Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals

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Abstract

The potential of short rotation coppice (SRC) for phytoremediation of contaminated sites and simultaneous generation of bio-energy by conversion of the produced biomass has been discussed in previous publications. The current study compares five species of *Salix* spp. in their ability to extract and accumulate heavy metals (Cd, Cr, Cu, Ni, Pb, Zn) in harvestable plant parts. The species used in this study were: *Salix dasyclados* 'Loden', *Salix triandra* 'Noir de Villaines', *Salix fragilis* 'Belgisch Rood', *Salix purpurea* × *Salix daphnoides* 'Bleu', *Salix schwerinii* 'Christina'. The comparison was conducted in a pot experiment in open air over the course of a growing season on three different soil types: a moderately contaminated dredged sediment derived surface soil (A1), a heavily polluted sediment derived surface soil (A2) and a sandy soil with moderately elevated concentrations due to atmospheric deposition by historic smelter activities (A3). In addition, the effects of soil treatment with 2.5 mmol kg^{-1} ethylene diamine disuccinate (EDDS) on metal accumulation in stems and leaves were evaluated for one *Salix* clone (Loden). Of the five clones tested, Christina, Loden and Belgisch Rood exhibited the highest Cd and Zn concentrations and therefore deserve further attention in field validation. A first estimation of the order of magnitude of Cd and Zn that could potentially be annually extracted with these clones, resulted in 5–27 kg ha^{-1} for Zn and 0.25–0.65 kg ha^{-1} for Cd, based on the soil type. However, biomass production in field situations will mainly determine metal removal. Treatment with EDDS exhibited distinct differences in inducing metal removal on the various soils. Uptake of Cd and Zn could not be enhanced in A1, whereas in A2 and A3 removal of these metals could be increased by 50–100% after treatment. In all soils EDDS treatment also had a distinct effect on Cu uptake.

Keywords: Phytoextraction; Short rotation coppice; Willow; *Salix*; EDDS; Heavy metals

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1. Introduction

Tree species have a range of characteristics, which make them possible candidates for application in phytoremediation approaches. In particular, *Salix* spp. have been suggested as they are fast to propagate, achieve high annual biomass production and generally possess a high tolerance against metal pollution. They have been proposed both for stabilisation and removal of contaminants from contaminated soils.

A vegetative cover can be considered as a self-sustaining cap composed of soil and plants. They can form an alternative to composite clay or plastic layer caps (Ettala et al., 1988; Schnoor, 2000; Nixon et al., 2001). The plants control erosion and reduce seepage of water. The canopy of dense short rotation coppice (SRC) willow stands intercepts 25–30% of the rainfall during the growing season, evaporating it to the air afterwards. In addition, water use by willows varies between 6 and 12 g dry weight produced per 1 L of water transpired (Perttu, 1998). Several studies have also demonstrated that many species or clones of *Salix* have the capacity to accumulate elevated levels of Cd and Zn in aboveground biomass compartments (Landberg and Greger, 1996; Lunácková et al., 2003; Rosselli et al., 2003). This capacity may lead to ecotoxicological risks but might also be applied for removing metals from the topsoil. Punshon et al. (1995) and Punshon and Dickinson (1997) suggested that *Salix* might be sufficiently tolerant to decrease the plant-available heavy metal load in contaminated soils, while still maintaining high yields in a phytoremediation system. The obtained biomass might subsequently be used for bio-fuel production (Hammer et al., 2003). The ability to cultivate willows on dredged sediments has been

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demonstrated in field experiments (Meers et al., 2003, 2005a; Vervaeke et al., 2001, 2003).

In the current study, the heavy metal phytoextraction potential of five willow clones was compared. The species used in this study were: *Salix dasyclados* 'Loden', *Salix triandra* 'Noir de Villaines', *Salix fragilis* 'Belgisch Rood', *Salix purpurea* × *Salix daphnoides* 'Bleu', *Salix schwerinii* 'Christina'. In addition, the effect of a mobilising chelator on induced heavy metal uptake was evaluated in *S. dasyclados* 'Loden'. The selected soil amendment was ethylene diamine disuccinate (EDDS). EDDS is a naturally occurring chelator (Nishikiori et al., 1984; Goodfellow et al., 1997). Schowanek et al. (1997) described a high 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. Meers et al. (2005b) observed half lives for heavy metal mobilisation ranging between 3.8 and 7.5 days in unplanted pots and between 4.5 and 6.6 days in pots planted with *Helianthus annuus*. The metal chelating ability, accompanied with the short activity time span in the soil due to rapid biodegradation, makes EDDS suitable to consider as soil amendment for enhanced phytoextraction purposes (Lestan and Grcman, 2002; Meers et al., 2005b).

2. Material and methods

2.1. Soil collection

Three Belgian soils and sediments were collected for this experiment: (A1) a moderately contaminated dredged sediment derived surface soil from the disposal site of Meigem (A2) a sediment derived surface soil from Gavere, originating from an overbank sedimentation zone with similar soil characteristics as the Meigem soil, yet with higher total content of Cd and Zn (A3) a soil collected at a site in Balen contaminated by historical smelter activity (atmospheric deposition) and currently used for phytoextraction field experiments (A3).

2.2. Soil characterisation

Soil conductivity was measured with a WTW LF 537 electrode (Wissenschaftlich-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 and Schuell, Dassel, Germany). To determine pH-H_2O, 10 g of air-dried soil was allowed to equilibrate in 50 ml of deionised water for 24 h. The pH of the supernatant was then measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA). 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 by loss-on-ignition at 550 °C (2 h). The grain size distribution of the soil samples was determined using laser diffractometry (Coulter LA200, 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).

Nutritional state of the three soils was evaluated. To this end, the extraction values of Ca, Mg, K, Na and P were assessed in the NH_4OAc–EDTA extractions, accounting for soil cation exchange capacity (Van Ranst et al., 1999). Phosphorus was determined colorimetrically using the method of Scheel (Van Ranst et al., 1999). An adapted Kjeldahl digestion was used for determination of total N content (Bremner, 1996).

Pseudo total soil content of heavy metals was estimated by *aqua regia* digestion (Van Ranst et al., 1999). Heavy metal (Cd, Cu, Cr, Ni, Pb, Zn) analysis in the digestion and the various extractions was subsequently performed, using inductively coupled plasma–optical emission spectrometry (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Different extraction procedures were used to characterise metal mobility in the soils under study (Table 1). All extraction protocols were performed on air dried and ground soil, with the exception of the rhizon soil samplers (MOM-type; Eijkelkamp Agrisearch, Giesbeek, the Netherlands). Rhizons were fitted in the pots and soil moisture was sampled at the actual soil moisture content (averaged between 2 and 3 of field capacity and field capacity).

Three single extraction procedures were used to gain insight in bioavailable soil fractions of heavy metals. An unbuffered $CaCl_2$ (0.01 M) solution was employed to extract exchangeable metals at ionic strength similar to that of the soil solution. A buffered extraction solution (pH 7) with higher ionic strength (1 M NH_4OAc) was also used for extracting exchangeable metals. The acetic acid also serves to mimic mobilisation by light molecular organic acids present in the rhizosphere (Van Ranst et al., 1999). The extraction solution is buffered at around pH 7 to avoid carbonate dissolution for use in environmental studies (Baker et al., 1994; Gommy et al., 1998; Van Ranst et al., 1999). A mixed EDTA (ethylenediaminetetra acetic acid)–NH_4OAc

Table 1
Extraction procedures used to characterise metal content and mobility in the soils used in this experiment

Extraction solution	Liquid:solid	Equilibration time	Fraction
Rhizon soil moisture samplers	At field capacity	–	Soil solution
0.01 M $CaCl_2$	5:1	2 h	Soil solution + weak exchangeable
1 M NH_4OAc (pH 7.0)	30:1	Column displacement	Exchangeable + weak complexable
0.5 M NH_4OAC, 0.5 M HOAc, 0.02 M EDTA (pH 4.65)	5:1	30 min	Exchangeable + complexable
Aqua regia	100:1	24 h	Pseudo total

extraction solution was used in accordance with Lakanen and Ervio (1971). Complexation by EDTA and acetic acid simulates complexing behaviour by root exudates, whereas NH_4^+ is capable of desorbing the exchangeable soil fraction and the pH simulates rhizosphere acidity. EDTA-extractions are widely used in ecochemical evaluations, more particularly those involving plant uptake.

2.3. *Plant experiment*

The screening consisted out of a pot experiment in open air, in which the five clones were planted in three different soils (3 kg dry soil per pot) and allowed to grow from May until November. Per clone and per soil three replicates were planted, giving a total of 45 pots. In each pot, three cuttings (20 cm) of equal diameter (0.8–1.2 cm) were inserted. The pots were randomised to avoid micro-climatic variations. To avoid limiting effects in regards with nutritional state, the pots were fertilised four times over the course of the growing season (twice in July, once in August, once in September) with a fertilizer consisting out of: 14% N (10% NO_3–N, 4% NH_4–N), 4% P_2O_5, 28% K_2O, 3% MgO, 0.02% B, 0.001% Mo. Of this formula, 0.4 g were dissolved in 200 ml water and applied to each pot in each of the four fertilisation treatments. In November, the plants were harvested, brushed to remove soil particles, rinsed with deionised water and oven dried at 60 °C. Subsequently the samples were separated in leaves and stems, after which each fraction was weighed to ascertain dry weight biomass production. For the purposes of this study the term 'stem' refers to the new shoot tissue sprouting from the cuttings. It therefore excludes foliar material and the cuttings themselves. Stem and leaf samples were ground using a Culatti DCFH 48 grinder and sieved with a 1 mm sieve. Plant samples were ashed at 450 °C and dissolved in nitric acid before element analysis using Flame-AAS (Varian SpectrAA 10, Palo Alto, CA, USA).

In a separate, simultaneous experiment, the effect of soil amendment with ethylene diamine disuccinate (EDDS) on induced metal uptake was evaluated in one of the clones (*S. dasyclados* 'Loden'). For this purpose, 7.5 mmol of Na_3–EDDS was applied to the top of each treated pot, divided over three separate doses (2.5 mmol dissolved in 200 ml each) which were spread over a period of 1 week in early September (last dose 8 weeks before harvest). Effects of EDDS on plant uptake were compared with uptake by untreated control plants.

2.4. *Statistical analysis*

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. Levene's test was used to ascertain homogeneity of the variances between the various treatments. When non-equal variances were observed, the Dunnett's T3 adaptation was used to screen for significant differences. Two-way ANOVA was used to check for factor interactions between soil type and clone and between soil type and soil treatment with EDDS. Tukey pairwise multiple comparison was used to compare all plant performances, and to check for the statistical significance of observed differences ($\alpha = 0.05$). In case of interaction between soil type and soil treatment with EDDS, metal concentrations were compared between the treated and untreated soil with the *t*-test.

3. Results

3.1. *Soil characterisation*

Table 2 presents the soil composition for the three soils used in this experiment. The sediment derived soils (A1, A2) exhibit higher levels of organic matter and clay than sandy surface soil (A3). This is also reflected in a higher cation exchange capacity (CEC). Carbonate content in the two dredged sediments is also elevated, resulting in a soil pH buffered around neutral to slightly alkaline levels. Soil texture according to USDA classification was Silty clay loam for A1, Clay loam for A2 and Loamy sand for the A3.

The soil nutritional state was estimated from the NH_4OAc–EDTA extraction and the soil specific cation exchange capacity according to Van Ranst et al. (1999). According to that system, the nutritional state for P was considered to be normal to high in A3 and very high in the other two soils. Available Ca was very high in all soils. Mg was normal A3, high for A2 and very high for A1. Available K was high for A2 and very high for A3 and A1. Available Na was very high for all three soils. Although the nutritional state was normal to very high for all macro-nutrients in all soils, additional fertilisation was applied over the course of the growing season to prevent nutrient limiting effects on growth and metal uptake.

Concentrations of metals extracted using the various extraction procedures is presented in Table 3. From the perspective of soil pollution levels, Cd and Zn are of particular interest. Content of Ni and Cu is rather low in all soils, while Pb exhibits slightly

Table 2
Soil composition and nutritional state of the three soils used in this study

	Meigem (A1)	Gavere (A2)	LUC (A3)
pH-H_2O	7.5 ± 0.0	7.6 ± 0.0	6.6 ± 0.1
EC ($\mu S\ cm^{-1}$)	109.5 ± 5.1	170.3 ± 11.5	55.8 ± 5.4
OM (%)	9.8 ± 0.2	13.2 ± 0.1	3.9 ± 0.2
$CaCO_3$ (%)	8.0 ± 0.3	6.6 ± 0.8	2.8 ± 0.5
CEC (cmol(+) kg^{-1})	23.5 ± 0.4	25.8 ± 0.2	7.1 ± 0.5
Sand (%)	6	20	86
Silt (%)	55	45	9
Clay (%)	39	35	5
N_{tot} ($mg\ kg^{-1}$)	2898 ± 86	3574 ± 40	1018 ± 63
P_{tot} ($mg\ kg^{-1}$)	331 ± 13	262 ± 12	147 ± 37
$P_{NH_4OAc–EDTA}$ ($mg\ kg^{-1}$)	290 ± 14	247 ± 4	105 ± 9
Ca_{tot} ($mg\ kg^{-1}$)	31388 ± 1139	36104 ± 1996	4654 ± 236
Mg_{tot} ($mg\ kg^{-1}$)	583 ± 1	488 ± 13	164 ± 1
K_{tot} ($mg\ kg^{-1}$)	842 ± 130	1039 ± 84	439 ± 4
Na_{tot} ($mg\ kg^{-1}$)	95 ± 22	341 ± 15	52 ± 2
$Ca_{NH_4OAc–EDTA}$ ($mg\ kg^{-1}$)	21371 ± 1364	16095 ± 64	1024 ± 27
$Mg_{NH_4OAc–EDTA}$ ($mg\ kg^{-1}$)	243 ± 20	190 ± 4	40 ± 2
$K_{NH_4OAc–EDTA}$ ($mg\ kg^{-1}$)	423 ± 57	338 ± 26	121 ± 4
$Na_{NH_4OAc–EDTA}$ (mg)	54 ± 6	114 ± 6	11 ± 0.8

Table 3
Extracted concentrations for Cd, Cr, Cu, Ni, Pb, and Zn with *aqua regia*, NH_4OAc–EDTA, NH_4OAc, $CaCl_2$ and rhizons

	Meigem (A1)	Gavere (A2)	LUC (A3)
Aqua regia (mg kg^{-1})			
Cd	8.0 ± 0.5	25 ± 2.8	5.5 ± 1.1
Cr	161 ± 11	440 ± 52	12 ± 2.4
Cu	111 ± 7.9	54 ± 6.3	28 ± 5.6
Ni	49 ± 3.0	26 ± 3.2	4.4 ± 0.9
Pb	134 ± 9.1	183 ± 18	206 ± 46
Zn	765 ± 51	1160 ± 69	275 ± 50
NH_4OAc–EDTA (mg kg^{-1})			
Cd	6.2 ± 0.4	22 ± 0.6	4.3 ± 0.2
Cr	2.9 ± 0.1	12 ± 1.5	0.1 ± 0.0
Cu	72 ± 4.6	32 ± 3.4	12 ± 0.7
Ni	11 ± 0.6	2.8 ± 0.3	0.4 ± 0.1
Pb	97 ± 5.5	126 ± 3.1	113 ± 7.9
Zn	413 ± 28	398 ± 13	160 ± 10
NH_4OAc (mg kg^{-1})			
Cd	2.1 ± 0.1	7.4 ± 0.2	1.9 ± 0.1
Cr	0.3 ± 0.0	0.7 ± 0.0	<0.1
Cu	2.1 ± 0.1	3.2 ± 0.1	<1.5
Ni	2.9 ± 0.5	1.3 ± 0.5	0.6 ± 0.2
Pb	0.63 ± 0.36	1.5 ± 0.5	7.9 ± 1.5
Zn	42 ± 3.8	32 ± 3.4	50 ± 4.5
$CaCl_2$ (μg kg^{-1})			
Cd	38 ± 12	89 ± 4.2	392 ± 16
Cr	12 ± 2.9	63 ± 3.8	2.2 ± 1.4
Cu	257 ± 3.7	2145 ± 186	89 ± 3.7
Ni	238 ± 109	194 ± 61	51 ± 10
Pb	18 ± 0.49	44 ± 20	191 ± 18
Zn	894 ± 39	1597 ± 182	17740 ± 775
Rhizon (μg l^{-1})			
Cd	2.4 ± 1.1	19 ± 4.0	11 ± 2.2
Cr	3.3 ± 2.3	17 ± 4.0	1.9 ± 0.5
Cu	70 ± 14	38 ± 11	40 ± 13
Ni	75 ± 34	44 ± 28	22 ± 14
Pb	4.7 ± 8.1	1.9 ± 1.2	12 ± 13
Zn	144 ± 65	114 ± 56	1254 ± 243

elevated levels in A2 and A3 and Cr is only increased in A2. Although A3 has the lowest total content of Zn, it has the highest observed levels in the rhizon, $CaCl_2$ and NH_4OAc extractions. Higher mobility in the soil as reflected by extractability with weak extractants may indicate higher relative availability for plant uptake. Similarly, A3 had the highest concentrations of Cd in the soil solution and in the $CaCl_2$ single extractions whereas it had the lowest total content.

3.2. *Biomass production*

Total dry weight production per willow species and soil type was subject to high relative standard deviations. Average dry weight production per pot was significantly lower in the A3 sandy soil (4.0 ± 2.4 g) when compared to the other two soils (6.9 ± 1.9 g). The lower productivity in A3 can either be due to nutritional deficiencies, in spite of fertilisation, or due to toxic effects induced by higher observed mobility of Cd, Pb and Zn in comparison to the other soils. No significant differences were observed between the various species within a given soil. Belgisch Rood and Bleu did achieve somewhat lower biomass productions than the other three species for all soils under investigation, yet the observed differences were not considered to be statistically significant ($\alpha = 0.05$). The provider of the plant material (Salix Devos Inc., Eksaarde, Belgium) reported lower overall productivity for Belgisch Rood in comparison with the other species in field scale comparisons under non-polluted conditions. On the other hand, Vervaeke et al. (2001) reported significantly higher biomass production for Belgisch Rood in comparison to Noir de Villaines in field experiments with mixed stands on dredged sediments. Vandecasteele et al. (2005) observed no significant growth effects for clones Belgisch Rood and *S. viminalis* Aage over a broad range of pollutant concentrations in the soil (0.9–41.4 mg kg^{-1} Cd, 188–2422 mg kg^{-1} Zn), indicating high pollution tolerance of *Salix* spp. High tolerance of *Salix* spp. for Cd and Zn was also observed by Punshon (1996). In the evaluation of the effect of EDDS on heavy metal uptake by Loden, no significant effect of the soil treatment was observed on plant growth.

3.3. *Plant uptake of heavy metals*

Table 4 presents shoot concentrations of Cr, Cu, Ni and Pb. These metals are present in the soil at concentrations which were not deemed to be ecologically relevant in reference to local legislative criteria. A2 was the only soil in which total Cr was increased. As can be observed in Table 4, shoot accumulation of these metals was low and insufficient to consider for phytoextraction of these metals.

Zinc concentrations in stems and leaves of the five species under study are presented in Fig. 1. Concentrations in the willow stems varied between 84 and 254 mg kg^{-1} when grown on soil A1, between 175 and 304 mg kg^{-1} on A2, and between 332 and 591 mg kg^{-1} on A3. Each time, Christina and Loden contained the highest concentrations. For the willows on A1, foliar Zn concentrations varied between 250 and 589 mg kg^{-1} with Christina and Loden exhibiting the highest levels. Foliar concentrations in willows grown on A2 varied between 497 and 1080 mg kg^{-1} and between 1067 and 2882 mg kg^{-1} when grown on A3 with Christina and Belgisch Rood accumulating the highest concentrations.

Two-way ANOVA, using soil type and clone as factors, revealed there was no significant interaction between both factors for Zn ($p = 0.05$). This was the case for both the foliar concentrations and concentrations in the stems. In other words, the clones behaved similarly in regards to Zn accumulation for the three soils. Based on accumulation in aerial plant parts, phytoavailability of Zn in the various soils under investigation decreased in the order: A3 > A2 > A1. The same order was observed for both stems and foliar concentrations. Post hoc analysis revealed the differences between these three soils to be significant for Zn in the stems ($\alpha = 0.05$).

Results of two-way ANOVA for foliar and stem concentrations of Zn suggests that all clones behave similarly compared to each other in all three soil types. Of all clones under study, Christina accumulated the highest levels of Zn in stem and

Table 4
Cr, Cu, Ni and Pb concentration in stems and leaves of the five willow species under study (mg kg^{-1}); intervals indicate standard deviation ($n = 3$)

Clone	Soil	Cr	Cu	Ni	Pb
Stem (mg kg^{-1})					
Bleu	A1	0.93 ± 0.19	4.8 ± 0.3	0.78 ± 0.18	1.2 ± 0.07
Bleu	A2	0.62 ± 0.18	3.7 ± 0.9	0.72 ± 0.16	2.0 ± 0.60
Bleu	A3	2.2 ± 1.4	5.5 ± 2.4	0.41 ± 0.02	2.1 ± 0.43
Chris	A1	0.58 ± 0.04	6.5 ± 0.9	0.80 ± 0.09	2.7 ± 0.54
Chris	A2	0.85 ± 0.33	7.2 ± 1.6	0.49 ± 0.40	2.3 ± 1.2
Chris	A3	0.69 ± 0.09	8.1 ± 1.9	0.56 ± 0.09	3.9 ± 1.8
Loden	A1	1.1 ± 0.11	9.5 ± 3.0	1.3 ± 0.10	2.2 ± 0.50
Loden	A2	1.2 ± 0.40	9.8 ± 1.7	0.50 ± 0.40	1.7 ± 0.12
Loden	A3	0.80 ± 0.20	8.7 ± 0.0	1.9 ± 1.5	3.4 ± 0.31
Noir	A1	0.69 ± 0.08	2.6 ± 1.0	0.32 ± 0.12	0.46 ± 0.34
Noir	A2	0.90 ± 0.10	2.1 ± 0.3	0.57 ± 0.10	2.2 ± 0.70
Noir	A3	0.76 ± 0.29	2.8 ± 0.9	0.49 ± 0.05	2.6 ± 0.50
Rood	A1	1.7 ± 1.4	3.9 ± 1.9	1.1 ± 0.78	1.4 ± 0.82
Rood	A2	0.98 ± 0.29	6.7 ± 0.5	1.2 ± 0.29	2.9 ± 0.70
Rood	A3	1.1 ± 0.40	3.7 ± 1.0	0.55 ± 0.14	0.89 ± 0.23
Leaves (mg kg^{-1})					
Bleu	A1	4.1 ± 0.6	9.0 ± 1.5	2.5 ± 0.40	11.9 ± 2.1
Bleu	A2	10.3 ± 6.0	10.3 ± 4.8	3.7 ± 1.5	7.3 ± 5.4
Bleu	A3	6.6 ± 2.8	6.7 ± 5.3	2.0 ± 2.0	3.6 ± 2.6
Chris	A1	1.1 ± 0.36	8.2 ± 0.7	2.9 ± 0.30	18.4 ± 2.2
Chris	A2	0.70 ± 0.10	5.7 ± 1.3	1.3 ± 0.80	12.1 ± 1.6
Chris	A3	0.86 ± 0.03	12.1 ± 5.1	2.7 ± 2.1	12.1 ± 0.60
Loden	A1	1.5 ± 0.20	8.2 ± 3.2	1.8 ± 0.60	7.1 ± 1.5
Loden	A2	1.1 ± 0.90	5.8 ± 3.3	0.80 ± 0.40	7.7 ± 3.3
Loden	A3	1.8 ± 1.2	10.9 ± 1.5	2.1 ± 0.90	13.5 ± 2.4
Noir	A1	0.51 ± 0.05	7.1 ± 5.0	3.8 ± 2.7	4.5 ± 2.7
Noir	A2	<DL	5.3 ± 0.1	0.60 ± 0.10	8.3 ± 3.0
Noir	A3	0.70 ± 0.10	6.1 ± 0.1	1.10 ± 0.50	4.3 ± 3.8
Rood	A1	3.0 ± 2.8	9.1 ± 4.7	3.2 ± 1.9	9.6 ± 3.9
Rood	A2	1.7 ± 0.90	8.6 ± 1.2	0.88 ± 0.55	13.2 ± 3.4
Rood	A3	3.4 ± 2.2	16.0 ± 1.2	3.0 ± 2.0	13.1 ± 3.0

Chris: Christina; Noir: Noir de Villaines; Rood: Belgisch Rood; <DL: below instrumental detection limit (<0.41 mg kg^{-1} for Cr).

leaves. Loden and Belgisch Rood also exhibited elevated levels whereas Bleu and Noir de Villaines had the lowest concentrations. The order of foliar Zn over all three soils, including significant differences according to the Tukey post hoc tests (letters in parentheses), was Bleu (a) < Noir de Villaines (ab) < Loden (abc) < Belgisch Rood (bc) < Christina (c). Analogous analysis for stem concentrations of Zn revealed the following order and significance of the observed differences according to Tukey: Bleu (a) < Belgisch Rood (a) < Noir de Villaines (ab) < Loden (bc) < Christina (c).

Fig. 2 presents the Cd contents in stems and leaves of the five clones under study. Cadmium concentration in the stems of the five clones was between 3.9 and 11.9 mg kg^{-1} when grown on A1, between 16.8 and 39.5 mg kg^{-1} when grown on A2, and between 4.7 and 20.0 mg kg^{-1} when grown on A3. Lowest accumulation was observed on A1, indicating that the “plant-availability” of Cd was lowest in this dredged sediment derived surface soil. On A2 and A3, Cd accumulation was highest in the Belgisch Rood and Loden clones, whereas on A1 highest levels were observed in the Christina and Loden clones. Foliar Cd concentrations varied between 7.1 and 16.1 mg kg^{-1} on A1, 20.4 and 103.0 mg kg^{-1} on A2 and 8.6 and 44.0 mg kg^{-1} on A3. Again, the lowest levels for all clones were observed for the plants on A1. The concentrations for A1 are insufficient to consider for phytoextraction purposes. Foliar content was highest in the Loden and Christina clones on A1, in Christina and Belgisch Rood on A2 and in Belgisch Rood and Christina on A3. Cadmium accumulation in Bleu and Noir de Villaines was lower in comparison with the other clones.

Unlike as was observed for Zn, two-way ANOVA indicated a significant interaction between soil and clone, both for foliar and stem concentrations of Cd. This implies that the five clones behaved differently in comparison to each other on the different soils. Post hoc analysis within each soil type separately revealed that in soil A1, the differences in foliar accumulation between the five clones were not statistically significant. In A2, the soil with highest pool of phytoavailable Cd, the order in foliar concentrations was Noir de Villaines (a) < Bleu (ab) < Loden (ab) < Christina (ab) < Belgisch Rood (b). Analogously, in A3 the observed order was Noir de Villaines (a) < Bleu (a) < Loden (ab) < Christina (ab) < Belgisch Rood (b). For accumulation of Cd in the stems, post hoc analysis again revealed no significant differences in soil A1, while in A2 and A3 the order was Bleu (a) < Noir de Villaines (ab) < Christina (ab) < Loden (b) < Belgisch Rood (b).

3.4. *Effect of EDDS on heavy metal uptake*

Towards the end of the growing season, pots containing the three soils under study and planted with *S. dasyclados* ‘Loden’ were treated with 7.5 mmol EDDS to temporarily enhance the phytoavailability of the heavy metals and by consequence stimulate their uptake. Figs. 3 and 4 exhibit shoot metal concentration (stem and leaves) with and without treatment with EDDS.

Effects of EDDS application were most pronounced in leaves and stems for Cu and Zn, while significant increases in foliar uptake were also observed for Pb and Ni (Figs. 3 and 4). Cd and Cr uptake was affected to a lesser extent. Uptake of Cr, Ni and Pb by EDDS-treated plants was deemed to be insufficient to consider for phytoextraction applications and will not be discussed in further detail.

Significant increases of Cd accumulation in both stems and leaves of plants treated with EDDS were observed in A3 and A2, but not in A1. In A2, Cd concentrations in stem and leaves reached levels of up to 60 mg kg^{-1}. Stem Cd concentrations increased by 60% for soil A2 in comparison to the control, while leaf Cd content increased by 35%. For A3 the relative increases after treatment were, respectively, 97% for Cd in the stems and 45% for foliar Cd concentrations. Zinc uptake was significantly affected by EDDS treatment in soils A2 and A3, with increases by 96–107% in A2 and 29–47% in A3. Again, no significant effect was observed in A1 for this metal.

Effects of EDDS on Cu uptake were more pronounced in the leaves than in the stems. Foliar Cu concentrations increased by a factor 3.3 in A1, 3.5 in A2 and 7.1 in A3. The Cu concentration in the stems increased by a factor 1.4–1.9 for all soils. In particular, the extraction performance in A3 is remarkable: relatively high extraction levels are achieved in a soil with low total content.

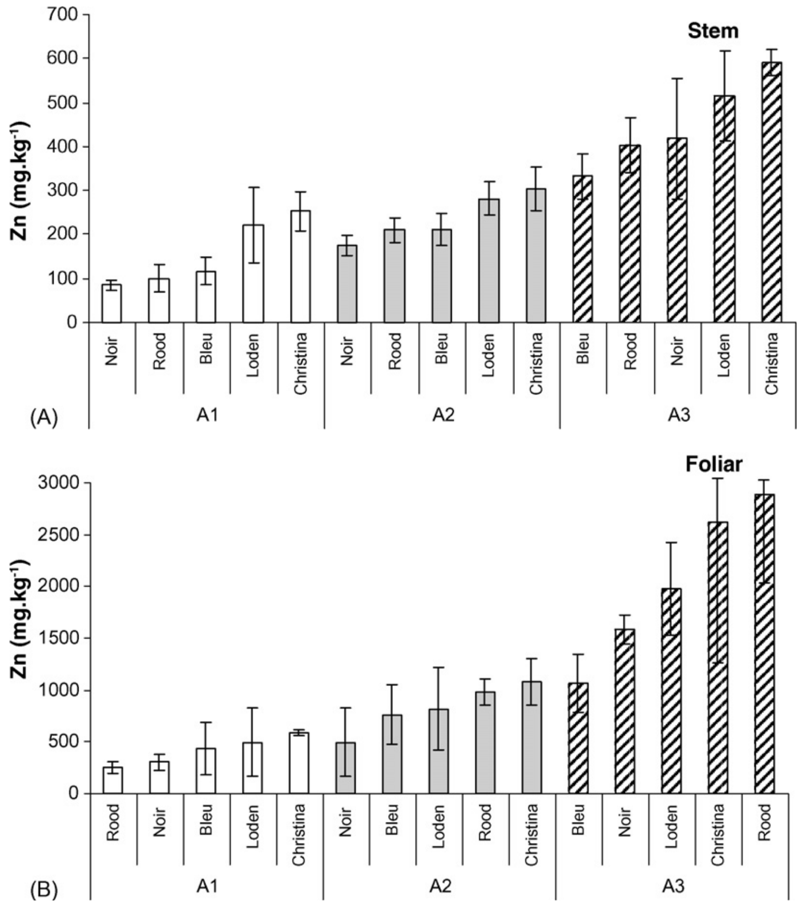

Fig. 1. Zinc concentration in (A) stems and (B) leaves of the five willow species under study (mg kg^{-1}) (*Note*: Chris: Christina, Noir: Noir de Villaines, Rood: Belgisch Rood). Error bars indicate standard deviation ($n = 3$).

4. Discussion

4.1. Heavy metal bioavailability in the soil

Uptake of Cr, Cu, Ni and Pb did not vary sufficiently and was not high enough for further examination regarding phytoavailability of these metals over the three soils.

Two-way ANOVA revealed that Zn phytoavailability as reflected by stem and leaf concentrations varied significantly in the order A3 > A2 > A1. There was a lower biomass production for the willows on soil A3, and comparison of plant concentrations is partly biased. The variation in phytoavailability for Zn between the three soils was not reflected in Zn extracted by the stronger *aqua regia*, NH_4OAc–EDTA or NH_4OAc extraction procedures. However, Zn extractable by the weaker extraction based on the dilute salt solution $CaCl_2$ followed the same order as plant concentrations in stem or leaves. Meers et al. (2003) also observed good correlations between foliar concentrations of Zn and $CaCl_2$ extracted levels. Zn recovered in the soil solution (rhizon) could distinguish between availability in A3 versus the other soils, yet could not distinguish in availability between A1 and A2. These data suggest that in this study $CaCl_2$ and rhizon extractions provided better measures for Zn phytoavailability than the more aggressive NH_4OAc–EDTA and NH_4OAc extractions or than the pseudo-total content as estimated by *aqua regia* digestion. $CaCl_2$ extractable Zn in A1 was of the same order of magnitude as extractable Zn in a previous field experiment (424–658 mg kg^{-1}) (Meers et al., 2003). Foliar concentrations of Zn in Loden in that field experiment were also within the same range as levels observed for Loden grown on A1 in the pot screening: 686 ± 80 mg kg^{-1} in the field experiment compared to 617 ± 366 mg kg^{-1} in the pot experiment.

For Cd, plant uptake occurred in the order A2 > A3 > A1, with only rhizon extractions exhibiting a similar order in Cd extractability. This implies that soil solution extraction provides a good measure for both Zn and Cd phytoavailability assessment.

4.2. Comparative screening

Soil concentrations of Cr, Cu, Ni and Pb were low and not considered ecologically relevant in relation to legislative criteria. This was also reflected in low shoot accumulation of these metals. Because of the lack of difference between shoot concentrations by the various clones in the soils under study, these metals are not discussed in further detail.

Meers et al. (2003) observed that in a field screening *S. dasyclados* Loden extracted significantly more Zn from dredged sediment than *Salix viminalis* Orm and *Salix schwerinii* × *Salix viminalis* Tora. The current study further accentuates the

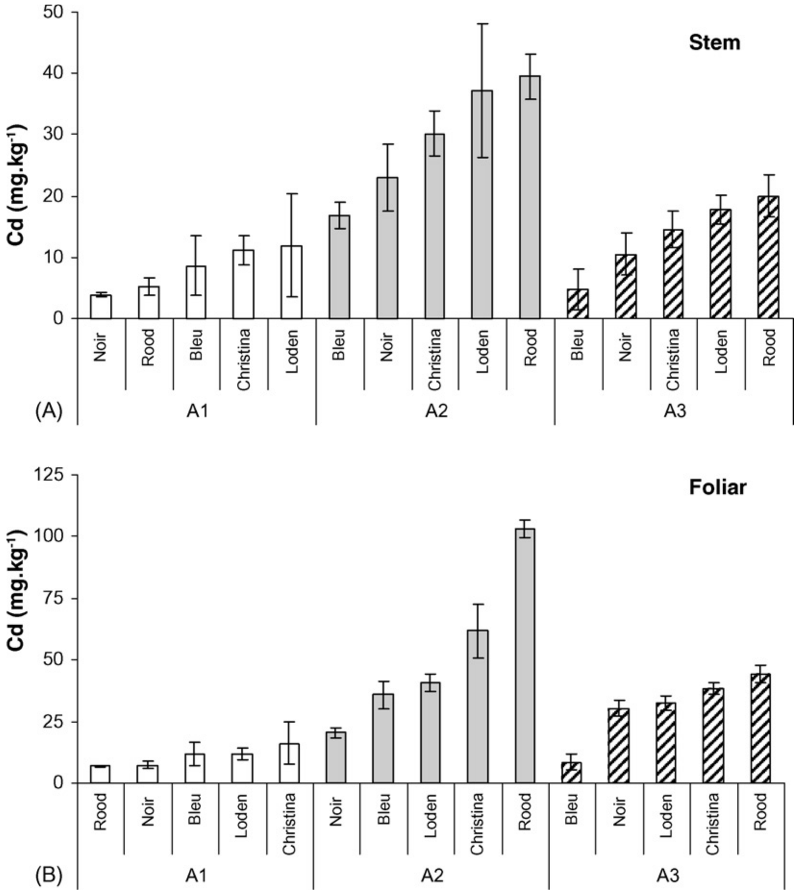

Fig. 2. Cadmium concentration in (A) stems and (B) leaves of the five willow species under study (mg kg^{-1}) (*Note*: Chris: Christina, Noir: Noir de Villaines, Rood: Belgisch Rood). Error bars indicate standard deviation ($n = 3$).

importance of Loden. The only clone which exhibited a decisively (yet insignificantly) higher uptake of Zn was Christina. Stem Zn concentrations were on average 10 ± 3% higher in Christina than in Loden, while foliar Zn in Christina were on average 34 ± 38% higher. In a field comparison between Noir de Villaines and Belgisch Rood, Vervaeke (2004) observed consistently higher accumulation levels for Zn in wood and leaves of Belgisch Rood. This is also in agreement with respective results from the current screening.

Just as for Zn, three clones (Loden, Christina and Belgisch Rood) could be distinguished from the other two in Cd uptake in stems and leaves. Uptake in the stems was generally higher for Loden in the three soils than in any of the other species, with the exception of Belgisch Rood on A2. Bertholdsson (2002) also reported that of 90 tested willow clones, Loden proved to be most promising for application in the phytoextraction of Cd. In addition, this clone was found to extract higher amounts of Cd and Zn than *Salix viminalis* Orm and *Salix schwerinii* × *Salix viminalis* Tora in a field screening comparing these three species (Meers et al., 2003). In the current study, foliar accumulation of Cd was consistently higher for Belgisch Rood than for Loden with concentrations ranging between 1.1 and 2.0 times those in Loden for the three soils. For Christina, foliar concentrations were 1.1 and 3.0 times higher than Loden in A3 and A2, respectively. The current data are in agreement with findings by Vervaeke (2004) who observed consistently higher Cd accumulation by Belgisch Rood than by Noir de Villaines in field experiments with willow stands of 1–6 years in age. They observed elevated concentrations in both clones, with foliar concentrations up to 90 mg kg^{-1} for both species and concentrations in stem and bark of up to 80 and 25 mg kg^{-1}, respectively. Vandecasteele et al. (2005) compared *S. fragilis* Belgisch Rood to *S. viminalis* Aage in a pot experiment with six different sediment derived soils, covering a broad range of Cd contamination. No significant differences were observed between both clones. Shoot accumulation depended strongly on soil content and attained concentrations of up to 50–70 mg kg^{-1} for stems and leaves.

4.3. *Effect of EDDS on heavy metal uptake*

Two-way ANOVA with soil treatment and soil type as factors revealed there was significant interaction between both factors for Cd and Zn concentrations in the stem and for Cu and Ni in the leaves. This implies that the effects of EDDS on induced heavy metal uptake are soil specific. This can be explained by the fact that chelator effect in the soil depends on physico-chemical characteristics such as pH and CEC as well as on concentrations of competitive cations such as other trace metals, divalent exchangeable bases (Ca, Mg) and more abundant metals such as Al and Fe.

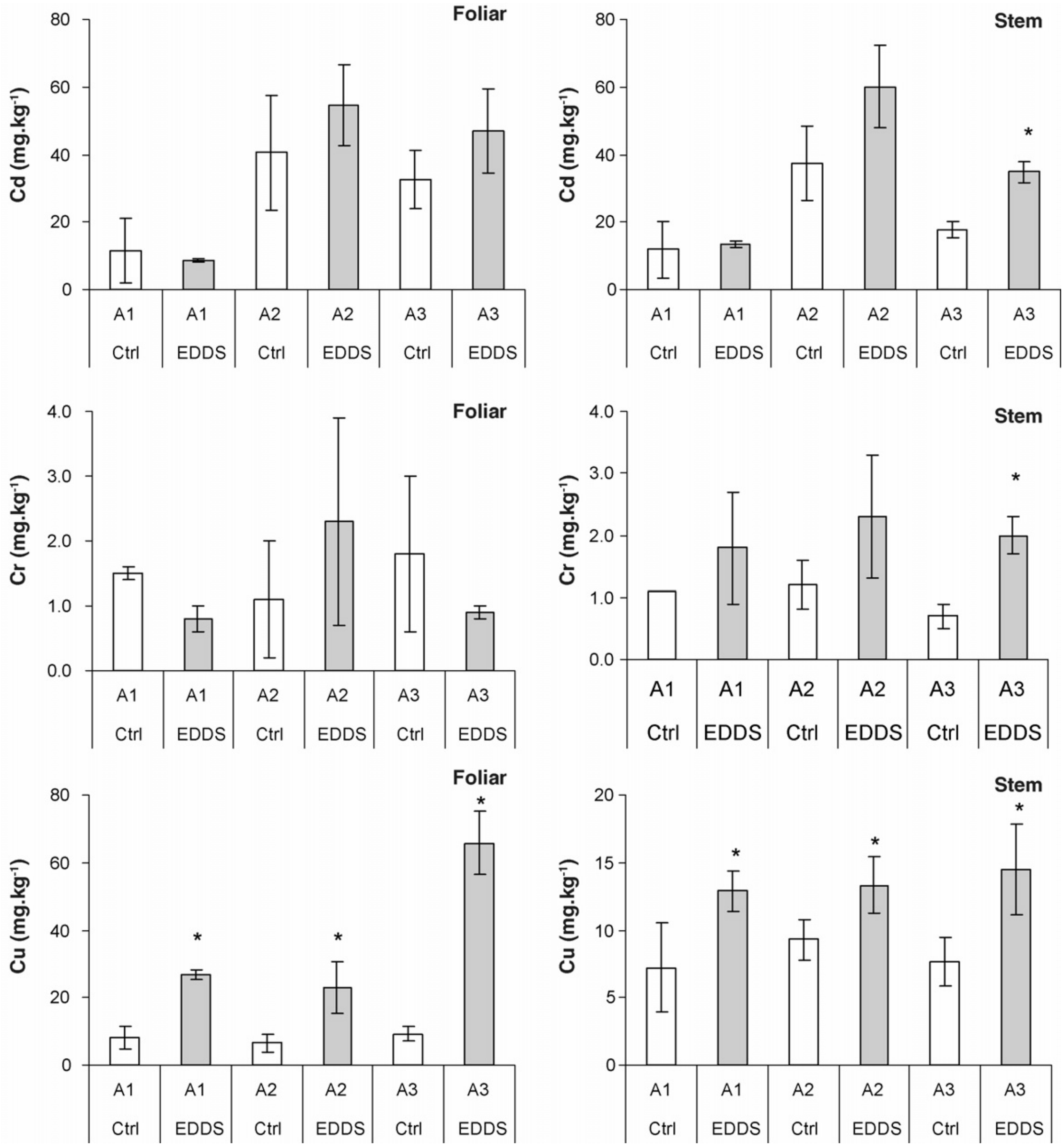

Fig. 3. Concentrations of Cd, Cr and Cu in stems and leaves of *S. dasyclados* 'Loden' with (gray bars) or without (white bars) treatment with 7.5 mmol EDDS per pot; asterisks indicate significant increases compared to the untreated control ($\alpha = 0.05$). Error bars indicate standard deviation ($n = 3$).

Recently, several studies have been conducted on the potential use of soil amendments to enhance metal uptake by willow crops. Robinson et al. (2000) investigated the impact of the chelating agents EDTA, DTPA and NTA on metal accumulation. Results were not encouraging with severe stress symptoms and growth depression being observed. Klang-Westin and Perttu (2002) investigated whether increased fertilisation could increase metal removal. Increases observed in Cd accumulation were small and in most cases insignificant. Kos and Lestan (2004) reported that out of four amendments tested (EDTA, DTPA, EDDS, citric acid) EDDS was most effective in enhancing Cu accumulation by *Brassica rapa* with increases in shoot accumulation with a factor 3.3 when grown on a natural (unspiked) soil with relatively low total Cu content (163 mg kg^{-1}). A similar observation was made by Meers et al. (2005b,c) in *Brassica rapa*, *Helianthus annuus*, *Zea mays* and *Cannabis sativa*. Analogously, in the current study EDDS appeared to significantly increase uptake of Cu.

The current results indicate that treatment with short-lived chelating agent EDDS might significantly enhance removal of Cd, Cu and Zn by *S. dasyclados*. However, the effects were found to be very soil specific. This accentuates the need for preliminary pot experiments before effective implementation in the field to ascertain the suitability of chelators for enhanced phytoextraction. In soil A1, EDDS treatment appears to be inefficient in successfully increasing metal removal by willows. In the other two soils, more significant effects were observed.

Results indicated that differences in metal uptake between clones and the effect of EDDS treatment were soil-specific. Before phytoremediation is applied in the field, site-specific

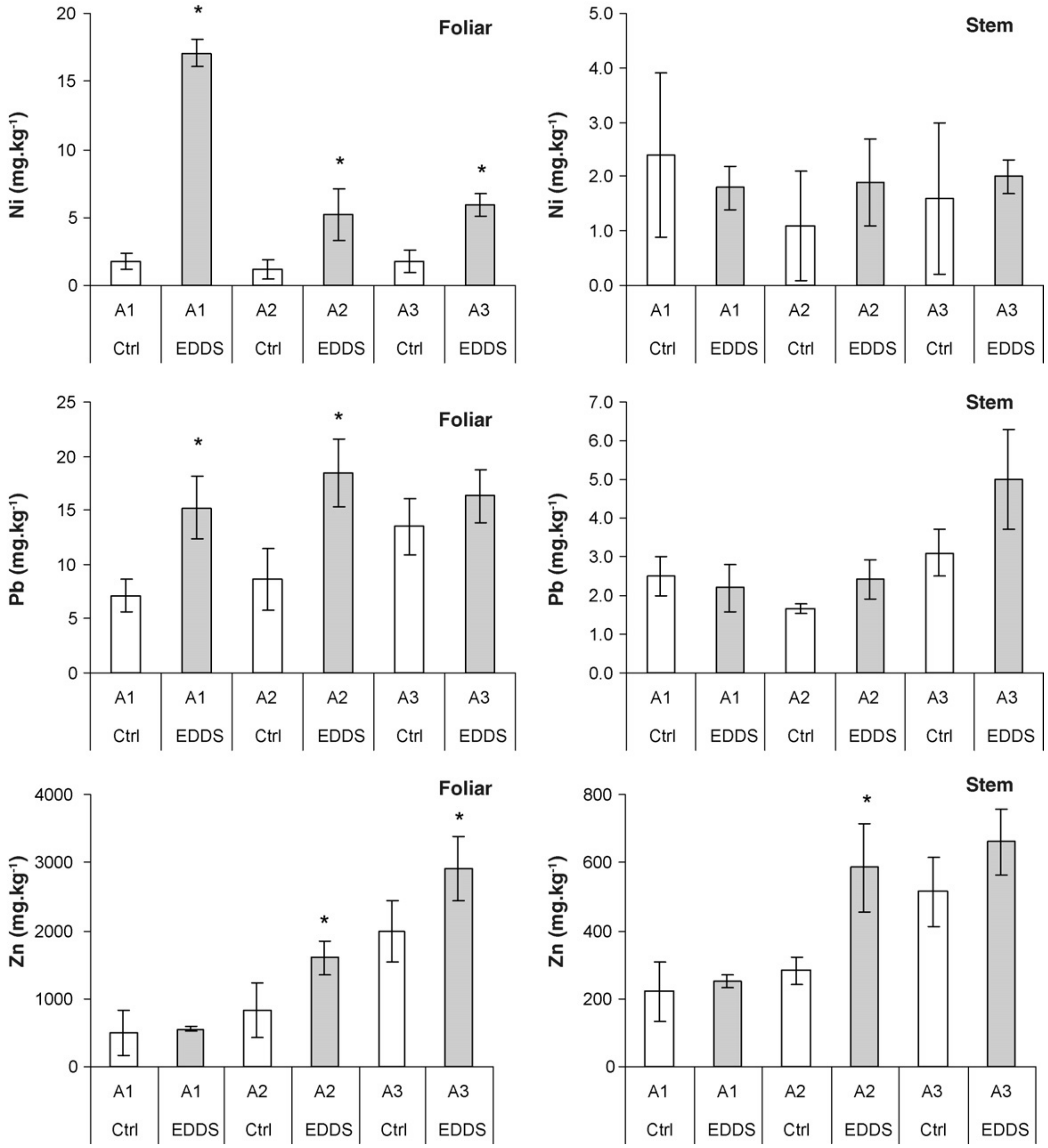

Fig. 4. Concentrations of Ni, Pb and Zn in stems and leaves of *S. dasyclados* 'Loden' with (gray bars) or without (white bars) treatment with 7.5 mmol EDDS per pot; asterisks indicate significant increases compared to the untreated control ($\alpha = 0.05$); Error bars indicate standard deviation ($n = 3$).

screenings, i.e. soil-specific pot experiments may help to select suitable clones and may evaluate the effect of EDDS application. It is of particular interest for future research to attempt to correlate observed shoot levels in pot experiments and laboratory scale phytoavailability assessments to observed levels under field conditions. The resulting interrelationships will be species and clone dependent. In this manner an attempt can be made to extrapolate predicted extraction performance in the field based on preliminary tests.

4.4. *Implications for phytoextraction*

Several studies have shown that *Salix* spp., with *S. viminalis* and *S. dasyclados* in particular, exhibit the capacity to accumulate high levels of Cd and Zn (Brieger et al., 1992; Punshon and Dickinson, 1997; Hammer et al., 2003; Meers et al., 2003, 2005a). This corresponds well with findings in the current study. Only the uptake of Cd and Zn by the various willow species appeared acceptable for potential application in phytoextraction of the contaminated soils under study. However, the soils only contained rather low levels of Ni and Cu, making lack of accumulation inconclusive with respect to applicability of willows for these metals. Pulford et al. (2001) evaluated the potential of *Salix* for phytoextraction of Cr on experimental sites with high levels of Cr (1630–1788 mg kg^{-1}) and concluded that even at high concentrations of available Cr, *Salix* was incapable of absorbing and translocating Cr in sufficient amounts to consider for phytoremediation purposes. In the same study, Zn was demonstrated to be freely distributed throughout the tree after uptake.

Concentrations observed in the leaves were generally higher than concentrations in the stems for all metals under investigation. Numerous studies reported that, in regards with compartmentalisation of heavy metals in aerial plant parts of trees, accumulation of heavy metals occurs predominantly in actively growing tissues (Pulford and Watson, 2003). Riddell-Black (1994) observed foliage concentrations to be greater than stem concentrations for four willow species grown on sludge amended soil. Vervaeke (2004) also found systematically higher concentrations of heavy metals in leaves than in wood for *S. fragilis* and *S. triandra* grown on a moderately contaminated dredged sediment derived surface soil. Klang-Westin and Eriksson (2003) estimated that the percentage of foliar Cd, expressed as relative fraction of total shoot content, varied between 21% and 48%. Meers et al. (2003, 2005a) also reported a high relative importance of heavy metals in foliage amounting up to levels of 49% and 62% of total aboveground content for Cd and Zn, respectively, for willows grown on moderately contaminated dredged sediments. This implies that in order to optimize phytoextraction and export of heavy metals, foliar material would also have to be removed. This can be performed by collecting the foliar material after it has fallen, or by harvesting the willows while they are still carrying leaves.

However, Sennersby-Forrse et al. (1992) stated that cutting willows while actively growing may result in physiological disorders and may severely affect resprouting. Stump mortality due to repeated harvests at higher frequency can be another cause for decreasing yields (Harrington and DeBell, 1984). Kopp et al. (1997) reported significantly lower yields in 3 years of annual willow harvesting compared to the observed production when a 3-year rotation period is applied. Furthermore, results from research in Sweden suggest that rotation lengths from 4 to 6 years result in larger annual increments (Willebrand et al., 1993). On the other hand, it can be argued that shorter rotation periods could be efficiently employed in more temperate regions. Hammer et al. (2003) reported no negative effects of annual harvesting before the start of leaf senescence in a long-term trial (5 years). They demonstrated that *Salix* could be clearfelled each year prior to leaf fall and still produce an increasing annual biomass. Dickinson and Pulford (2005) claim that sufficient evidence has accumulated that raises expectations that clean-up of Cd-contaminated land can be achieved through cultivation and harvest of selected clones of short-rotation coppice willow within a realistic crop lifecycle. They stated that repeated harvest before leaf fall is a prerequisite for efficient removal of Cd. Field quantification of the effects of annual harvest on biomass and heavy metal removal therefore require further study in light of optimizing phytoextraction efficiency.

In particular on A3, Zn extraction was satisfactory for phytoextraction purposes. However, biomass production in the pot trial was 40% lower for this soil than for A1 and A2, which may reduce the phytoextraction capacity on this soil. Foliar concentrations in Christina, Loden and Belgisch Rood ranged between 1980 and 2882 mg kg^{-1}, while the soil itself only contained low levels of pseudo-total Zn (275 ± 50 mg kg^{-1}). Although extrapolating to field conditions is a hazardous exercise, it is useful to gain an insight as to the order of magnitude of the expected metal mass to be extracted annually. Uptake of Zn by Loden in pots with A1 was similar to observed concentrations in a field experiment conducted on moderately contaminated dredged sediments with low natural phytoavailability and comparable composition (Meers et al., 2003). In that particular field experiment, average bark Zn concentrations were 319 mg kg^{-1}, wood Zn concentrations were 66 mg kg^{-1} and foliar Zn concentrations were 686 mg kg^{-1}. The annual extraction of Zn by Loden in that experiment was estimated at about 5 kg ha^{-1} from the topsoil layer (25 cm). The uptake of Zn into the stems and leaves by Loden in pots containing A3 was 2.5–4.0 times higher than the uptake in the pots with A1. Uptake by Christina was in general a factor of 1.1–1.3 higher than by Loden. Based on the relative uptake, an annual extracted mass of Zn by the best performing accumulator Christina on soil A3 might be achieved in the general order of 14–27 kg ha^{-1}. This corresponds with an estimated average decrease in the soil with 4.0–7.7 mg kg^{-1}. However, this preliminary projection needs to be addressed with some caution and is aimed at ascertaining the general order of expected extraction efficiency. Further field validation would be required to ascertain a more exact mass balance, especially for A3, since a lower biomass production was observed for the studied clones.

Foliar Cd concentrations in Loden grown on A3, A2 and A1 were 2–5.5 fold higher than in the field experiment described in Meers et al. (2003, 2005a). Estimated annual decrease of Cd in the topsoil layer in that experiment was estimated at 0.036 mg kg^{-1} per year. If the extracted mass in the field would relate in a similar manner then the ratios described above, the workable extraction interval for A3, A2 and A1 would be in the range 0.068–0.15–0.19 mg kg^{-1} for A1, A3 and A2, respectively. However, biomass production in field situations will mainly determine metal removal. Klang-Westin and Eriksson (2003) estimated an annual Cd removal when employing willows for phytoextraction in the range of 5–17 g ha^{-1}. This would imply an average decrease with 0.001–0.005 mg kg^{-1} in the topsoil layer (25 cm). Hammer et al. (2003) reported removal of 170 g ha^{-1} after 5 years on a calcareous soil and 47 g ha^{-1} after 2 years on an acidic soil. Vervaeke (2004) concluded that only Cd was extracted in sufficient amounts from dredged sediments to consider for phytoextraction purposes.

5. Conclusion

In this paper, three distinctly different soils were used: a slightly contaminated dredged sediment derived surface soil (Meigem; A1), a sediment derived surface soil with elevated levels of Cd, Cr and Zn (Gavere; A2) and a sandy soil with moderate contamination of Cd (LUC; A3). Only Cd and Zn uptake by the various clones was considered sufficient to consider for phytoextraction purposes, especially for the polluted soil A3.

Of the five clones tested, Christina, Loden and Belgisch Rood deserve further attention in field validation. A first estimation of the order of magnitude of Cd and Zn that could potentially be annually extracted with these clones, resulted in 5–27 kg ha^{-1}

for Zn and 0.25–0.65 kg ha^{-1} for Cd, with distinct differences observed between the various soil types. This corresponds with reductions in the topsoil layer (25 cm) with 1.4–8 mg kg^{-1} for Zn and 0.07–0.20 mg kg^{-1} for Cd, depending on the soil type and pollution level. However, adequate field validation is required to ascertain phytoextractable levels more exactly, as lower biomass production was observed for the sandy soil.

Treatment with EDDS exhibited distinct differences in inducing metal removal on the various soils. Uptake of Cd and Zn could not be enhanced in A1, whereas in A2 and A3 removal of these metals could be increased by 50–100% after treatment. In all soils EDDS treatment also had a distinct effect on Cu uptake. The relatively high Cu removal from A3, which contains low soil concentrations of total Cu, after EDDS application might be of particular interest for future phytoextraction prospects.

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References

Baker, A.J.M., Reeves, R.D., Hajar, A.S.M., 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. and C. Presl. (Brassicaceae). New Phytol. 127, 61–68.

Bertholdsson, N.-O., 2002. Bioremediation of Composted Sludge with *Salix*. OST 837 Action Fourth WG2 Workshop, Bordeaux, France, April 25–27.

Bremner, J.M., 1996. Nitrogen-total. In: Bartels, J.M., Bigham, J.M. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series No. 5, pp. 1085–1122.

Brieger, G., Wells, J.R., Hunter, R.D., 1992. Plant and animal species and heavy metal content in fly ash ecosystems content in fly ash ecosystem. Water, Air, Soil Pollut. 63, 87–103.

Dickinson, N.M., Pulford, I.D., 2005. Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ. Int. 31, 609–613.

Ettala, M.O., Yrjönen, K.M., Rossi, E.J., 1988. Vegetation coverage at sanitary landfills in Finland. Waste Manage. Res. 6, 281–289.

Gommy, C., Perdrix, E., Galloo, J.-C., Guillermo, R., 1998. Metal speciation in soil: extraction of exchangeable cations from a calcareous soil with a magnesium nitrate solution. Int. J. Anal. Chem. 72, 27–45.

Goodfellow, M., Brown, A.M., Cai, J., Chu, J., Collins, M.D., 1997. *Amycolatopsis japonicum* sp. nov., an actinomycete producing (*S*,*S*)-*N*,*N*^{t}-ethylenedisuccinic acid. Syst. Appl. Microbiol. 20, 78–84.

Hammer, D., Kayser, A., Keller, C., 2003. Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Manage. 19, 187–192.

Harrington, C.A., DeBell, D.S., 1984. Effects of irrigation, pulp mill sludge, and repeated coppicing on growth and yield of black cottonwood and red alder. Can. J. For. Res. 14, 844–849.

Klang-Westin, E., Perttu, J., 2002. Effects of nutrient supply and soil cadmium on cadmium removal by willow. Biomass Bioenerg. 23, 415–426.

Klang-Westin, E., Eriksson, J., 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil 249, 127–137.

Kopp, R.F., Abrahamson, L.P., White, E.H., Burns, K.F., Nowak, C.A., 1997. Cutting cycle and spacing effects on a willow clone in New York. Biomass Bioenerg. 12, 313–319.

Kos, B., Lestan, D., 2004. Chelator induced phytoextraction and in situ washing of Cu. Environ. Pollut. 132, 333–339.

Lakanen, E., Ervio, R., 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agric. Fenn. 123, 223–232.

Landberg, T., Greger, M., 1996. Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl. Geochem. 11, 175–180.

Lestan, D., Grcman, H., 2002. Chelate enhanced Pb phytoextraction: plant uptake, leachin and toxicity. In: Proceedings of the 17th WCSS World Congress of Soil Science, Symposium, vol. 42, Paper No. 1701, Bangkok (Thailand).

Lunácková, L., Masarovicová, E., Kráová, K., Stresko, V., 2003. Response of fast growing woody plants from family *Salicaceae* to cadmium treatment. B Environ. Contam. Toxicol. 70, 576–585.

Meers, E., Vervaeke, P., Tack, F.M.G., Lust, N., Verloo, M., Lesage, E., 2003. Field trial experiment: phytoremediation with *Salix* sp. on a dredged sediment disposal site in Flanders. Belgium. Remediat. J. 13, 87–97.

Meers, E., Lamsal, S., Vervaeke, P., Hopgood, M., Lust, N., Tack, F.M.G., Verloo, M.G., 2005a. Assessment of heavy metal bioavailability in a moderately contaminated dredged sediment disposal site and its potential for phytoextraction by *Salix viminalis*. Environ. Pollut. 137, 354–364.

Meers, E., Samson, D., Hopgood, M., Ruttens, A., Tack, F.M.G., 2005b. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58, 1011–1022.

Meers, E., Ruttens, A., Hopgood, M., Lesage, E., Tack, F.M.G., 2005c. Potential of *Brassica rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. Chemosphere 61, 561–572.

Nishikiori, T., Okuyama, A., Naganawa, Takita, T., Hamada, M., Takeuchi, T., Aoyagi, T., Umezawa, H., 1984. Production by actinomycetes of (*S*,*S*)-*N*,*N*^{t}-ethylenediamine-disuccinic acid, an inhibitor of phospholipidase C. J. Antibiot. 37, 426–427.

Nixon, D.J., Stephens, W., Tyrrel, S.F., Brierley, E.D.R., 2001. The potential for short rotation energy forestry on restored landfill caps. Bioresour. Technol. 77, 237–245.

Perttu, K.L., 1998. Environmental justification for short rotation forestry in Sweden. Biomass Bioenerg. 15, 1–6.

Pulford, I.D., Watson, C., McGregor, S.D., 2001. Uptake of Chromium by trees: prospects for phytoremediation. Environ. Geochem. Health 23, 307–311.

Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by Trees—a review. Environ. Int. 29, 529–540.

Punshon, T., Dickinson, N.M., 1997. Acclimation of *Salix* to metal stress. New Phytol. 137, 303–314.

Punshon, T., Lepp, N.W., Dickinson, N.M., 1995. Resistance to copper toxicity in some British willows. J. Geochem. Explor. 52, 259–266.

Punshon, T., 1996. Heavy metal resistance in *Salix*. Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy at the Liverpool John Moores University, School of Biological and Earth Sciences, Liverpool.

Riddell-Black, D., 1994. Heavy metal uptake by fast growing willow species. In: Aronsson, P., Perttu, K. (Eds.). Willow Vegetation Filters for Municipal Wastewaters and Sediments: A Biological Purification System. Proceedings of a Study Tour, Conference and Workshop in Sweden. Swedish University of Agricultural Sciences, Section of Short Rotation Forestry, Rapport 50, June 5–10, pp. 133–144.

Robinson, B.H., Mills, T., Petit, D., Fung, L., Green, S.R., Clothier, B.E., 2000. Natural and induced cadmium-accumulation in poplar and willow: implications for phytoremediation. Plant Soil 227, 301–306.

Rosselli, W., Keller, C., Boschi, K., 2003. Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256, 65–272.

Schnoor, J.L., 2000. Phytostabilization of metals using hybrid poplar trees. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. John Wiley & Sons, New York, NY, USA, pp. 133–150.

Schowanek, D., Feijtel, T.C.J., Perkins, C.M., Hartman, F.A., Federle, T.W., Larson, R.J., 1997. Biodegradation of [*S*,*S*], [*R*,*R*] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere 34, 2375–2391.

Sennersby-Forrse, L., Ferm, A., Kauppi, A., 1992. Coppicing ability and sustainability. In: Mitchell, C.P., Robinson, F.J.B., Hinckley, T., Sennerby-Forsse, S. (Eds.), Ecophysiology of Short Rotation Crops. Elsevier Applied Science, pp. 146–184.

Vandecasteele, B., De Vos, B., Tack, F.M.G., 2002. Heavy metal contents in surface soils along the upper Scheldt river (Belgium) affected by historical upland disposal of dredged materials. Sci. Total Environ. 290, 1–14.

Vandecasteele, B., Meers, E., Vervaeke, P., De Vos, B., Quataert, P., Tack, F.M.G., 2005. Growth and trace metal accumulation of two *Salix* clones on sediment-derived soils with increasing contamination levels. Chemosphere 58, 995–1002.

Van Ranst, E., Verloo, M., Demeyer, A., Pauwels, J.M., 1999. Manual for the Soil Chemistry and Fertility Laboratory. Ghent University, Faculty Agricultural and Applied Biological Sciences, p. 243.

Vervaeke, P., Luyssaert, S., Mertens, J., De Vos, B., Speleers, L., Lust, N., 2001. Dredged sediment as a substrate for biomass production of willow trees established using the SALIMAT technique. Biomass Bioenerg. 21, 81–90.

Vervaeke, P., Luyssaert, S., Mertens, J., Meers, E., Tack, F.M.G., Lust, N., 2003. Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ. Pollut. 126, 275–282.

Vervaeke, P., 2004. Phytoremediation of Land Disposed Contaminated Dredged Sediments: Fate of Heavy Metals. Thesis Submitted in Fulfilment of the Requirements for Degree of Doctor (PhD) in Applied Biological Sciences. University of Ghent, Faculty of Agricultural and Applied Biological Sciences, Belgium, 222 pp.

Willebrand, E., Ledin, S., Verwijst, T., 1993. Willow coppice systems in short rotation forestry—effects of planting design, rotation length and clonal composition on biomass production. Biomass Bioenerg. 4, 323–331.