As, Cd, Pb and Zn uptake by *Salix* spp. clones grown in soils enriched by high loads of these elements

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ABSTRACT

As, Cd, Pb and Zn accumulation in the aboveground biomass of seven clones of *Salix* spp. and changes in element uptake by plants after element addition to soil were studied in a pot experiment. Unpolluted Chernozem (Suchdol) as a control and soils with addition of As (100 mg/kg), Cd (40 mg/kg) and either Pb (2000 mg/kg) (Suchdol-Pb) or Zn (2000 mg/kg) (Suchdol-Zn) were used for the experiment. Significant differences were found in the accumulation of elements between willow clones and also between different element additions to the soil. Although As and Cd uptake slightly increased in Suchdol-Zn soil compared to Suchdol-Pb soil, the element removal from soil was significantly higher in Suchdol-Pb soil due to a significant reduction of aboveground biomass yield in Suchdol-Zn soil caused by Zn phytotoxicity. The yield reduction decreased the uptake of plant-available elements by biomass, thus higher plant-available portions of As and Cd were found in Suchdol-Zn soil. Element removal from soil was more dependent on element contents in willow tissues in Suchdol-Pb soil than in Suchdol-Zn soil, where willow plants exhibited physiological symptoms of phytotoxicity.

Keywords: soil; arsenic; cadmium; lead; zinc; *Salix* spp.; element removal; phytotoxicity

Phytoremediation is a method for *in situ* cleanup of contaminated soil. Green plants play a major role in achieving this goal (Baker and Brooks 1989, Baker et al. 1994, Salt et al. 1998, Wenzel et al. 1999). Phytoextraction presents direct uptake of heavy metals by plants and theit translocation to aboveground biomass. Willows meet main requirements for suitable phytoextraction because of their high biomass production, metal tolerance, and high concentrations of heavy metals in aboveground biomass (Dickinson et al. 1994, Greger and Landberg 1995). Willows have the capacity of efficient uptake of macro- and micro- nutrients and high evapotranspiration, which is reflected in their high productivity (Perttu and Kowalik 1997). Under environmental conditions of the Czech Republic *Salix* spp. can reach annual yield of dry biomass about 10 t/ha (Weger and Havlickova 2002). Willow plants have different capabilities to accumulate different heavy metals among the clones. The mechanisms behind the accumulation, transport, and tolerance are specific of each of the different metals (Greger and Landberg 1999). *Salix* spp. was shown as shoot accumulators of Cd and Zn in comparison with other wetland species growing on old submerged mine tailings that restricted translocation of elements to shoots (Stoltz and Greger 2002). Landberg and Greger (1994) reported that the accumulation or exclusion of heavy metals by willows did not depend on their tolerance. However, Landberg and Greger (1996) found that clones from polluted areas had higher accumulations of Cd, Cu, and Zn in their roots, and the transport of those metals to shoots was more restricted than in clones from an unpolluted area. The species that accumulate and tolerate large concentrations of metals in aboveground harvestable tissues would clearly be appropriate to reclaim contaminated soils (Punshon et al. 1995). Willows acclimation to toxic metals can be achieved by gradually increasing concentrations of heavy metals over 128 days rather than by short-term single pre-treatment. Increased resistance (especially to Cd) is attributable to reduced uptake (Punshon and Dickinson 1997). The interaction between Cd and Zn is either antagonistic or synergistic. Cd competes with Zn in forming protein complexes, a negative association between the two was found. However, soil Zn induces dissociation of Cd sorbed onto the binding sites as a result of competition for these sites and increases Cd in solution. Such synergism was found also for Pb and Zn (Adriano 2001).

MATERIAL AND METHODS

Bioavailability of As, Cd, Pb and Zn and accumulation of these elements by *Salix* spp. were investigated in a pot experiment. Unpolluted Chernozem (Suchdol locality) was used as a control soil. Amounts of 100 mg As/kg, 40 mg Cd/kg and either 2000 mg Pb/kg (Suchdol-Pb) or 2000 mg Zn/kg (Suchdol-Zn) were added into this soil. As, Cd, Pb and Zn were supplied to the soil as Na_2HAsO_4, $CdCl_2$, $Pb(CH_3COO)_2$ and $Zn(CH_3COO)_2$, respectively. Soils with substances were thoroughly mixed, and immediately used in the experiment. Mean total contents of As, Cd, Pb and Zn and plant available portions of these

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Table 1. Total contents (mg/kg) and plant-available portions (%) of As, Cd, Pb and Zn in experimental soils

Soil	As		Cd		Pb		Zn	
	total	plant-available	total	plant-available	total	plant-available	total	plant-available
Suchdol-Pb	118	0.28	40.4	0.82	2029	0.05	87.1	< 0.023
Suchdol-Zn	118	1.64	40.4	1.04	29.3	< 0.031	2087	0.39
Suchdol	18.0	0.48	0.42	0.72	29.3	< 0.031	87.1	< 0.023

elements in experimental soils are summarised in Table 1. Seven clones of high biomass production willows from Silva Tarouca Research Centre for Landscape and Decorative Horticulture 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 in the experiment. The willow collection is well documented and of known provenance. Willows were planted in 5-litre plastic pots with 5 kg of air-dried soil for five and a half months. Soil moisture was regularly checked and kept at 60% of MWHC. Four replications were used for each clone (three 20cm cuttings in one pot). After harvest, the aboveground biomass was separated into leaves and twigs, checked for fresh and dry biomass, ground and analysed. The plant material was decomposed by a modified dry ashing procedure in the mixture of oxidising gases ($O_2 + O_3 + NO_x$) in APION Dry Mode Mineralizer (Miholová et al. 1993). Total soil element concentration was determined after two-step decomposition, using APION in the first step and wet digestion by a mixture of $HF + HNO_3$ in the second step (Mader et al. 1998). The soil samples were taken in the middle of the vegetation period and extracted with 0.01 mol/l $CaCl_2$ (Novozamsky et al. 1993). Element concentrations in the digests were determined by atomic absorption spectrometry (VARIAN SpectrAA-400, Varian, Australia) in flame (Cd, Pb and Zn), flameless (Pb), and hydride generation (As) measurement modes, respectively. 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 (Fisher's *LSD* procedure method) and linear regression model were used for statistical evaluation of the data using Statgraphics Plus 5.0.

Table 2. Contents of As, Cd, Pb and Zn (mg/kg dry weight) in leaves and twigs in seven clones of *Salix* spp. (*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)

Soil	Clone	As		Cd		Pb		Zn	
		leaves	twigs	leaves	twigs	leaves	twigs	leaves	twigs
Suchdol	S-218	1.04^{d}	0.281^{c}	3.21^{de}	2.18^{c}	0.805^{a}	0.797^{b}	141^{c}	193^{c}
	S-150	1.40^{e}	0.253^{bc}	2.60^{cd}	1.46^{abc}	3.48^{c}	1.19^{c}	35.6^{a}	112^{b}
	S-519	0.604^{c}	0.129^{ab}	1.45^{ab}	1.13^{ab}	0.952^{ab}	0.446^{ab}	68.6^{b}	75.1^{a}
	S-464	0.561^{bc}	0.135^{ab}	0.929^{a}	1.70^{bc}	0.871^{a}	0.412^{a}	37.7^{a}	80.8^{a}
	S-141	0.333^{a}	0.159^{abc}	1.94^{bc}	0.913^{a}	0.897^{ab}	0.605^{ab}	138^{c}	80.4^{a}
	S-406	0.361^{ab}	0.116^{a}	2.91^{de}	1.42^{abc}	1.38^{b}	0.542^{ab}	74.1^{b}	112^{b}
	S-391	0.376^{ab}	0.218^{abc}	3.66^{e}	1.11^{ab}	0.469^{a}	0.784^{b}	144^{c}	80.5^{a}
Suchdol-Pb	S-218	8.78^{b}	2.21^{bc}	76.8^{b}	41.9^{b}	8.09^{a}	16.6^{bc}	58.1^{a}	47.9^{ab}
	S-150	6.72^{b}	1.16^{ab}	53.4^{ab}	23.8^{a}	23.2^{b}	11.5^{ab}	62.1^{a}	34.4^{a}
	S-519	2.49^{a}	1.01^{a}	35.1^{a}	21.3^{a}	8.44^{a}	9.63^{a}	66.1^{a}	34.2^{a}
	S-464	3.22^{a}	2.72^{c}	36.7^{a}	19.0^{a}	6.86^{a}	12.5^{ab}	43.6^{a}	34.9^{a}
	S-141	2.37^{a}	0.994^{a}	52.9^{ab}	24.0^{a}	26.8^{b}	11.8^{ab}	153^{b}	44.8^{ab}
	S-406	3.56^{a}	0.766^{a}	75.2^{b}	48.8^{b}	20.1^{b}	20.5^{c}	67.0^{a}	69.2^{b}
	S-391	3.26^{a}	1.22^{ab}	70.8^{b}	41.1^{b}	23.5^{b}	18.6^{c}	121^{b}	60.5^{b}
Suchdol-Zn	S-218	5.96^{b}	4.08^{a}	84.1^{a}	34.8^{ab}	1.68^{a}	0.276^{a}	2105^{ab}	592^{ab}
	S-150	7.84^{c}	2.89^{c}	81.8^{a}	42.7^{b}	2.30^{ab}	1.04^{ab}	1552^{a}	452^{a}
	S-519	4.00^{a}	1.75^{ab}	75.8^{a}	32.7^{ab}	1.90^{a}	0.573^{ab}	3631^{c}	517^{ab}
	S-464	4.62^{ab}	3.21^{c}	55.1^{a}	21.7^{a}	2.72^{ab}	0.686^{ab}	1581^{a}	507^{ab}
	S-141	3.67^{a}	1.24^{a}	63.1^{a}	38.6^{b}	4.47^{b}	2.85^{bc}	2221^{b}	761^{bc}
	S-406	3.34^{a}	2.09^{b}	89.7^{a}	38.8^{b}	3.68^{ab}	4.55^{c}	3664^{c}	1033^{c}
	S-391	3.52^{a}	1.64^{ab}	134^{b}	66.0^{c}	8.10^{c}	2.67^{bc}	2639^{b}	983^{c}

Clones with identical alphabetic signs are not significantly different at the 95% confidence level, $n = 4$

RESULTS AND DISCUSSION

A general trend of significantly increasing concentrations of heavy metals in aboveground biomass was found in all tested willow clones as a result of addition of metals to the soils in comparison with unpolluted soil. Comparison of individual elements indicated much higher accumulation of Cd and Zn in aboveground biomass than that of As and Pb and it is in accordance with findings of Stoltz and Greger (2002). The clonal variations in accumulation of heavy metals in leaves and twigs are demonstrated in Table 2. It is obvious that each clone has a different ability to accumulate higher amounts of specific heavy metals. Significant differences were found in the accumulation of different metals between willow clones and also between different element additions to the soil. These results confirm observations of Riddell-Black (1994) and Greger and Landberg (1999). Arsenic was predominantly accumulated by leaves of clones S-218 and S-150 (*Salix* × *smithiana*) in all investigated soils. Cadmium was mostly accumulated by leaves of clone S-391 (*S.* × *rubens*) in all investigated soils. On the other hand, the lowest content of Cd was found in leaves of clone S-464 (*S. alba*). The lead concentration in plant biomass was lower compared to Cd and Zn, and translocation of this element from twigs to leaves was limited in both cases, with and without addition of Pb to the soil. *Salix* spp. showed high ability to accumulate zinc in Suchdol-Zn soil. The higher content of Zn was found in leaves of clones S-519 and S-406 (*S. viminalis* and *S. dasyclados*). Larger translocations of Cd and Zn from twigs to leaves were found in soils when Cd and Zn were added. Nissen and Lepp (1997) found evident Zn accumulation in eight willow species and general trend of exclusion of Cu. Zn contents decreased in the order leaves > bark > twigs > wood.

It is obvious from the results in Figure 1 that Zn addition caused an increase in As content in leaves of five out of the seven clones (not statistically significant due to high variations of replications) whereas in twigs As content was higher in all seven clones (statistically significant in four clones) compared to the soil with Pb addition. On the other hand, Zn addition caused a higher accumulation of Cd in leaves of all clones (statistically significant in two clones) whereas higher Cd content in twigs was found only in five clones (statistically significant in three clones) in comparison with Cd content in clones grown in the soil with Pb addition. An exposure

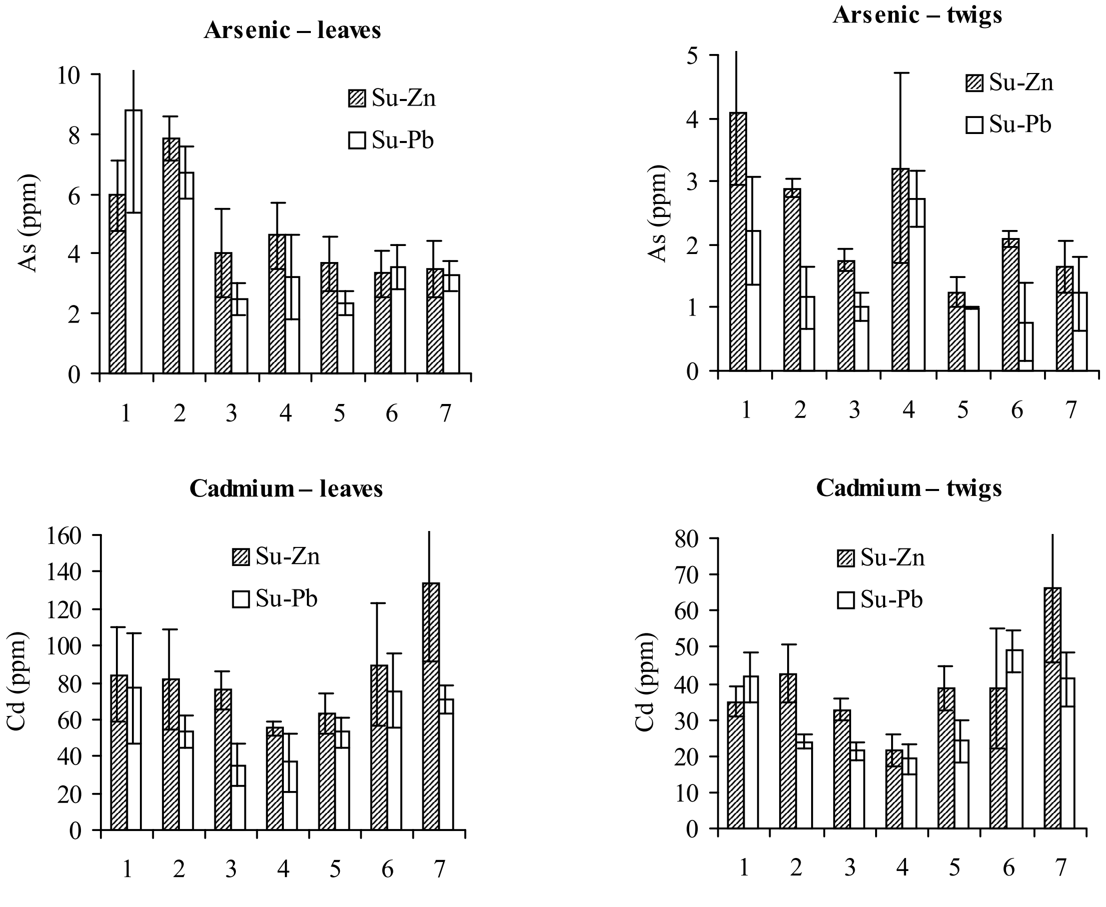

Figure. 1 As and Cd (mg/kg dry weight) contents in leaves and twigs of seven willow clones planted in the soil supplied with Zn (Su-Zn) and Pb (Su-Pb)

x axis = clone No.: 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

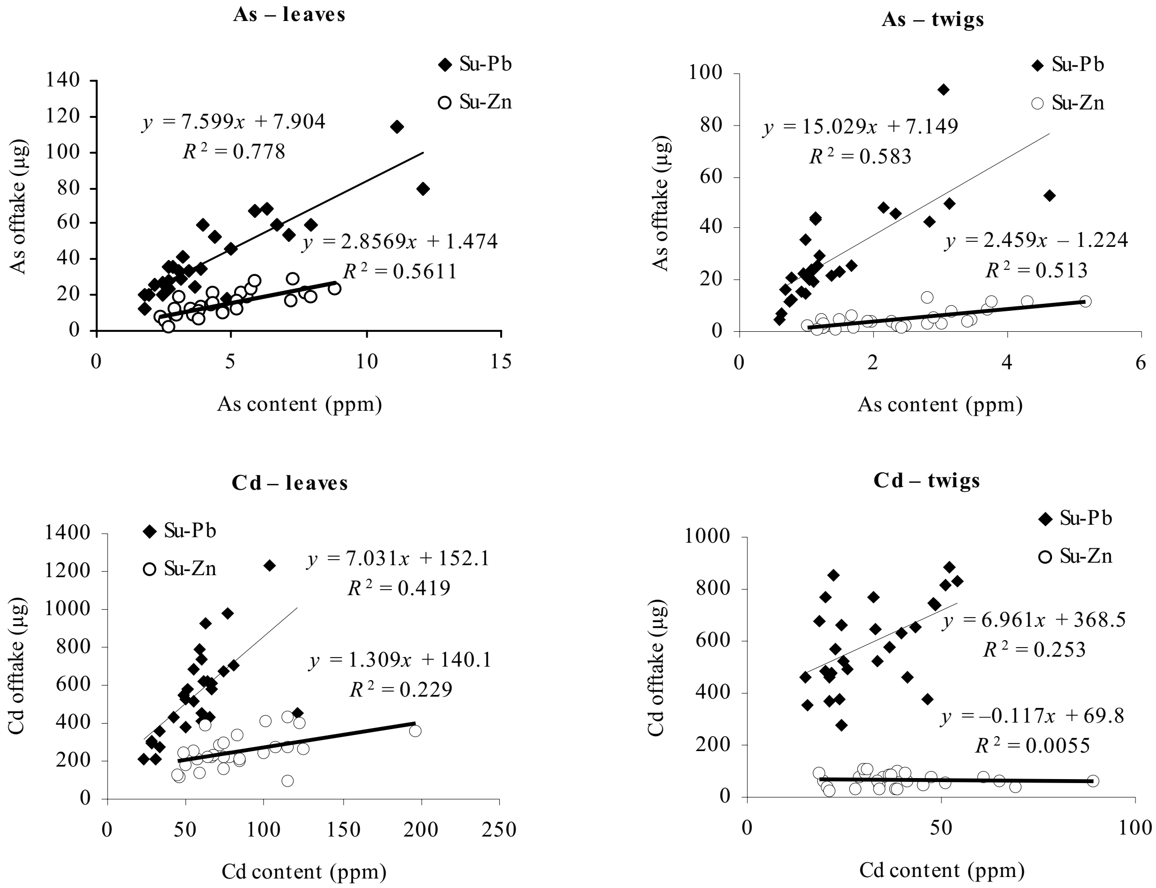

Figure 2. Relationship between As and Cd contents in leaves or twigs (mg/kg dry weight) and As and Cd removal (offtake, µg) by harvested biomass from pots after one vegetation period

to dual combinations of metals in the solution culture increased Cd and Zn content in willow roots when supplied with Cu, but not when it was supplied with Zn. Root-bound Cu increased in all combinations (Punshon and Dickinson 1997). However, these element interactions are necessary to compare biomass yields. In Suchdol-Zn soil total high Zn content and its considerable plant available portion had phytotoxic effects and caused a significant reduction in the yield of aboveground biomass, chlorosis, and partial foliage abscission that could have an effect on the uptake of elements by plants. Pb addition caused a much lower yield reduction than Zn addition. Yields of leaf and twig biomass in control soil and in soils with addition of elements are shown in Table 3. Günthardt-Goerg and Vollenweider (2002) found that the heavy metal stress caused extended necrosis of lower epidermis. Zn appeared to be responsible for visible symptoms on poplar leaves.

The yield reduction feasibly decreased the plant-available portion of elements taken up by biomass in the soil. Higher plant-available portions of As and Cd were found in Suchdol-Zn soil in the middle of vegetation period (Table 1). Eriksson and Ledin (1999) reported that *Salix* cultivation reduced the amount of plant-available Cd in the soil whereas total contents were influenced only slightly. An amount of elements removal from the con-

Table 3. Yield of total (leaves + twigs) dry biomass (g) of seven willow clones (*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)

Soil	S-218	S-150	S-519	S-464	S-141	S-406	S-391
Suchdol-Pb	21.8^b	28.1^b	46.0^b	29.5^b	34.7^b	28.0^b	24.8^b
Suchdol-Zn	5.8^a	4.1^a	6.4^a	6.3^a	5.8^a	3.8^a	3.3^a
Suchdol	49.4^c	42.3^c	57.5^c	61.8^c	36.0^b	32.4^b	39.8^c

Clones with identical alphabetic signs are not significantly different at the 95% confidence level, $n = 4$

taminated soil is most important for phytoextraction purposes (Wenzel et al. 1999). Based on the results in Figure 2, total removal of elements depends not only on the heavy metal concentration in plants but also on total biomass production. Although Cd content in leaves was slightly higher in Suchdol-Zn soil, total removal of Cd by leaves was much higher in Suchdol-Pb soil, where the yield of biomass was not influenced by Zn addition. Analogous results were obtained for As content in leaves and twigs. Removal of As and Cd by leaves or twigs were found significantly higher in Suchdol-Pb soil than in Suchdol-Zn soil. Although Cd content in twigs of willows planted in Suchdol-Zn soil markedly ranged from 18.5 to 89.4 mg/kg, Cd removal by biomass was influenced only slightly. Higher variations in Cd removal by twig biomass were found in the soil supplied with Pb (from 274 to 886 μg Cd) whereas Cd contents were found to be in the range from 15.0 to 52.1 mg/kg. Moreover, it is evident from highly differing slopes of linear correlation (Figure 2) that the element removal is more dependent on element content in biomass planted in Suchdol-Pb soil, where willow plants grew without any physiological symptoms of phytotoxicity, than in the soil supplied with Zn. The relationship between As and Cd contents and their removal by leaves showed a higher correlation coefficient than the correlation of both elements in willow twigs. Arsenic always showed closer relations in comparison with cadmium, especially with Cd in twigs, where Cd content did not correlate with its removal in both soils (Suchdol-Zn and Suchdol-Pb).

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REFERENCES

Adriano C.D. (2001): Trace elements in terrestrial environments. Springer, New York, USA.

Baker A.J.M., Brooks R.R. (1989): Terrestrial plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery, *1*: 81–126.

Baker A.J.M., Mc Grath S.P., Sidoli C.M.D., Reeves R.D. (1994): The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Res. Conserv. Recyc., *11*: 41–49.

Dickinson N.M., Punshon T., Hodkinson R.B., Lepp N.W. (1994): Metal tolerance and accumulation in willows. Willow vegetation filters for municipal waste waters and sludges. A biological purification system. Proc. Conf. Sweden, Sect. Short Rot. Forest., Uppsala: 121–127.

Eriksson J., Ledin S. (1999): Changes in phytoavailability and concentration of cadmium in soil following long term *Salix* cropping. Wat. Air Soil Pollut., *114*: 171–184.

Greger M., Landberg T. (1995): Use of willow clones with high Cd accumulating properties in phytoremediation of agricultural soils with elevated Cd levels. Proc. 3rd Int. Conf. Biogeochemistry of trace elements. Contaminated soils, Paris: 505–511.

Greger M., Landberg T. (1999): Use of willow in phytoextraction. Int. J. Phytorem., *1*: 115–123.

Günthardt-Goerg M.S., Vollenweider P. (2002): Cellular injury, heavy metal uptake and growth of poplar, willow and spruce influenced by heavy metals and soil acidity. COST Action 837, 4th WG2 Worksh. Risk assessment and sustainable land management using plants in trace element-contaminated soils: 103–104.

Landberg T., Greger M. (1994): Can heavy metal tolerant clones of *Salix* be used as vegetation filters on heavy metal contaminated land? Willow vegetation filters for municipal waste waters and sludges. A biological purification system. Proc. Conf. Sweden, Sect. Short Rot. Forest., Uppsala: 133–144.

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.

Mader P., Száková J., Miholová D. (1998): Classical dry ashing of biological and agricultural materials. Part II. Losses of analytes due to their retention in an insoluble residue. Analysis, *26*: 121–129.

Miholová D., Mader P., Száková J., Slámová A., Svatoš Z. (1993): Czechoslovak biological certified reference materials and their use in the analytical quality assurance system in a trace element laboratory. Fresenius J. Anal. Chem., *51*: 256–260.

Nissen L.R., Lepp N.W. (1997): Baseline concentrations of copper and zinc in shoot tissues of a range of *Salix* species. Biomass Bioenerg., *12*: 115–120.

Novozamsky J., Lexmond T.M., Houba V.J.H. (1993): A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int. J. Environ. Anal. Chem., *51*: 47–58.

Perttu K.L., Kowalik P.J. (1997): *Salix* vegetation filters for purification of waters and soils. Biomass Bioenerg., *12*: 9–19.

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.

Riddell-Black D. (1994): Heavy metal uptake by fast growing willow species. Willow vegetation filters for municipal waste waters and sludges. A biological purification system. Proc. Conf. Sweden, Sect. Short Rot. Forest., Uppsala: 145–151.

Salt D.E., Smith R.D., Raskin I. (1998): Phytoremediation. Ann. Rev. Plant. Physiol. Plant. Mol. Biol., *49*: 643–668.

Stoltz E., Greger M. (2002): Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ. Exp. Bot., *47*: 271–280.

Weger J., Havlickova K. (2002): The first results of the selection of woody species for short rotation coppices in the transitional oceanic-continental climate of the Czech Republic. Proc. 12th Eur. Conf. Technol. Exhib. Biomass

for energy, industry and climate protection, Amsterdam: 107–110.
Wenzel W.W., Adriano D.C., Salt D., Smith R. (1999): Phytoremediation: A plant-microbe-based remediation system. ASA, CSSA, SSSA, Madison, WI, USA. Bioremediation of contaminated soils, Agron. Monogr. 37: 457–508.

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ABSTRAKT

Příjem As, Cd, Pb a Zn vrbami (*Salix* spp.) rostoucími na půdě s vysokým přídavkem těchto prvků

V nádobovém pokusu byly sledovány akumulace As, Cd, Pb a Zn v nadzemní biomase sedmi klonů *Salix* spp. a změny v příjmu těchto prvků rostlinami po jejich přídavku do půdy. Jako kontrolní půda byla použita černozem z lokality Suchdol, do níž bylo dodáno 100 mg As/kg, 40 mg Cd/kg a buď 2000 mg Pb/kg (Suchdol-Pb), nebo 2000 mg Zn/kg (Suchdol-Zn). Byly nalezeny výrazné rozdíly v akumulaci prvků mezi jednotlivými klony a také mezi různými přídavky prvků do půdy. Ačkoliv byl příjem As a Cd mírně zvýšen na půdě Suchdol-Zn v porovnání s půdou Suchdol-Pb, odběr prvků z půdy byl výrazně vyšší na půdě Suchdol-Pb kvůli značné výnosové depresi nadzemní biomasy na půdě Suchdol-Zn způsobené fytotoxicitou Zn. Redukce výnosu snížila odběr rostlinám přístupných forem prvků biomasou, takže na půdě Suchdol-Zn byly nalezeny vyšší podíly rostlinám přístupného As a Cd. Na půdě Suchdol-Pb byl odběr prvků z půdy více závislý na obsahu prvku v rostlinných tkáních než na půdě Suchdol-Zn, kde rostliny vrb vykazovaly fyziologické poruchy.

Klíčová slova: půda; arzen; kadmium; olovo; zinek; *Salix* spp.; odběr; fytotoxicita

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