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Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals

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Abstract

Phytoextraction has been proposed as an alternative remediation technology for soils polluted with heavy metals or radionuclides, but is generally conceived as too slow working. Enhancing the accumulation of trace pollutants in harvestable plant tissues is a prerequisite for the technology to be practically applicable. The chelating aminopolycarboxylic acid, ethylene diamine tetraacetate (EDTA), has been found to enhance shoot accumulation of heavy metals. However, the use of EDTA in phytoextraction may not be suitable due to its high environmental persistence, which may lead to groundwater contamination. This paper aims to assess whether ethylene diamine disuccinate (EDDS), a biodegradable chelator, can be used for enhanced phytoextraction purposes. A laboratory experiment was conducted to examine mobilisation of Cd, Cu, Cr, Ni, Pb and Zn into the soil solution upon application of EDTA or EDDS. The longevity of the induced mobilisation was monitored for a period of 40 days after application. Estimated effect half lives ranged between 3.8 and 7.5 days for EDDS, depending on the applied dose. The minimum observed effect half life of EDTA was 36 days, while for the highest applied dose no decrease was observed throughout the 40 day period of the mobilisation experiment. Performance of EDTA and EDDS for phytoextraction was evaluated by application to *Helianthus annuus*. Two other potential chelators, known for their biodegradability in comparison to EDTA, were tested in the plant experiment: nitrilo acetic acid (NTA) and citric acid. Uptake of heavy metals was higher in EDDS-treated pots than in EDTA-treated pots. The effects were still considered insufficiently high to consider efficient remediation. This may be partly due to the choice of timing for application of the soil amendment. Fixing the time of application at an earlier point before harvest may yield better results. NTA and citric acid induced no significant effects on heavy metal uptake.

Keywords: Phytoextraction; EDDS; EDTA; Heavy metals; *Helianthus annuus*

1. Introduction

Pollution of soils and sediments with organic and inorganic pollutants has been an unfortunate by-product of industrialisation. In Flanders, Belgium the most

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recent estimates for polluted sites requiring prioritory clean-up constitute for 21 000–28 000 ha or 1.4% of the total surface area (Ceenaeme et al., 2001). This number excludes exceedances of legal thresholds which are not deemed first priority. The underwater soils of Western European waterways are generally polluted due to current and historic industrial activity. Dredging activities for the maintenance of these waterways require annual removal of $4.5 \times 10^7 \mathrm{m}^3$ of dredged sediments of which $4–6 \times 10^6 \mathrm{m}^3$ require to be disposed of on land (Tack et al., 1995; Singh et al., 1998). This also generates a systematic form of soil pollution. The average cost of soil remediation in Flanders, runs up to an estimated \$390 per ton (based on data from De Naeyer, 2000) or \$1 365 000 per ha for the top soil layer (25 cm) alone.

Phytoremediation is the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates (EPA, 2000). It offers the prospect of being an attractive, economic and non-invasive alternative for a portion of the polluted soils (Salt et al., 1995). Phytoextraction is defined as a green technology that removes pollutants from contaminated soil by plant absorption and translocation to harvestable plant parts (Chaney, 1983; Baker et al., 1991). The technique appears more applicable for the remediation of heavy metals that are also essential nutrients (Cu and Zn) as these are taken up actively. It can also be applied to non-essential elements, such as Cd, Ni, and Pb, which are taken up unintentionally. Unintentional uptake is the result of physiological lapses in selectivity, in which non-essential elements are transported into the plant by regular nutrient uptake mechanisms.

In phytoextraction research, two main strategies can be identified. The first is the use of metal hyperaccumulating species (Baker et al., 1994; Brown et al., 1994; Kumar et al., 1995). Hyperaccumulators are naturally occurring species which contain more than $100 \mathrm{mg\,kg^{-1}}$ dry weight Cd, $1000 \mathrm{mg\,kg^{-1}}$ Co, Cu, Pb or Ni, or more than $10 000 \mathrm{mg\,kg^{-1}}$ dry weight Mn or Zn (Dahmani-Muller et al., 2000). The general drawback on the use of metal hyperaccumulators, is their low biomass production. A second phytoextraction approach involves the use of high biomass producing species, such as sunflower (*H. annuus*), and chemically enhancing their shoot levels to increase the removal efficiency. A number of soil amendments have been reported in literature which could render soil trace metals more phytoavailable, among which ethylene diamine tetraacetate (EDTA) has taken a predominant place (Cooper et al., 1999; Epstein et al., 1999; Shen et al., 2002). However, in recent years the use of persistent aminopolycarboxylic acids (APCAs), such as EDTA, has made scientists and legislation alike frown upon the entire technology of enhanced phytoextraction. This compound is recalcitrant to biodegradation and is, therefore, characterised by a high environmental persistence (Bucheli-Witschel and Egli, 2001; Egli, 2001). Its prolonged presence in the soil, combined with its ability to chelate and mobilise heavy metals, dramatically increase risks of leaching.

Ethylene diamine disuccinate (EDDS) is an APCA that is produced naturally by a number of microorganisms (Goodfellow et al., 1997; Nishikiori et al., 1984). Schowanek et al. (1997) describe a good degree of biodegradability for EDDS, with observed half lives ranging from 2.5 days in a soil experiment to 4.6 days in an unacclimated Sturm test. The metal chelating ability, accompanied with the short activity timespan in the soil due to rapid biodegradation make this substance a promising substrate for use as soil amendment for enhanced phytoextraction purposes.

The plant species used for the enhanced phytoextraction experiment in this study, is *Helianthus annuus*. The species is known for its high biomass yield. Madejon et al. (2003) observed a shoot dry weight production for *H. annuus* of $24 \mathrm{t\,ha^{-1}}$ at a shoot density of 60 000 plants per hectare grown on heavy metal contaminated soil (Aznalcollar mine spill, Spain). Murillo et al. (1999) observed no growth depression for *H. annuus* cultivated on the same Aznalcollar soil substrate in comparison to unpolluted controls. Cabelguenne et al. (1999) reported a production of up to $13 \mathrm{t\,ha^{-1}}$ for *H. annuus* in France. The EU-project Phyles estimates an annual biomass production per hectare for *H. annuus* at $10 \mathrm{t\,ha^{-1}}$ for the purposes of phytoextraction of heavy metals (Phyles, 2004). In addition, it is tolerant to soil pollution with heavy metals (Murillo et al., 1999; Madejon et al., 2003). The species has been described or proposed for phytoextraction of heavy metals (Chen and Cutright, 2001; Van der Lelie et al., 2001) and radionuclides (Lee et al., 2002; Soudek et al., 2004).

The aim of this paper is to compare the performance of the naturally occurring biodegradable chelator, EDDS, with the recalcitrant synthetic chelator, EDTA, for phytoextraction purposes. The 40 d monitoring of heavy metals in the soil solution of dredged sediment and their presence in the shoot tissues of *H. annuus* following chelator application will be discussed.

2. Material and methods

2.1. *Sediment analysis*

The soil substrate used in our experiments was a dredged sediment derived surface soil, which had been in an oxidised state for several years prior to sample collection. The sediment was collected at the dredged sediment disposal site at Meigem (Belgium), currently in use as a (non-agricultural) grassland. Due to the general state of pollution in Western European waterways, sediments dredged from these waterways contain elevated levels of organic and inorganic pollutants.

Table 1
General soil properties and total metal content of the dredged sediment derived soil used in the pot experiments; pH-H_2O: actual soil acidity, EC: electrical conductivity, $CaCO_3$: soil carbonate content, OM: organic matter content, CEC: cation exchange capacity; intervals denote standard deviation ($n = 3$)

pH-H_2O	7.1 ± 0.1	Cd (mg kg^{-1})	8.8 ± 0.8
EC (mS cm^{-1})	509 ± 18	Cu (mg kg^{-1})	120 ± 17
Clay (%)	38	Cr (mg kg^{-1})	177 ± 23
Silt (%)	42	Ni (mg kg^{-1})	61 ± 9
Sand (%)	21	Pb (mg kg^{-1})	178 ± 33
$CaCO_3$ (%)	10.7 ± 0.4	Zn (mg kg^{-1})	907 ± 118
OM (%)	7.5 ± 0.9		
CEC (cmol(+) kg^{-1})	22.9 ± 1.4		

All soil samples were analysed in triplicate for general soil properties and total metal content (Table 1). Soil conductivity was measured using a WTW LF 537 electrode (Wissenschafltich Technischen Werkstäten, Weilheim, Germany) after equilibration for 30 min in deionised water at a 5:1 liquid:solid ratio and subsequent filtering (white ribbon; Schleicher & Schuell, Dassel, Germany). To determine actual soil pH, 10 g of air-dried soil was allowed to equilibrate in 50 ml of deionised water for 24 h. For potential soil pH, 50 ml of 1 M KCl was added to 10 g of air-dried soil and allowed to equilibrate for 10 min. The pH of the mixture was then measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA), calibrated using pH 4.0 and pH 7.0 standards. Total carbonate content present in the sediment was determined by adding a known excess quantity of sulphuric acid and back-titrating the excess with sodium hydroxide (Van Ranst et al., 1999). Organic matter was determined using the method described by Walkley & Black (Allison, 1965). The grain size distribution of the soil samples was determined using laser diffractometry (Coulter LS200, Miami, FL, USA) (Vandecasteele et al., 2002). The cation exchange capacity (CEC) of the sediment was determined by first saturating the soil matrix with NH_4^+, then desorbing the NH_4^+ by K^+ and measuring the quantity of the NH_4^+ in the leachate (Van Ranst et al., 1999). Ammonia nitrogen was determined by adding 3 g of MgO to 20 ml of sample and subsequent distillation using a Kjeltec 1002 Distilling Unit (Foss, Höganäs, Sweden). The distillate was captured in a 250 ml erlenmeyer containing 20 ml of 0.3 M boric acid. Finally, the captured ammonia was titrated with 0.01 M HCl in the presence of a methyl red and bromocresol green mixed indicator. Field capacity was determined by adding an excess of water to 400 g of dry weight soil. The pots were assumed to be at field capacity when formation of further droplets at the bottom of the pot after free percolation has fully ended. The pots were then re-weighed and field capacity estimated. Soil metal content was determined after *aqua regia* digestion (Van Ranst et al., 1999). Analysis was subsequently performed, using inductively coupled plasma-optical emission spectrometry (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA).

2.2. Soil solution experiment

Pots containing 450 g of dry weight soil were brought to 2/3 field capacity and fitted with Rhizon-MOM for soil solution extraction (MOM-type Rhizons; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Subsequently the pots were treated with: 0.8 mmol, 1.6 mmol, 2.4 mmol, 4.0 mmol Na-EDDS or 0.8 mmol, 1.6 mmol, 4.0 mmol Na-EDTA. Control pots were treated with deionised water. Soil solution was extracted at regular time intervals: in the EDDS-treated pots after 1 d, 4 d, 14 d and 30 d; in the EDTA-treated pots after 1 d, 7 d, 25 d and 40 d. The soil solution samples were analyzed for Al, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Zn using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA). All experiments were conducted in quadruplicate.

2.3. Plant experiment

Pots containing 450 g of dry weight dredged sediment were sown with three *H. annuus* 'giganteus' seeds. After germination, the plants were thinned to 1 per pot. The plants were allowed to grow in a greenhouse during the spring months (March–June), without additional lighting. The soil moisture content was kept constant at 2/3 of field capacity. After 10 weeks of growth, the pots were treated with the soil amendments outlined in Table 2. Soil treatment closer towards the harvest was preferred as opposed to pre-sow or post-germination treatment to avoid detrimental growth depressions. As observed in Meers

Table 2
Treatments used in the plant experiment (applied 1 week before harvest); T0 presents the untreated control, certain treatments (T1, T5, T6, T8, T10, T14) received a second application 3 days after the initial treatment, the high loads of NH_4 citrate (T11, T12) were evenly spread over four consecutive days

T0	Control	
T1	0.8 + 0.8 mmol	EDDS
T2	1.6 mmol	
T3	2.4 mmol	
T4	4 mmol	
T5	2 + 2 mmol	
T6	4 + 4 mmol	
T7	0.8 mmol	EDTA
T8	0.8 + 0.8 mmol	
T9	1.6 mmol	
T10	2 + 2 mmol	
T11	25 mmol	NH_4 citrate
T12	100 mmol	
T13	0.8 mmol	NTA
T14	0.8 + 0.8 mmol	

et al. (2004), phytotoxic effects induced by metal mobilisation in a pre-sow or post-germination treatment considerably limited overall metal extraction due to severely reduced biomass production. Soil treatment was performed by dissolving the amendments in deionised water and applying the solutions to the top of the pots. In addition to EDDS and EDTA, NTA and NH_4 citrate were evaluated. For EDTA and EDDS, Na-salts were used. Treatments T1, T5, T6, T8, T10 and T14 received a 2nd application 3 days after the first. The NH_4 citrate treatment was spread over four consecutive days. One week after treatment, all plants were harvested, oven-dried (60 °C) and weighed. All treatments were performed in triplicate. Shoot samples were ground using a Culatti DCFH 48 grinder and sieved with a 1 mm sieve. Samples were then ashed at 450 °C, dissolved in 6 M HNO_3 on a hot plate (150 °C for 30 min). The resulting solution was passed through a blue ribbon filter (Schleicher & Schuell, Dassel, Germany) and the resulting filtrate was then analysed for heavy metals using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA).

In order to determine post-harvest mobility of heavy metals in the pots, the pots were kept moist at 2/3 field capacity and fitted with Rhizon-MOM for soil solution extraction (MOM-type Rhizons; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Two weeks and five weeks after harvest, soil solution samples were collected and analysed for levels of heavy metals.

In a second, follow-up plant experiment, the kinetics of plant absorption of mobilised metals were assessed by harvesting plants at varying time periods after treatment. Pots were filled with 2.5 kg of sediment and sown with *H. annuus* 'giganteus'. Soil moisture content was kept at 2/3 field capacity. The pots were grown outside during the summer (May–September). After 3 months of growth, 7.5 mmol EDTA was applied to the top of the pots, dissolved in deionised water. Subsequently plants were harvested 1, 2, 3 or 4 weeks after treatment and analyzed for metal content.

2.4. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. Dunnet's pairwise multiple comparison *t* test was used to compare all plant performances against the control mean, and to check for the statistical significance of observed differences.

3. Results

3.1. Soil solution experiment

Observed concentrations of heavy metals in the soil solution, following treatment with EDDS and EDTA are presented in Fig. 1. Levels of Cr have not been included because they were not affected significantly by any of the treatments. Levels observed in the control pots have been indicated in bold. The control levels varied only a little over time: for Zn 0.19 ± 0.08 $mg\,l^{-1}$, for Cu 0.20 ± 0.19 $mg\,l^{-1}$, for Cd 0.03 ± 0.02 $mg\,l^{-1}$ and for Ni 0.26 ± 0.08 $mg\,l^{-1}$. Over time, decreases in metal mobilisation were observed for the pots treated with EDDS. However, no such observation could be made for EDTA. The observed decreases were associated to the respective biodegradability of these substances. For EDDS, the induced effects were largely dissipated 30 days after treatment, while for EDTA no significant decrease could be observed even after 40 days.

Mobilisation of Zn was initially comparable for both chelators, but decreased over time in EDDS treated pots only. Cu mobilisation by EDDS appeared to be independent of applied dose with induced levels between 130 and 180 $mg\,l^{-1}$. Mobilisation of Cu in EDTA treated pots was substantially lower and dose dependent, varying between 18 and 86 $mg\,l^{-1}$ in the lowest and highest treatments. Cd was only to a small extent mobilised by EDDS, while mobilisation by EDTA was substantially higher. Ni was initially more mobilised by EDDS than by EDTA. However, levels in EDTA treated pots increased over time whereas they decreased in pots treated with EDDS.

3.2. Enhanced phytoextraction experiments

3.2.1. Plant uptake

Shoot concentrations of Zn, Cu, Cd and Ni after treatment with soil amendments are presented in Fig. 2. Levels observed in the untreated control were: 96 ± 6 $mg\,kg^{-1}$ for Zn, 8.3 ± 0.9 $mg\,kg^{-1}$ for Cu, 1.4 ± 0.2 $mg\,kg^{-1}$ for Cd and 2.5 ± 0.4 $mg\,kg^{-1}$ for Ni. Zn uptake was significantly enhanced ($p < 0.05$) by most of the EDDS-treatments and the two highest EDTA treatments (1.6 mmol, 2 + 2 mmol). At the 0.1 level, increases observed for T3 and T8 were also considered significant. NTA and NH_4 citrate did not appear to enhance Zn uptake significantly. Cu uptake was significantly enhanced by all EDDS treatments, but by none of the EDTA, NTA or NH_4 citrate treatments. Cd uptake was only significantly enhanced in the 2 + 2 mmol EDDS and the 1.6 mmol EDTA treatments. Again, no effects were observed for the treatments with NTA or NH_4 citrate. Ni uptake was significantly enhanced at the 0.05 level by NTA and by 5 out of 6 EDDS treatments. The 6th EDDS treatment (4 + 4 mmol) induced elevated levels, which proved to be significant at the 0.1 level. For all treatment, any observed increased shoot contents appear to be independent of the applied dose of the soil amendment. This metal accumulation pattern may suggest that either this plant can only be

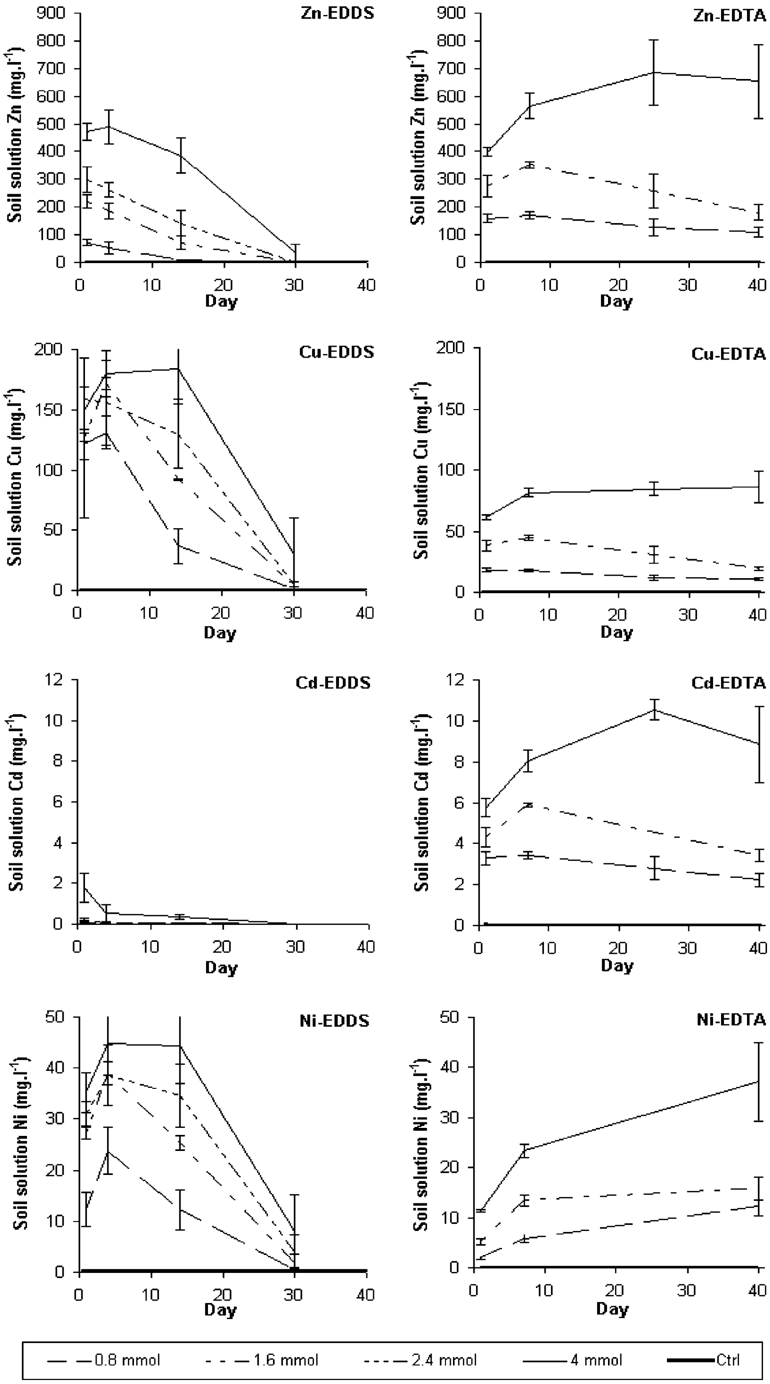

Fig. 1. Concentrations of heavy metals in the soil solution after treatment with 4 mmol, 2.4 mmol, 1.6 mmol or 0.8 mmol EDDS (left) or with 4 mmol, 1.6 mmol or 0.8 mmol EDTA (right); bold line indicates levels in the untreated control, intervals denote standard deviation ($n = 4$).

induced to accumulate up to the observed levels or that the limiting factor in our experiment concerned the plant physiological kinetics for absorption and translocation.

In the second case, the rate of mass transfer from soil to shoot should be taken into account when choosing the optimal moment of soil treatment.

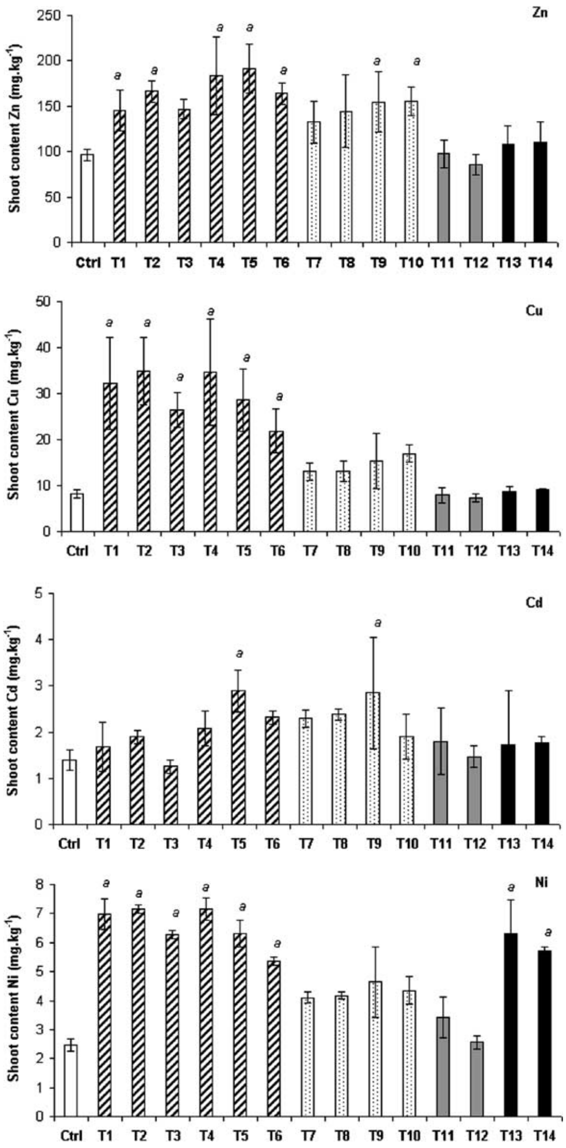

Fig. 2. Heavy metal content in shoots of *H. annuus* 'giganteus' following treatment with soil amendments: white = control, striped = EDDS, dotted = EDTA, gray = NH_4 citrate, black = NTA (for detailed description treatments, cfr. Table 2); intervals denote standard deviation ($n = 3$).

The average dry weight production per plant was 3.7 ± 0.8 g and was not significantly influenced by any of the treatments. This was attributed to the fact that the soil amendments were applied only one week before harvest. The longer the time period between treatment and harvest, the greater the growth depression as caused by metal toxicity may be. Heavy metal (Cu, Cd, Ni, Zn) extraction on a molar base was 6.3 µmol per pot in the control, 8.0–15.1 µmol per pot in the EDDS treatments and 8.8–10.6 µmol per pot in the EDTA treatments. Levels for NTA and NH_4 citrate were similar to those observed in the control. These extraction values suggest that only a fraction of the mobilised metals, as described in Section 3.1, are effectively absorbed by the plant and subsequently translocated to the shoot. To estimate the relative importance of the effectively absorbed fraction of mobilised metals, the maximum mobilised mmolar levels observed in the soil solution experiment were compared to the mass extracted per plant in pots receiving identical doses of chelators. For example in the treatment with the highest observed total metal extraction (1.6 mmol EDDS), only 2.2% of mobilised Zn, 0.8% of mobilised Cu and 7.3% of mobilised Ni were recovered in the shoots of *H. annuus*. However, it can be argued that these levels may increase by prolonging the exposure time of the plants or in other words by fixing the time of (EDDS) application at an earlier time before harvest (cfr. also Section 3.2.3).

3.2.2. Post-harvest effects

Prolonged mobilisation of heavy metals after harvest is highly undesirable as the absence of an actively transpiring crop may result in percolation of mobilised metals. Therefore, the effects induced by soil amendments should be largely gone by the time of harvest or at least attenuate rapidly thereafter. To examine the decrease in metal mobilisation, the soil solution was extracted 14 and 34 days after harvesting the plants. Control values observed in the pots did not differ significantly from those observed in the soil solution experiment (cfr. Section 3.1).

Two weeks after harvest and three weeks after initial soil treatment, the soil solutions of the EDDS-treated pots still contained elevated levels of heavy metals. Zn content varied from $35 \pm 3\,\mathrm{mg\,l^{-1}}$ in the lowest EDDS treatment (T1), over $155 \pm 74\,\mathrm{mg\,l^{-1}}$ (2 + 2 mmol) to $410 \pm 217\,\mathrm{mg\,l^{-1}}$ in the highest EDDS treatment (4 + 4 mmol). Likewise, Zn levels in EDTA-treated pots were elevated and varied from $251 \pm 9\,\mathrm{mg\,l^{-1}}$ in the lowest treatment (0.8 mmol), over $353 \pm 107\,\mathrm{mg\,l^{-1}}$ in the pots treated with 1.6 mmol EDTA and to $595 \pm 146\,\mathrm{mg\,l^{-1}}$ in the highest treatment (2 + 2 mmol). Levels in the NTA or NH_4 citrate treatments were not significantly higher than those observed in the untreated controls. Thirty-four days after harvest, levels of heavy metals had diminished in all pots, suggesting biodegradation or physicochemical removal of the amendments from the soil solution. Levels observed in the EDDS treated pots ranged from $2.9 \pm 1.9\,\mathrm{mg\,l^{-1}}$ in the lowest EDDS treatment (0.8 + 0.8 mmol), over $10.7 \pm 11.7\,\mathrm{mg\,l^{-1}}$ (2 + 2 mmol) to $56 \pm 38\,\mathrm{mg\,l^{-1}}$ in the highest EDDS treatment (4 + 4 mmol). Zn levels in the EDTA treated pots 5 weeks after harvest, ranged from $86 \pm 21\,\mathrm{mg\,l^{-1}}$ in the lowest treatment (0.8 mmol), over $176 \pm 68\,\mathrm{mg\,l^{-1}}$

in the pots treated with 1.6 mmol EDTA and to 239 ± 54 mg l^{-1} in the highest treatment (2 + 2 mmol).

3.2.3. Accumulation kinetics

Fig. 3 exhibits the observed dry weight biomass and the shoot content of Zn, Cu, Cd and Ni in shoots of *H. annuus* 'giganteus' after 1–4 weeks following treatment with EDTA. The selected dose was 7.5 mmol, applied to 2.5 kg of dredged sediment after 3 months of growth. The heavy metal levels in the untreated control did not vary significantly over 4 weeks. The observed shoot concentrations levels in the control pots were: 0.8 ± 0.2 mg kg^{-1} Cd, 18.1 ± 1.5 mg kg^{-1} Cu, 0.8 ± 0.2 mg kg^{-1} Cr, 2.2 ± 0.6 mg kg^{-1} Ni, and 146 ± 12 mg kg^{-1} Zn. These concentrations were significantly higher than the levels observed for the control pots in the greenhouse experiment, particularly for Cu and Zn. This was attributed to differences in season effects and light conditions. A significant increase in heavy metal content was observed only 3 weeks after application. After 4 weeks, concentrations appeared to decline again, although in our experiment this decline was not observed to be significant. Dry weight in the control pots increased over time and was 3.1 ± 0.1 g after 4 weeks. Biomass production of EDTA treated pots did not increase over time and remained constant at 2.4 ± 0.1 g.

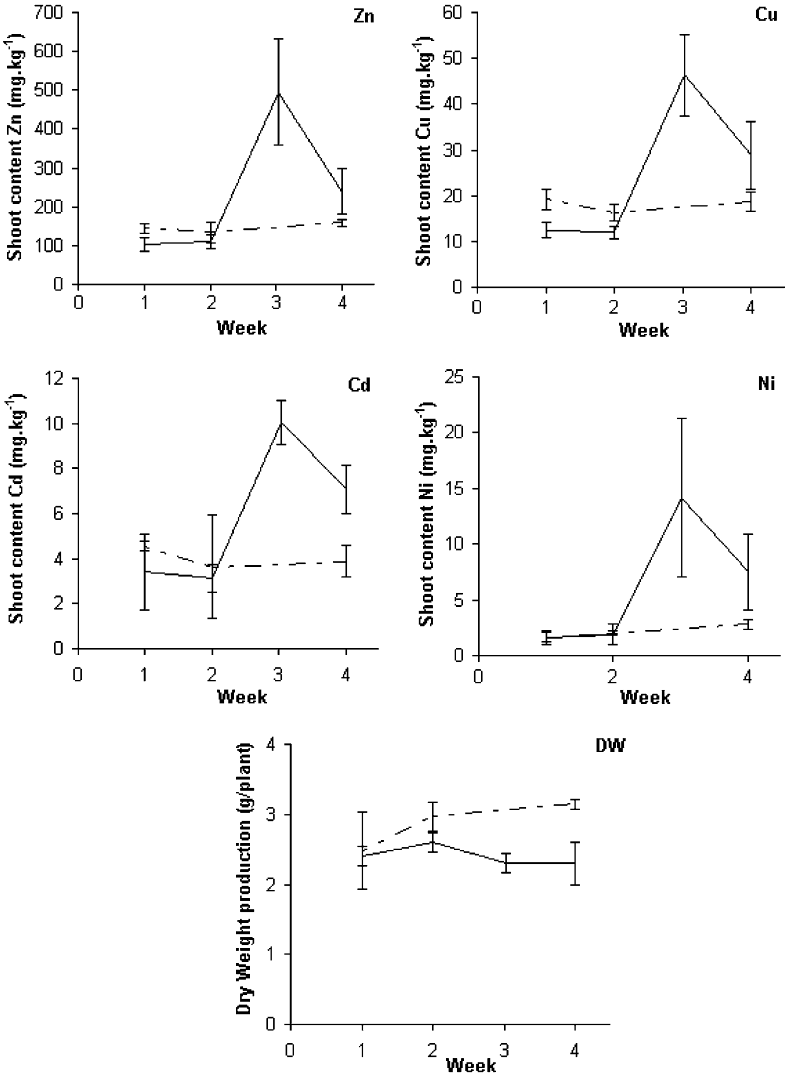

Fig. 3. Shoot concentration of heavy metals and shoot dry weight observed in *Helianthus annuus* 'giganteus' after addition of EDTA to the soil (7.5 mmol/3 kg soil); dashed line indicates levels for the untreated control; intervals denote standard deviation ($n = 3$).

4. Discussion

4.1. Soil solution experiment

4.1.1. Heavy metal mobilisation

Soil solution levels of Cd, Cu, Cr, Ni and Zn were evaluated for a period of 40 days after soil treatment with EDDS or EDTA. Cr was not mobilised by either soil amendment under study. Cu mobilisation by EDDS appeared to be dose independent, with the lowest dose (0.8 mmol) mobilising equal amounts than the highest dose (4 mmol). Zn on the other hand was increasingly mobilised with increasing dosage of EDDS. Cd was only slightly influenced, while Pb remained below the detection limit at all times. Effects on Ni were comparable to those observed for Cu. This pattern suggests that EDDS first scavenges the Cu- and Ni-complexable pool and that surplus chelator molecules move to the next available metal. This observed selectivity for Cu, Zn and Ni and the lack thereof for Cr, Pb and Cd has important repercussions for enhanced phytoextraction of soils contaminated with Cu and Ni. At the lowest applied dose 63% of the mobilised metals were Cu, 25% Zn and 12% Ni. At the highest dose the balance shifts to 67% Zn, 26% Cu and 7% Ni. The additional chelator molecules applied with increasing dosage are therefore used for mobilisation of Zn, while Ni and Cu mobilisation appear to have reached saturated levels at lower doses. The ligand effect, defined as the mmolar amount of metals mobilised per mmol chelator applied to the soil varied between 0.44 mmol at the highest dose to 0.65 mmol at the lowest applied dose. Therefore, the efficiency decreased at increasing dose of EDDS.

Cu mobilisation by EDTA was significantly and substantially lower than was observed for EDDS: at the lowest applied dose, EDTA was able to induce soil solution levels of 18 mg l^{-1}, while an equal dose of EDDS resulted in the mobilisation of 131 mg l^{-1} Cu. Mobilisation by EDTA proved to be dose dependent, unlike as was observed for EDDS treatments. This suggests, that no saturation of Cu complexation was observed within the range of EDTA applied in our experimental setup. Initial mobilisation of Zn was comparable to that induced by EDDS, yet Zn levels continued to increase over time to levels significantly exceeding those observed for EDDS. Cd was more explicitly mobilised by EDTA than by EDDS to levels ranging between 3 and 11 mg l^{-1} in the lowest and highest dose of EDTA. Similarly, Pb was mobilised by EDTA to levels ranging between 3–49 mg l^{-1}, while Pb levels in the EDDS treatments remained below the detection limit. The proportional distribution of metal mobilisation after EDTA treatment was as follows: 85 ± 1% Zn, 10 ± 1% Cu, 3.4 ± 0.2% Ni, 0.9 ± 0.1% Cd, 0.5 ± 0.5% Pb. This distribution pattern did not differ significantly between the various applied doses of EDTA. For every mmol EDTA added per kg soil, an additional 0.48–0.63 mmol of heavy metals were mobilised into the soil solution. The ligand effect for EDTA is therefore similar to the one described for EDDS.

Distinct differences in mobilisation patterns by both amendments could be observed: Zn was comparable for both substances, Cu was mobilised more by EDDS than by EDTA, Cd and Pb were mobilised more by EDTA than by EDDS and initial mobilisation of Ni was highest for EDDS but was overtaken by EDTA as time progressed.

4.1.2. Effect half life

Effect half life is defined as the decrease of heavy metal mobilisation by soil amendments over time. It can be calculated by substituting C_t by $C_0/2$ in the following equation (1):

$$C_t = C_0 \cdot e^{-kt} \tag{1}$$

with C_0, the concentration of mobilised heavy metals at initial mobilisation (mM); C_t, the concentration of mobilised heavy metals at time t (mM); k, the mobilisation decrease constant (≈chelator degradation constant), t, the time since treatment (d).

To solve the equations obtained from the observed soil solution levels, the equation was first log-transformed into Eq. (2). Subsequent linear regression allowed ascertaining the k-values for each treatment. Finally, these values resulted in the calculation of the corresponding observed effect half life.

$$\mathrm{Ln}(C_t) - \mathrm{Ln}(C_0) = -kt \tag{2}$$

Table 3 exhibits the k-values, corresponding $t_{1/2}$ values and observed correlation coefficients of the linear regression as presented by Eq. (2) for all treatments. For all treatments, mobilisation initially continued to increase the first days. The decrease in ligand effect therefore was calculated based from the 2nd sampling point onwards. This lag-phase, over which soil solution levels will increase rather than diminish should be taken into account. For EDDS this lag-phase will be <4 days, for

Table 3
Observed decrease in ligand effect (metal mobilisation) as described by Eq. (2) for pots treated with EDDS and EDTA and the correlation coefficient of the linear correlation; k = decrease constant (dimensionless), $t_{1/2}$ = half life (d)

		k	$t_{1/2}$	R^2
EDDS	4.0 mmol	0.09	7.5	0.89
	2.4 mmol	0.15	4.7	0.92
	1.6 mmol	0.16	4.2	0.95
	0.8 mmol	0.18	3.8	0.99
EDTA	4.0 mmol	0	∞	–
	1.6 mmol	0.019	36	0.99
	0.8 mmol	0.012	59	0.85

EDTA <7 days. The increase within this initial time period is assumed to be caused by the fact that distribution of the soil amendment over the soil profile and subsequent mobilisation of heavy metals are more slow-working than the 24 h incubation period respected in our experimental setup. After this small initial increase, a strong and steady decrease was observed for EDDS. Ligand effect half lives varied between 3.8 and 7.5 days, which makes the effect short-lived. The rate of decrease appeared to be dependent of applied dose: the lowest dose exhibited the steepest relative decrease. This was attributed to potential additional metal toxicity at higher mobilisation levels, causing slightly lower microbial activity and therefore lower biodegradation rates. For EDTA, the highest applied dose (4.0 mmol) exhibited no decrease in mobilisation in the 40 days after application. In the lower doses, slow decrease could be observed with half lives ranging from 36 to 59 days. The longevity of the ligand effect is attributed to the environmental persistence of this substance.

Ligand effect half life is a crucial parameter when considering the implementation of enhanced phytoextraction procedures. Short living substances such as EDDS can be used to dramatically increase extraction by a standing crop. The induced effect dissipates sufficiently fast to prevent downward motion of mobilised metals, while it endures sufficiently long to enhance phytoextraction. The use of soil amendments in this context requires the maintenance of the following hydrological balances (Eqs. (3) and (4)).

$$E + T > P \tag{3}$$

with E, the soil water evaporation (ml m^{-2}); T, the plant transpiration (ml m^{-2}); P, the climatological precipitation (ml m^{-2}).

$$P_{\text{event}} < (\text{FC} - M) \times W \tag{4}$$

with P_{event}, a single event or string of events with increased precipitation (ml^{-2}); FC, the field capacity (ml kg^{-1}); M, the moisture content (ml kg^{-1}); W, the weight of treated top soil layer (kg m^{-2}).

When performed towards the end of the growing season, the crop will exhibit a strong active evapotranspiration. In temperate regions, such as Western Europe, evapotranspiration exceeds climatological precipitation during the summer months. This implies the prevention of downward motion of water and hence the percolation of mobilised metals. The condition stated in Eq. (3) can therefore be met in large areas of the world. Any weather system can display short outbursts of increased volumes of rainfall (storm events). The system is buffered against such events by the difference between actual soil moisture content in the top soil layer and the field capacity of the soil. Only when this difference is bridged by additional water input over a short time span, sufficiently short not to be able to be compensated by plant transpiration, then the mobilised metals run the risk of being leached to underlying soil layers. At a half life of 3.8 days, 94% of the ligand effect will have been dissipated within a time span of 15 days. At a field capacity of 500 ml kg^{-1} in clayey soils and 3 500 000 kg ha^{-1} in the top 25 cm layer, a single raining event would require to deposit 58.3 l per m^2 (or 5.8 cm) to bridge the difference between field capacity and 2/3 of field capacity. It can therefore be concluded that enhanced phytoextraction is feasible with short-lived substances that allow control of the relevant parameters over this short engineered window of metal mobilisation. The ability to implement a waterscreen on small sites may provide for additional safeguards.

From the above, it becomes apparent that long-lived substances, such as EDTA, are inappropriate for the use in enhanced phytoextraction: its longevity will cause elevated metal mobility, even long after harvest. After the crop has been removed from the site the hydrological conditions stated in Eqs. (3) and (4) can no longer be guaranteed. Practically, this will imply leaching of metals in the autumn and winter months and consequential contamination of groundwater.

4.2. Enhanced phytoextraction experiments

4.2.1. Plant uptake

None of the treatments significantly affected biomass production. This was attributed to the short time span between treatment and harvest. Heavy metal content in the control plants was: 96 ± 6 mg kg^{-1} for Zn, 8.3 ± 0.9 mg kg^{-1} for Cu, 1.4 ± 0.2 mg kg^{-1} for Cd and 2.5 ± 0.4 mg kg^{-1} for Ni.

Zn uptake in EDDS and EDTA treated pots was 150–200% than that in the untreated controls. Application of NTA or NH$_4$ citrate did not result in significant increases. Although significant, the observed increases are not considered sufficient for optimal enhanced phytoextraction. At a dry weight production of 25 t ha^{-1} y^{-1}, concentrations of 200 mg kg^{-1} Zn in the shoot suggest an annual reduction of Zn in the top soil layer (25 cm) of merely 1.4 mg kg^{-1}. For optimal performance, >500 mg kg^{-1} Zn in the shoots is desirable. However, the level of uptake in our experiment may be unduly low because of the short time span between soil treatment and harvest (7 days). Longer periods may be required, both in light of sufficient time for redistribution of the amendment throughout the top soil profile (cfr. Section 4.1) and in light of plant limiting plant kinetics for root absorption and subsequent translocation (cfr. Section 4.2.3). However, the time of application will also be function of climatological conditions (cfr. Section 4.1.2). Furthermore, the optimal moment will be a compromise between (a) postponing the treatment to minimize potential growth depression induced by metal toxicity upon increased phytoavailability and

(b) fixing time of application at an earlier time to guarantee sufficient exposure time for uptake and to prevent post-harvest mobility of heavy metals.

Cu uptake was significantly affected only by EDDS: observed shoot levels were 265–422% that of the untreated control. Levels of >30 mg kg^{-1} Cu were no exception. Even so, average shoot levels of this order of magnitude suggest an annual decrease of only 0.2 mg kg^{-1} in the top soil layer in a high biomass producing crop. Interestingly, Cu mobilisation and uptake appeared to be dose independent for soils treated with EDDS. This suggests that even at the lowest dosage, all complexable Cu was selectively mobilised. Only when all complexable Cu was chelated, the other elements, such as Zn, were thought to be progressively mobilised. Mobilisation of Cu by equal doses of EDTA proved to be lower than observed for EDDS. Also, for EDTA the mobilisation was dose-dependent.

Cd uptake was only deemed significant in the highest EDDS treatment and the second highest EDTA treatment. At observed shoot levels of maximum 2.9 mg kg^{-1}, Cd accumulation was not deemed sufficient for phytoextraction purposes. Annual removal of 0.1 mg kg^{-1} from the top soil layer would require shoot levels of up to 20 mg kg^{-1} in a crop with a yield of 25 t ha^{-1} y^{-1}. Ni uptake was significantly enhanced in EDDS treated pots and pots treated with NTA with shoot levels of 217–290% of those observed in the control. Again, higher levels will be required to consider effective phytoextraction.

4.2.2. Post-harvest effects

Only a limited fraction of mobilised metals was effectively absorbed and translocated by the plant: in the treatment with the highest observed total metal extraction (1.6 mmol EDDS), only 2.2% of mobilised Zn, 0.8% of mobilised Cu and 7.3% of mobilised Ni are recovered in the shoots of *H. annuus*. This is another reason why amendments and their effects should be short-lived: only a fraction of the mobilised metals are extracted from the soil solution, the remaining portion must be re-stabilised by biodegradation of the ligand. Post-harvest effects are highly undesirable because the absence of an actively transpiring crop greatly increase the risk for percolation of the mobilised metals.

Post-harvest decreases in ligand effect occurred more rapidly than in the soil solution experiments. This effect was more pronounced for EDTA than for EDDS. The higher rate of ligand degradation was attributed to a culmination of two things: (i) microbial activity was expected to be higher in pots which had contained plants for 11 weeks and (ii) soil temperature in the greenhouse experiment was expected to be slightly higher than the soil solution experiment, which was performed at room temperature. The ligand effect half life in pots treated with 4 mmol EDDS was 5.2 days as compared to 7.5 days in the soil solution experiment. In addition, pots treated with double this amount still exhibited a reasonably low ligand effect half life of 6.6 days. Decreases in pots treated with 0.8 + 0.8 mmol EDDS had more or less similar half life values than were observed for the soil solution experiment: 4.5 days as opposed to 4.2 days. Decrease rates in EDTA-treated pots were substantially higher in the plant experiment than in the soil experiments described in 4.1, with *k*-values ranging from 0.034–0.052 as compared to 0–0.019. This resulted in half lives ranging from 13 to 20 days. The rate of decrease was observed to be dose dependent, with the lowest EDTA-dose exhibiting the steepest relative decrease in ligand effect. For NTA and NH_4 citrate, heavy metal concentrations in the soil solution 2 weeks after harvest did not differ significantly from the controls.

4.2.3. Accumulation kinetics

Based on the dose independent shoot accumulation of heavy metals as observed in Fig. 2, it was assumed that plant physiological kinetics may induce an additional limiting factor. It is plausible that the rate of mass transfer of mineral components from soil to root and root to shoot may operate within set boundaries as well. Or in other words, that in the one week between treatment and harvest in our experiment, the plants could only absorb and translocate the observed mass of minerals. Although grown on the same sediment, shoot levels of essential heavy metals in the control pots were significantly higher than those observed in the greenhouse pot experiment: 143 ± 19 mg kg^{-1} for Zn and 18 ± 2 mg kg^{-1} for Cu. This was attributed to longer day lengths and higher light intensity in the summer months as compared to the previous experiment which took place in spring. Mineral nutrition is a light driven process (Raven et al., 1999), therefore conditions during summer may be more optimal for effective mineral extraction of essential heavy metals. Shoot levels of the non-essential heavy metals in the controls of the pot experiment during summer did not differ significantly from the concentrations observed during the spring greenhouse experiment (as discussed in Section 4.2.1).

The experiment revealed that during the first two weeks after application, shoot levels of heavy metals did not increase in comparison to the control, while a rapid increase was observed after 3 weeks. The subsequent drop in shoot levels after 4 weeks was found to be statistically insignificant. However the observed drop deserves further research to ascertain whether complexed metals could potentially be remobilised from the shoot upon initial accumulation. The observed accumulation pattern suggests that plants may require a sufficient exposure time for optimal effect. This should be taken into consideration when applying soil treatments towards the end of the growing season.

5. Conclusion

Strong mobilisation of heavy metals was observed for both chelators. However, there were distinct differences between both amendments: Zn mobilisation was comparable for both substances, Cu was mobilised more by EDDS than by EDTA, Cd and Pb were mobilised more by EDTA than by EDDS and initial mobilisation of Ni was highest for EDDS but was overtaken by EDTA as time progressed. The mobilisation effect decreased rapidly for the soils treated with EDDS, with half lives of 3.8–7.5 days, depending on the applied dose. The effect half life observed in EDTA treated soils ranged between 36 days and infinity (no degradation observed within 40 days of monitoring). The strong persistence of EDTA makes it unsuitable for use in phytoextraction under normal field conditions.

In the plant experiment, none of the treatments significantly affected biomass production of *H. annuus*. This was attributed to the short time span between treatment and harvest (7 days). Significant increases in plant uptake were observed for Zn in all EDDS-treated pots and in the highest doses of EDTA. For Cu significant increases were only observed in the EDDS-treated pots, for Cd in one of the EDDS and one of the EDTA treatments and for Ni in all pots treated with either EDDS or NTA. However, none of the observed increases were sufficiently convincing under the current experimental conditions to consider for field application of phytoextraction. This may potentially be caused by a lack of exposure time to the induced metal mobility with only 7 days between treatment and harvest. A kinetics experiment validated this possibility: accumulation levels in the shoot of *H. annuus* revealed that the enhanced increase became significant 3 weeks after treatment. Future experiments will fix the moment of treatment at an earlier time before harvest. Post-harvest mobility of heavy metals in the soil solution was evaluated 2 and 5 weeks after harvest. The observed rate of decrease in the ligand effect was slightly more rapid in the plant experiment than was observed in the soil solution experiment. Post-harvest half lives ranged from 4.5 to 6.6 days in the EDDS treated pots and from 13 to 20 days in EDTA treated pots. The relatively strong decrease pattern for EDTA is unexpected and deserves further attention. The rate is probably still slightly too slow in regards to potential risks for leaching, but is much faster than observed in the soil experiment.

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