10.2 (*38*). ANOVA was carried out on log transformed data except for dry weight analysis. Differences were considered significant if $p = <0.05$.

Results

Plant Dry Weight. Plant shoots showed adverse effects to the addition of EDDS. Two days after the addition of EDDS to the soil the shoots started to show signs of toxicity and by 3 days they were necrotic. EDDS also seemed to reduce the shoot dry weight (Table 2S, Supporting Information). In previous hydroponics investigations no signs of toxicity were seen for 500 μM EDDS (*23*). In the pot experiments the concentrations of EDDS in soil solution were around 25 mM, about 50 times greater than in the hydroponics experiment. Necrosis and loss of dry weight has also been noted previously for smaller additions (5 mmol kg^{-1}) of EDDS (*20*) and EDTA (*17*, *18*, *27*).

The influence of initial soil pollution on plant dry weight was evident. Shoot dry weight was not adversely affected by the Cu treatment and showed only a small reduction in the Zn treatment compared to the control (Table 2S, Supporting Information), but was greatly reduced in the ZnCu treatment ($p = 0.002$). The root dry weight showed a similar trend (Table 2S, Supporting Information). Both shoot and root dry weights were much smaller on the heavily contaminated Soils 2 and 4 than on the lightly contaminated Soil 3 ($p = <0.001$). We suspect that the high availability of Zn (Table 3S, Supporting Information) was the cause for the low growth of sunflowers in Soils 2 and 4.

Solubilized Metals. Soil solution Cu and Zn were both significantly increased 1 day (Figure 1a and b) and 4 days after the addition of 10 mmol kg^{-1} EDDS to Soil 1 ($p = <0.001$). Soil solution was only extracted from the bare pots because it was not possible to extract solution from the planted pots after 1 day due to water consumption of the plants. In the second sampling 3 days later, it was possible to extract some solution from some planted pots 2 h after adding water to the soil. The soil solution metal concentrations from these planted and the bare pots were compared using ANOVA.

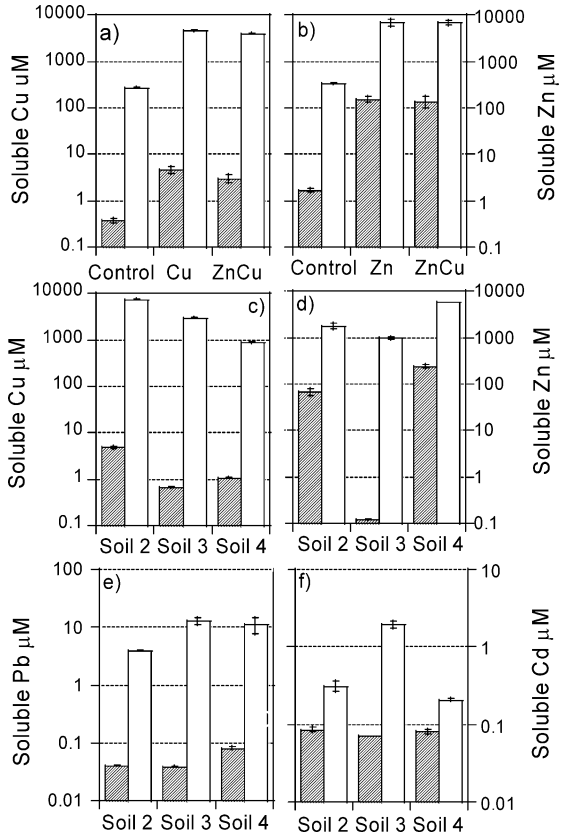

FIGURE 1. Soil solution concentration of heavy metals. Soil 1 Cu (a) and Zn (b) and Soils 2−4 Cu (c), Zn (d), Pb (e), and Cd (f), 24 h after addition of H_2O (diagonally striped bars) or EDDS (white bars). Error bars are standard errors.

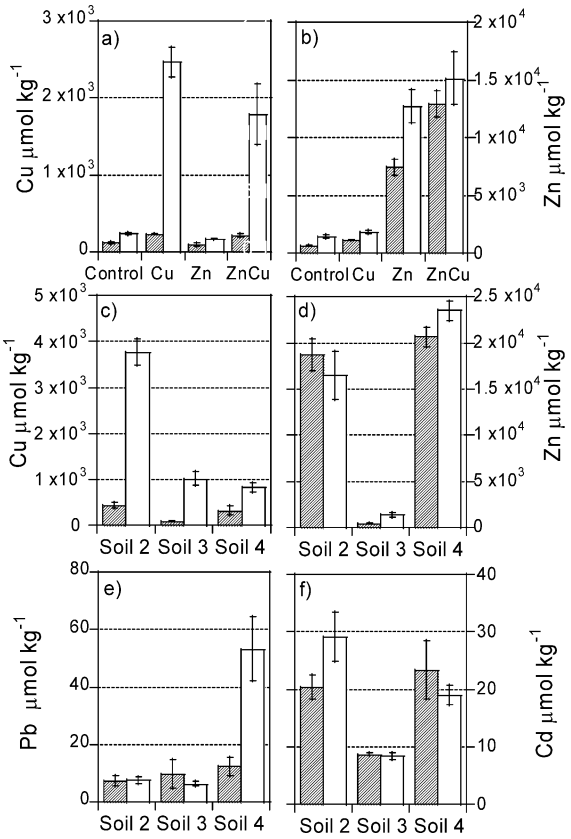

FIGURE 2. Shoot uptake in Soil 1 of Cu (a) and Zn (b) and in Soils 2−4 of Cu (c), Zn (d), Pb (e), and Cd (f), in the absence (diagonally striped bars) and presence (white bars) of EDDS. Error bars are standard errors.

Only the Cu concentrations differed significantly between bare and planted pots for the control and EDDS treatments. All other treatments and metals proved to be not significantly different due to the large variation among replicate samples. In light of this, we feel the use of the soil solution from the bare pots for data analysis is justified.

The addition of EDDS to soil dramatically enhanced the soil solution concentrations for Cu (factor 840−4260), Zn (factor 23−50 for Soils 1, 2, and 4, factor 8000 for Soil 3), Pb (factor 100−315), and Cd (factor 2.5−38) (Figure 1). The pH of soil solutions in the EDDS treatments was around neutral for Soil 1 and 3 and significantly more acidic (pH 5.2−5.5) for soils 2 and 4 (Table 4S, Supporting Information).

Plant Metal Uptake. Shoot Cu uptake was significantly enhanced by EDDS in all soil 1 treatments but especially in the Cu (factor 11) and ZnCu (factor 8) treatments ($p = <0.001$) (Figure 2a). Cu uptake from the ZnCuEDDS treatment was less than from the CuEDDS treatment. Zn shoot uptake was only enhanced by EDDS in the ZnEDDS treatment (factor 1.7) ($p = 0.035$) and was not significantly enhanced ($p = 1.0$) in the ZnCuEDDS treatment compared to the respective metal only treatments (Figure 2b). In the absence of EDDS, Zn uptake from the ZnCu treatment was much greater than from Zn treatment, while in the presence of EDDS Zn uptake in these two treatments was not significantly different ($p = 1.0$).

In the field contaminated soils shoot Cu was enhanced by EDDS in all treatments (Figure 2c). Zn shoot uptake was not increased in the presence of EDDS in soils that were heavily contaminated with Zn. Uptake was only enhanced in Soil 3, which was lightly contaminated (factor 3, $p = <0.001$). Pb shoot uptake was increased by EDDS (factor 4.3, $p = 0.025$) in Soil 4, the only soil substantially contaminated with Pb (Figure 2e). Cd shoot uptake was not significantly enhanced in the presence of EDDS (Figure 2f).

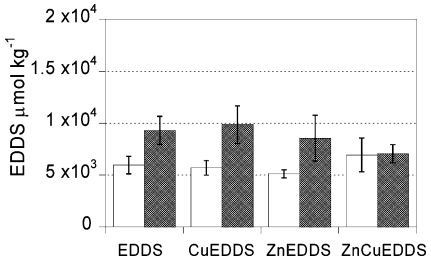

FIGURE 3. EDDS uptake in shoots (white bars) and roots (diamond bars) from Soil 1. Error bars are standard errors.

Metal concentrations in the roots were generally not influenced by the addition of EDDS to soil (Table 5S, Supporting Information).

EDDS Uptake. EDDS was detected in shoots and roots of sunflowers from all treatments where EDDS was added to the soil. The concentrations of EDDS in the shoots and roots were not significantly different between treatments, but in most cases root EDDS was greater than shoot EDDS (Figure 3).

Metal Uptake Versus EDDS Uptake. In the Soil 1 control treatment with EDDS, more EDDS was present in the shoots than metals (Figure 4a). For the CuEDDS and ZnCuEDDS treatments equal amounts of metals were found in the shoots compared to EDDS. This was the same as seen for Cu and Zn in hydroponics experiments (*23*). In all field contaminated soils roughly equal quantities of metal and EDDS were taken up (Figure 4b).

Speciation of EDDS. EDDS not complexed to Cu, Zn, and Fe(III) ranged between 35 and 70% in Soil 1 (Table 1). Model calculations by Hauser et al. (*24*) have shown that Ca, Mg, and Mn are the main additional ions that complex EDDS in

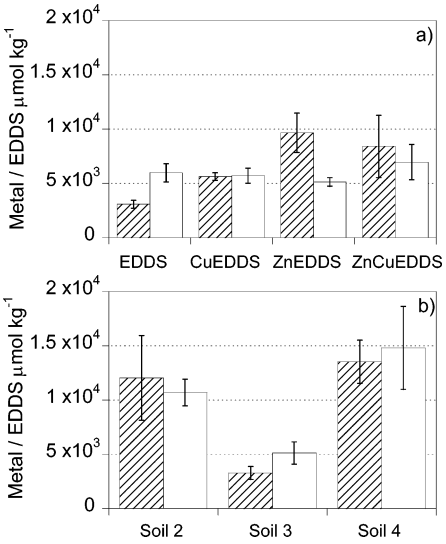

FIGURE 4. Metal (diagonally striped bars) and EDDS (white bars) uptake in shoots from EDDS treatments after the addition of EDDS: (a) Soil 1, (b) Soils 2−4. Error bars are standard errors.

TABLE 1. Speciation of Soil Solution from Soil 1, Taken 4 days after EDDS Addition[a]

treatment	"free" EDDS[b]	CuEDDS	ZnEDDS	FeEDDS
EDDS	70 ± 2.9	2.1 ± 0.5	2.5 ± 0.4	25 ± 2.0
CuEDDS	56 ± 7.6	31 ± 7.2	1.2 ± 0.2	12 ± 0.8
ZnEDDS	35 ± 1.2	1.7 ± 0.1	50 ± 0.8	14 ± 0.4
ZnCuEDDS	38 ± 1.5	27 ± 2.8	28 ± 3.2	7.2 ± 0.8

[a] Values represented as a percentage of the total EDDS concentration. [b] Free EDDS and complexes with Ca, Mg, and Mn.

addition to heavy metals. The EDDS treatment had the largest free EDDS concentration, followed by the CuEDDS treatment, and then the treatments containing Zn. CuEDDS ranged between 27 and 31% in the treatments with added Cu and 1.7 and 2.1% in the non-Cu treatments. Likewise ZnEDDS was 1.2−2.5% in non-Zn treatments. ZnEDDS was greater in the ZnEDDS treatment (50%) than in the ZnCuEDDS treatment (28%). FeEDDS decreased with increasing heavy metal contamination.

Relationship Soil Solution to Plant Uptake. Figure 5 shows the relationship between metal uptake by sunflowers and the solubilized metal concentration in soil solution. Dissolved Cu in the absence of EDDS was very low and a clear linear relationship was observed in the presence of EDDS ($R^2 = 0.98$) (Figure 5a). Shoot accumulation of Zn as a function of total dissolved metals was clearly reduced in the presence of EDDS and again a linear relationship was observed, although the larger variation of the Zn-uptake reduced the correlation coefficient ($R^2 = 0.88$) (Figure 5b). Calculations were also carried out to see the effect of the calculated metal uptake value prior to EDDS addition on this relationship. In this 70 or 90% of the H_2O treatment values were subtracted from the EDDS treatment values instead of 80%. For Cu there was virtually no change in slope or R^2 value compared to results for the original calculations. For Zn there was a slope change of about 20% but the R^2 values were still around 0.8, so the relationship remained linear. Uncertainties in metal uptake prior to the EDDS addition have therefore no influence (for Cu) or only a slight influence (for Zn) on the result.

Discussion

EDDS Uptake. The measurement of EDDS in roots and shoots clearly proves that substantial uptake of EDDS had taken

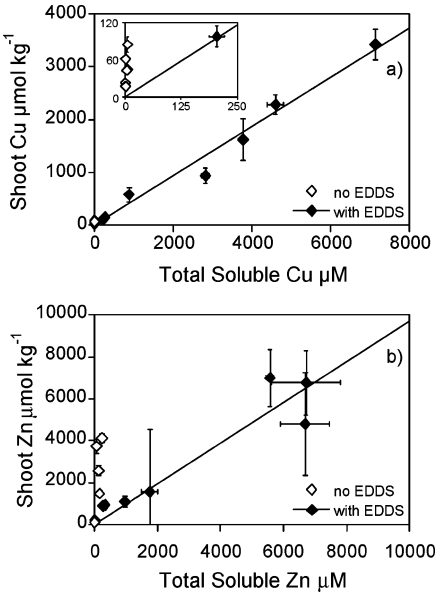

FIGURE 5. Uptake of Cu (a) and Zn (b) into the shoots in the absence and presence of EDDS related to the solubilized metal concentration in soil solution.

place. Whereas the root EDDS concentrations were similar to that observed in hydroponics (about 4000 μmol/kg) (*23*), the shoot concentration in the pot was about 30 times higher than in hydroponics. However, the EDDS concentration in the soil solution was also about a factor of 50 higher in the pot experiments. This suggests that shoot EDDS uptake increases in proportion to the dissolved EDDS whereas root uptake does not. One possible explanation for the root behavior might be that EDDS is absorbed to the roots and has already become saturated at lower concentrations of EDDS.

The fact that accumulation of metals and EDDS in the shoots was approximately equal indicates that metals were taken up in the complexed form or that all EDDS was complexed once inside the plant. At the pH of our experiment free EDDS and divalent cation−EDDS complexes possess the same charge (minus 2) and therefore should behave in the same way. In soil solution, 35−70% of the EDDS was not bound to Cu, Zn, or Fe. This means that extra metals must have been sequestered and transported to the location where complexation took place. This could have come from the metals adsorbed and taken up into the roots in the time before EDDS was added which were then complexed by EDDS within the roots and taken up as metal complexes. Alternatively, trace levels of free Cu, Zn, or Fe in the soil solutions could have been taken up very efficiently by a selective uptake mechanism and then complexed by free EDDS in the plant.

Only in the EDDS-only control treatment of Soil 1 was the metal uptake less than the EDDS uptake. The majority of EDDS in solution of that treatment was uncomplexed due to the low levels of metals in the soil.

Metal Uptake in the Presence of EDDS. The linear relationship between metal uptake in the presence of chelant and soil solution concentrations for Cu and Zn is a new finding for EDDS. Although one EDDS study carried out soil solution metal analysis, they did not make the link directly to plant uptake (*21*). One previous study found a nonlinear relationship between Zn shoot content and soluble Zn in the presence of EDTA. However the shoot concentration was not corrected for Zn taken up before the addition of EDTA to soil (*33*). Two other studies have also found linear relationships between Pb uptake and soil solution concentrations in the presence of chelants (*7*, *9*). This linearity and the presence of EDDS in the sunflower shoots are evidence that the chelates are

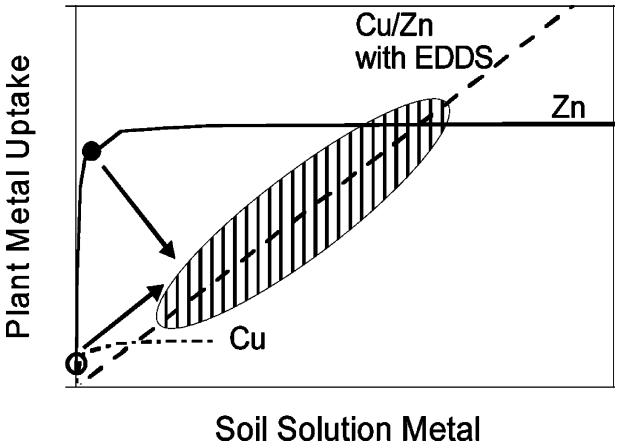

FIGURE 6. Schematic illustrating how the effects of EDDS on Cu and Zn uptake into plant shoots can be explained by the change from selective uptake of metals to nonselective uptake of metal−EDDS complexes. Solid line, selective uptake of Zn; dot−dash line, selective uptake of Cu; dashed line, unselective uptake of Cu and Zn bound to EDDS. The black and open circles represent solution concentrations of Zn and Cu, respectively, in the absence of EDDS, and the shaded area represents metal concentrations in the presence of EDDS.

indeed taken up by the passive apoplastic pathway into the xylem and are then transported to the plant shoots.

The different relationships strongly suggest that the uptake of essential metals in the absence and presence of chelating agents is dominated by different mechanisms. The effects of chelants such as EDDS on metal uptake can therefore be explained by a shift of the main transport route from the symplastic pathway (selective uptake) to the apoplastic pathway (nonselective uptake) (*5*, *28*, *29*).

Figure 6 shows a schematic illustrating this hypothesis for the uptake of Cu and Zn. In the absence of EDDS uptake mainly occurs by a selective uptake mechanism. By adding EDDS, the Zn soil solution concentration is increased but the plant uptake decreases. This is because the free Zn concentration decreases and thus selective uptake is greatly reduced and the unselective uptake of metal−EDDS complexes (dashed line) is less efficient than the selective mechanism. Cu uptake in the absence of EDDS (dash−dot line), although selective, is less efficient than that for Zn. By adding EDDS the solution concentration increases to a level above the intercept of the two lines representing the two different mechanisms of Cu uptake. This means that shoot Cu uptake in the presence of EDDS is greater than that in the absence of EDDS. Another factor adding to this situation is that the soil solution concentration of Cu in the absence of EDDS (dot on the Cu line) is much less than that for Zn, making the increase in solution on the addition of EDDS greater than for Zn.

Factors Influencing Chelant-Assisted Phytoextraction. Obviously speciation of metals and competition for the chelants in the soil solution play an important role in determining the uptake of contaminating metals from multi-metal contaminated soils. The strength of the metal complexes of the chelants and the composition of the soil determine the speciation of the chelant in soil solution, which in turn is related to the metals taken up. In multi-metal contaminated soils solubilized metal concentrations can be very different from those in singly polluted soils.

The CuEDDS complex is very strong (log K 18.4 (*39*)) and not much competition with other metals is observed. Our results for Cu uptake are indeed equal or better than those of most other studies (*21*, *22*, *34*). It has previously been shown that EDDS is better in solubilizing Cu around neutral pH than other chelants due to weak competition with Ca (*40*).

ZnEDDS is weaker (log K 13.4 (*39*)) than CuEDDS and therefore competition starts to play a role. For Zn we obtained a lower increase on the addition of EDDS than in other studies. In these other EDDS studies total soil Zn was always much greater than soil Cu with which it must compete for EDDS and which has a higher stability with EDDS.

PbEDDS is quite a weak complex (log K 12.7 (*39*)) compared to PbEDTA (log K 18.0 (*39*)) and much more competition is expected. Our Pb accumulation is indeed lower than that in soils treated with EDTA (*7*, *11*) and also other EDDS experiments (*16*−*20*). Our Pb contaminated soil was contaminated with Zn to a similar degree, while other EDDS studies and most studies on phytoextraction of Pb used soil that had Pb as the main contaminant. In our soil Pb mobilization was limited due to competition of Zn and Pb for EDDS. This shows that without knowing the actual solubilized metal concentration, studies with different soils cannot be directly compared.

A hurdle for phytoremediation might be the growth of the plant before and after chelant addition. High concentrations of chelants are known to decrease the growth of plants (*17*, *18*, *20*) and this was also observed in our study. However, this need not be a problem if they are added shortly before harvest. On the other hand, a high biomass at the point of chelant addition is important to provide a high transpirational flow for chelate uptake. The high bioavailability of metals in our multi-metal contaminated soils limited plant growth. This shows that only moderately contaminated soils or soils with low bioavailability of the contaminating metals could be decontaminated in this way.

Acknowledgments

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Note Added after ASAP Publication

This article was released ASAP on March 14, 2006 with errors in the author affiliation. The correct version was posted on March 15, 2006.

Supporting Information Available

Tables of initial soil characteristics; shoot and root biomass of sunflowers; inital soil "available" Cu and Zn concentrations; soil solution pH of EDDS treatments 4 days after EDDS addition, bare pots; root metal concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration

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Table 1S. Initial soil characteristics.

Soil	Soil type	Treatment	pH ($CaCl_2$)	C_{org} %	$CaCO_3$ %	Cu mg kg^{-1}	Zn mg kg^{-1}	Pb mg kg^{-1}	Cd mg kg^{-1}	Ni mg kg^{-1}
1	Luvisol	**Control**	6.42	0.94	0.16	26	94	30	< 0.5	25.1
1		**Cu**	6.38	0.94	0.16	430	94	29	< 0.5	25.9
1		**Zn**	6.30	0.94	0.16	25	596	28	< 0.5	24.8
1		**ZnCu**	6.32	0.94	0.16	409	594	28	< 0.5	26.4
2	Eutric Regosol		5.65	2.73	0.48	537	786	66	2.07	46
3	Calcaric Regosol		6.87	1.79	1.74	111	191	39	0.53	32
4	Haplic Luvisol		5.07	1.24	0.49	52	515	386	0.7	21

Table 2S. Shoot and root biomass of sunflowers.

Soil	Treatment	Shoot dry weight (g) ± SE	Root dry weight (g) ± SE
Control (1)	H_2O	7.50 ± 0.96	0.24 ± 0.43
Control (1)	EDDS	4.52 ± 0.46	0.66 ± 0.20
Cu (1)	H_2O	8.00 ± 0.58	2.05 ± 0.04
Cu (1)	EDDS	4.76 ± 0.11	0.78 ± 0.04
Zn (1)	H_2O	4.69 ± 1.70	1.04 ± 0.20
Zn (1)	EDDS	2.82 ± 0.39	0.60 ± 0.06
ZnCu (1)	H_2O	1.59 ± 0.29	0.58 ± 0.14
ZnCu (1)	EDDS	1.44 ± 0.46	0.46 ± 0.16
2	H_2O	0.44 ± 0.22	0.14 ± 0.06
2	EDDS	0.53 ± 0.10	0.14 ± 0.03
3	H_2O	8.32 ± 0.89	2.52 ± 0.12
3	EDDS	5.24 ± 0.73	1.57 ± 0.30
4	H_2O	0.47 ± 0.32	0.22 ± 0.15
4	EDDS	0.36 ± 0.12	0.10 ± 0.02

Table 3S. Initial soil 'available' (0.1M $NaNO_3$ extractable) Cu and Zn concentrations

Soil	Cu mg kg^{-1}	Zn mg kg^{-1}
Control (1)	<0.25	<0.25
Cu (1)	0.32	<0.25
Zn (1)	<0.25	3.15
ZnCu (1)	0.28	2.84
Soil 2	1.3	16.66
Soil 3	0.56	<0.25
Soil 4	<0.25	19.34

Table 4S. Soil solution pH of EDDS treatments 4 day after EDDS addition, bare pots.

Soil	Treatment	pH
Control (1)	EDDS	6.56 ± 0.08
Cu (1)	EDDS	6.20 ± 0.03
Zn (1)	EDDS	6.46 ± 0.07
ZnCu (1)	EDDS	6.21 ± 0.20
Soil 2	EDDS	5.48 ± 0.09
Soil 3	EDDS	7.36 ± 0.01
Soil 4	EDDS	5.28 ± 0.13

Table 5S. Root metal concentrations.

Soil	Treatment	Cu mg/kg ± SE	Zn mg/kg ± SE	Pb mg/kg ± SE	Cd mg/kg ± SE
Control (1)	H_2O	219 ± 26	884 ± 110	—	—
Control (1)	EDDS	326 ±9	1675 ± 199	—	—
Cu (1)	H_2O	3747 ± 265	926 ± 73	—	—
Cu (1)	EDDS	5581 ± 572	1290 ± 36	—	—
Zn (1)	H_2O	283 ± 14	12549 ± 2455	—	—
Zn (1)	EDDS	272 ± 18	13222 ± 810	—	—
ZnCu (1)	H_2O	2941 ± 220	10444 ± 1102	—	—
ZnCu (1)	EDDS	2883 ± 292	9872 ± 344	—	—
2	H_2O	6531 ± 855	18074 ± 695	55 ± 6.7	74 ± 5.4
2	EDDS	7180 ± 889	12093 ± 644	54 ± 4.5	72 ± 9.4
3	H_2O	1023 ± 105	1048 ± 169	21 ± 4.0	< 8.8 ± 0.05
3	EDDS	1975 ± 240	2338 ± 123	34 ± 2.0	24 ± 1.2
4	H_2O	869 ± 102	22975 ± 3474	483 ± 36	< 79 ± 32
4	EDDS	2102 ± 577	15558 ± 2901	773 ± 13	< 61 ± 17