Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils

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ABSTRACT

The Cd and Zn accumulation and phytoextraction potential of seven willow clones was investigated in a pot experiment for two vegetation periods. Heavily polluted Fluvisol-Litavka, moderately contaminated Cambisol-Pribram, and unpolluted control Chernozem-Suchdol were used. Significant differences were found in Cd and Zn accumulation between the willow clones. Cd and Zn were transferred from roots to aboveground tissues and all tested clones confirmed higher Cd and Zn accumulation in leaves than in twigs. Cd and Zn amounts removed by willow leaves were the highest from the most polluted soil (up to 83% Cd and 71% Zn of total removal). Therefore the harvest of leaves is necessary if willows are planted for heavy metal phytoextraction. Although the extremely high Zn contamination of Fluvisol-Litavka significantly reduced biomass production, willows planted in this soil showed the highest Zn removal because of extremely high Zn accumulation (max. 5061 ppm in leaves). Clones planted in moderately contaminated soil achieved the highest Cd removal. Clones showed different abilities to remove Cd and Zn, which was dependent on soil type and contamination level. Remediation factors were determined less than 1% for Zn in the heavily polluted soil and also unsatisfactory for Cd. However, it was shown that willows were suitable phytoextractors of moderately contaminated soil. About 20% of Cd and 4% of Zn were removed by harvested biomass from the total content of soil after two vegetation periods.

Keywords: cadmium; zinc; phytoextraction; removal; *Salix*; willow; contamination; soil

Willow is suitable as a heavy metal phytoextractor due to high element accumulation, high heavy metal transport to the shoots and high biomass production (Greger and Landber 1999). However, out of the tested metals (Cr, Ni, Cu, Zn, Cd and Pb) higher aboveground contents than in the soil were found only for Cd and Zn (Riddell-Black 1994). A large number of species and hybrids of *Salix* spp. suggests wide genetic variability within the genus and some species are known to colonise contaminated soil. For example *S. alba*, *S. viminalis*, *S. cinerea* and *S. caprea* naturally invade polluted dredged sediment disposal sites (Vandecasteele et al. 2002). Therefore, there is an opportunity for genotype selection to survive, revegetate, and potentially also to accumulate metals in tissues which could then be harvested to remediate contaminated soils (Dickinson et al. 1994). Willow species and clones have different tolerance to particular metals. Tolerance to heavy metals was generally confirmed in some clones while others were tolerant to only one or two of the examined metals (Landberg and Greger 1994). Significant differences were found in the uptake of metals and between willow varieties and clones (Landberg and Greger 1994, Riddell-Black 1994, Nissen and Lepp 1997, Greger 1999, Vysloužilová et al. 2002). Clones that accumulate relatively high amounts of metals are desirable if soil remediation is to be achieved by phytoextraction and biomass harvesting (Pulford and Watson 2002). In general, the Czech Republic has relatively good climatic and soil conditions for willow biomass production. The average primary production is estimated to range from 8 to 17 tonnes of dry biomass per hectare and year (Weger and Havlickova 2002). However, extremely high Zn soil contamination has phytotoxic effects and reduces biomass yield (Vysloužilová et al. 2003). It confirms a suggestion that phytoextraction is the best suited for low- and medium-contaminated agricultural soils and not for highly contaminated sites such as mining areas (Greger and Landberg 1999, Pulford and Watson 2002). After harvest, contaminated willow biomass can be used as wood fuel if heavy metals are recovered from smoke gases and ashes are properly handled (Perttu and Kowalik 1997).

MATERIAL AND METHODS

The accumulation of Cd and Zn and phytoextraction potential of *Salix* spp. were investigated in a pot experiment when these soils were used: heavily polluted Fluvisol from the alluvium of the Litavka river (main contamination was caused by the floods of water contaminated by wastes from smelter setting pits), Cambisol Pribram with moderate contamination mainly by atmospheric emissions from the same smelter (Kovohutě Příbram) and unpolluted Chernozem (Suchdol locality). Mean total contents and available amounts of Cd and Zn in soils are summarized in Table 1. Seven clones of willows with high biomass production from Silva Tarouca

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Table 1. Total and 0.01 mol/l $CaCl_2$ extractable concentration of elements in experimental soils

Soil	Soil type	Cd total (mg/kg)	Cd extractable (%)	Zn total (mg/kg)	Zn extractable (%)
Litavka	Fluvisol	30.5	12.6	3718	5.87
Pribram	Cambisol	4.73	6.56	180	0.29
Suchdol	Chernozem	0.42	0.92	87.1	0.08

Research Institute for Landscape and Ornamental Gardening in Průhonice (*S.* × *smithiana* S-218, *S.* × *smithiana* S-150, *S. viminalis* S-519, *S. alba* S-464, *S. alba* Pyramidalis S-141, *S. dasyclados* S-406, *S.* × *rubens* S-391) were used for the experiment. The willow collection is well documented and of known provenance and all clones were chosen on the basis of their already tested high biomass production. Willows were planted in 5-litre plastic pots with 5 kg of air-dry soil for two vegetation periods, from April 2001 to September 2002. Soil moisture was regularly controlled and kept at 60% of MWHC. Four replications of each treatment were used (three 20 cm long cuttings per pot). Aboveground biomass was harvested only after first vegetation period, whereas after the second period root biomass was collected as well. Aboveground biomass was separated into leaves and twigs, roots were thoroughly washed by demineralised water, biomass was checked for fresh and dry biomass, ground and analysed. Plant material was decomposed by a modified dry ashing procedure in a mixture of oxidizing gases ($O_2 + O_3 + NO_x$) in APION Dry Mode Mineraliser (Miholová et al. 1993). Total element concentration in soils was determined after two-step decomposition, using APION in the first step and wet digestion with a mixture of $HF + HNO_3$ in the second step (Mader et al. 1998). Metal availability in soil was determined by 0.01 mol/l $CaCl_2$ extraction at a 1:10 ratio (w/v) (Novozamsky et al. 1993). In 2001, the element concentrations in the digests were determined by atomic absorption spectrometry (VARIAN SpectrAA-400) in flame (Cd, Pb and Zn), flameless (Pb), and hydride generation (As) measurement modes, respectively. The measurements in 2002 were carried out by inductively coupled plasma optical emission spectrometry (VARIAN Vista-Pro). The measurements were performed in trace element laboratories of Departments of Chemistry and Agrochemistry at the Czech University of Agriculture in Prague. Certified reference materials RM 12-02-03 Lucerne and RM 7001 Light Sandy Soil were applied for quality assurance of analytical data. One-way analysis of variance and multiple-samples comparison (Fisher's *LSD* procedure method) were applied for statistical evaluation of the data using Statgraphics Plus 5.0.

RESULTS AND DISCUSSION

Cd and Zn accumulation in plants

The tested willow clones confirmed high aboveground Cd and Zn accumulation potentials. By comparison of individual aboveground parts of plants, Cd and Zn were more intensively accumulated in leaves than in twigs in

Table 2. Minimum and maximum Cd and Zn contents in leaves, twigs and roots (mg/kg dry weight) and minimum significant differences (at the 95% confidence level) between seven willow clones and experimental soils

Soil	2001				2002					
	leaves		twigs		leaves		twigs		roots	
	Cd	Zn	Cd	Zn	Cd	Zn	Cd	Zn	Cd	Zn
Litavka										
min	5.0	1074	4.9	400	9.0	997	7.3	471	44.2	1664
max	108	5061	75.6	1623	204	4484	94.0	2055	132	4046
d min	10.6	663	14.5	270	47.7	769	18.2	401	54.9	1540
Pribram										
min	28.1	178	15.0	42.0	9.6	117	11.4	98.8	13.0	148
max	113	668	50.2	145	62.9	709	28.2	222	33.5	365
d min	17.6	112	11.3	23.6	6.8	66.7	3.6	28.7	10.8	152
Suchdol										
min	0.6	28.8	0.2	53.5	1.1	120	0.6	48.0	0.9	84.6
max	4.6	153	2.5	203	4.4	348	20.2	232	1.9	213
d min	0.8	19.0	0.8	30.9	0.8	44.7	6.9	49.1	0.8	61.2

accordance with investigations of Riddell-Black (1994). Cd and Zn were not retained in roots and were transferred to aboveground plant tissues in all tested clones. Cd and Zn contents in roots were approximately as high as in willow shoots planted in all types of soils. On the other hand, Landberg and Greger (1994) cultivated 103 different clones in solution culture and found that the net Cd and Zn transport to shoots ranged between 1 and 72% of the total metal uptake. The clonal variation of Cd and Zn accumulation, varying between the elements, plant parts and types of soil, is demonstrated in Table 2. The highest clonal variation in accumulation was found for Cd in leaves of *Salix* spp. planted on heavily polluted Fluvisol-Litavka. The highest Cd content was approximately 20 times higher than the minimum content in leaves and 15 times higher in twigs in both years. Differences in the Zn and Cd accumulation in *Salix* tissues for all tested soils were lower on moderately contaminated Cambisol-Pribram and unpolluted Chernozem-Suchdol, about 5 times. Greger (1999) found that the Cd uptake capacity of 70 *Salix* genotypes could differ by as much as 43 times between the clones with the highest and lowest values. Although the total Cd and Zn contents and available amounts of these elements in soil were much higher in Fluvisol-Litavka than in Cambisol-Pribram, Cd contents in leaves and twigs of *Salix* spp. planted on these two soils were not significantly different in the first vegetation period. After the second harvest the Cd content in leaves and twigs grown in Fluvisol-Litavka was slightly higher than in Cambisol-Pribram. On the other hand, Zn content in aboveground biomass of *Salix* spp. planted on Fluvisol-Litavka was much higher than on Cambisol-Pribram in both years. It shows the higher ability of willow tissues to take up Zn compared to Cd. Cd and

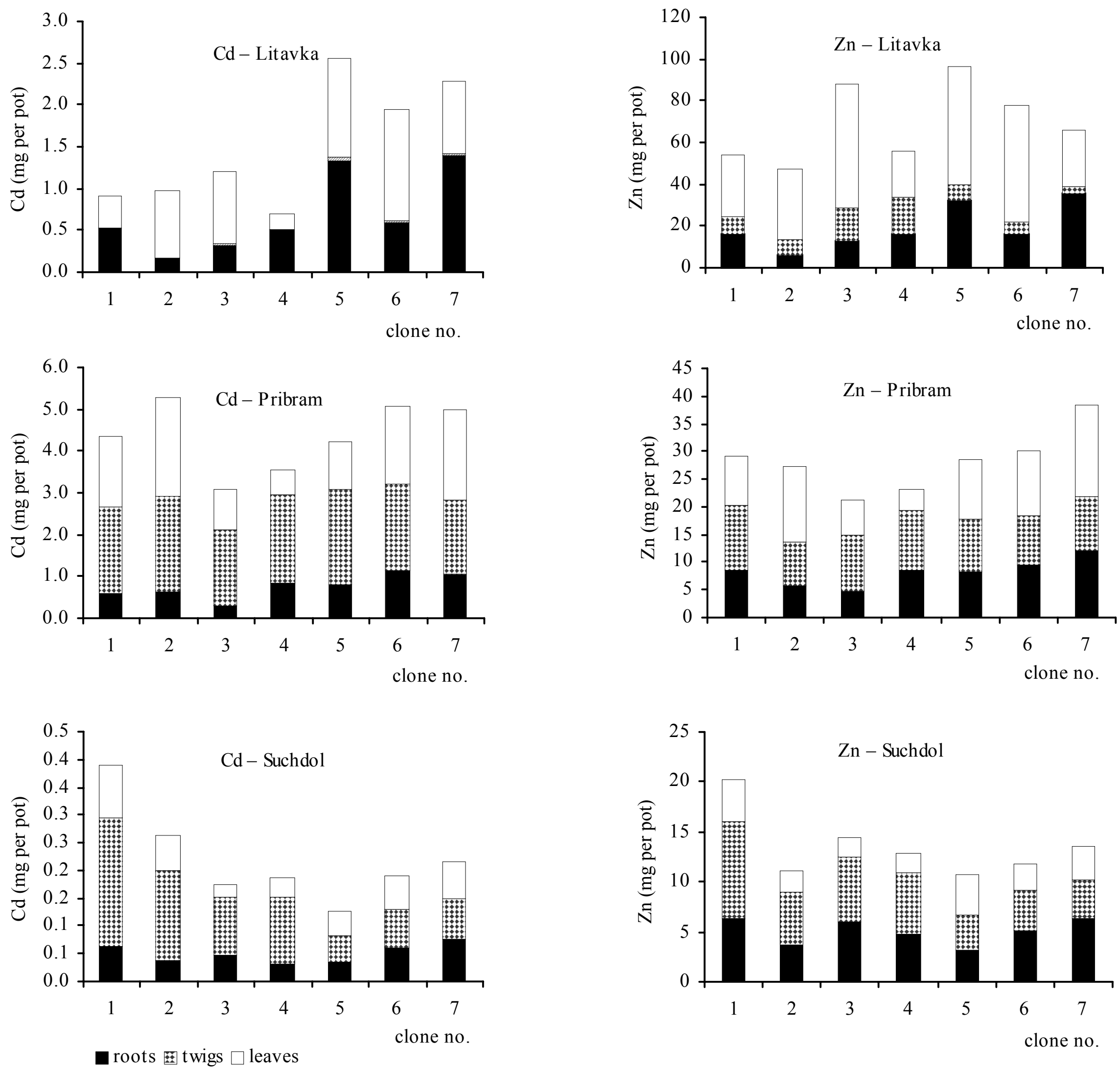

Figure 1. Total Cd and Zn removal (mg of element per pot) by roots, twigs and leaves of willows planted on tree types of soil (heavily contaminated Fluvisol-Litavka, moderately contaminated Cambisol-Pribram and unpolluted Chernozem-Suchdol) for two vegetation periods (1. *S.* × *smithiana* S-218, 2. *S.* × *smithiana* S-150, 3. *S. viminalis* S-519, 4. *S. alba* S-464, 5. *S. alba* Pyramidalis S-141, 6. *S. dasyclados* S-406, 7. *S.* × *rubens* S-391)

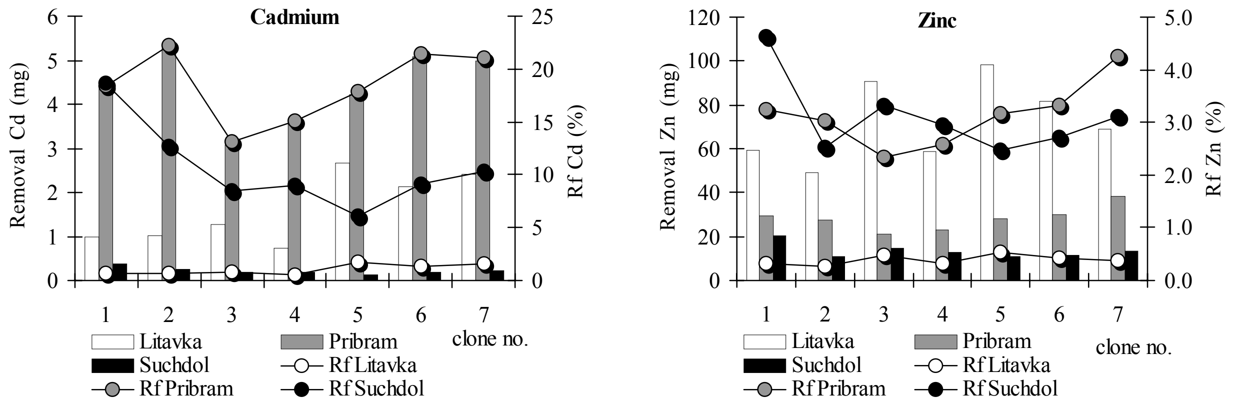

Figure 2. Total removal – y axis left (mg of element per pot) and total remediation factors – y axis right (% of element removed by harvested biomass from total content of elements in soil) of seven clones of *Salix* spp. (1. *S.* × *smithiana* S-218, 2. *S.* × *smithiana* S-150, 3. *S. viminalis* S-519, 4. *S. alba* S-464, 5. *S. alba* Pyramidalis S-141, 6. *S. dasyclados* S-406, 7. *S.* × *rubens* S-391)

Zn contents in the tissues of *Salix* spp. planted on control Chernozem-Suchdol were lower in comparison with contaminated Fluvisol-Litavka and Cambisol-Pribram.

Cd and Zn removal by plants

Extremely high contamination of Fluvisol-Litavka resulted in phytotoxic effects and caused chlorosis, partial defoliation and significant yield reduction of aboveground biomass in all seven willow clones in both years. Therefore the phytoextraction potential of *Salix* spp. planted on this soil largely decreased. Yield reduction was higher in the first year of cropping than in the second one. The root biomass determined after the second harvest was also reduced by contamination. Higher production of both aboveground and root biomass was found in willow clones planted in moderately contaminated Cambisol-Pribram in comparison with those planted in the unpolluted Chernozem-Suchdol. This fact indicates that willows have a specific demand for nutrient supply and high tolerance to moderate contamination of soil by heavy metals. The clones did not show any significant differences in yield production grown in one type of soil.

Total Cd and Zn removal of harvested willow leaves, twigs and root biomass planted in three types of soils in both years is shown in Figure 1. It is obvious from this figure that the removal potential of willow clones varied between the types of soil and contamination level. The most suitable clone for Cd and Zn phytoextraction from the most polluted Fluvisol-Litavka was found to be S-141 *S. alba* Pyramidalis, whereas the lowest removal was measured in clone S-464 *S. alba*. Another clone S-391 *S.* × *rubens* was the most suitable for Cd and Zn phytoextraction from moderate contaminated Cambisol-Pribram. The lowest Cd and Zn removal from this soil was achieved by clone S-519 *S. viminalis*. Clearly, the best clone for Cd and Zn phytoextraction from unpolluted Chernozem-Suchdol was S-218 *S.* × *smithiana*, and clone S-141 *S. alba* Pyramidalis was shown as the least suitable, in spite of being the most suitable for Fluvisol-Litavka. Clones showed different abilities to remove Cd and Zn, which depended on soil type and contamination level. On the other hand, Greger and Lanberg (1999) reported that the uptake ability of the clone is stable. By comparison of identical clones cultivated at three different locations in Sweden and after hydroponics cultivation they found that the order of the clones was still the same in all cases, based on increasing Cd content in stems. However, the interactions between root processes, soil properties and the dynamics of the associated microbial population significantly alter heavy metal uptake by plants (Gobran et al. 2001). Stoltz and Greger (2002) observed differences in uptake and translocation properties of the same plant species between field-grown plants and plants grown in hydroponics. It is also obvious from Figure 1 that in the most polluted Fluvisol-Litavka most of Cd and Zn was removed by leaves. The highest amount of Cd and Zn removed by leaves was 83 and 71%, by twigs only 2 and 30%, and by roots 73 and 53%, respectively. A higher amount of Cd and Zn removed by twigs was determined in moderately contaminated Cambisol-Pribram. Up to 60% Cd and 47% Zn were found to be removed by twigs. The highest amount of Cd and Zn (maximum 64 and 49%) was removed by twigs from unpolluted Chernozem-Suchdol. Cd and Zn amounts removed by leaves increased with heavy metal pollution of soil, whereas opposite results were obtained for Cd and Zn amounts removed by twigs. Consequently, harvest of leaves is necessary if willows are planted for heavy metal phytoextraction from contaminated soils.

Generally, the highest total Cd removal was achieved on Cambisol-Pribram because of the highest yield production together with high Cd accumulation in willow tissues, whereas the highest Zn removal was achieved on Fluvisol-Litavka. It was influenced by the much higher Zn accumulation in willow tissues (above all in leaves), despite of the significant yield reduction in comparison with Cambisol-Pribram. The lowest Cd and Zn removal was determined in uncontaminated Chernozem-Suchdol (Figure 2). The phytoextraction potential of willow clones in Figure 2 is expressed by the remediation factor Rf (%) that indicates amounts of elements removed by harvested biomass from the total content of elements in the pot soil. Although Zn removal from Fluvisol-Litavka is highest, the remediation factor is lowest (below 1%) because of the extremely high total Zn content in soil. Slightly higher, but still unsatisfactory Rf (up to 1.8%) was achieved for Cd for this soil. The significantly highest remediation factors for Cd were determined on moderately contaminated Cambisol-Pribram. Maximally 22.3% of Cd in total was removed by S-150 *S.* × *smithiana* from this soil after two vegetation periods and 4.3% of Zn by S-391 *S.* × *rubens*. Similar remediation factors of Zn were obtained for Chernozem-Suchdol, which ranged from 2.3 to 4.6%. The first step in phytoremediation of extremely contaminated soil should be its phytostabilisation and phytoimmobilisation that provide desirable soil conditions, thereby giving a possibility of healthy growth of willow plants and good results of phytoextraction.

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ABSTRAKT

Schopnost fytoextrakce kadmia a zinku u sedmi klonů *Salix* spp. pěstovaných na půdách kontaminovaných těžkými kovy

V dvouletém nádobovém vegetačním pokusu byla sledována fytoextrační účinnost sedmi klonů vrb a jejich schopnost přijímat Cd a Zn. K pokusu byly použity extrémně kontaminovaná fluvizem Litavka, středně kontaminovaná kambizem Pribram a jako kontrola nekontaminovaná černozem Suchdol. Mezi jednotlivými klony a použitými zeminami byly nalezeny významné rozdíly v akumulaci Cd a Zn. Cd a Zn byly transportovány z kořenů do nadzemních pletiv a všechny testované klony potvrdily jejich vyšší akumulaci v listech než ve větvích. Nejvyšší podíl Cd a Zn odebraných listy byl nalezen u vrb pěstovaných na nejvíce kontaminované půdě (až 83% Cd a 71% Zn z celkového odběru). Proto je nezbytná sklizeň vrb pěstovaných pro fytoremediační účely včetně listů. Ačkoliv extrémně vysoká kontaminace fluvizemě Litavka významně snížila produkci biomasy, bylo vrbami pěstovanými na této půdě odebráno nejvíce Zn díky extrémně vysoké akumulaci Zn (max. 5061 ppm v listech). Cd bylo nejvíce odebráno klony pěstovanými na středně kontaminované půdě. Klony vykázaly odlišnou schopnost odebírat Cd a Zn v závislosti na půdním typu a úrovni kontaminace. Remediační faktory pro Zn na silně kontaminované půdě byly stanoveny pod 1% a rovněž získané hodnoty Rf pro Cd byly neuspokojivé. Vrby se však ukázaly jako vhodné fytoextraktory středně kontaminované půdy, kde bylo po dvou letech vegetace odebráno sklizenou biomasou okolo 20% Cd a 4% Zn z jejich celkového obsahu v půdě.

Klíčová slova: kadmium; zinek; fytoextrakce; odběr; *Salix*; vrba; kontaminace; půda

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