Journal of Hazardous Materials 181 (2010) 455–459

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effects of IDSA, EDDS and EDTA on heavy metals accumulation in hydroponically grown maize (*Zea mays*, L.)

Zhongqiu Zhao[a,c,*], Meizhu Xi[a], Guangyu Jiang[a], Xiaona Liu[a], Zhongke Bai[a,c], Yizong Huang[b]

[a] *School of Land Science and Technology, China University of Geosciences, Beijing 100083, PR China*
[b] *Department of Soil Environmental Sciences, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China*
[c] *Key Laboratory of Land Consolidation and Rehabilitation, The Ministry of Land and Resources, Beijing 100035, PR China*

ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form 23 April 2010
Accepted 9 May 2010
Available online 13 May 2010

Keywords:
Chelants
Heavy metals
Uptake
Distribution
Apoplast
Symplast

ABSTRACT

Heavy metals contamination of soil is a widespread global problem. Chelant assisted phytoextraction has been proposed to improve the efficiency of phytoextraction which involves three subsequent levels: transfer of metals from the bulk soil to the root surfaces, uptake into the roots and translocation to the shoots. However, most studies focused on the first level. A hydroponic experiment, which addresses the latter two levels, was conducted to study the effects of EDTA, EDDS and IDSA on the uptake and the distribution of Pb, Zn, Cu and Cd in the apoplast and the symplast of roots of maize (*Zea mays*, L.). The concentrations of the metals (with exception of Zn) in the shoots were increased significantly by addition of all the chelants. EDTA was most effective for Pb uptake and IDSA was interestingly most effective for Cd uptake. Pb in the roots with EDTA was mostly distributed in the apoplast, while Zn, especially with IDSA, was mostly located in the symplast. The results indicated that, the capacity of chelant to enhance the nonselective apoplastic transport of metal may be most important for chelant enhanced phytoextraction.

1. Introduction

Heavy metals contamination of soil is a widespread global problem. Contaminated soil can be remediated by physical, chemical or biological techniques [1], but the traditional physical or chemical methods can be very costly and also destructive to the soil [2]. Phytoextraction, one of biological techniques, has been proposed as an environmentally friendly in situ remediation technology for soils contaminated with heavy metals [3–5]. However, the efficiency of metal extraction is generally conceived as too slow due to the limitations of hyperaccumulator plants such as low biomass and very slow growth rate. Chelant assisted phytoextraction has been proposed to improve the efficiency of phytoextraction [6–8]. The chelating aminopolycarboxylic acid, ethylene diamine tetraacetate (EDTA), has been proven to enhance plant accumulation of heavy metals [9–11]. However, the use of EDTA in phytoextraction is found not to be suitable due to its high environmental persistence or un-biodegradabe property, which may lead to secondary contamination [12]. EDTA and the formed EDTA–metal complexes have low biodegradability and high solubility in soil, resulting in an elevated risk of adverse environmental effects due to metal mobilization and long persistence [8,13]. In this respect, the amount of metal taken up by plants has been reported to be much less than the amount mobilized from the soil during EDTA-induced metal phytoextraction [14]. Ethylene diamine disuccinate (EDDS), a biodegradable chelant, has been proposed as an alternative environmentally friendly phytoextraction assister to be used for enhanced phytoextraction purposes [10,15]. EDDS has been shown to be easily biodegradable [16], to form strong complexes with transition metals and radionuclides [17], to cause a much smaller leaching of metal down the soil profile than EDTA [18], and to be less toxic to soil microorganisms [18]. Recently, a new biodegradable chelant, iminodisuccinic acid (IDSA) has been marketed. IDSA has the same aspartic acid derivative as EDDS and good biodegradability [19], so it may be another environmentally friendly alternative after EDDS for persistent EDTA.

Phytoremediation involves three subsequent levels: transfer of metals from the bulk soil to the root surfaces, uptake into the roots and translocation to the shoots. Hydroponic experiments address the latter two levels [2,20]. It is mostly a soil chemical process at the first level. The metals are desorbed from the solid soil bulk and enter the soil solution, then were transferred to the root surface, called bioavailability of metals, which affects the latter two processes and was affected by various soil environments significantly. Most studies focused on the first level which is understood relatively clear [11,21,22]. For the latter two levels or phases, however,

---

* Corresponding author at: School of Land Science and Technology, China University of Geosciences, No. 29 Xueyuan Rd, Haidian District, Beijing 100083, P.R. China. Tel.: +86 10 82323490; fax: +86 10 82321807.
E-mail address: zhongqiuzhao@163.com (Z. Zhao).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.05.032

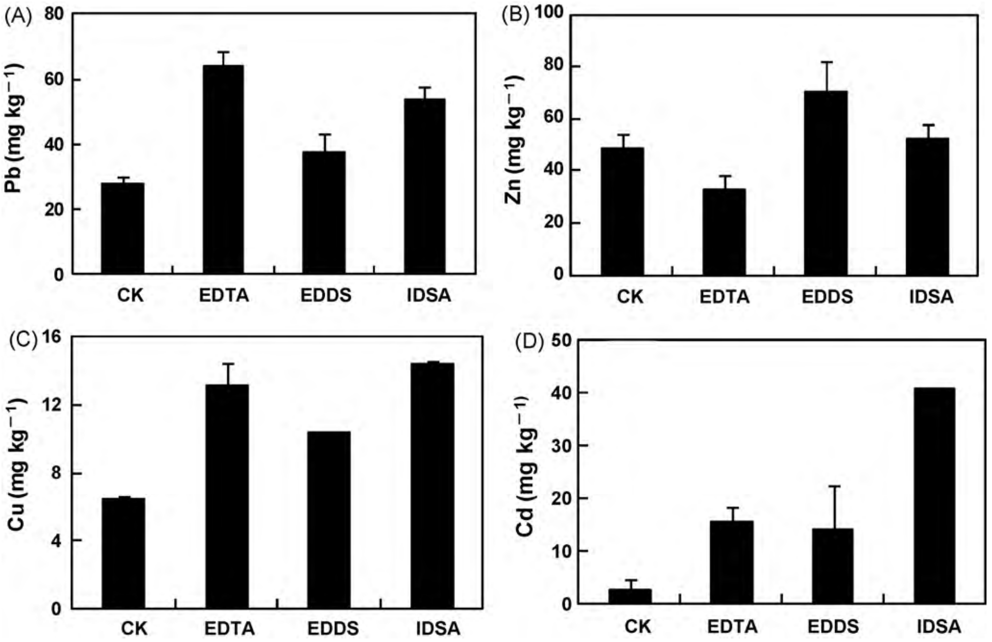

Fig. 1. Shoot uptake of Pb (A), Zn (B), Cu (C) and Cd (D) by maize from nutrient solution in the presence of EDTA, EDDS and IDSA. Results shown are mean values, error bars are standard error.

it is not very clear yet, especially how the metals uptake and distribution in plant parts (e.g. apoplast and the symplast in roots), etc. under chelating environment [5].

There are two parallel transport pathways for metals through the root cortex toward the shoot: one pathway of active transport from cell to cell in the symplast (selective transport across membranes) and another pathway of passive transport by diffusion and convection through the apoplast, namely cell walls and intercellular spaces [23,24]. Cellular active uptake way is highly selective for essential metals such as Cu and Zn as free metal ions, and nonessential metals such as Cd, there is limited cellular uptake through this pathway. The apoplastic pathway is discontinuous, being interrupted by the endodermis, the innermost layer of cells of the cortex. In the radial and transverse walls of the endodermis, hydrophobic incrustations of suberin such as the Casparian band obstruct the passive transfer of solutes into the shoot. But the endodermis is an imperfect barrier for apoplastic transport. At the root apex the Casparian strip is not yet fully developed and, thus, allows apoplastic transport to reach the shoot [25,26].

It has been hypothesized that metal may enter the roots at breaks in the root endodermis and the Casparian strip, and be rapidly transported to the shoots. With a high dissolved metal concentration, the nonselective uptake in the presence of chelants would exceed selective uptake along the symplastic pathway [26]. There is debate whether some metal complexes are taken up into plants [27], but it is generally assumed that anionic metal–ligand complexes are not bioavailable [28]. This is contradicted by the observed increase in metal uptake in the presence of high concentrations of chelant [25,29–31]. While there were few evident reports about this hypothesis and which pathway dominate the metals transport to the shoot under the chelants because of the limitation of measures to identify apoplastic and symplastic elements [32]. After investigating various fraction methods in both monocot and dicot to try to identify apoplastic and symplastic fraction of metals (Co), Reid and Liu [32] proposed a simple freeze–thaw approach to distinguish between the two fractions.

The objectives of this study were to investigate the effects of EDTA, EDDS and IDSA on the uptake and the distribution of heavy metals (Pb, Zn, Cu and Cd) in the apoplast and the symplast in roots of maize (*Zea mays*, L.) by hydroponic experiment, implying the nonselective and selective uptake of heavy metals under chelants environment.

2. Materials and methods

2.1. Nutrient solution and plant culture

Seeds of maize (*Z. mays*, L.) were sterilized in 10% H_2O_2 for 10 min followed by thorough washing in deionised water, then germinated on moist filter paper for 2 days. Geminated seeds were transferred to moist perlite and cultivated for about 5 days. The seedlings were then removed from the perlite and were washed carefully under tap water to remove any adhering particles. Seedlings were then transferred to PVC pots containing 1100 ml nutrient solution. Modified Hoagland nutrient solution contained (in mmol L^{-1}): KNO_3, 1.33; $Ca(NO_3)_2$, 1.33; $MgSO_4$, 0.5; KH_2PO_4, 0.44; in micromol L^{-1}, $FeSO_4{\cdot}7H_2O$, 50; $CuSO_4$, 0.5; $MnSO_4$, 2.5; H_3BO_3, 5; Na_2MoO_4, 0.25; $CoSO_4$, 0.09; NaCl, 50. The solution was modified by using $FeSO_4{\cdot}7H_2O$ instead of NaFe(III) EDTA to avoid interference between another complexing agent and EDDS. The seedlings were grown in a growth chamber for 3 weeks before they were exposed to metal and chelant solutions, with 14/10 h light/dark cycles. Light intensity was around 280 μES^{-1}. The nutrient solution was renewed twice a week and aerated continuously. Pots were randomly arranged every day during the growth period.

2.2. Experimental design

After 3 weeks of growth the plants were transferred into the experimental solution, in which all micronutrients were added other than the metals under study and KH_2PO_4 were omitted to avoid precipitation of Cu and Pb phosphate. Four metals (Pb 150 μmol L^{-1}, Zn 150 μmol L^{-1}, Cu 100 μmol L^{-1} and Cd 20 μmol L^{-1}) were added in combination and three chelants (EDTA, EDDS and IDSA) with 500 μmol L^{-1} were added alone. Each treatment had four replicates. The seedlings were grown in the experimental solution for 1 week.

2.3. *Plant analysis*

2.3.1. *Metals uptake*

At harvest the shoots and roots were separated. The shoots were washed with deionised water and dried at 70 °C until constant weight and the roots were used for determination of metals distribution between root apoplast and symplast. The oven-dried shoot samples finely ground in a stainless steel miller. Sub-samples of between 100 and 250 mg were digested in 5 ml high-purity HNO_3 for metal analysis. The digest was diluted to 25 ml in high-purity water. The concentrations of Pb, Zn, Cu and Cd in the solution were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima 2000 DV, PerkinElmer, USA).

2.3.2. *Metals distribution in root apoplast and symplast*

In order to distinguish the selective symplastic transport and nonselective apoplastic transport influenced by different chelants, the metal distributions in apoplast and symplast were measured. Roots were desorbed using a modified desorption procedure as described in Reid and Liu [32] and Zhang et al. [33].

After being treated with metals and chelants in hydroponic solution, roots were excised. The entire root system from different chelants addition was rinsed using 5 mmol L^{-1} $CaCl_2$ to remove the metals adsorbed to the root surface. Then they were desorbed in 5 mmol L^{-1} $CaCl_2$, which was changed every 5 min. After 20 min, most of the apoplastic metals were removed, the roots were rapidly frozen in liquid nitrogen to disrupt cell membranes and desorption was continued for 40 min. The metals released in the first 20 min desorption plus the metals remaining in the root (mainly binding or precipitated in the root cell wall) after the freeze–thaw was considered to be apoplastic fraction. The metal released following the freeze–thaw was considered symplastic fraction.

2.4. *Statistical analysis*

All statistical analysis (ANOVA) was carried out with SPSS 11.5. Differences at $P < 0.05$ level were considered statistically significant.

3. Results

3.1. *Metals uptake*

The hydroponic experiment carried out with all the studied metals was to evaluate the effects of chelants on the overall ability of maize to uptake metals and to accumulate them in the shoots. Pb, Zn, Cu and Cd uptake by maize as affected by EDTA, EDDS and IDSA was shown in Fig. 1. Generally, the concentrations of the metals (with exception of Zn) in the shoots were increased significantly by addition of EDTA, EDDS and IDSA compared to the control. The accumulation of Pb and Cu with EDTA and IDSA was higher than EDDS significantly (Fig. 1A and C) ($P < 0.05$). EDTA was most effective for Pb uptake which was 2.3 times higher than for the control. Cu uptake was 2.1 times, 1.6 times and 2.2 times for EDTA, EDDS and IDSA addition compared to the control, respectively. For the uptake of Zn, there was no significant difference between the treatments and the control (Fig. 1B). Interestingly, IDSA was dramatically effective for Cd uptake which was 17.2 times higher than that of the control ($P < 0.001$), and 2.7 times and 3.0 times higher than that of EDDS and EDTA treatments, respectively (Fig. 1D) ($P < 0.05$).

3.2. *Metals distribution in root apoplast and symplast*

In order to detect the selective symplastic transport and nonselective apoplastic transport in the presence of chelants, the metal distributions in apoplast and symplast were measured as shown in Fig. 2. Pb in the roots with EDTA addition was mostly distributed in the apoplast, the concentration of which was about 5-folds higher than that in the symplast ($P < 0.05$) (Fig. 2A), and the content in the symplast was also much higher than the control and other treatments. The distribution ratio of Cu was not significantly affected by chelants addition compared to the control, although the whole contents in the roots were influenced. Interestingly, Zn in the roots with IDSA treatment was much higher than those with other treatments

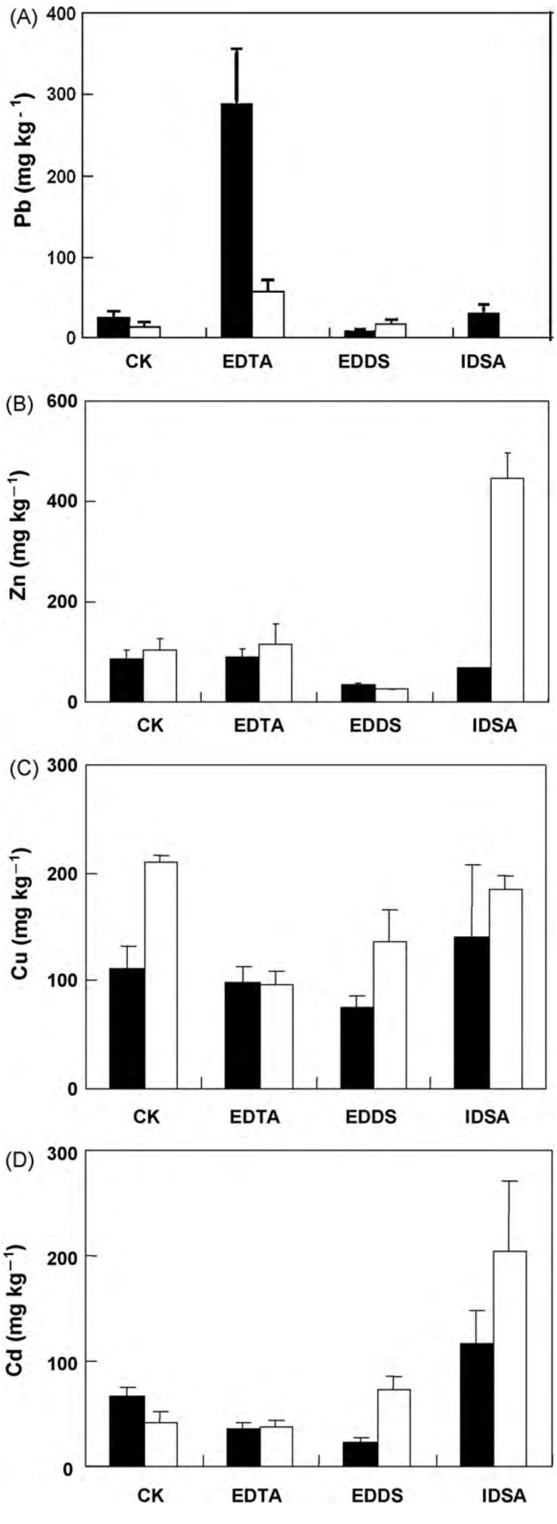

Fig. 2. Distribution of Pb (A), Zn (B), Cu (C) and Cd (D) in apoplast (black bar) and symplast (white bar) in the roots of maize from nutrient solution in the presence of EDTA, EDDS and IDSA. Results shown are mean values, error bars are standard error.

($P < 0.001$), and was mostly distributed in the symplast with less distributed in the apoplast. Zn concentration in symplast with IDSA reached 446 mg kg^{-1} FW, 17.2 times higher than the control and 4.5 and 3.9 times higher than EDTA and EDDS treatments, respectively, and 6.6 times higher than in the apolast. Cd concentrations in both apoplast and symplast were significantly increased in the presence of IDSA.

4. Discussion and conclusions

It was hypothesized that the formation of negatively charged complexes prevented free metals from binding to the cation exchange sites in the cell walls of the roots and allowed them to enter into the cells by a nonselective apoplastic mechanism [34]. The detection of EDDS in roots, shoots and xylem sap of sunflower grown either in soil or in hydroponics by Tandy et al. [2,5] is an indication that metal complexes or free are taken up into the xylem and then translocated to the above-ground parts. In this experiment, our results were in agreement with above reports. Levels of Pb, Zn, Cu and Cd in shoot of hydroponically grown maize were elevated by addition of EDTA, EDDS and IDSA in comparison with the control. EDTA was most effective for Pb accumulation in accordance with many other reports [11,15,35–37].

In most cases, the EDTA treatment was superior in solubilizing soil Pb for root uptake and translocation into shoot [11,15,35–37] due to its strong chemical affinity for Pb ($\log Ks = 17.88$) [38]. And evidence showed that, the accumulation of Pb in plant shoots was correlated with the formation of the Pb–EDTA complex which was the major form of Pb absorbed and translocated by plants [29,37]. In the case of hydroponic environment of present experiment, EDTA was also most effective for Pb root uptake and translocation into the shoot (Fig. 2A). In roots, the Pb was mainly distributed in apolast either with chelants or with control. It was in evidence that, in the presence of chelants especially EDTA, the transport of Pb to the shoot was mainly via apolast system with both apoplastic and symplastic transport enhanced. It has been widely accepted that uptake of chelants and their metal complexes occurs via the apoplastic pathway [25,29–31]. Disruption of the endodermis could facilitate plant–metal accumulation by allowing the free passage of chelated metals into the stele. The endodermis may also be damaged when high concentrations of chelants were added to the solution.

A simplified schematic of the accumulation of Cu, Zn, and Pb in shoots or translocation from roots to shoots in the absence and presence of chelating agents based on actual pot and hydroponic experiments [2,5] proposed by Nowack et al. [26] showed that, in the absence of chelant, Zn and Cu accumulation is governed by uptake of free metal ions in the symplastic pathway, which is efficient at low solution concentrations, and only a little Pb is taken up into the shoots. If the same metal concentrations in solution were chelated, then the uptake would occur through the apoplastic pathways and Cu and Zn uptake would be reduced while Pb uptake would be strongly increased. It is a clear explanation that why there is an increase in Pb uptake in the presence of the chelant in most cases. But there is few hydroponic data show a decrease in Cu and Zn uptake in the presence of chelant [2,34,39]. It is suggested that, with a high dissolved metal concentration, the nonselective uptake in the presence of chelant would exceed selective uptake along the symplastic pathway for both essential and nonessential metals. So the translocation of both essential and nonessential metals to the shoots would increase [26]. In the present experiment, levels of Pb with EDTA and Cd with IDSA (both of which had a high accumulation in shoots) in the apoplast of roots were much higher than the control and other chelant treatments, especially for Pb with EDTA (Fig. 2A and D), indicating that the nonselective apoplastic uptake dominated the translocation of Pb and Cd from root to shoot. At the same time, the contents of Pb with EDTA and Cd with IDSA in the symplast were also significantly higher than the control and other treatments, which suggested that, in the presence of EDTA or IDSA, the sympastic uptake of Pb or Cd may be also enhanced. The probable reason was that, EDTA and IDSA enhanced the capacity of across membrane transport of Pb and Cd or the complexes Pb–EDTA and Cd–IDSA had a higher activity to be transported across membrane.

Our results with Cu and Zn uptake showed that, Cu levels in the shoot of maize were significantly increased by all the three chelants addition. For Zn uptake, however, Zn levels were decreased or not significantly changed compared to the control, which was in the few cases (Fig. 1B and C). In roots, interestingly, a much higher percent of Zn occurred in the symplast with IDSA treatment, which was evidently different from the control and other treatments (Fig. 2B). This suggested that, in the presence of IDSA, the selective uptake by symplastic pathway may be still the main way for essential Zn transport, and IDSA may enhance the across membrane transport of Zn, the mechanism of which needs further study.

In conclusion, in the presence of chelants, the high accumulation of nonessential Pb with EDTA and Cd with IDSA in shoots may be mainly attributed to the nonselective apoplastic pathway transport, and the uptake of essential Zn, not significantly changed, may be still mainly through the selective symplastic transport. On the point of accumulation effectiveness of phytoextration, the capacity of chelants to enhance the nonselective transport of metals may be most important for metal phytoextraction. Our results suggested that the new biodegradable IDSA may be potentially useful chelant for Cd contaminated soil.

Acknowledgements

The work was supported by the Key (Key grant) Project of Chinese Ministry of Education (No. 109036), the National Natural Science Foundation of China (No. 20807039) and the National Science and Technology Supporting Plan of the Eleventh Five-year (2008BAB38B07). The authors express great gratitude to Prof. Y.G. Zhu for providing valuable instruction and comments during the experiment.

References

[1] S. McEldowney, D.J. Hardman, S. Waite, Treatment technologies, in: S. McEldowney, J. Hardman, S. Waite (Eds.), Pollution Ecology and Biotreatment Technologies, Longman, Singapore Publishers, Singapore, 1993.
[2] S. Tandy, R. Schulin, B. Nowack, The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers, Chemosphere 62 (2006) 1454–1463.
[3] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Ann. Rev. Plant Phys. 49 (1998) 643–668.
[4] A.G. Khan, C. Kuek, T.M. Chaudhry, C.S. Khoo, W.J. Hayes, Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation, Chemosphere 41 (2000) 197–207.
[5] S. Tandy, R. Schulin, B. Nowack, Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration, Environ. Sci. Technol. 40 (2006) 2753–2758.
[6] M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents, Environ. Sci. Technol. 31 (1997) 860–865.
[7] C. Garbisu, J. Hernández-Allica, O. Barrutia, I. Alkorta, J.M. Becerril, Phytoremediation: a technology using green plants to remove contaminants from polluted areas, Rev. Environ. Health 17 (2002) 173–188.
[8] I. Alkorta, J. Hernández-Allica, J.M. Becerril, I. Amezaga, I. Albizu, M. Onaindia, Chelate-enhanced phytoremediation of soils polluted with heavy metals, Rev. Environ. Health 3 (2004) 55–70.
[9] S.P. McGrath, F.J. Zhao, E. Lombi, Phytoremediation of metals, metalloids, and radionuclides, Adv. Agron. 75 (2002) 1–56.
[10] B. Kos, D. Leštan, 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 (2003) 403–411.
[11] E. Meers, A. Ruttens, M.J. Hopgood, D. Samson, F.M.G. Tack, Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals, Chemosphere 58 (2005) 1011–1022.

[12] B. Nowack, Environmental chemistry of aminopolycarboxylate chelating agents, Environ. Sci. Technol. 36 (2002) 4009–4016.
[13] I. Alkorta, J. Hernández-Allica, J.M. Becerril, I. Amezaga, I. Albizu, C. Garbisu, Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic, Rev. Environ. Health 3 (2004) 71–90.
[14] Y.H. Chen, X.D. Li, Z.G. Shen, Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process, Chemosphere 57 (2004) 187–196.
[15] C.L. Luo, Z.G. Shen, L. Lou, X.D. Li, EDDS and EDTA-enhanced phytoextraction of metals from artificially contaminated soil and residual effects of chelant compounds, Environ. Pollut. 144 (2006) 862–871.
[16] E. Meers, F.M.G. Tack, M.G. Verloo, Degradability of ethylenediaminedisuccinic acid (EDDS) in metal contaminated soils: implications for its use soil remediation, Chemosphere 70 (2008) 358–363.
[17] P.W. Jones, D.R. Williams, Chemical speciation used to assess [S,S′]-ethylenediaminedisuccinic acid (EDDS) as a readily-biodegradable replacement for EDTA in radiochemical decontamination formulations, Appl. Radiat. Isotopes 54 (2001) 587–593.
[18] H. Grčman, D. Vodnik, S. Velikonja-Bolta, D. Leštan, Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced lead phytoextraction, J. Environ. Qual. 32 (2003) 500–506.
[19] J. Jäkärä, R. Aksela, Benign and effective: Kemira has conducted intensive studies to find chelating agents for pulp bleaching applications that are effective and environmentally benign, Pulp Paper Int. 48 (2006) 28–31.
[20] Z.Q. Zhao, Y.G. Zhu, Y.L. Cai, Effects of zinc on cadmium uptake by spring wheat (*Triticum aestivem*, L.): long-time hydroponic study and short-time ^{109}Cd tracing study, J. Zhejiang Univ. Sci. 6A (7) (2005) 643–648.
[21] M.W.H. Evangelou, U. Bauer, M. Ebel, A. Schaeffer, The influence of EDDS and EDTA on the uptake of heavy metals of Cd and Cu from soil with tobacco *Nicotiana tabacum*, Chemosphere 68 (2007) (2007) 345–353.
[22] A. Cao, A. Carucci, T. Lai, P.L. Colla, E. Tamburini, Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria, Eur. J. Soil Biol. 43 (2007) 200–206.
[23] E. Steudle, C.A. Peterson, How does water get through roots? J. Exp. Bot. 49 (1998) 775–788.
[24] U.W. Stephan, Intra- and intercellular iron trafficking and subcellular compartmentation within roots, Plant Soil 241 (2002) 19–25.
[25] S.H. Crowdy, T.W. Tanton, Water pathways in higher plants. I. Free space in wheat leaves, J. Exp. Bot. 21 (1970) 102–111.
[26] B. Nowack, R. Schulin, B.H. Robinson, Critical assessment of chelant-enhanced metal phytoextraction, Environ. Sci. Technol. 40 (2006) 5225–5232.
[27] D.R. Parker, J.F. Pedler, Z.A.S. Ahnstrom, M. Resketo, Re-evaluating the free-ion activity model of trace metal toxicity toward higher plants: experimental evidence with copper and zinc, Environ. Toxicol. Chem. 20 (2001) 899–906.
[28] D.R. Parker, R.L. Chaney, W.A. Norvell, Chemical equilibrium models: application to plant nutrition research, in: Chemical Equilibrium and Reaction Models, Soil Science Society of America, American Society of Agronomy, Madison, WI, 1995.
[29] A.D. Vassil, Y. Kapulnik, I. Raskin, D.E. Salt, The role of EDTA in lead transport and accumulation by Indian mustard, Plant Physiol. 117 (1998) 447–453.
[30] R.N. Collins, G. Merrington, M.J. McLaughlin, C. Knudsen, Uptake of intact zinc–ethylenediaminetetraacetic acid from soil is dependent on plant species and complex concentration, Environ. Toxicol. Chem. 21 (2002) 1940–1945.
[31] P.F. Bell, M.J. McLaughlin, G. Cozens, D.P. Stevens, G. Owens, H. South, Plant uptake of ^{14}C-EDTA, ^{14}C-citrate and ^{14}C-histidine from chelator-buffered and conventional hydroponic solutions, Plant Soil 253 (2003) 311–319.
[32] R. Reid, J.H. Liu, Measurement of trace metal influx in plants: a case study with Co, Funct. Plant Biol. 31 (2004) 941–947.
[33] X.H. Zhang, A.J. Lin, Y.L. Gao, R.J. Reidd, M.H. Wong, Y.G. Zhu, Arbuscular mycorrhizal colonisation increases copper binding capacity of root cell walls of *Oryza sativa* L. and reduces copper uptake, Soil Biol. Biochem. 41 (2009) 930–935.
[34] K. Wenger, S.K. Gupta, G. Furrer, R. Schulin, The role of nitrilotriacetate in copper uptake by tobacco, J. Environ. Qual. 32 (2003) 1669–1676.
[35] B. Kos, D. Leštan, Soil washing of Pb, Zn and Cd using biodegradable chelator and permeable barriers and induced phytoextraction by *Cannabis sativa*, Plant Soil 263 (2004) 43–51.
[36] C.L. Luo, Z.G. Shen, X.D. Li, Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS, Chemosphere 59 (2005) 1–11.
[37] A.L. Epstein, C.D. Gussman, M.J. Blaylock, U. Yermiyahu, J.W. Huang, Y. Kapulnik, C.S. Orser, EDTA and Pb–EDTA accumulation in *Brassica juncea* grown in Pb-amended soil, Plant Soil 208 (1999) (1999) 87–94.
[38] A.E. Martell, R.M. Smith, R.J. Motekaitis, NIST Critically Selected Stability Constants of Metal Complexes, Version 6.0, NIST, Gaithersburg, MD, 2001.
[39] G.J. Taylor, C.D. Foy, Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum*, Can. J. Bot. 63 (1985) 1271–1275.