Phytoextraction of lead, zinc and cadmium from soil by selected plants

B. Kos, H. Grčman, D. Leštan

Biotechnical Faculty, University of Ljubljana, Slovenia

ABSTRACT

The Pb, Zn and Cd phytoextraction potential of 14 different plants was assessed in a chelate induced phytoextraction experiment. In the used soil heavy metals mainly reside in carbonate, organic matter, and residual soil fractions. The addition of a chelate, 5 mmol/kg ethylenediamine-tetracetic acid (EDTA), increased the proportion of phytoavailable Pb, Zn and Cd in the soil (dissolved in soil solution and exchangeable from soil colloids), and also their uptake by tested plants up to 48 times (*Sinapis alba*), 4.6 times (*Raphanus sativus oleiformis*), and 3.3 times (*Amaranthus spp*.), respectively, compared to the control. The biodegradable chelate ethylenediamine-disuccinic acid (EDDS) was generally less effective (tested on a selection of 4 plant species), except for *Cannabis sativa*. In a treatment with 10 mmol/kg EDDS, Pb, Zn and Cd concentrations of 1053 ± 125, 211 ± 16 and 5.4 ± 0.8 mg/kg, respectively, were measured in the biomass of *Cannabis sativa* and were 105, 2.3 and 31.7 times higher, respectively, than in the control treatment. The calculated Pb phytoextraction potential of *Cannabis sativa* amounted to 26.3 kg/ha.

Keywords: lead; zinc; cadmium; soil remediation; phytoextraction; chelates

The pollution of agricultural land in the EU and associated European states by heavy metals is widespread. Heavy metals are one of the most prevalent agents causing public health problems, entering the body in food (i.e. crops grown on heavy metal contaminated soils), ingestion of soil or inhalation of dust. An increasing body of evidence suggests that soil organisms, vitally important for soil health and fertility, are sensitive to heavy metal stress (Dahlin et al. 1997) and that the biological diversity of the soil is reduced by heavy metal contamination (Giller et al. 1998).

In future, the availability of arable land may decrease because of stricter environmental laws limiting food production on contaminated land (Council Directive 86/278/EEC 1986). National farmers and consumer organizations in EU and Associated Countries do not recognize organic/ecological farming on soils contaminated by heavy metals any longer. Furthermore, the use of sludge amendments, as the most available source to compensate for the organic matter loss, is restricted if the soil is already contaminated by heavy metals (Council Directive 86/278/EEC 1986).

Remediation of polluted soils is essential for sustainable use of agricultural land. Phytoextraction, the use of plants for extraction of heavy metals from contaminated soils, has emerged as a promising method for remediation of low to medium polluted soil (Salt et al. 1995). Some ubiquitous heavy metals, especially Pb, have however limited availability for plant uptake due to complexation with solid soil fractions (Rieuwerts et al. 1998). For example, in every contaminated soil we have examined with sequential extractions, no or a very small fraction of total soil Pb was present in a form (in soil solution or exchangeable from soil colloids) directly available to plants (Leštan and Grčman 2001). Recent evidence suggests that the addition of chelates to the soil (i.e. ethylenediamine-tetracetic acid (EDTA) and structural analogues) increases the phytoavailability of Pb and other heavy metals by forming water-soluble chelate-heavy metal complexes (Huang et al. 1997). The main drawback of chelate induced phytoextraction is that EDTA forms chemically and microbiologically stable complexes that pose a threat of groundwater contamination (Grčman et al. 2001). Kos and Leštan (2003) showed that the leaching of heavy metal complexes through the soil profile could be efficiently prevented by using ethylenediamine-disuccinic acid (EDDS) instead of EDTA, and placing a horizontal permeable barrier below the layer of treated soil. Barriers were composed of reactive materials that facilitate microbiological degradation of EDDS-heavy metal complexes and retain the released heavy metals. EDDS is an easily biodegradable, low-toxic chelate with a strong chemical affinity to Pb and other heavy metals and produces benign degradation products (Jaworska et al. 1999).

Since phytoextraction is a long-term technology, it is imperative to keep fields undergoing phytoremediation productive to achieve economically viable and socially acceptable decontamination. Industrial plants, i.e. energy crops or crops for bio-diesel production, are therefore the prime candidates as phytoextraction plants. The use of energy and/or bio-diesel crops as heavy metal phy-

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toextraction plants would give contaminated soil a productive value and decrease remediation costs.

The objective of our study was to evaluate the Pb, Zn and Cd phytoextraction potential (defined as the total metal extraction from soil) of a selection of potential energy (*Cannabis sativa*, *Sorghum vulgare*, *Arundo donax*), biodiesel (*Brassica napus*, *Raphanus sativus oleiformis*, *Sinapis alba*) and other plants (*Amaranthus* spp., *Linum usitatissimum*, *Trifolium pratense*, *Trifolium repens*, *Medicago sativa*, *Zea mays*, *Brassicca rapa*). Phytoextraction was induced by the addition of commonly used EDTA and a new biodegradable chelate, EDDS, into the soil. Soil availability for plants and fractionation of Pb, Zn and Cd in the soil before and after chelate addition were assessed by sequential extractions.

MATERIAL AND METHODS

Soil properties

Soil samples were collected from the 0–30 cm surface layer at an industrial site of a former Pb and Zn smelter in Mežica valley in Slovenia. The following soil properties were determined: pH ($CaCl_2$) 6.8, organic matter (determined with the Walkley-Black method) was 5.2%, total N (Kjeldahl digestion) was 0.25%, P and K (determined as P_2O_5 and K_2O after extraction with 0.4 mol/l acetic acid and 0.1 mol/l ammonium lactate) were 90 and 38 mg/kg, respectively. The particle-size distribution (sedimentation method) was 55.4% sand, 12.0% coarse silt, 18.9% fine silt, and 13.7% clay. Total amounts of heavy metals in soil (atomic absorption spectroscopy (AAS) after digestion with *aqua regia*) included 1100 mg Pb/kg, 800 mg Zn/kg, and 5.5 mg Cd/kg. The soil texture was sandy loam.

Sequential extraction

A modified analytical procedure according to Tessier et al. (1979) was used to determine the fractionation of Pb, Zn and Cd into six fractions, before and after addition of 5 mmol/kg EDTA (applied in a 50 ml/kg water solution) to the soil columns (Leštan and Grčman 2001). The final fractional recovery of Pb, Zn and Cd was calculated after summing the recoveries of all six steps of sequential extractions, and the error of final recovery therefore increased accordingly. This is also presumably the reason for a wide range of recoveries: 84.2% for Pb, 104.5% for Zn, and 161.2% for Cd.

Experimental set up

The influence of EDDS (Octel, Cheshire), EDTA (Fluka) on Pb, Zn and Cd plant uptake was tested in soil column experiments with four replications for each treatment: control with no chelate addition (all plants), 5 mmol/kg EDTA addition (all plants), 5 mmol/kg EDDS addition (selection of 4 plants), and 10 mmol/kg EDDS addition (*Cannabis sativa*). We tested 14 different plants: oilseed rape (*Brassica napus* var. *napus*), amaranth (*Amaranthus* spp.), hemp (*Cannabis sativa*), flax (*Linum usitatissimum*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), lucerne (*Medicago sativa*), maize (*Zea mays* cv. Raissa and *Zea mays* cv. Matilda), oil radish (*Raphanus sativus oleiformis*), white mustard (*Sinapis alba*), sorghum (*Sorghum vulgare*), Chinese cabbage (*Brassicca rapa* var. *pekinensis*), giant reed (*Arundo donax*).

In a greenhouse experiment, all plants except *Amaranthus* spp. and *Brassicca rapa* var. *pekinensis* were grown in 28 cm high and 32 cm diameter columns filled with 22 kg of air-dry 4-mm sieved soil. *Amaranthus* spp. was grown in 13 cm high and 14 cm diameter columns filled with 2 kg of air-dried soil and *Brassicca rapa* var. *pekinensis* was grown in 20 cm high and 15 cm diameter columns filled with 3.5 kg of air-dried soil. All plants except *Arundo donax*, which was cultured from rhizomes, were cultured from seeds. Columns were used to prevent soil saturation and were equipped with a leachate collection device. Soils in all treatments were fertilized with 100 mg/kg N and K as $(NH_4)_2SO_4$ and K_2SO_4, respectively. Plants were watered twice a week with up to 50 ml/kg soil of tap water.

EDTA (EDDS) was applied in 50 ml/kg soil water solution in a single dose of 5 (10) mmol/kg soil, 4 weeks after *Amaranthus* spp., *Raphanus sativus oleiformis*, *Sorghum vulgare* and *Brassicca rapa* var. *pekinensis* seed germination, 11 weeks after *Brassica napus* var. *napus, Cannabis sativa*, *Zea mays* cv. Raissa, *Zea mays* cv. Matilda and *Sinapis alba* seed germination, 15 weeks after *Linum usitatissimum*, *Trifolium pratense*, *Trifolium repens* and *Medicago sativa* seed germination, and 15 weeks after the development of shoots of *Arundo donax*. The aboveground tissues were harvested 5–7 days after chelate amendment by cutting the stem 1 cm above the soil surface. The tissues were dried at 60°C until a constant weight was achieved.

Heavy metal determination

Shoot tissues were collected and thoroughly washed with deionised water. They were dried to a constant weight and ground in a titanium centrifugal mill. Metal concentrations in plant tissue samples (290–310 mg dry weight) were determined using an acid (65% HNO_3) dissolution technique with microwave heating and analyzed by Flame-AAS. Heavy metal concentrations in leachates were determined by Flame-AAS. Controls of the analytical procedure were performed using blanks and reference materials (BCR 60 and BCR 141R, Community Bureau of Reference, for plant and soil) that were treated identically to experimental samples. Two measurements of heavy metals were performed for each sample.

Phytoextraction potential

The phytoextraction potential was calculated from soil and plant heavy metal concentrations and dry biomass plant yield, as the total amount of heavy metal extracted per ha of soil, in a single phytoextraction cycle, and expressed as kg/ha. Biomass yields of plants were obtained from literature data.

RESULTS AND DISSCUSSION

The rate of element uptake by plant is substantially affected by plant species grown on different soils (Tlustoš et al. 2001). Plant availability of certain heavy metals depends on soil properties such as soil pH and cation exchange capacity and on the distribution of metals among several soil fractions. The fractionation of Pb, Zn and Cd in control soil and in soil treated with 5 mmol/kg EDTA is shown in Table 1. Pb was found mostly bound to carbonate, soil organic matter and residual fraction. A significant association of Pb with the soil carbonate phase was also reported by Li and Thornton (2001) and a strong association with organic matter by Kabata-Pendias and Pendias (1992). Most of Zn was found bound to organic matter and particularly to the residual fraction. Kabala and Singh (2001), and Rivero et al. (2000) also reported for agricultural calcareous soil that Zn was mostly concentrated in the residual fraction. Cd was predominantly concentrated in carbonate and residual fraction. It should be emphasised that very different distribution patterns have been reported by other authors, depending on the soil and contamination type.

As expected, the addition of EDTA increased the portion of Pb, Zn and Cd in the first two labile fractions, mostly at the expense of the carbonate fraction (Table 1). The effect of EDTA addition was more important for the fractionation of Pb than for Zn and Cd. This can be explained by the higher stability constant of the Pb-EDTA complex (log Ks = 17.88) compared to Zn-EDTA (log Ks = 16.44) and Cd-EDTA (log Ks = 16.36) complexes (Bucheli-Witschel and Egli 2001).

On the day of chelate application to soil *Zea mays* cv. Raissa and *Zea mays* cv. Matilda were in the juvenile vegetative growth phase, *Amaranthus* spp., *Raphanus sativus oleiformis*, *Sorghum vulgare*, *Brassicca rapa*, and *Arundo donax* were in the adult vegetative growth phase, and *Brassica napus*, *Cannabis sativa*, *Sinapis alba*, *Linum usitatissimum*, *Trifolium pratense*, *Trifolium repens* and *Medicago sativa* were at the beginning of the reproductive growth phase. Chelate addition had no observable toxic effect on *Sorghum vulgare*. Visual symptoms of toxicity (necrotic lesions) were observed on the leaves of *Zea mays* cv. Raissa, *Zea mays* cv. Matilda, *Arundo donax*, *Brassica napus*, *Trifolium pratense*, *Trifolium repens* and *Medicago sativa*. The addition of chelate resulted in rapid senescence of *Amaranthus* spp., *Raphanus sativus oleiformis*, *Brassicca rapa*, *Cannabis sativa*, *Sinapis alba*, and *Linum usitatissimum*.

The analysis of plant material indicated that the addition of EDTA to the soil generally increased the concentration of heavy metals in the aboveground parts of the tested plants (Table 2). The results indicated a more than 48-times increase in Pb (*Sinapis alba*), up to 4.6-times increase in Zn (*Raphanus sativus oleiformis*), and up to 3.3-times increase in Cd (*Amaranthus* spp.) compared to control treatments. The greater ability of EDTA to enhance the plant uptake of Pb against that of Zn and Cd was also reported earlier (Blaylock et al. 1997) and is presumably related to the formation of the Pb-EDTA complex, which is dominant over other heavy metal complexes in most soils between pH 5.2 and 7.7 (Sommers and Lindsay 1979).

Phytoextraction of Zn from the control soil was high into all plants (Table 2). This is not surprising since many plants are known to hyperaccumulate Zn. Some hyperaccumulators, e.g. *Alyssum* and *Thlaspi* spp., can accumulate > 1% of Zn in their dry biomass (Baker and Walker 1990). Chelate addition has a lesser effect on both phytoextraction (Table 2) and fractionation of Zn into labile fractions than of Pb (Table 1). For example, Zn concentration in *Sinapis alba* (484.93 mg/kg), the best Zn accumulator, was only 7.6% higher in the EDTA treatment than in the control treatment.

Table 1. Fractionation of Pb, Zn and Cd in soil before (control) and after the addition of 5 mmol/kg EDTA: soluble in soil solution (1), exchangeable from soil colloids to soil solution (2), bound to carbonate (3), bound to Fe and Mn oxides (4), bound to organic matter (5), and residual fraction (6); means of four replications are presented ± *s.d.*

Fraction	Control			Addition of 5 mmol/kg EDTA		
	Pb (%)	Zn (%)	Cd (%)	Pb (%)	Zn (%)	Cd (%)
1	0.12 ± 0.02	0.00	nd	12.79 ± 3.19	1.27 ± 0.10	5.79 ± 0.54
2	nd	0.23 ± 0.03	nd	1.95 ± 0.84	0.22 ± 0.10	nd
3	36.68 ± 1.28	8.72 ± 0.85	35.19 ± 0.36	9.46 ± 2.53	1.34 ± 0.13	7.11 ± 0.40
4	0.38 ± 0.03	2.95 ± 0.21	7.77 ± 0.26	nd	0.72 ± 0.09	7.11 ± 0.20
5	51.03 ± 1.59	28.31 ± 0.94	11.81 ± 0.60	52.10 ± 9.11	35.02 ± 7.50	13.63 ± 1.62
6	11.78 ± 0.36	59.79 ± 1.87	45.23 ± 0.79	23.70 ± 2.85	61.42 ± 7.49	66.35 ± 1.82

Table 2. Concentrations of Pb, Zn and Cd in the aboveground tissues of tested plants in response to the addition of 0 (control) and 5 mmol/kg EDTA (EDTA 5); the phytoextraction potential was calculated on the basis of dry biomass yield of plants obtained from the literature; means of four replications are presented

Plant	Dry biomass (t/ha)	Pb uptake (mg/kg)		PP* (kg/ha)	Zn uptake (mg/kg)		PP* (kg/ha)	Cd uptake (mg/kg)		PP* (kg/ha)
		control	EDTA 5		control	EDTA 5		control	EDTA 5	
Brassica napus var. *napus*	2–5[1)]	< 10	93.92	0.33	99.88	151.48	0.53	1.84	3.21	0.011
Amaranthus sp.	1–3[2)]	< 10	396.17	0.79	127.23	253.88	0.51	2.69	8.91	0.018
Cannabis sativa	20–30[3)]	< 10	220.58	5.51	90.36	105.07	2.63	0.17	0.76	0.019
Linum usitatissinum	5–7[3)]	< 10	332.05	1.99	60.16	116.79	0.70	4.55	8.11	0.049
Trifolium pratense	3–4[4)]	< 10	397.74	1.39	107.69	179.65	0.63	0.51	3.07	0.011
Trifolium repens	1–2[4)]	< 10	434.32	0.65	51.31	167.99	0.25	0.26	3.27	0.005
Medicago sativa	3.5–4.5[4)]	< 10	107.28	0.43	57.51	92.55	0.37	2.20	3.75	0.015
Zea Mays cv. Raissa	16–22[4)]	11.10	49.50	0.94	174.15	225.98	4.29	6.20	4.29	0.082
Zea Mays cv. Matilda	16–22[4)]	< 10	72.80	1.38	193.04	321.03	6.10	1.57	2.61	0.050
Raphanus sativus oleiformis	2–3.5[1)]	< 10	197.19	0.54	87.31	402.69	1.11	3.50	5.70	0.016
Sinapis alba	2–3.5[1)]	< 10	479.71	1.32	484.93	524.68	1.44	4.66	7.93	0.022
Sorghum vulgare	10–16[1)]	< 10	67.38	0.88	101.02	122.96	1.60	6.11	6.12	0.080
Brassicca rapa var. *pekinensis*	2–5[5)]	< 10	130.3	0.46	64.2	100.9	0.35	2.27	3.23	0.011
Arundo donax	20[6)]	< 10	26.95	0.54	106.99	71.44	1.43	2.92	4.02	0.080

PP = phytoextraction potential
*calculation is based on the mean value of dry biomass yield
[1]Kramberger (1999), [2]Myers (1996), [3]Jevtić (1986), [4]Čergan et al. (2001), [5]Černe (1998), [6]Duke (1983)

The accumulation of Cd into the biomass of tested plants was low in both control and EDTA amended soil (Table 2). However, this was mostly due to the low concentration of Cd in the soil. Cd is highly interchangeable between different soil fractions: the carbonate form of Cd is susceptible to pH changes, particularly in the rhizosphere during plant growth, and the adsorption constant of the Cd-organic matter complex is low. Cd is therefore only loosely bound to soil particles and, except for Cd associated with the residual fraction, can be easily taken up by plants (Ramos et al. 1994, Chlopecka et al. 1996).

The results of EDTA induced heavy metal fractionation in soil and plant uptake indicated Pb as a prime heavy metal candidate for chelate induced phytoextraction. The effectiveness of phytoextraction depends on the total metal extraction indicated by the phytoextraction potential (Table 2). However, one must bear in mind that phytoextraction potentials presented in Table 2 are based on the biomass yield of fully grown crops and are probably higher than actual since older plants are likely to concentrate less heavy metals than younger one used in our study. Hemp (*Cannabis sativa*), a potential energy and fiber crop with the highest Pb phytoextraction potential, oilseed rape (*Brassica napus*), a crop for bio-diesel production, and two other crops: Chinese cabbage (*Brassica rapa*) which was used as a standard test plant in our phytoextraction experiments, and amaranth (*Amaranthus* spp.), were employed in a further induced phytoextraction experiment with EDDS as chelate. EDDS permits environmentally safer induced phytoextraction than EDTA. The addition of 5 mmol/kg EDDS was substantially less effective for Pb plant uptake, except for *Cannabis sativa* where an increase in Pb biomass concentration of 42% over the EDTA treatment was measured (Tables 2 and 3). The use of a higher 10 mmol/kg EDDS concentration further increased Pb, Zn and Cd concentrations in *Cannabis sativa* to 1053 ± 125, 211 ± 16, and 5.4 ± 0.8 mg/kg, respectively. The Pb concentration was more than 100-times higher than in the control treatment and resulted in a Pb phytoextraction potential of 26.3 kg/ha.

Even in the most efficient treatment (*Cannabis sativa*, 10 mmol/kg EDDS), the percentage of Pb phytoextracted in a single cycle was only approx. 0.6% of the total Pb present in the upper 30 cm of soil. The achieved Pb concentration in *Cannabis sativa* is far from the Pb concentrations required for efficient soil remediation within a reasonable time span. Theoretically, Pb concentrations exceeding 1% of dry plant biomass (10 times higher than obtained) would be required to reduce soil Pb concentrations from initial 1100 to 300 mg/kg Pb (the limit set by 86/278/EEC), over approx. 10–15 years.

Some other authors reported much higher Pb concentrations in plants induced by chelate application. Blaylock et al. (1997) used 3 weeks old seedlings and measured more than 15 000 mg Pb/kg in the dry weight of shoots of *Brassica juncea* after an addition of 10 mmol/kg EDTA. Huang et al. (1997) determined 8960 mg/kg and 2410 mg Pb/kg in two weeks old pea and corn shoots transplanted into a soil substrate pre-treated with 1.5 mmol EDTA/kg. Possible reasons for the higher Pb

Table 3. Concentrations of Pb, Zn and Cd in the aboveground tissues of selected plants in response to the addition of 5 mmol/kg EDDS; the phytoextraction potential was calculated on the basis of dry biomass yield of plants obtained from the literature; means of four replications are presented

Plant	Pb uptake (mg/kg)	Phytoextraction potential* (kg/ha)	Zn uptake (mg/kg)	Phytoextraction potential* (kg/ha)	Cd uptake (mg/kg)	Phytoextraction potential* (kg/ha)
Brassica napus var. *napus*	43.08	0.15	154.09	0.54	3.55	0.012
Amaranthus sp.	14.32	0.03	326.83	0.65	6.75	0.014
Cannabis sativa	382.79	9.57	147.34	3.68	1.74	0.044
Brassicca rapa var. *pekinensis*	56.72	0.20	118.23	0.41	3.42	0.012

*calculation is based on the mean value of dry biomass yield

concentrations reported by these authors compared to our results are: the use of very young plants, favourable fractionation of Pb in soil (achieved by artificial soil contamination by heavy metals), and the experimental set up in which no losses of the chelate-Pb complex due to leaching occurred.

The remediation of polluted agricultural lands is just one, but an absolutely necessary prerequisite for the sustainable development of rural communities. Induced phytoextraction technologies for removal of Pb and other heavy metals are still in the early phase of development. However, through development of (industrial) plants with (genetically) increased phytoextraction potential, they may offer a viable remediation solution in future.

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ABSTRAKT

Fytoextrakce olova, zinku a kadmia vybranými druhy rostlin z kontaminovaných půd

Fytoextrakční potenciál 14 rostlinných druhů vůči olovu, zinku a kadmiu byl sledován v pokusu s modifikovanou přístupností prvků aplikací chelátů. V použité půdě jsou těžké kovy převážně vázány v karbonátech, na organické hmotě a v reziduální frakci. Přídavek 5 mmol/kg kyseliny ethylendiaminotetraoctové (EDTA) zvýšil podíl rostlinám přístupného Pb, Zn a Cd v půdě a také jejich příjem rostlinami. *Sinapis alba* akumuloval 48krát více sledovaných prvků, *Raphanus sativus oleiformis* 4,6krát a *Amaranthus* spp. 3,3krát více ve srovnání s kontrolními variantami. Biodegradabilní chelát ethylendiamindisukcinové kyseliny (EDDS) byl celkově méně efektivní (hodnocen pro čtyři druhy rostlin) s výjimkou *Canabis sativa*. Ve variantě ošetřené 10 mmol/kg EDDS byly v rostlinách *Canabis sativa* stanoveny obsahy Pb, Zn a Cd 1053 ± 125, 211 ± 16 a 5,4 ± 0,8 mg/kg. Tyto obsahy byly 105krát, 2,3krát a 31,7krát vyšší než obsahy prvků v rostlinách pěstovaných na kontrolních variantách. Vypočtený remediační potenciál *Canabis sativa* dosahoval 26,3 kg Pb/ha.

Klíčová slova: olovo; zinek; kadmium; remediace půdy; fytoextrakce; cheláty

Corresponding author:

Assist. Prof. Domen Leštan, Agronomy Department, Biotechnical faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
tel.: + 386 1 423 1161, fax: + 386 1 423 1088, e-mail: domen.lestan@bf.uni-lj.si