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INFLUENCE OF [S, S]-EDDS ON PHYTOEXTRACTION OF COPPER AND ZINC BY *ELSHOLTZIA SPLENDENS* FROM METAL-CONTAMINATED SOIL

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*Two pot experiments were conducted to investigate the time course effects of the (S, S)-N, N'-ethylenediamine disuccinic acid (EDDS) addition to contaminated soil on the uptake of Cu and Zn by the Cu accumulator *Elsholtzia splendens* and on plant Cu and Zn concentrations at different growth stages. EDDS increased the amounts of Cu and Zn soluble in the soil, taken up by plants, concentrated in the xylem sap, and translocated from roots to stems and leaves. The increase in soil-soluble metals, especially Cu, resulted in a corresponding increase in metal concentrations in the xylem sap and leaves. The addition of EDDS to the soil increased plant Cu and Zn concentrations, especially in the leaves, and changed the proportions of Cu and Zn taken up by different plant parts. The proportions of Cu and Zn taken up by the roots were higher than by the leaves of control plants, but EDDS-treated plants showed the opposite trend. EDDS exerted greater effects at the end of the vegetative growth stage than at the start of the flowering or reproductive stages.*

KEY WORDS ethylenediamine disuccinic acid (EDDS), *Elsholtzia splendens*, growth stage, Cu uptake, Zn uptake

INTRODUCTION

Copper is an essential transition metal that is involved in many physiological processes in plants. The biochemical toxicity of Cu is derived from its effects on the structure and function of biomolecules such as DNA and on membranes and proteins, either directly or through oxygen-radical mechanisms. The major sources of Cu release to the terrestrial environment are mining operations, agriculture, solid waste, and sludge from wastewater treatment works (IPCS, 1998). Zn can also show toxicity when present at high concentrations (Kamal *et al.*, 2004; Nahmani and Lavelle, 2002).

Most of the Cu and Zn deposited in soils is adsorbed to iron and manganese oxides, organic matter, or carbonate minerals. Relatively small fractions of Cu and Zn are soluble and mobile in soil and, therefore, potentially phytoavailable; this severely limits plant uptake unless a chelant is applied. Edaphic factors influencing the fate of heavy metals in soils include soil pH, oxides, redox potential, charged surfaces, organic matter, and cation exchange capacity. Synthetic chelants have been used to artificially enhance heavy metal solubility in the soil solution from the soil solid phase and, thus, to increase heavy metal phytoavailability. The [S, S]-isomer of ethylenediamine disuccinic acid (EDDS) was isolated from culture filtrates of the actinomycete *Amicolatopsis japonicum* (Goodfellow *et al.*, 1997) and is readily biodegradable (Schowanek *et al.*, 1997). At neutral pH, the addition of EDDS gave the best compromise between extraction efficiency of Cu and Zn and loss of Ca and Fe, so that unwanted extraction of major ions from the soil was minimal (Tandy *et al.*, 2004). The applied EDDS solution extracted Cu and Zn from exchangeable, mobile, Mn oxides and organic soil fractions (Tandy *et al.*, 2004).

Chelant-enhanced phytoremediation, in which a chelant is applied to the soil to form a chelate with the target metal(s), has been proposed as an effective tool for the extraction of heavy metals from soils by non-hyperaccumulating plants. However, potential side effects related to the addition of chelants, such as metal leaching to groundwater, have led to health, safety, and environmental concerns. Many synthetic chelants and their complexes with heavy metals can have negative effects on the environment (Bucheli-Witschel and Egli, 2001) and are poorly photo-, chemo-, and biodegradable in soils (Bucheli-Witschel and Egli, 2001; Egli, 2001; Kari and Giger, 1996; Nortemann, 1999). EDDS, a structural isomer of EDTA, is a promising new alternative synthetic chelant. The [S, S]-isomer is not only readily biodegradable, but also usually performs as well as EDTA (Grčman *et al.*, 2003; Luo, Shen and Li, 2005; Meers *et al.*, 2005; Schowanek *et al.*, 1997; Tandy *et al.*, 2004; Ultra *et al.*, 2005; Vandevivere *et al.*, 2001). EDDS performs better than EDTA at solubilizing Cu and Zn from soils at pH 7 at equimolar ratios of chelating agent to metals (Hauser *et al.*, 2005). A colorimetric assay, IC-ICP-MS, GC, and HPLC are the most powerful tools to determine EDDS in soils and plants (Ammann, 2002; Knepper, 2003; Metsarinne, Tuhkanen and Aksela, 2001; Takahashi *et al.*, 1997, 1999; Tandy *et al.*, 2005; Vandevivere *et al.*, 2001).

Elsholtzia splendens is a Cu-tolerant and accumulating species that occurs in mine areas and can accumulate Cu at a concentration of $3417 \mu\text{g g}^{-1}$ in the shoots when grown in a nutrient solution containing $1000 \mu\text{mol L}^{-1}$ Cu (Jiang *et al.*, 2003; Yang *et al.*, 1998; Yang, Yang, and Römheld, 2002). However, some studies have indicated that *E. splendens* does not hyperaccumulate Cu, but instead behaves as a typical Cu excluder (Jiang, Yang, and He, 2004; Song *et al.*, 2004). Synthetic chelants may be used to facilitate the uptake of heavy metals by plants and the translocation of the metals to the aboveground parts. Applying [S, S]-EDDS to enhance Cu accumulation by *E. splendens* from soil may, therefore, be a

Table 1 Selected properties of the soil used in the pot experiments; intervals denote standard deviation (n = 3)

Physicochemical property	
pH (H ₂ O)	6.3 ± 0.0
Electrical conductivity at 25°C (ms cm ⁻¹)	466 ± 40
Total N (%)	0.2 ± 0.0
Total P (%)	0.1 ± 0.0
Total K (%)	2.4 ± 0.2
Available N (mg kg ⁻¹)	164 ± 1
Available P (mg kg ⁻¹)	28.8 ± 0.9
Available K (mg kg ⁻¹)	98.0 ± 5.7
Organic matter (%)	4.4 ± 1.0
Cation exchange capacity (cmol (+) kg ⁻¹)	12.4 ± 0.3
Total Metal Concentrations (mg kg ⁻¹)	
Cu	199 ± 6
Zn	1374 ± 44
Pb	274 ± 8
Cd	3.6 ± 0.1
NH ₄ NO ₃ -Extractable Metal Concentrations (mg kg ⁻¹)	
Cu	3.3 ± 0.1
Zn	4.5 ± 0.0
Pb	0.1 ± 0.0
Cd	0.1 ± 0.0

viable environmental technology. However, there is little information on the best growth stage of the plants at which to apply the chelant to the soil for maximum metal uptake. The aims of the present study were to identify how EDDS activates heavy metals in soil and enhances plant heavy metal uptake, and which plant growth stage is the most effective for EDDS application. Concentrations of heavy metals in the soil solution and their presence in *E. splendens* were monitored following the chelant application. The effects of EDDS addition on Cu and Zn accumulation at different plant growth stages were also compared.

MATERIALS AND METHODS

Soil Properties

The 0–20-cm surface layer of an alluvial soil (fluvio-marine yellow soil) was sampled from the suburbs of Hangzhou city, Zhejiang province, China. This site had a history of several decades of soil contamination by particulate emissions from adjacent copper smelters. Freshly collected soil was airdried and passed through a 2-mm nylon sieve before use. Selected properties of the soil analysed by standard methods (Lu, 2000) are shown in Table 1.

Plant Growth

Seeds of *E. splendens* were collected from a copper mine site in Zhuji county, Zhejiang province, east China, in December 2004. Seeds were selected for uniformity and washed several times with deionized water. They were germinated at room temperature (20–25°C) on wet filter paper. The seedlings were transplanted into pots containing soil equivalent to 1.25 kg (oven-dry basis). Basal fertilizers were applied at 460 mg N per pot

as $\text{CO}(\text{NH}_2)_2$ and 230 mg P per pot as KH_2PO_4 . Soil moisture was maintained at about 70% of water-holding capacity by the addition of deionized water after weighing. Plants were grown in a greenhouse under the following conditions: 14-h photoperiod; 25°C day/20°C night regimen, relative humidity 65–70%; illumination 60 W m^{-2} .

Experimental Setup

Experiment 1: Time course of plant metal uptake. After growth for 2 wk, the seedlings were thinned to four plants per pot. After the seedlings had grown for 4 mo, subsets of pots were allocated to each of the following treatments, with four replicate pots per treatment: 1) no EDDS applied to the soil (control); 2) 3.0 mmol EDDS (as Na_3EDDS) applied per kg soil (+EDDS). The plants were harvested and soil samples were collected 3, 7, 21, and 28 d after application of the chelant.

Experiment 2: Effects of EDDS on Cu and Zn uptake at different stages of growth. After growth for 2 wk, the seedlings were thinned to three plants per pot. On days 110 (the end of the vegetative growth stage) and 135 (the start of flowering or the reproductive stage) after transplanting, EDDS was applied in 100 ml of deionized water at a rate of 3 mmol kg^{-1} soil. There were three replicate pots of the control (Control St) and five replicates of the EDDS treatment (+EDDS St). The plants were harvested and soil samples were collected 10 d after the EDDS addition.

Collection of Xylem Sap

The stems of *E. splendens* were cut using stainless steel scissors about 1 cm above the soil surface. Xylem sap was collected between 9 AM and 3 PM, as described by Jiang and Zhu (1999), filtered immediately (nylon, 0.45 μm), and frozen at -80°C before analysis.

Soil Analysis

Soil's water-holding capacity was determined according to Lu (2000). Soil available Cu was extracted with 1 M NH_4NO_3 (Deutsches Institute für Normung [DIN], 1995). Metals in the NH_4NO_3 extracts were determined by inductively coupled plasma-atomic emission spectroscopy (IRIS Advantage, Thermo Element, Waltham, MA, USA).

Plant Chemical Analysis

The plants were separated into roots, stems, and leaves. Roots were washed sequentially in deionized water, a solution of 20 mmol L^{-1} Na_2EDTA , for 30 min to remove extra-cellular metals, and finally deionized water. Washed root, stem, and leaf samples were dried at 80°C for 48 h, ground, and digested with HNO_3 and HClO_4 . The elemental concentrations were determined by atomic absorption spectrophotometer (Varian SpectrAA 220 FS, Varian, Palo Alto, CA, USA) (Zhao, McGrath, and Crosland, 1994). Blanks and plant standard reference materials were included in each analytical batch for quality assurance.

Chlorophyll Estimation

Leaf tissues (0.25 g fresh weight [FW]) were homogenized with acetone containing 1% Tris (pH 8.0) and the chlorophyll content was determined according to Cleon (1974).

The absorbance of the extracts was measured at 663 and 645 nm using a UV-visible spectrophotometer (Helios γ , Thermospectronic, Cambridge, UK).

Extraction and Determination of EDDS

The leaf tissues were harvested, frozen at -80°C and lyophilized, ground to a powder, extracted with deionized water (2 g/10 ml) and sonicated for 2 h. The extracts were then centrifuged at 3000 rpm for 3 min at room temperature and the supernatants were removed.

The leaf extracts and xylem sap (1 ml) were derived by CuSO_4 (1 ml) to a total volume of 2 ml. CuSO_4 was used to convert all metal-EDDS complexes to Cu-EDDS for efficient detection at 254 nm. The samples were then filtered through a $0.45\text{-}\mu\text{m}$ nylon membrane filter (Nalgene, syringe filter) prior to analysis. The samples were analyzed using high-performance liquid chromatography (Agilent Model 1100, Agilent, Santa Clara, CA, USA) equipped with a UV detector at 254 nm, reverse phase Agilent-Zorbax SB-C18 ($5\text{ }\mu\text{m}$, $4.6 \times 150\text{ mm}$). The mobile phase consisted of a 0.03-M acetate buffer at pH 4.0 with 20% tetrabutyl ammonium hydroxide (40 ml/L) as a counter ion and was filtered through a $0.45\text{-}\mu\text{m}$ nylon membrane (Bergers and de Groot, 1994; Epstein *et al.*, 1999; Tandy *et al.*, 2005; Vassil *et al.*, 1998).

Statistical Analysis

Analysis of variance was performed using DPS version 3.11 (Tang and Feng, 2002). Tukey's multiple range test was used to determine the statistical significance ($p = 0.05$) of the difference between EDDS treatment and control. The relationship was assessed using the Pearson correlation test performed using the SPSS version 10.0 software package.

RESULTS AND DISCUSSION

Plant Heavy Metal Concentrations

EDDS application to the soil increased the concentrations of Cu and Zn in the leaves of the test plants in both experiments (Figure 1, Table 2). In Experiment 1, foliar Cu and Zn concentrations increased significantly about 5 times ($p < 0.05$) after EDDS treatment, while root concentrations after treatment with soil amendments did not appear to be enhanced significantly at any harvest time (Figure 1). After the addition of EDDS, the Cu and Zn concentrations in shoots (especially Cu) were increased by prolonging the exposure time of the plants to the EDDS. Three weeks after the EDDS application, its concentration appeared to decline. This may have been due to degradation of the EDDS (Tandy *et al.*, 2006), but in our experiment the apparent decline was not significant.

In Experiment 2, the efficiency of the increase in plant Cu uptake was more significant than that of Zn. Plant Cu uptake differed at different growth stages, with over $387\text{ }\mu\text{g pot}^{-1}$ in the leaves, on average, at the early stage (110 d), which was 190 times greater than that in the controls. However, only about $68\text{ }\mu\text{g pot}^{-1}$ Cu was taken up by the leaves at the later growth stage (135 d), which was 23 times greater than that in the controls (Table 2). At the later growth stage, the plants grew more slowly and the transpiration rate was lower. Thus, the effects of EDDS on plant metal uptake at the later growth stage were smaller than at the earlier stage. The EDDS addition had significant effects on foliar Cu concentrations at both growth stages as compared with the controls ($F = 131.69$, $p < 0.001$; $F = 11.189$,

Table 2 Cu and Zn concentrations and the total Cu and Zn removals by roots and aboveground parts of *E. splendens* at different times after application of EDDS to the soil

Days after sowing	Treatment	Items	Cu			Zn		
			Root	Stem	Leaf	Root	Stem	Leaf
110	Control St	Concentration (mg kg ⁻¹)	56.0 a	7.40 a	2.00 b	218 a	188 a	73.4 b
		Uptake amount (μg metal pot ⁻¹)	387 a	150 a	46.4 b	1833 a	3818 a	1727 b
		Efficiency of remediation	0.16	0.06	0.02	0.11	0.22	0.10
	+EDDS St	Concentration (mg kg ⁻¹)	20.5 a	27.7 a	387 a	148 a	196 a	232 a
		Uptake amount (μg metal pot ⁻¹)	170 a	716 a	6076 a	890 a	3176 a	3677 a
135	Control St	Efficiency of remediation	0.07	0.29	2.44	0.05	0.18	0.21
		Concentration (mg kg ⁻¹)	144 a	1.70 b	3.80 b	523 a	177 a	62.3 a
		Uptake amount (μg metal pot ⁻¹)	1107 a	62.2 a	163 b	4605 a	5836 a	2671 a
	+EDDS St	Efficiency of remediation	0.45	0.03	0.07	0.27	0.34	0.16
		Concentration (mg kg ⁻¹)	31.7 b	4.60 a	68.4 a	207 b	184 a	169 a
		Uptake amount (μg metal pot ⁻¹)	278 b	126 a	1929 a	1802 b	5282 a	4792 a
		Efficiency of remediation	0.11	0.05	0.78	0.10	0.31	0.28

Within each column, mean values followed by the same letter are not significantly different by LSD at the 5% level.

Figure 1 Concentrations of Cu and Zn in roots, stems, leaves, and xylem sap of *E. splendens* grown on contaminated soil after treatment with EDDS. Vertical bars: standard error ($n = 4$).

Figure 2 Concentrations of Cu (A) and Zn (B) in xylem sap of *E. splendens* at 110 and 135 d (Control St: n = 3; +EDDS St: n = 5).

$p < 0.001$). Furthermore, the Cu concentrations in leaves after 110 d were significantly higher than after 135 d in control plants ($F = 23.488$, $p < 0.001$), while the EDDS treatment showed the opposite trend ($F = 96.307$, $p < 0.001$). In control plants, the Cu uptake by the roots represented more than 60% of the total Cu uptake by the whole plants, but in the EDDS treatment the value was over 80% (Table 2).

Total Cu and Zn concentrations in the xylem sap increased after adding EDDS (Figures 1 and 2). The rate of increase in Cu concentration was higher than that of Zn in xylem sap (Figure 2). Leaf Cu concentrations were related to xylem Cu concentrations ($r^2 = 0.721$, $p < 0.01$, $n = 21$) in Experiment 1. The Cu and Zn concentrations in the xylem sap of EDDS-treated plants were significantly higher than control plants at the early stage (110 d) ($F = 112.154$, $p < 0.001$; $F = 98.000$, $p < 0.05$). The plants had much stronger transpiration at the earlier growth stage than later and the concentration of EDDS in the xylem sap was higher at this growth stage. The plants may have been at greater risk of injury from the high mobility of soil heavy metals, but at the later stage (135 d) EDDS had no significant effect on Cu and Zn concentrations in the xylem sap ($F = 0.998$, $p = 0.4231$; $F = .589$, $p = 0.2489$).

EDDS in Shoots

EDDS was detected in both the xylem sap and leaves of *E. splendens*. After the addition of EDDS, the concentrations of EDDS in the xylem sap decreased at later stages of growth, but foliar concentrations continued to increase (Figure 3). EDDS concentrations in the xylem sap were related to foliar EDDS concentrations ($r^2 = -0.728$, $p < 0.05$, $n = 11$). This may be explained by the EDDS concentration decreasing along with the decline in transpiration rate while the foliar EDDS concentration increasing growth period and increasing plant biomass.

The EDDS concentration in xylem sap at 110 d was higher than at 135 d, a trend similar to the foliar concentrations (Figure 4), but neither of these trends was significant ($F = 1.289$, $p = 0.3197$; $F = 2.387$, $p = 0.1830$).

Figure 3 The EDDS concentration in xylem sap (mg L⁻¹) and leaves (mg kg⁻¹ DW) after adding EDDS. Vertical bars: standard error (n = 4).

Heavy Metal Mobilization

The dynamics of Cu and Zn mobilization in soils with the addition of EDDS are presented in Figure 5. In the EDDS treatment, the quantities of Cu and Zn extracted with NH₄NO₃ were higher by about 90- and 4-fold, compared with controls at each harvest. NH₄NO₃-extractable Cu was related to xylem sap Cu concentrations and foliar Cu concentrations ($r^2 = 0.632$, $p < 0.01$; $r^2 = 0.837$, $p < 0.01$, n = 11).

In the second experiment, NH₄NO₃-extractable Cu and Zn in the EDDS treatment were significantly higher than in the controls at the early stage of growth (110d) (F =

Figure 4 The EDDS concentration in xylem sap and leaves after adding EDDS at different growth stages. Vertical bars: standard error (n = 5).

Figure 5 Soil NH_4NO_3 -extractable Cu (A) and Zn (B) concentrations. Vertical bars: standard error ($n = 4$).

180.518, $p < 0.001$; $F = 340.873$, $p < 0.001$). At the later stage (135 d) the plants were growing more slowly and would have had lower nutrient requirements, and the effect of EDDS was not significant ($F = 3.370$, $p = 0.1403$; $F = 1.252$, $p = 0.3140$) (Figure 6). NH_4NO_3 -extractable Cu was related to foliar Cu concentration ($r^2 = 0.706$, $p < 0.05$, $n = 9$).

Toxicity of Chelant or Metal–Chelate to *E. splendens*

In the first experiment, visual symptoms of toxicity were observed as necrotic lesions on leaves when chelants had been applied. EDDS treatment led to a decrease in both total chlorophyll and chlorophyll A contents, but the decrease in chlorophyll B was not significant when compared to the control (Table 3). Chlorophyll A content decreased with increasing Cu concentrations in the soil and leaves as compared to the controls. These results indicate that when the *E. splendens* plants were exposed to Cu contamination, the

Figure 6 Soil NH_4NO_3 -extractable Cu (A) and Zn (B) concentrations at 110 and 135 d. Vertical bars: standard error (Control St: $n = 3$; +EDDS St: $n = 5$).

Table 3 Effects of EDDS on mean chlorophyll content of *E. splendens* (mg g⁻¹ FW) (n = 4)

Chlorophyll	Treatment	Days after EDDS addition			
		3	7	21	28
Chl A	Control	1.56 a	1.18 a	0.988 a	1.09 a
	+EDDS	1.11 b	0.926 b	0.766 b	0.945 a
Chl B	Control	0.607 a	0.478 a	0.391 a	0.449 a
	+EDDS	0.453 b	0.396 a	0.336 a	0.407 a
Chl (A+B)	Control	2.16 a	1.66 a	1.38 a	1.54 a
	+EDDS	1.56 b	1.32 b	1.10 b	1.35 a

Within each column, mean values followed by the same letter are not significantly different by LSD at the 5% level.

Cu had a stimulatory effect on chlorophyll content. The addition of EDDS also resulted in plant growth inhibition, with decreasing biomass of roots and leaves compared with the control (Figure 7). Root biomass was correlated with foliar biomass ($r^2 = 0.565$, $p < 0.05$, $n = 32$). A lower content of chlorophyll and reduced biomass were the typical “symptoms” of Cu toxicity.

In the second experiment, similar symptoms were observed. The dry weight of *E. splendens* decreased with the addition of EDDS (Table 4). In the EDDS treatment, the biomass of leaves declined significantly compared with the control at both growth stages ($F = 13.422$, $p < 0.05$; $F = 30.775$, $p < 0.05$).

General Considerations

Enhancement of plant heavy metal concentrations and translocation by EDDS largely depends on the time elapsed after the EDDS addition and the growth stage. EDDS is readily degradable (Schowanek *et al.*, 1997; Vandevivere *et al.*, 2001). After application to soil, EDDS concentrations have a lag time of about 7 d before the degradation of EDDS begins (Tandy *et al.*, 2006). In our experiments, soil NH_4NO_3 -extractable Cu and Zn increased over the first 7 d after the addition of the EDDS but declined later (Figure 5), while the concentration of EDDS in xylem sap declined sharply after 7 d (Figure 3). This may have been due to rapid degradation of EDDS and a decreasing proportion of Cu or Zn EDDS complexes, resulting in a sharp decrease in plant Cu and Zn uptake. This indicates that as

Figure 7 Effects of EDDS on dry biomass of roots, stems, and leaves of *E. splendens* (g pot⁻¹). Vertical bars: standard error (n = 4).

Table 4 Effects of EDDS on mean dry biomass of roots, stems, and leaves of *E. splendens* (g pot⁻¹) (control: n = 3; EDDS: n = 5)

Treatment	110 d after sowing				135 d after sowing			
	Root	Stem	Leaf	Shoot	Root	Stem	Leaf	Shoot
Control St	8.24 a	20.4 a	23.5 a	43.9 a	7.56 a	32.1 a	42.6 a	74.7 a
+EDDS St	6.22 a	17.7 a	16.7 b	34.1 b	8.31 a	30.4 a	28.7 b	59.0 b

Within each column, mean values followed by the same letter are not significantly different by LSD at the 5% level.

the available heavy metals declined, the concentration in the xylem sap showed a similar trend.

Cu and Zn concentrations in the shoots were higher in the EDDS treatment than the control (Figure 1). The translocation factor (TF) for metals within a plant was expressed as the ratio of metal (shoot)/metal (root) to show metal translocation properties from roots to shoots (Stoltz and Greger, 2002). When plants were harvested 110 d after sowing, the plant Cu TF increased by 0.08 in the “Control St” treatment to 9.95 in the “+EDDS St” treatment, with values of 0.58 and 1.46, respectively. After 135 days’ growth, the EDDS addition had a smaller enhancement effect on TF, from 0.02 to 1.12 for Cu and from 0.21 to 0.85 for Zn. Total Cu and Zn concentrations in xylem sap increased significantly with increasing Cu and Zn within the roots (Figure 1). It appears that the capacity of the whole plant to extract Cu and Zn from the polluted soil relied mainly on acquisition from the soil by the roots, and translocation from roots to shoots relied on the concentrations of Cu and Zn in the xylem sap. In chicory and tomato plants, as the root Cu concentrations increase, xylem sap Cu concentrations increase in an exponential manner, which indicates weaker binding of Cu by roots as the amount of root-bound Cu increases. As xylem Cu concentrations increase, sites for binding Cu on the surface of xylem vessels become limited (Liao *et al.*, 2000a). This may be normal behavior in most plants that are not hyperaccumulators.

At both growth stages, Cu concentrations in leaves were higher in the EDDS treatment than in the control, while the roots showed the opposite trend (Table 2). The addition of EDDS has been reported to increase heavy metal uptake by plants (Grčman *et al.*, 2003; Kos and Lestan, 2003a, b; Meers *et al.*, 2005). In our experiments, the addition of EDDS increased Cu and Zn concentrations in the plants, especially in the leaves, and changed the proportion of Cu and Zn in each plant part (Table 2). The proportion of Cu and Zn in the roots was higher than in the leaves in the control plants, while in EDDS treatment the opposite was found. The high metal concentrations found in the roots in the treatment without ligand may have resulted primarily from binding in the apoplasm of the root cortex, rather than from “true” uptake into the root cells. The reduction in metal concentrations in the roots by the addition of ligand may then be explained simply by the prevention of metal sorption to the cation exchange sites in the apoplast due to the formation of negatively charged metal complexes (Wenger, Tandy, and Nowack, 2005). Greater concentrations of Cu and Zn in the shoots is more appropriate for phytoremediation, allowing removal of the heavy metals from contaminated soils by harvesting the aboveground plant parts. The concentrations of Cu and Zn in the roots were generally higher than in the shoots of *E. splendens* without EDDS. At the early growth stage (110d), the plants had higher Cu concentrations in the leaves and had much less Cu in the roots than at the late growth stage (135d). Zn concentrations showed similar trends, but the effects were not significant. This

indicates that *E. splendens* has different nutrient element requirements at different growth stages and that other studies have shown growth stage effects on plant concentrations of heavy metals (Jiang, Yang, and He, 2004). In terms of the efficiency of remediation, adding EDDS after 110 d may be more effective than after 135 d (Table 2).

The mineral composition of the xylem generally reflects the content of elements in the growth substrate. Liao *et al.* (2000 a) used a copper-sensitive electrode to measure the Cu content of chicory and tomato xylem sap and found that more than 99.8% of all xylem sap Cu was in complexed form. Increased Cu concentrations in the rooting media induced selective synthesis of certain amino acids that have high stability constants with Cu (Liao *et al.*, 2000b). After addition, the EDDS may compete with amino acids in the xylem sap as a ligand for Cu ($\log K_a = 18.45$) and for Zn ($\log K_a = 13.49$) (Whitburn, Wilkinson, and Williams, 1999). In our experiments, the concentrations of Cu and Zn were independent on the concentration of EDDS, presumably because cations, especially the heavy metals Cd, Pb, Fe, Mn, and Ni, may affect Cu and Zn speciation. Nickel has the second highest stability constant with EDDS, which is about 0.43 log units lower than that of Cu with EDDS (Whitburn *et al.*, 1999). The detection of EDDS in xylem sap and leaves implies that EDDS may play an important role in the transport and accumulation of heavy metals in *E. splendens*.

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REFERENCES

- Ammann, A.A. 2002. Determination of strong binding chelators and their metal complexes by anion-exchange chromatography and inductively coupled plasma mass spectrometry. *J. Chromatography A* **947**, 205–216.
- Bergers, P.J. and de Groot, A.C. 1994. The analysis of EDTA in water by HPLC. *Water Res.* **28**, 639–642.
- Bucheli-Witschel, M. and Egli, T. 2001. Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiol. Rev.* **25**, 69–106.
- Cleon, W.R. 1974. *Plant Physiology Manual*. Belmont, CA, Wadsworth.
- Deutsches Institute für Normung. 1995. *Soil Quality Extraction of Trace Elements with Ammonium Nitrate Solution*. Berlin, Germany, Beuth Verlag.
- Egli, T. 2001. Biodegradation of metal-complexing aminopolycarboxylic acids. *J. Biosci. Bioeng.* **92**, 89–97.
- Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., and Orser, C.S. 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant Soil* **208**, 87–94.
- Goodfellow, M., Brown, A.B., Cai, J.P., Chun, J.S., and Collins, M.D. 1997. *Amicolatopsis japonicum* sp. Nov., an actinomycete producing (S, S)-N, N'-ethylenediaminedisuccinic acid. *Syst. Appl. Microbiol.* **20**, 78–84.
- Grčman, H., Vodnik, D., Velikonja-Bolta, S., and Lestan, D. 2003. Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced: Lead phytoextraction. *J. Environ. Qual.* **32**, 500–506.

- Hauser, L., Tandy, S., Schulin, R., and Nowack, B. 2005. Column extraction of heavy metals from soils using the biodegradable chelating agent EDDS. *Environ. Sci. Technol.* **39**, 6819–6824.
- IPCS (International Programme on Chemical Safety). 1998. *Environmental Health Criteria No. 200—Copper*. Geneva, Switzerland, World Health Organization.
- Jiang, D.A. and Zhu, C. 1999. *Plant Physiology Experimental Guiding*. Chengdu, China, Chengdu University of Science and Technology Press.
- Jiang, L.Y., Yang, X.E., and He, Z.L. 2004. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. *Chemosph.* **55**, 1179–1187.
- Jiang, L.Y., Yang, X.E., Ye, Z.Q., and Shi, W.Y. 2003. Uptake, distribution and accumulation of copper in two ecotypes of *Elsholtzia*. *Pedosph.* **13**, 359–366.
- Kamal, M., Ghaly, A.E., Mahmoud, N., and Cote, R. 2004. Phytoaccumulation of heavy metals by aquatic plants. *Environ. Int.* **29**, 1029–1039.
- Kari, F.G. and Giger, W. 1996. Speciation and fate of ethylenediaminetetraacetate (EDTA) in municipal wastewater treatment. *Water Res.* **30**, 122–134.
- Knepper, T.P. 2003. Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment. *Trends Anal. Chem.* **22**, 708–724.
- Kos, B. and Lestan, D. 2003a. Influence of a biodegradable ([S, S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. *Plant Soil* **253**, 403–411.
- Kos, B. and Lestan, D. 2003b. Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers. *Environ. Sci. Technol.* **37**, 624–629.
- Liao, M.T., Hedley, M.J., Woolley, D.J., Brooks, R.R., and Nichols, M.A. 2000a. Copper uptake and translocation in chicory (*Cichorium intybus* L. cv. Grasslands Puna) and tomato (*Lycopersicon esculentum* Mill. Cv. Rony) plants grown in NFT system. I. Copper uptake and distribution in plants. *Plant Soil* **221**, 135–142.
- Liao, M.T., Hedley, M.J., Woolley, D.J., Brooks, R.R., and Nichols, M.A. 2000b. Copper uptake and translocation in chicory (*Cichorium intybus* L. cv. Grasslands Puna) and tomato (*Lycopersicon esculentum* Mill. Cv. Rony) plants grown in NFT system. II. The role of nicotianamine and histidine in xylem sap copper transport. *Plant Soil* **223**, 243–252.
- Lu, R.K. 2000. *Analytical Methods for Soil and Agrochemistry*. Beijing, China, Agricultural Science and Technology Press.
- Luo, C.L., Shen, Z.G., and Li, X.D. 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosph.* **59**, 1–11.
- Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., and Tack, F.M.G. 2005. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosph.* **58**, 1011–1022.
- Metsarinne, S., Tuhkanen, T., and Aksela, R. 2001. Photodegradation of ethylenediaminetetraacetic acid (EDTA) and ethylenediamine disuccinic acid (EDDS) within natural UV radiation range. *Chemosph.* **45**, 949–955.
- Nahmani, J. and Lavelle, P. 2002. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. *Eur. J. Soil Biol.* **38**, 297–300.
- Nortemann, B. 1999. Biodegradation of EDTA. *Appl. Microbiol. Biotechnol.* **51**, 751–759.
- Schowaneck, D., Feijtel, T.C.J., Perkins, C.M., Hartman, F.A., Federle, T.W., and Larson, R.J. 1997. Biodegradation of [S, S], [R, R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. *Chemosph.* **34**, 2375–2391.
- Song, J., Zhao, F.J., Luo, Y.M., McGrath, S.P., and Zhang, H. 2004. Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. *Environ. Pollut.* **128**, 307–315.
- Stoltz, E. and Greger, M. 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. *Environ. Exp. Bot.* **47**, 271–280.

- Takahashi, R., Fujimoto, N., Suzuki, M., and Endo, T. 1997. Biodegradabilities of ethylenediamine-N, N'-disuccinic acid (EDDS) and other chelating agents. *Biosci. Biotechnol. Biochem.* **61**, 1957–1959.
- Takahashi, R., Yamayoshi, K., Fujimoto, N., and Suzuki, M. 1999. Production of (S, S')-ethylenediallaine-N, N' -disuccinic acid from ethylenediamine and fumaric acid by bacteria. *Biosci. Biotechnol. Biochem.* **63**, 1269–1273.
- Tandy, S., Ammann, A., Schulin, R., and Nowack, B. 2006. Biodegradation and speciation of residual SS-ethylenediaminedisuccinic acid (EDDS) in soil solution left after soil washing. *Environ. Pollut.* **142**, 191–199.
- Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., and Nowack, B. 2004. Extraction of heavy metals from soils using biodegradable chelating agents. *Environ. Sci. Technol.* **38**, 937–944.
- Tandy, S., Schulin, R., Suter, M.J.F., and Nowack, B. 2005. Determination of [S, S']-ethylenediamine disuccinic acid (EDDS) by high performance liquid chromatography after derivatization with FMO. *J. Chromatography A* **1077**, 37–43.
- Tang, Q.Y. and Feng, M.G. 2002. *DPS Data Processing System for Practical Statistics*. Beijing, China, Science Press.
- Ultra, V.U., Yano, A., Iwasaki, K., Tanaka, S., Kang, Y., and Sakurai, K. 2005. Influence of chelating agent addition on copper distribution and microbial activity in soil and copper uptake by brown mustard (*Brassica juncea*). *Soil Sci. Plant Nutr.* **51**, 193–202.
- Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C.J., and Schowanek, D.R. 2001. Biodegradation of metal- [S, S]-EDDS complexes. *Environ. Sci. Technol.* **35**, 1765–1770.
- Vassil, A.D., Kapulnik, Y., Raskin, I., and Salt, D.E. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol.* **117**, 447–453.
- Wenger, K., Tandy, S., and Nowack, B. 2005. Effects of chelating agents on trace metal speciation and bioavailability. In: *Biogeochemistry of Chelating Agents*, ACS Symposium Series Vol. 910, pp. 204–224. (Nowack, B. and VanBriesen, J.M., Eds.). Washington, DC, American Chemical Society.
- Whitburn, J.S., Wilkinson, S.D., and Williams, D.R. 1999. Chemical speciation of ethylenediamine-N, N'- disuccinic acid (EDDS) and its metal complexes in solution. *Chem. Speciation Bioavailability* **11**, 85–93.
- Yang, M.J., Yang, X.E., and Römheld, V. 2002. Growth and nutrient composition of *Elsholtzia splendens* Nakai under copper toxicity. *J. Plant Nutr.* **25**, 1359–1375.
- Yang, X.E., Shi, W.Y., Fu, C.X., and Yang, M.J. 1998. Copper hyperaccumulators of Chinese native plants: characteristics and possible use for phytoremediation. In: *Sustainable Agriculture for Food, Energy and Industry*, pp. 484–489. (Bassam, N.E., Ed.). London James and James.
- Zhao, F.J., McGrath, S.P., and Crosland, A.R. 1994. Comparison of three wet digestion methods for the determination of plant sulphur by inductively coupled plasma atomic emission spectroscopy (ICP-AES). *Commun. Soil Sci. Plant Anal.* **25**, 407–418.