

Potential of *Brassic rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils

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Abstract

Remediation of soil pollution is one of the many current environmental challenges. Anthropogenic activity has resulted in the contamination of extended areas of land, the remediation of which is both invasive and expensive by conventional means. Phytoextraction of heavy metals from contaminated soils has the prospect of being a more economic in situ alternative. In addition, phytoextraction targets ecotoxicologically the most relevant soil fraction of these metals, i.e. the bioavailable fraction. Greenhouse experiments were carried out to evaluate the potential of four high biomass crop species in their potential for phytoextraction of heavy metals, with or without with the use of soil amendments (EDTA or EDDS). A calcareous dredged sediment derived surface soil, with high organic matter and clay content and moderate levels of heavy metal pollution, was used in the experiments. No growth depression was observed in EDTA or EDDS treated pots in comparison to untreated controls. Metal accumulation was considered to be low for phytoextraction purposes, despite the use of chelating agents. The low observed shoot concentrations of heavy metals were attributed to the low phytoavailability of heavy metals in this particular soil substrate. The mobilising effects induced by EDTA in the soil were found to be too long-lived for application as a soil amendment in phytoextraction. Although EDDS was found to be more biodegradable, higher effect half lives were observed than reported in literature or observed in previous experiments. These findings caution against the use of any amendment, biodegradable or otherwise, without proper investigation of its effects and the longevity thereof.

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1. Introduction

Soil pollution by heavy metals is a widespread problem posing considerable threats to the environment. In Flanders (Belgium), current estimates of hazardous contaminated black spots requiring priority clean up, reach

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28 000 ha or 1.4% of the total surface area (Ceenaeme et al., 2001). Remediation of this area proposes to be a very expensive endeavour. Based on overall costs of the first four years of the Flemish soil remediation decree (1996–2000) divided by actual remediated mass of soil, the cost can be estimated at \$410 per ton soil (De Naeyer, 2000). Due to high costs involved, local legislation has granted itself a period of 40 years (until 2036) to remediate the priority black spots. In the meantime, these lands remain barren, posing continued risks. In addition, other more moderately contaminated sites, which may still pose environmental risks, are excluded from immediate remediation. Remediation with conventional engineering methods can thus become prohibitively expensive (Salt et al., 1995; Lasat, 2000). Dredging activities for the maintenance of waterways in Flanders produce $4-6 \times 10^6 \text{ m}^3 \text{ years}^{-1}$ of sediments requiring disposal on land (Tack et al., 1995; Singh et al., 1998). Due to the historically polluted state of Western European waterways, these sediments generally contain elevated concentrations of heavy metals. Both sediment disposal on land and natural sedimentation processes therefore generate additional pollution on a systematic basis.

Phytoremediation is a process that uses living green plants for the in situ risk reduction of contaminated soil, sludge, sediments and groundwater through contaminant removal, degradation or containment (EPA, 1998). Phytoextraction is the removal of components from the soil by plants (Cunningham and Berti, 1993; Garbisu and Alkorta, 2001). Plants extract metals, such as Co, Cu, Mn, Mo, Ni and Zn to meet their mineral nutritional demands (Lasat, 2000). In addition to the active uptake of these essential micronutrients, non-essential elements such as Cd and Pb can be taken up unintentionally. Intensive plant culturing and export of the produced biomass will therefore result in a gradual decrease of metal content in the top soil layer. The overall removal of the target contaminants is determined by the product of the annual harvestable biomass and the metal content contained within this biomass. Two distinct different strategies have emerged, seeking to optimise the potential of phytoextracting crops: (i) the use of natural metal hyperaccumulators and (ii) the use of amendment induced hyperaccumulation. Although capable of achieving very high concentrations of heavy metals in the shoot, hyperaccumulators tend to have low annual biomass productions ultimately limiting their applicability. The induction of hyperaccumulation in agronomic crops with high annual biomass yields has been reported by Cunningham and Ow (1996), Huang et al. (1997) and Blaylock et al. (1997).

Ethylene diamine tetraacetate (EDTA) has received much attention as a soil amendment in phytoextraction research. It is a complexing agent that has been used in agriculture as an additive in micronutrient fertilizers since the 1950s (Wallace et al., 1992; Bucheli-Witschel

and Egli, 2001). Despite the reported potential of EDTA for induced phytoextraction, it has a number of important drawbacks. It exhibits poor biodegradability in the environment (Schowanek et al., 1997; Bucheli-Witschel and Egli, 2001; Jones and Williams, 2002), which in combination with its high affinity for heavy metal complexation, results in increased risks for leaching (Grcman et al., 2001). Ethylene diamine disuccinate (EDDS) is a naturally occurring chelator (Nishikiori et al., 1984; Goodfellow et al., 1997). Schowanek et al. (1997) describe a high degree of biodegradability for EDDS, with observed half lives ranging from 2.5 d in a soil experiment to 4.6 d in an unacclimated Sturm test. The metal chelating ability, accompanied with the short activity time span in the soil due to rapid biodegradation, make EDDS suited to consider as soil amendment for enhanced phytoextraction purposes.

This paper aims to assess the potential of four agronomic plant species with high annual biomass yield (*Brassica rapa*, *Cannabis sativa*, *Helianthus annuus*, *Zea mays*) for phytoextraction of heavy metals from moderately contaminated, calcareous dredged sediment derived, surface soils. The extent to which accumulation in the shoot can be enhanced by EDDS or EDTA is also evaluated. Mobilisation effects induced by these amendments and the longevity of these effects were investigated as well. The moment of soil treatment with these amendments was chosen pre-harvest rather than pre-sow, based on previous experience (Meers et al., 2004a,b; Lesage et al., in press).

2. Material and methods

2.1. Soil characterisation

The soil was air dried, ground in a hammer cross beater mill and sieved through 1 mm filter before analysis or use in the various experiments. Soil conductivity was measured with a WTW LF 537 electrode (Wissenschaftlich-Technischen Werkstätten, 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 pH-H₂O, 10 g of air-dried soil was allowed to equilibrate in 50 ml of deionised water for 24 h. For determination of pH-KCl, 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 supernatant was then measured using a pH glass electrode (Model 520A, Boston, MA, USA). Total carbonate content 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 and Black (Allison, 1965). The particle size distribution of the soil samples was determined

using laser diffractometry (Coulter LS200, Miami, FL, USA) with the clay fraction defined as the 0–6 μm fraction (Vandecasteele et al., 2002). This fraction was found to correspond with the 0–2 μm fraction using the conventional pipette method. Likewise, 6–63 μm was used as the silt fraction and 63–2000 μm as the sand fraction. 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). Field capacity was determined by adding an excess of water to 400 g 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 had fully ended. The pots were then re-weighed and field capacity estimated. Soil metal concentrations were determined after *aqua regia* digestion (Van Ranst et al., 1999). Analysis was subsequently performed, using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA). All analyses were carried out in triplicate. For mass balance calculations of extracted heavy metals, the mass of the top 25 cm soil layer was estimated with apparent soil density of 1.4 kg dm^{-3} or 3.5 10^6 kg ha^{-1} .

2.2. Soil amendment effect on metal mobilisation

To assess the effects of EDTA and EDDS on metal mobilisation into the soil solution, small plastic pots containing 100 g of air dry soil were treated with 0.1, 0.3 or 0.5 mmol of the soil amendments dissolved in 43.6 ml H_2O . This corresponds with a final dose of 1, 3, and 5 mmol kg^{-1} of the chelating agents. The selected volume of solution corresponds with the field capacity of the soil. The pots were kept closed to avoid evaporation, except at frequent time intervals to allow aeration and/or soil sampling. Homogenised soil was collected from the plastic pots after 1 day, 1 week and 3 weeks to evaluate soluble soil fractions of heavy metals. A sample corresponding with 10 g of air dry soil was removed from the plastic containers after mixing the soil. The sample was subsequently mixed with 50 ml of deionised water, incubated overnight while shaken and finally filtered (white ribbon; Schleicher & Schuell, Dassel, Germany). The filtrate was analysed for metals using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA).

2.3. Plant uptake of heavy metals

The comparison of the four agronomic species, with or without application of EDDS or EDTA, was performed as a greenhouse screening. This implies that the findings derived from this study will require field validation. However, the use of mobilising agents, such as EDDS and EDTA, in field trials is sensitive and requires adequate greenhouse evaluation prior to upscaling to the field scale.

Pots were filled with 1 kg of air dried soil and brought to 2/3 of field capacity with deionised water (280 ml per pot). The bottoms of the pots were sealed off to avoid leaching of mobilised heavy metals. Subsequently, seeds of *H. annuus* ‘Giganteus’, *C. sativa* ‘Chameleon’, *B. rapa* ‘Durmelander’ and *Z. mays* ‘Impact’ were sown at a density of 10 seeds per pot. In subsequent weeks after germination, plants were thinned to 1 plant per pot (3 pots per treatment).

Considering the long duration of the pot experiment (11 weeks), all pots were fertilised with a synthetic fertiliser solution to avoid limiting nutritional conditions. Fertilisation in this context is somewhat ambiguous: on one hand nutrient deficiencies may limit the uptake of other inorganic components from the soil (such as heavy metals), on the other hand addition of nutrient salts may influence the chemical availability of heavy metals within the soil matrix. In previous screenings with agronomic crops on this particular soil substrate, the application of the nutrient solution used in this experiment proved not to have significant effects on heavy metal uptake by the plants. To validate this finding in the current experimental setup, compared heavy metal accumulation was compared in fertilised versus unfertilised plants in a separate yet simultaneous experiment, using the same soil substrate. No significant differences in shoot metal concentrations were observed in the fertilised versus the unfertilised pots. The nutrient solution contained 5 g NH_4NO_3 , 2.5 g $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 2.5 g K_2SO_4 and 2.5 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per l of deionised water. Per pot, 4 \times 25 ml of the nutrient solution were applied in consecutive days. The pots, containing 1 kg of air dry soil, were maintained at a constant soil moisture content of 2/3 of the field capacity and cultivated in a greenhouse at a day/night regime of 14 h light per day (forced light). The pot experiment was performed during November–January. To maintain the soil moisture content at desired constant levels, losses in pot weight due to evapotranspiration were compensated on a daily basis.

After 7 weeks of growth, the pots were treated with either deionised water or 3 mmol EDDS or EDTA. Previous experiments suggested that soil treatment should preferably be at least 3 weeks before harvest for optimal uptake. The soil treatments were dissolved in deionised water and applied to the top of the pot. The bottom of the pots was shut watertight to prevent leaching. After another 4 weeks, or 11 weeks after sowing, the plants were harvested, oven dried at 60 $^\circ\text{C}$ and weighed to determine dry weight biomass production. Shoots were ground using a cross hammer beater mill and sieved with a 1 mm sieve. Samples were then ashed at 450 $^\circ\text{C}$ and dissolved in 6 M HNO_3 on a hot plate. The resulting solution was filtered (blue ribbon; Schleicher & Schuell, Dassel, Germany) and the resulting filtrate was analysed for heavy metals using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA).

To make a crude first estimate of the general phytoextraction potential of the various agronomic crops, we first assess attainable biomass production as reported in literature, subsequently multiply this by observed shoot concentrations with or without induced accumulation of heavy metals and finally divide this by the weight of the top soil layer to ascertain achievable annual extraction levels. This extrapolation is only intended to make a calculated estimation on the order of magnitude of potentially phytoextractable metals. Validation in the field would however be required for actual in situ application of the technique.

2.4. Statistical analysis

All analytical determinations were performed in triplicate. Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. Dunnett's pairwise multiple comparison *t*-test was used to compare all soil treatments against the control mean, and to check for the statistical significance of observed differences ($\alpha = 0.05$). LSD one way ANOVA was used to compare biomass production between different plant species ($\alpha = 0.05$). Quality assessment of the *aqua regia* digestion of soils was evaluated on reference sample BCR 141 (Cd $14.0 \pm 0.4 \text{ mg kg}^{-1}$; Cu $46.9 \pm 1.8 \text{ mg kg}^{-1}$; Cr $138 \pm 5 \text{ mg kg}^{-1}$; Ni $94 \pm 5 \text{ mg kg}^{-1}$; Pb $51.3 \pm 0.2 \text{ mg kg}^{-1}$; Zn $270 \pm 8 \text{ mg kg}^{-1}$). Observed results were: $13.3 \pm 0.1 \text{ mg kg}^{-1}$ for Cd, 42.0 ± 1.1 for mg kg^{-1} Cu, $131.0 \pm 1.2 \text{ mg kg}^{-1}$ for Cr, $90.1 \pm 0.1 \text{ mg kg}^{-1}$ for Ni, 49.8 ± 2.0 for Pb mg kg^{-1} , $264 \pm 3 \text{ mg kg}^{-1}$. Quality assessment of the plant extractions was evaluated on a certified ryegrass sample BCR 281 ($0.12 \pm 0.003 \text{ mg kg}^{-1}$ Cd; $9.65 \pm 0.38 \text{ mg kg}^{-1}$ Cu; $2.1 \pm 0.4 \text{ mg kg}^{-1}$ Cr; $3.00 \pm 0.17 \text{ mg kg}^{-1}$ Ni; $2.38 \pm 0.11 \text{ mg kg}^{-1}$ Pb; $31.5 \pm 1.4 \text{ mg kg}^{-1}$ Zn). Observed concentrations were: $0.12 \pm 0.01 \text{ mg kg}^{-1}$ for Cd, 8.95 ± 0.25 for mg kg^{-1} Cu, $1.75 \pm 0.12 \text{ mg kg}^{-1}$ for Cr, $2.81 \pm 0.14 \text{ mg kg}^{-1}$ for Ni, 2.08 ± 0.16 for Pb mg kg^{-1} , $29.0 \pm 1.5 \text{ mg kg}^{-1}$ for Zn.

3. Results

3.1. Soil characterisation

The soil substrate used in the pot experiments was a calcareous dredged sediment derived surface soil, which had been in an oxidised state for more than 8 years prior to sampling for this research. Due to the general state of pollution in Western European waterways, sediments dredged from these waterways contain elevated concentrations of organic and inorganic pollutants. Soil properties and total metal concentrations are presented in Table 1. The levels of Cd and Zn were considered to be slightly elevated. Legal criteria for re-use of this

Table 1

General properties of the soils under study: soil actual acidity (pH-H₂O), soil potential acidity (pH-KCl), electrical conductivity (EC), grain size distribution, soil carbonate content (CaCO₃), organic matter (OM), cation exchange capacity (CEC) and heavy metal concentration; intervals denote standard deviations ($n = 3$)

pH-H ₂ O	–	7.4 ± 0
pH-KCl	–	7.0 ± 0
EC	$\mu\text{S cm}^{-1}$	1153 ± 32
Clay	%	22
Silt	%	28
Sand	%	50
CaCO ₃	%	8.5 ± 0.3
OM	%	4.7 ± 0.7
CEC	$\text{cmol}(+) \text{ kg}^{-1}$	8.2 ± 0.5
Cd	mg kg^{-1}	5.6 ± 0.2
Cu	mg kg^{-1}	90 ± 5
Cr	mg kg^{-1}	123 ± 7
Fe	mg kg^{-1}	6359 ± 813
Mn	mg kg^{-1}	221 ± 14
Ni	mg kg^{-1}	41 ± 2
Pb	mg kg^{-1}	108 ± 5
Zn	mg kg^{-1}	575 ± 28

dredged sediment as soil substrate are, respectively (dependent of organic matter and clay content) 500 mg kg^{-1} for Zn and 2.5 mg kg^{-1} for Cd.

3.2. Amendment effect on metal mobilisation

Table 2 presents metal mobilisation into the soil solution after soil treatment with varying doses of EDDS or EDTA.

3.3. Plant uptake of heavy metals

Fig. 1 exhibits dry weight biomass production and shoot accumulation of Cd, Cu, Ni, Pb and Zn with or without the use of soil amendments. Biomass production (dry weight) in the control was approximately 24 g per plant for *Z. mays*, 10 g for *C. sativa*, 8 g for *B. rapa*, and 4 g for *H. annuus*. Dry weight yield was substantially lower than what one may anticipate under optimal conditions in field grown crops. In particular, the lower growth for *H. annuus* (5.5 times lower than *Z. mays*) may be caused by the fact that this species reacts strongly to the natural seasonal light regime, regardless of additional synthetic light.

3.3.1. Zinc

Zn concentrations varied between 34 and 192 mg kg^{-1} in the untreated controls, with *Z. mays* having the lowest concentrations and *B. rapa* the highest. Treatment with either EDDS or EDTA resulted in increased shoot concentrations of Zn for *C. sativa*,

Table 2

Heavy metal mobilisation into the soil solution (μM) and evolution in time, following soil treatment with varying doses (1, 3, 5 mmol kg^{-1}) of EDDS or EDTA; intervals denote standard deviations ($n = 3$)

	Days	Control	EDDS			EDTA		
			1 mmol kg^{-1}	3 mmol kg^{-1}	5 mmol kg^{-1}	1 mmol kg^{-1}	3 mmol kg^{-1}	5 mmol kg^{-1}
Zn	1	7.2 ± 0.4	442 ± 107	2007 ± 56	2234 ± 43	694 ± 73	1939 ± 114	2656 ± 235
	7	11.3 ± 7.2	595 ± 84	1529 ± 138	1812 ± 646	696 ± 101	1310 ± 206	1948 ± 185
	21	8.6 ± 0.9	10 ± 4	1215 ± 74	1822 ± 65	367 ± 14	2057 ± 66	1493 ± 19
Cu	1	6.6 ± 0.2	372 ± 100	974 ± 25	614 ± 29	63 ± 6	258 ± 9	353 ± 12
	7	6.2 ± 2.7	404 ± 39	444 ± 23	453 ± 36	36 ± 8	47 ± 8	113 ± 21
	21	3.1 ± 0.2	111 ± 51	345 ± 53	470 ± 31	26 ± 2	227 ± 2	75 ± 2
Cd	1	0.06 ± 0.01	0.07 ± 0.01	0.33 ± 0.21	0.98 ± 0.25	10.3 ± 2.0	22 ± 2	28 ± 4
	7	0.07 ± 0.04	0.05 ± 0.01	0.13 ± 0.01	0.18 ± 0.04	9.2 ± 1.2	16 ± 3	22 ± 2
	21	0.08 ± 0.01	0.07 ± 0.04	0.12 ± 0.01	0.07 ± 0.00	1.3 ± 0.1	26 ± 1	16 ± 1
Pb	1	0.1 ± 0.0	0.3 ± 0.0	0.8 ± 0.8	1.2 ± 0.8	3.6 ± 0.3	34 ± 5	69 ± 4
	7	0.4 ± 0.4	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	2.4 ± 0.6	5 ± 0	12 ± 1
	21	0.3 ± 0.1	0.7 ± 0.3	0.4 ± 0.0	0.3 ± 0.1	2.0 ± 0.3	10 ± 0	7 ± 2
Ni	1	3.3 ± 0.2	62 ± 5.5	199 ± 3.4	146 ± 3	6.7 ± 0.5	27 ± 1	52 ± 2
	7	2.9 ± 1.0	171 ± 18.4	196 ± 3.5	205 ± 19	23 ± 4	57 ± 5	109 ± 8
	21	1.7 ± 0.1	27 ± 11.5	237 ± 10	244 ± 4	103 ± 8	181 ± 5	161 ± 3

H. annuus and *B. rapa* up to the range of 243–342 mg kg^{-1} . This implies relative increases, compared to the untreated control, of, respectively, 2.6–3.4 for *C. sativa*, 1.6–1.9 for *H. annuus* and 3.4–3.5 for *B. rapa*. These increases were significant for both treatments in *C. sativa* and for the EDDS in *H. annuus* and *B. rapa*. Shoot concentrations in the monocotyl species *Z. mays* were enhanced with a factor 2.3–3.5 up to concentrations of 78–117 mg kg^{-1} . These increases were significant for both treatments at the 0.05 level (Dunnet's multiple comparison *t*-test).

3.3.2. Copper

Shoot concentrations of Cu varied between 2.7 and 15 mg kg^{-1} , with the lowest concentrations again observed for *Z. mays*, while *C. sativa* exhibited the highest concentrations. Treatment with EDDS or EDTA had distinctly different effects on shoot accumulation of Cu. As has been observed in the soil experiment, EDDS mobilised Cu more than did EDTA (Table 2). This is also reflected in higher observed shoot concentrations in EDDS treated plants in comparison to EDTA. EDDS treated *C. sativa* extracted significantly more Cu than both EDTA and the untreated control. For *H. annuus* and *B. rapa* the significance of the induced effects were in the order EDDS > EDTA > control. Observed increases for *Z. mays* were statistically insignificant.

3.3.3. Cadmium

Cd concentrations in the shoot varied between 0.3 and 1.8 mg kg^{-1} , with the highest concentrations ob-

served for *H. annuus* and the lowest for *Z. mays*. Treatment with EDTA or EDDS had distinctly different effects on shoot accumulation of Cd, with EDTA inducing higher accumulation levels than EDDS. Higher mobilisation of Cd by EDTA than by EDDS in the soil was also observed in Table 2. Shoot concentrations following treatment with EDTA increased to 9.7 mg kg^{-1} in *B. rapa*, 4.4 mg kg^{-1} in *H. annuus*, 3.4 mg kg^{-1} in *C. sativa* and 0.6 mg kg^{-1} in *Z. mays*. For plants treated with EDDS these concentrations were 6.6 mg kg^{-1} in *B. rapa*, 2.1 mg kg^{-1} in *H. annuus*, 1.0 mg kg^{-1} in *Z. mays* and 0.7 mg kg^{-1} in *C. sativa*. The treatment effects were found to be significant only for EDTA treated *B. rapa*.

3.3.4. Lead

Concentrations of Pb in the shoots of untreated controls varied between 0.3 and 2.4 mg kg^{-1} with the highest concentrations observed in *B. rapa* and the lowest observed in *Z. mays*. Concentrations were enhanced more by EDTA than they were by an equal dose of EDDS. The higher degree of mobilisation of Pb in the soil by EDTA has already been described above. Shoot concentrations in EDTA treated pots were 10.2 mg kg^{-1} for *H. annuus*, 8.4 mg kg^{-1} for *B. rapa*, 7.3 mg kg^{-1} for *C. sativa* and 2.4 mg kg^{-1} for *Z. mays*. Pots treated with EDDS resulted in shoot concentrations of 5.2 mg kg^{-1} for *B. rapa*, 2.2 mg kg^{-1} for *C. sativa*, 1.4 mg kg^{-1} for *Z. mays* and 1.2 for *H. annuus*. The effects of EDTA were significant for all plants, except *Z. mays*. Effects of EDDS were only significant for *B. rapa*.

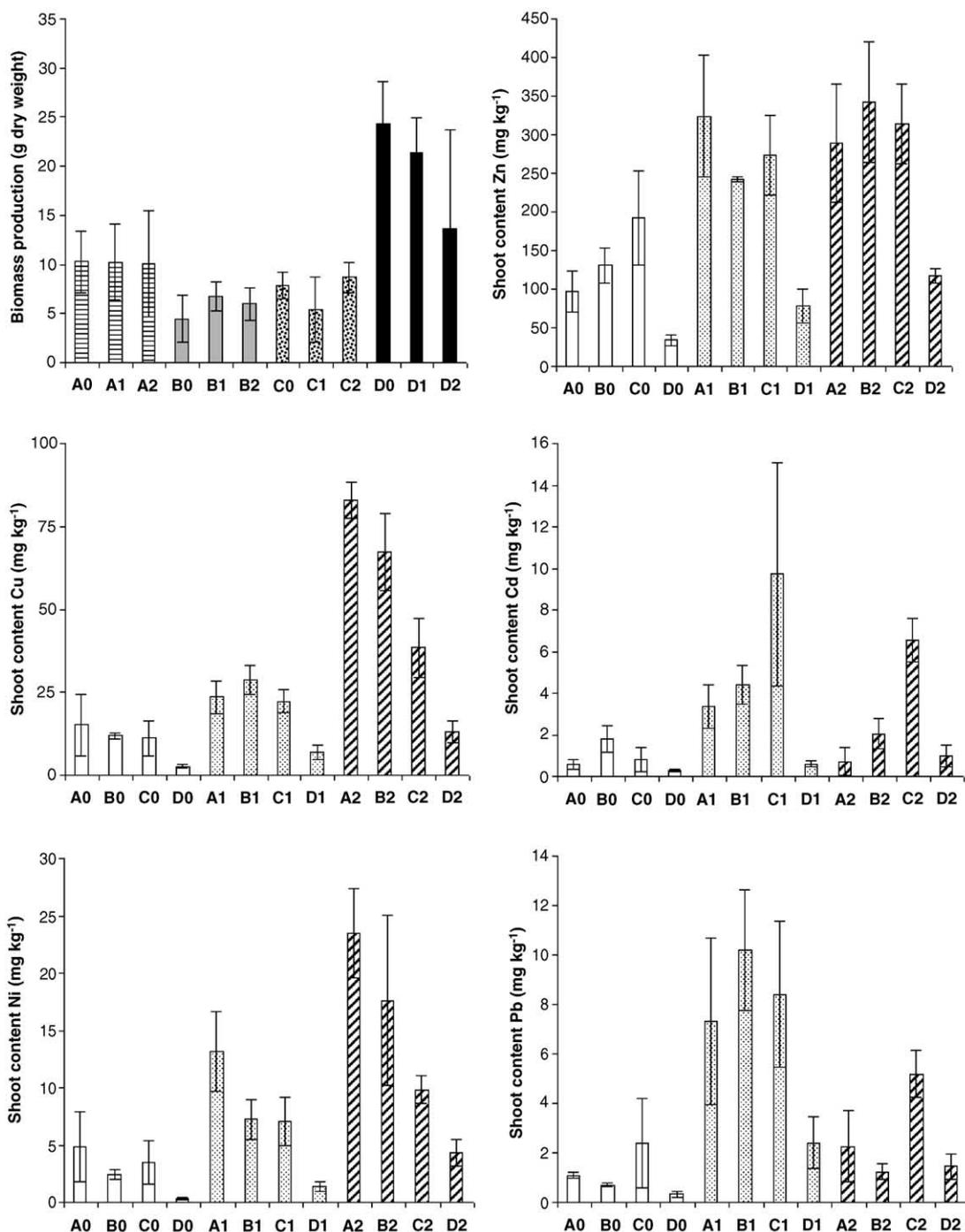


Fig. 1. Biomass production (g dry weight) and shoot concentrations of Zn, Cu, Cd, Ni and Pb (mg kg^{-1} ; dry weight) for: (A) *C. sativa*, (B) *H. annuus*, (C) *B. rapa*, (D) *Z. mays*; treated with: (0) deionised water (ctrl), (1) 3 mmol kg^{-1} EDTA, (2) 3 mmol kg^{-1} EDDS; intervals denote standard deviation ($n = 3$).

3.3.5. Nickel

Concentrations of Ni in the shoots of the control plants varied between 0.3 and 4.8 mg kg^{-1} , with *C. sativa*

exhibiting the highest concentrations and *Z. mays* the lowest. Uptake was stimulated more by EDDS than by EDTA. This is also reflected in higher mobilisation of

Ni by EDDS (Table 2). EDDS enhanced Ni content up to 23 mg kg⁻¹ in *C. sativa*, 18 mg kg⁻¹ in *H. annuus*, 10 mg kg⁻¹ in *B. rapa* and 4 mg kg⁻¹ in *Z. mays*. For *C. sativa* this implies an increase by a factor 4.8 in comparison to the control. EDTA treated plants exhibited lower accumulation levels than EDDS treated plant: 13 mg kg⁻¹ for *C. sativa*, 7 mg kg⁻¹ for *H. annuus* and *B. rapa*, 1.4 mg kg⁻¹ for *Z. mays*. Treatment effects were significant in the order EDDS > EDTA > control for *C. sativa* and *H. annuus* and EDDS > EDTA, control for *B. rapa*. Effects in *Z. mays* were found to be insignificant.

4. Results

4.1. Amendment effect on metal mobilisation

The evolution in time allows us to estimate ligand effect half life. The ligand effect, defined as the mmolar concentration of heavy metals mobilised into the soil solution per mmol chelator added to the soil was very high for both amendments and did not differ significantly at the 0.05 level: 845 ± 232 mmol mmol⁻¹ for EDDS, and 724 ± 80 mmol mmol⁻¹ for EDTA. However, the mobilisation patterns themselves differed distinctly between both chelators. EDDS mobilised more Cu and Ni than EDTA, while EDTA mobilised more Pb and Cd than EDDS. Zn mobilisation was similar between both amendments. Neither amendment affected Cr mobility (data not shown in table). This is in contrast with findings by Chen and Cutright (2001) who reported Cr-mobilisation after application of EDTA or HEDTA (hydroxyethylenediaminetriacetic acid) to a contaminated soil. The release of Cd and Pb by EDDS was low compared to Cu, Ni or Zn. The chelator preference for these last metals is an important footnote when considering selective mobilisation for enhanced phytoextraction purposes. The higher observed mobilisation of Ni and Cu by EDDS could not be explained by their respective stability constants with the two chelators: Log $K = 18.7$ and 18.4 for Cu–EDTA and Cu–EDDS, respectively and Log $K = 18.5$ and 16.8 for Ni–EDTA and Ni–EDDS. These stability constants would suggest equal or better mobilisation of Cu and Ni by EDTA. The higher mobilisation of these elements by EDDS in the current experiment can be explained by lower affinity (based on stability constants) of EDDS for competitor ions such as Ca (Log $K_{Ca-EDDS} = 4.2$; Log $K_{Ca-EDTA} = 10.6$), Mg (Log $K_{Mg-EDDS} = 5.8$; Log $K_{Mg-EDTA} = 8.8$), Fe(III) (Log $K_{Fe-EDDS} = 22.0$; Log $K_{Fe-EDTA} = 25.0$) and Mn (Log $K_{Mn-EDDS} = 9.0$; Log $K_{Mn-EDTA} = 13.8$). Values for the stability constants used were taken from Bucheli-Witschel and Egli (2001). Ca and Mg form weaker complexes with both chelators compared with heavy metals, but the smaller stability is compensated

by their higher concentrations in the soil. The effect of competition was deemed higher for EDTA than for EDDS, resulting in more specific Cu mobilisation by EDDS under the current experimental conditions. Heil et al. (1994) observed interference for soil leaching remediation of Pb by Ca and to a lesser extent Zn, Mn and Mg in alkaline soils and by Fe in acidic soils. In this context as well, competition for ligand binding plays a vital role.

Of equal importance as the mobilisation ability or pattern, is the subsequent decrease of ligand effect after treatment. Indeed, the induced effects should be engineered to be effective, specific but also sufficiently short lived. The effects should suffice to significantly and considerably enhance shoot accumulation, but should dissipate rapidly thereafter to avoid post-harvest mobility and leaching. EDTA is a known persistent metal chelator, whereas EDDS has been proven to be biodegradable in a variety of tests (Schowanek et al., 1997). The observed ligand effect half life of the 1 mmol kg⁻¹ EDDS treatment was 4.7 d. These results are in line with previous experiences with pot experiments either with or without the presence of plants (Meers et al., 2004b). Schowanek et al. (1997) observed a half life of EDDS in the soil of 2.5 d, making it very short lived under natural conditions. Chelation to heavy metals may in theory decrease EDDS degradability and therefore increase ligand effect half lives. In addition, this effect may be element-specific as some heavy metals may be more toxic to microbial degraders than other metals. To this date no specific data have been published on EDDS degradability as affected by chelated ions. With a half life of 4.7 d, the ligand effect is reduced to 1.5% of initial mobilisation within a period of 4 weeks. Fixing the time of soil treatment at 4 weeks before harvest should therefore suffice to prevent percolation through the soil profile of the mobilised metals. That is, provided that during this period crop evapotranspiration surpasses climatological precipitation within this time frame. In temperate regions, such as Western Europe, plant transpiration exceeds rainfall during the growing season, causing an upward water flow rather than downwards, induced by capillary effects in the soil matrix.

The observed ligand effect half life for 1 mmol kg⁻¹ EDTA was 21 d as opposed to the 4.7 d observed for an equal dose of EDDS. No decrease whatsoever was observed for the 3 mmol kg⁻¹ EDTA treatment, while for the 3 mmol kg⁻¹ EDDS treatment a half life of 24 d was observed. Wu et al. (2004) observed a drop to 10% of initial levels 29 d after application of 3 mmol kg⁻¹ EDTA in a soil experiment, as estimated from decreases in dissolved organic matter. This would correspond with a half life of 9 d. However, in none of our previous experiments, such a rapid decline could be observed in the soil. Observed half lives for 3 mmol kg⁻¹ and 5 mmol kg⁻¹ EDDS increased rapidly

to 24 and 83 d, respectively. This high persistence of the ligand effect at higher dosage was unexpected and completely contrary to previous findings both in planted and unplanted pot experiments (Meers et al., 2004b). The effect may have been caused by metal induced depression in microbial soils processes. On the other hand, the degradation may also have been (additionally) limited by the experimental design: the experiment was conducted in closed containers which were opened solely for the purposes of sampling, after, respectively, 1, 7 or 21 d. In retrospect, it is possible that the flow of oxygen thus supplied was insufficient for optimal microbial activity. However, when the effect of other biodegradable substances such as light molecular weight organic acids (a.o. citric acid, oxalic acid, acetic acid) was tested in a similar experimental setup to validate this hypothesis, the observed decrease was swiftly and effectively. Whether the observed longevity of the ligand effect is caused by metal induced microbial depression, by sub-optimal microbial ecological conditions or by a combination of both is secondary to the notion that even highly biodegradable compounds can become persistent if conditions are not optimal for their microbial degradation. This strongly cautions against unmonitored application of soil amendments for enhanced phytoextraction purposes.

Strict prerequisites for the use of mobilising agents are in this regard not only the maintenance of hydrological balances as determined by soil evaporation, plant transpiration, farmer irrigation, climatological precipitation, moisture content and its deficit in comparison to the field capacity, but also the strict monitoring of ligand effect kinetics. When observed decrease is insufficiently fast, measures may be required to remedy the problem. These may include: (i) postponing harvest, (ii) applying amendments which stimulate microbial activity (e.g. fast N and C source), (iii) applying fertilisers cation which can compete with the ligands for chelation and therefore decrease ligand effect as defined by mmol heavy metals per mmol of chelator, (iv) soil management techniques after harvest (e.g. ploughing for additional oxygen input), or (v) most drastically the implementation of a water screen until levels are normalized.

Ligand effect half life is not necessarily synonymous with ligand half life. Decreases in mobilisation patterns of heavy metals may also be caused by: (i) sorption of the chelate to soil particles or (ii) competition of other electropositive elements for chelation. Schowanek et al. (1997) observed low sorption of EDDS complexes to soil particles, which suggests that ligand degradation and shifts in chelation patterns are the primary cause for decreased metal solubility after initial mobilisation. The relative importance of the second mechanism has not been evaluated in this study. That shifts in chelation do play a role can be exemplified by the fact that although total mmolar concentration of heavy metals

in the soil solution generally decreased over time, Ni was progressively more mobilised over time. This effect was observed for both amendments.

4.2. Plant uptake of heavy metals

Biomass production per plant was significantly higher for *Z. mays* than any of the other species in the untreated controls at the 0.05 level. The other three agronomic crops did not differ significantly from each other. *H. annuus* had the lowest observed biomass production. The observed low growth response of *H. annuus* (5.5 times lower biomass than *Z. mays*) may be caused by the fact that this species reacts strongly to the natural seasonal light regime, regardless of additional forced light. Variations in shoot biomass production after treatment with either EDDS or EDTA were found not to be significant at the 0.05 level for any of the plants, except between EDDS-treated *Z. mays* (D2) and untreated (D0) or EDTA-treated *Z. mays* (D1).

4.2.1. Zinc

The highest extracted mass of Zn in untreated plants was observed for *B. rapa* with 1500 µg extracted per plant. This implies a decrease in the soil of 1.5 mg kg⁻¹ Zn. For the other species an extraction of 580 µg was observed for *H. annuus*, 819 µg for *Z. mays* and 991 µg for *C. sativa*. Treatment with EDDS or EDTA resulted in the highest extracted mass for *C. sativa* with concentrations 2.9–3.3 times those observed in the untreated control, resulting in an extraction of 2895–3313 µg per plant. With a factor of 2.8–3.5, the observed increases for *H. annuus* were of the same order, resulting in the extraction of 1633–2042 µg Zn. For *B. rapa*, no increase was observed after treatment with EDTA and an increase by a factor 1.8 was observed upon application of EDDS. Extraction by *Z. mays* increased 1.9–2.0-fold following treatment and resulted in extraction of 1594–1668 µg. These concentrations are comparable to the untreated *B. rapa* plants. When we compare extracted mmolar amounts by mobilised amounts after soil treatment (Table 2), we find that only 2.5% of mobilised Zn was effectively recovered in the shoot at the time of harvest. To prevent the remaining mobilised fraction from leaching, post-harvest mobility is undesirable. The estimation of the relative fraction of Zn being absorbed and translocated was performed, taking into account soil solution concentration with and without addition of chelation, soil moisture content, shoot concentrations and biomass production.

4.2.2. Copper

C. sativa proved to be most inducible for enhanced Cu accumulation after treatment with EDDS with concentrations up to 83 mg kg⁻¹. This constitutes for an increase with a factor 5.5 compared to the control. Cu

concentrations in shoots of EDDS treated *H. annuus* were slightly less (67 mg kg^{-1}), while *B. rapa* and *Z. mays* exhibited significantly lower shoot concentrations of 38 and 13 mg kg^{-1} , respectively. The observed level for *Z. mays* after treatment was even lower than the untreated *C. sativa* plants.

Extracted mass per plant was for the untreated plants highest in *C. sativa* with $156 \mu\text{g}$, followed by *B. rapa* with $87 \mu\text{g}$, *Z. mays* with $66 \mu\text{g}$ and *H. annuus* with $53 \mu\text{g}$. Treatment with EDDS enhanced these levels to $832 \mu\text{g}$ for *C. sativa*, $402 \mu\text{g}$ for *H. annuus*, $333 \mu\text{g}$ for *B. rapa* and $177 \mu\text{g}$ for *Z. mays*. Since our pot experiment was conducted with 1 plant per pot containing 1 kg of dry weight soil, these values also reflect the decrease per kg soil. Treatment with EDTA resulted in levels of $241 \mu\text{g}$ for *C. sativa*, $194 \mu\text{g}$ for *H. annuus*, $151 \mu\text{g}$ for *Z. mays* and $120 \mu\text{g}$ for *B. rapa*. Maximum 1.3–1.5% of Cu mobilised by EDDS or EDTA (Table 2) was also recovered in aboveground plant parts.

4.2.3. Cadmium

Extracted mass of Cd varied between 6.2 and $8.0 \mu\text{g}$ per plant in the untreated controls. Although EDTA enhanced shoot concentrations the most, *B. rapa* plants treated with EDDS exhibited the highest overall extraction with $57 \mu\text{g}$ per plant. EDTA-treated *B. rapa* plants exhibited slightly lower extraction with $53 \mu\text{g}$ per plant. The difference however, was not found to be significant. *C. sativa* and *H. annuus* treated with EDTA extracted 34 and $30 \mu\text{g}$ per plant, respectively. The same plants treated with EDDS only extracted 7 and $12 \mu\text{g}$ per plant, respectively. A maximum of 2.1% of Cd mobilised by EDTA was also recovered in the shoot. EDDS did not mobilise Cd to any substantial extent. Concentrations surpassing 100 mg kg^{-1} Cd in the shoot as observed by Chen and Cutright (2001) in *H. annuus* after soil treatment with EDTA, were not observed in the current experiment. This is attributed to the difference in soil substrate: while these authors used an acidic soil, spiked with heavy metals (50 mg kg^{-1} Cd), the soil properties and pollution level in our experiment tend to suppress phytoextraction.

4.2.4. Lead

The overall extracted mass, calculated by the produced biomass multiplied by the Pb content, varied between 3 and $19 \mu\text{g}$ per plant. The lowest extracted mass was observed for *H. annuus*, attributed to its low biomass production in our experiments. The highest extracted mass was observed for *B. rapa*. Treatment with EDTA resulted in the extraction of $75 \mu\text{g}$ by *C. sativa*, $69 \mu\text{g}$ by *H. annuus*, $51 \mu\text{g}$ by *Z. mays* and $45 \mu\text{g}$ by *B. rapa*. EDDS enhanced extraction up to $45 \mu\text{g}$ by *B. rapa*, $23 \mu\text{g}$ by *C. sativa*, $20 \mu\text{g}$ by *Z. mays* and $7 \mu\text{g}$ by *H. annuus*. A maximum of 1.1% of Pb mobilised by EDTA was recovered in the shoot. Schmidt (2003) reported that

plants extracted on average 1% of soluble Pb after addition of EDTA or related substances. EDDS did not substantially mobilise Pb in the soil. Cunningham and Ow (1996) and Huang et al. (1997) reported induced hyperaccumulation of Pb in *Z. mays* ($>10,000 \text{ mg kg}^{-1}$ shoot dry weight) upon soil application of EDTA. Similar Pb-concentrations were reported by Blaylock et al. (1997) in *B. juncea* upon treatment with EDTA. No such dramatic increases could be observed in any of the tested species. The difference is attributed to differences in phytoavailability of heavy metals between substrates and the pollution level of the test soil.

4.2.5. Nickel

Overall extracted mass per plant varied between 7 and $50 \mu\text{g}$ in the control, between 29 and $135 \mu\text{g}$ in the EDTA treated pots and between 58 and $235 \mu\text{g}$ per plant in pots treated with EDDS. Therefore, the use of EDDS combined with cultivation of *C. sativa* would result in a decrease of 0.235 mg kg^{-1} over a period of 11 weeks. A maximum of 1.3–1.7% of Ni mobilised in the soil by soil amendments were subsequently recovered in aboveground plant parts. The mobilised fraction, not taken up by the plant must return to more stable and less available soil fractions post-harvest. Chen and Cutright (2001) observed concentrations exceeding 150 mg kg^{-1} Ni in shoots of *H. annuus* upon treatment with EDTA. The difference in shoot accumulation between our and their experiment is mainly attributed to difference in soil substrate (pH, OM-content) and experimental conditions (spiked soil versus equilibrated sediment).

4.3. Implications for phytoextraction potential

To make a first crude estimation of the potential phytoextraction capacity of the various agronomic species for the heavy metals under investigation, uptake performance in the comparative pot screening is multiplied by potential biomass production and divided by the mass of the top soil layer. This (speculative) extrapolation is meant to grant more insight in the general order of magnitude of the annually extracted mass of metals, when adopting a phytoextraction procedure for site remediation. However, it does not allow for predictive modelling as this would require the determination of the exact field scale performance.

Biomass produced by phytoextracting crops is species and site dependent. Madejon et al. (2003) observed a shoot dry weight production for *H. annuus* of 24 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 t ha^{-1} for *H. annuus* in France. The EU-project

Phyles estimates an annual biomass production per hectare for *H. annuus* at 10 t ha⁻¹ for the purposes of phytoextraction of heavy metals (Phyles, 2004). Cabelguenne et al. (1999) observed an annual biomass production per hectare for *Z. mays* of up to 29 t ha⁻¹ in France. Yang et al. (2004) reported a production of 20–25 t ha⁻¹ in Iowa. Hallam et al. (2001) observed total dry matter biomass productions for *Z. Mays* for the purposes of energy-cropping of 7–14 t ha⁻¹ and 12–18 t ha⁻¹ at two separate test sites in Iowa. Phyles (2004) estimates an annual biomass production per hectare for *Z. mays* at 20 t ha⁻¹ for the purposes of phytoextraction of heavy metals. Cappelletto et al. (2001) observed a dry weight biomass yield of 10.5 t ha⁻¹ for *C. sativa* in Italy. Van der Werf et al. (1995) observed an annual yield of 12–19.4 t ha⁻¹ for the same species in the Netherlands.

For all species, productions of 10–25 t ha⁻¹ were observed. Table 3 estimates the extraction potential of the four species for two levels of biomass yield and with or without the use of soil amendments for enhanced shoot accumulation. The shoot concentrations used for the calculations were those observed in the greenhouse experiment and reflect phytoextractable levels from calcareous dredged sediments. Phytoavailability of heavy metals in the soil substrate under study is expected to be suppressed by the soil carbonates, neutral to slightly alkaline pH, elevated organic matter content and soil texture (clay content).

At a biomass production of 20 t ha⁻¹, untreated *Z. mays* would annually remove 0.2 mg kg⁻¹ Zn and negligible traces of the other metals. At either 10 or 20 t ha⁻¹ all other agronomic species would extract substantially more Zn (0.3–1.1 mg kg⁻¹ soil), but still negligible amounts of the other metals. Treatment with EDDS or EDTA would result in an annual re-

moval of 0.5–0.7 mg kg⁻¹ Zn by *Z. mays* or 0.8–2.0 mg kg⁻¹ by any of the other agronomic species. Cu removal would be stimulated most by application of EDDS, resulting in an estimated annual extraction of 0.1–0.5 mg kg⁻¹ by *C. sativa*, *B. rapa* and *H. annuus*. EDTA would be less effective, inducing removal of 0.06–0.16 mg kg⁻¹ by the same species. Removal of Ni was also stimulated more by EDDS (up to >0.1 mg kg⁻¹ soil) than by EDTA (<0.1 mg kg⁻¹ soil). Removal of Cd and Pb was insufficient to consider for phytoextraction, even after application of chelating soil amendments.

Z. mays exhibited biomass yields which corresponded with up to 2.5 times those observed in the other species. Practically this means, that although *Z. mays* exhibited distinctly lower accumulation of heavy metals in the shoots, it may still be applicable for phytoextraction purposes. However, in general the estimated phytoextraction potential of the four agronomic species with or without the use of soil amendments was still considered too low to make them practically suited for extraction of heavy metals from moderately contaminated calcareous sediments.

These results indicate the extraction performance in a pot experiment grown on calcareous sediment, containing elevated levels of clay and organic matter. Evaluating the performance of these agronomic species with or without EDDS in other soil types (e.g. more sandy, more acidic) is of particular interest for future research. Also, assessment of the four agronomic species with EDDS under field condition is required to reach a conclusive verdict on their applicability in conjunction with degradable amendments for calcareous dredged sediments. Without induced accumulation of heavy metals (i.e. with EDDS or other means), these plant species are not likely to extract sufficient amounts. Considering

Table 3

First (crude) estimate of annual potential extraction rate of Zn, Cu, Cd, Ni and Pb (mg kg⁻¹ soil) for: (A) *C. sativa*, (B) *H. annuus*, (C) *B. rapa*, (D) *Z. mays*; treated with: (0) deionised water (ctrl), (1) 3 mmol kg⁻¹ EDTA, (2) 3 mmol kg⁻¹ EDDS; intervals denote standard deviation ($n = 3$)

	Zn		Cu		Cd		Pb		Ni	
	10 t ha ⁻¹	20 t ha ⁻¹	10 t ha ⁻¹	20 t ha ⁻¹	10 t ha ⁻¹	20 t ha ⁻¹	10 t ha ⁻¹	20 t ha ⁻¹	10 t ha ⁻¹	20 t ha ⁻¹
A0	0.28	0.55	0.04	0.09	0.00	0.00	0.00	0.01	0.01	0.03
B0	0.37	0.75	0.03	0.07	0.01	0.01	0.00	0.00	0.01	0.01
C0	0.55	1.09	0.03	0.06	0.00	0.00	0.01	0.01	0.01	0.02
D0	0.10	0.19	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
A1	0.93	1.85	0.07	0.13	0.01	0.02	0.02	0.04	0.04	0.08
B1	0.69	1.39	0.08	0.16	0.01	0.03	0.03	0.06	0.02	0.04
C1	0.78	1.56	0.06	0.13	0.03	0.00	0.01	0.05	0.02	0.04
D1	0.22	0.45	0.02	0.04	0.00	0.00	0.01	0.01	0.00	0.01
A2	0.82	1.65	0.24	0.47	0.00	0.00	0.01	0.01	0.07	0.13
B2	0.98	1.96	0.19	0.38	0.01	0.01	0.00	0.01	0.05	0.10
C2	0.90	1.79	0.11	0.22	0.02	0.01	0.01	0.03	0.03	0.06
D2	0.33	0.67	0.04	0.07	0.00	0.04	0.00	0.01	0.01	0.02

its overall lack of degradability, field scale trials with EDTA are not required further: its use cannot be condoned for phytoremediation purposes for soils under natural conditions.

5. Conclusion

EDTA and EDDS demonstrated distinct differences in heavy metal mobilisation patterns: EDDS mobilised more Ni and Cu than EDTA, EDTA on the other hand mobilised more Cd and Pb than EDDS. These mobilisation patterns are assumed to be soil specific, as it depends on the presence of di- and trivalent cations and their competition for complexation to the chelators. Zn mobilisation was similar for both chelators. Ligand effect half life, defined as the decrease in heavy metal mobilisation in the period following soil treatment, varied considerably between the applied doses of EDDS. A swift decrease ($t_{1/2} = 4.7$ d) was observed for the lowest dose, while a quasi-persistence ($t_{1/2} = 83$ d) was observed for the highest. EDDS is considered to be a highly biodegradable compound (Schowanek et al., 1997), yet these data suggest that under less optimal conditions ligand and ligand effect persistence may increase dramatically. Ligand effect half life of EDTA remained above 20 d for all treatments, with no significant decrease observed for the 3 mmol kg⁻¹ dose. These results caution against the use of EDTA for phytoextraction and against the untested and/or unmonitored application of EDDS.

Pronounced effects on heavy metal accumulation were observed following treatment of the plants with EDTA or EDDS. These effects were in line with the findings in regards with metal mobilisation (cfr. supra): EDTA induced higher accumulation of Pb and Cd, while EDDS induced higher shoot concentrations of Cu and Ni. The effects on Zn uptake were similar for both chelators. However, the observed enhanced accumulation is considered insufficient for efficient removal of heavy metals from the calcareous clayey and moderately contaminated soil. Administered 4 weeks before harvest, no depression in growth was observed for either soil amendment. This is in contrast with previous experiments, in which the same substrate was treated with EDTA before sowing (Meers et al., 2004a). This confirms the hypothesis that soil amendments should be applied pre-harvest rather than pre-sow for optimal extraction. However, inappropriately postponing application increases the risk of post-harvest metal mobility and leaching. Appreciating the optimal time of application should therefore be a research topic in its own.

Z. mays exhibited by far the highest biomass productions, but the lowest shoot concentrations for all heavy metals. Because of its high biomass production and heavy metal tolerance, *Z. mays* may therefore be equally suited as the other crops for phytoextraction despite its

lower observed accumulation levels. In general, overall extraction of heavy metals from the moderately contaminated calcareous soil was considered too low for all species under evaluation to consider for practical application of phytoextraction. In order to evolve towards a suitable technique which can compete with conventional soil remediation techniques, either the extraction efficiency requires to be further increased or the produced biomass needs to be economically valorisable (e.g. bioenergy production, phytomining of rare elements).

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