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Heavy metal accumulation by willow clones in short-time hydroponics

J. Malá, H. Cvrčková, P. Máchová, J. Dostál, P. Šíma

Forestry and Game Management Research Institute, Strnady, Czech Republic

ABSTRACT: The differences in Pb and Cd accumulation capabilities of *Salix miyabeana*, *S. viminalis*, *S.* × *blanda*, and *S. elbursensis* derived from willows planted in 1997 in coal-ash settling localities in the Krušné hory Mts.,the Czech Republic, were determined. Plantlets micropropagated by organogenesis were grown in sterile hydroponic media supplemented with 0.5mM $Pb(NO_3)_2$ or 0.5mM $Cd(NO_3)_2 \cdot 4H_2O$. The samples of roots and aboveground parts were collected after 48, 72, 96, and 168 h of cultivation. Generally, substantially higher concentrations of accumulated Pb and Cd were identified in roots than in aboveground parts of all willow clones, even if clonal differences in their accumulation were detected. The results sufficiently confirmed the clonal differences in the uptake and translocation of heavy metals in the above-mentioned willows.

Keywords: heavy metals; micropropagation; phytoremediation; willow clones

Heavy metal accumulation in plants was described for the first time in 1865 in *Thlaspi caerulescens* growing on Zn contaminated soils (Sachs 1865). However, intensive research on the plant capability to accumulate metals started in the mid-20th century, when extraordinary levels of Ni were documented in *Alyssum bertolonii* growing on serpentine soils (Minguzzi, Vergnano 1948). Up to the present, there have been described more than 400 plant species possessing a capability to accumulate heavy metals in high concentrations (Baker et al. 2000). Many of these plant hyperaccumulators (Brooks et al. 1977) could be used for remediation of contaminated soils. Phytoremediation represents a lower cost method and offers great promises of commercial development (Chaney et al. 1997; Lombi et al. 2001; Schmidt 2003). Nowadays, it constitutes a major component in the environmental policy of industrial countries (McGrath 1998; Salt et al. 1998). The use of fast-growing tree species (McGrath et al. 2001; Arthur et al. 2005) is one of the most effective investigated remediation strategies. Of woody plants, research is preferentially focused on fast-growing phreatophytic birches, poplars, Scots pines and willows, which also achieve high biomass production (Soudek et al. 2004; Tlustoš et al. 2007; Baltrenaite, Vaitkute 2008). Considerable effort was exerted to study the metal uptake capacities of willows (Landberg, Greger 1996; Riddell-Black et al. 1997). Short-rotation coppice such as willow species is a perennial crop with the growth period up to approximately 25 years (Klang-Westin, Eriksson 2003) that can be advantageously explored for heavy metal uptake in contaminated coal-ash settling basins and similarly polluted soils (Riddell-Black 1994; Maxted et al. 2007). It can be used, moreover, as a CO_2^- neutral energy crop (Hammer et al. 2003; Demirbas 2005), which corresponds to emission targets set in 1997 by the Kyoto Protocol. Unfortunately, substantial variations in heavy metal uptake, accumulation and metal toxicity resistance were described among different willow species or their clones (Landberg, Greger 2002).

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The aim of this study was to compare Pb and Cd accumulation capacities of willow clones growing in field trials established in 1997 in a coal-ash settling locality in the Krušné hory Mts., the Czech Republic, and that are characterized by good and retarded growth in these conditions. To limit the genotype variations, the plantlets were micropropagated by organogenesis (Malá et al. 2006, 2007). To avoid the edaphon interference (Fernández et al. 2008), the micropropagated plantlets were cultured in semisterile short-time hydroponics.

MATERIALS AND METHODS

Selection of willow clones

Among the willows growing since 1997 in a coal-ash settling locality with equal ambient conditions (subsoil and soil water balance) situated in the Krušné hory Mts., (the Czech Republic) clones S 114 (*S. miyabeana*), S 522 (*S.* × *blanda*) and S 527 (*S. elbursensis*) with good growth characteristics (fulfilling mean criteria of increment size, biomass yield and mortality) and clone S 337 (*S. viminalis*) showing symptoms of growth retardation in these conditions were selected.

Hydroponics

Six micropropagated plantlets of each clone were cultured in 10 times diluted Murashige-Skoog solution (MS) (Murashige, Skoog 1962) supplemented with 0.5mM $Pb(NO_3)_2$ or with 0.5mM $Cd(NO_3)_2 \cdot 4H_2O$ (Kahle 1993; Kališová-Špirochová et al. 2003). The same numbers of control plants were cultured in 10 times diluted MS solution without metal salts. The pH value was adjusted to 5.6 by 1 N KOH in all solutions (all substances Sigma-Aldrich Co.). After 48, 72, 96, and 168 h of cultivation at 24°C under white fluorescent light (30 $\mu mol.m^{-2}.s^{-1}$) and 16 h photoperiod, the samples of root and aboveground parts were collected for analyses.

Determination of heavy metals

The samples were washed in deionized water to prevent the superficial adhesion of metal salts and dried at 80°C for 24 h and then they were ground to powder and 0.5 g samples were digested in 12 ml of a mixture of concentrated HNO_3 and concentrated H_2O_2 (5:1. v. v) (both Lach-Ner Ltd., Czech Republic) in digestion glass tubes for 20 min. Next, the tubes were closed and digestion was completed in two phases in MDS 2000 microwave equipment (CEM, USA). Digestion protocol: **Phase I** – 5 min, 70% of microwave power, pressure 275.8 kPa; 10 min, 70% of microwave power, 827.4 kPa; 10 min, 0% of microwave power, 0 kPa; **Phase II** – 5 min, 100% of microwave power, 275.8 kPa; 5 min, 100% of microwave power, 551.6 kPa; 10 min, 100% of microwave power, 827.4 kPa; 15 min, 100% of microwave power, 1,172.2 kPa; 10 min, 0% of microwave power, 0 kPa. After cooling, deionized water was used to adjust the final volume to 50 ml. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) (Varian, Australia) was used for determination of metal ion concentrations.

Table 1. Differences between willow clones in Pb content ($mg.kg^{-1}$ dry matter) in aboveground parts and roots of plantlets grown in 0.5mM of $Pb(NO_3)_2$ solution. No Pb presence was detected in the control. Different letters (a, b, c) indicate significant difference (factorial ANOVA, post-hoc Tukey's HSD test $P < 0.05$, $n = 6$)

Clone	48 h	72 h	96 h	168 h
Pb – root				
114	51,833$^{b, c}$	62,433^{b}	69,967$^{b, c}$	70,767^{b}
522	48,933^{b}	65,933$^{b, c}$	72,433^{c}	71,833^{b}
527	60,933^{c}	71,500^{c}	63,067^{b}	65,133^{b}
337	36,133^{a}	49,633^{a}	53,933^{a}	53,300^{a}
Pb – stem				
114	1,483^{b}	2,233^{b}	6,433^{b}	7,067^{c}
522	1,117$^{a, b}$	2,500^{b}	4,817^{b}	6,850^{c}
527	1,317$^{a, b}$	3,200^{c}	1,867^{a}	2,027^{b}
337	883^{a}	1,300^{a}	1,467^{a}	1,267^{a}

Statistic evaluation

The data were subjected to ANOVA test and Tukey's test (UNISTAT 5.6. program).

RESULTS

Accumulation of Pb

There were no differences in accumulation dynamics of Pb between clones 114 and 522 from the beginning to the end of the experiment. Mean Pb amounts in roots of clone 114 increased 1.4 times and in clone 522 they increased 1.5 times (Table 1). In both clones, the Pb amounts in aboveground parts were 10 times lower at the end of the experiment but the augmentation rate coefficients were higher: 4.7 times in clone 114 and 6.1 times in clone 522 (Table 1). In clone 527, mean Pb accumulation dynamics was very rapid. In roots and aboveground parts, a high amount of Pb was reached within 72 hours and then a significant decline of Pb uptake was documented up to the end of the experiment (Table 1). In aboveground parts, the Pb amounts were on average 33 times lower than in the roots.

The Pb accumulation dynamics of clone 337 was rather different from both previous clones: mean Pb amounts increased 1.4 times both in roots and aboveground parts only to 72 h but no further significant increase was observed. Mean Pb amounts in roots, however, were 39 times higher than in aboveground parts (Table 1). The aboveground parts rate coefficient was 4.8 lower than in the aboveground parts of clone 114 and 522.

Accumulation of Cd

No substantial differences in accumulation dynamics and amounts of accumulated Cd in roots among clones 114, 522 and 527 were observed. The accumulation increased linearly approximately 1.5 times from the beginning to the end of the experiment (Table 2). In aboveground parts, some differences were observed: the highest Cd accumulation after 48 h was observed in clone 522, the lowest (approximately a half) in clone 114. At the end of the experiment (168 h), the amounts of Cd were comparable in all clones (Table 2).

The dynamics of Cd accumulation was different in clone 337 in comparison with the other clones. Concentrations of accumulated Cd in roots were highest in 72 h and then they decreased until the end of the experiment. In aboveground parts, the Cd amounts remained without substantial changes from the beginning to the end of the experiment and they were on average 26 times lower than in roots.

DISCUSSION

Fast-growing willow species represent popular woody plants for the remediation of contaminated soils (Greger, Landberg 1999; Lombi et al. 2001; Stoltz, Greger 2002). Willow clones having a high ability to accumulate heavy metals without suffering any growth constraints are in the focus of research interests (Raskin et al. 1997; Lasat 2002). The willow plantlets used in these experiments originated from *S. miyabeana* S 114, *S.* × *blanda* S 522 and *S. elbursensis* S 527 clones characterized by opti-

Table 2. Differences between willow clones in Cd content (mg.kg^{-1} dry matter) in above ground parts and roots of plantlets grown in 0.5mM of Cd $(NO_3)_2 \cdot 4H_2O$ solution. No Cd presence was detected in the control. Different letters (a, b, c) indicate significant difference (factorial ANOVA, post-hoc Tukey's HSD test $P < 0.05$, $n = 6$)

Clone	48 h	72 h	96 h	168 h
Cd – root				
114	16,500^{a}	20,200^{c}	24,450^{c}	25,600^{c}
522	16,767^{a}	16,733^{b}	18,867^{b}	21,833^{b}
527	17,367^{a}	17,400^{b}	24,733^{c}	24,850^{c}
337	8,033^{b}	11,167^{a}	9,967^{a}	9,900^{a}
Cd – stem				
114	717^{b}	1,183^{b}	2,410^{c}	2,497^{c}
522	1,520^{c}	1,633^{c}	1,850^{b}	2,000^{b}
527	1,380^{c}	887^{b}	2,733^{c}	2,750^{c}
337	360^{a}	317^{a}	420^{a}	430^{a}

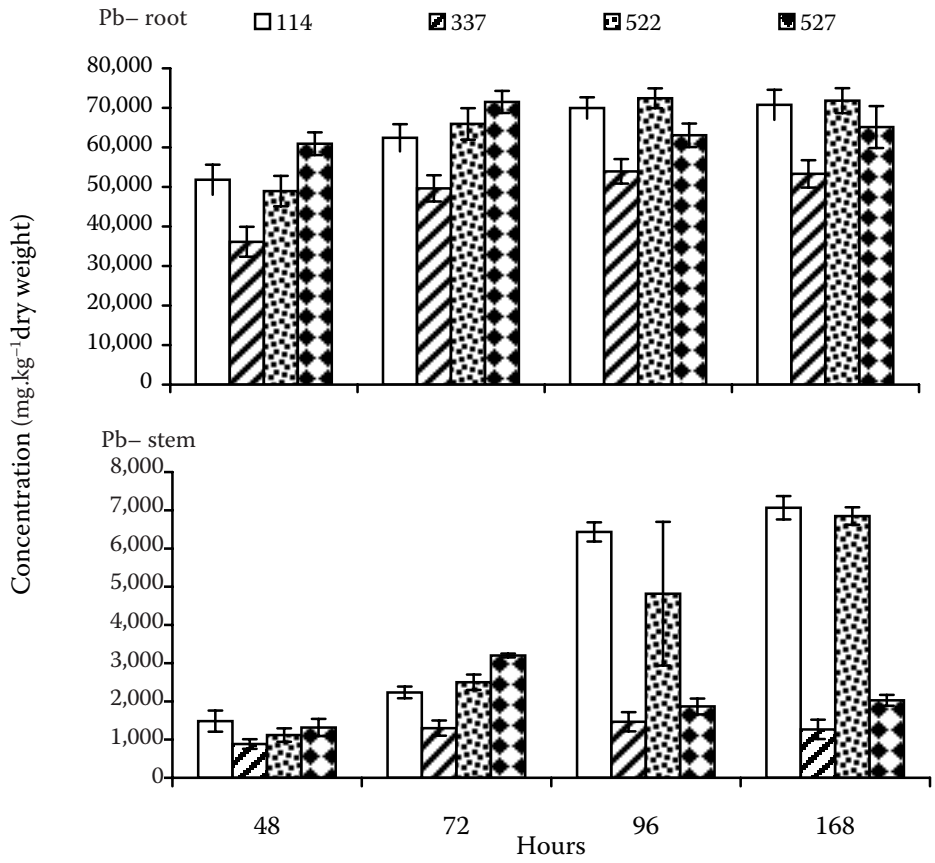

Fig. 1. Means values (standard error in parentheses) of Pb ($mg.kg^{-1}$ dry matter) in aboveground parts and roots of willow clones grown in 0.5mM of $Pb(NO_3)_2$ solution (n = 6). No Pb presence was detected in the control

mal growth, and from *S. viminalis* S 337 clone with growth retardation in the experimental plot. All clones were micropropagated by organogenesis *in vitro* in order to limit the genotype variations (Malá et al. 2006, 2007). Recently, it was documented that the mycorrhization could influence the accumulation of heavy metals (Fernández et al. 2008). To exclude the influence of edaphon, the micropropagated willow clones were cultured in semisterile short-time hydroponics (Marschner et al. 1996; Whiting et al. 2001). Concentrations of heavy metals and also the pH of the solutions were established according to Kahle (1993) and Kališová-Špirochová et al. (2003), who studied the accumulation of heavy metals by willows and other woody species grown in hydroponics. Though both Pb and Cd are toxic to the plant growth and only a small number of plant species were reported to accumulate these metals without signs of toxicity (Ma et al. 2005), neither changes in morphology and colour nor growth inhibition were observed in willow plantlets grown in our experimental design. Generally, all clones cultured in short-time hydroponics were able to accumulate Pb and Cd into roots and aboveground parts (Figs. 1 and 2) but remarkable clonal variations in the uptake and translocation of both heavy metals were observed. During the experiment, Pb and Cd accumulated preferentially in the roots in all clones. Practically, no differences in the accumulation of Pb in optimally growing clones were found but its amount in aboveground parts were substantially lower (Table 1). In the clone showing retarded growth the Pb accumulation dynamics was rather different. Pb amounts increased both in roots and aboveground parts only up to 72 h (Table 1). Similarly like in Pb, no differences in accumulation dynamics and amounts of accumulated Cd in roots in optimally growing the clones were observed (Table 2) but some variations of Cd amount in aboveground parts were recorded (see Results, Table 2). In the clone with retarded growth, like in the Pb accumulation, amounts of Cd in roots were the highest in 72 h but in aboveground parts no substantial changes were recorded during the experiment. All mean values of Pb and Cd concentrations in plant tissues determined in these experiments respond to observations of heavy metal accumulation by willow species grown in contaminated soils (Tlustoš at al. 2007). Similar lesser tolerance to heavy metals (lower capacity for their accumulation) in the clone with retarded growth was in a clone of *S. alba* grown in hydroponics for 14 days (Vassilev et al. 2005). Different distribution dynamics for Cd and Pb agrees also with results described in *S. fragilis* and *S. viminalis* grown in a greenhouse

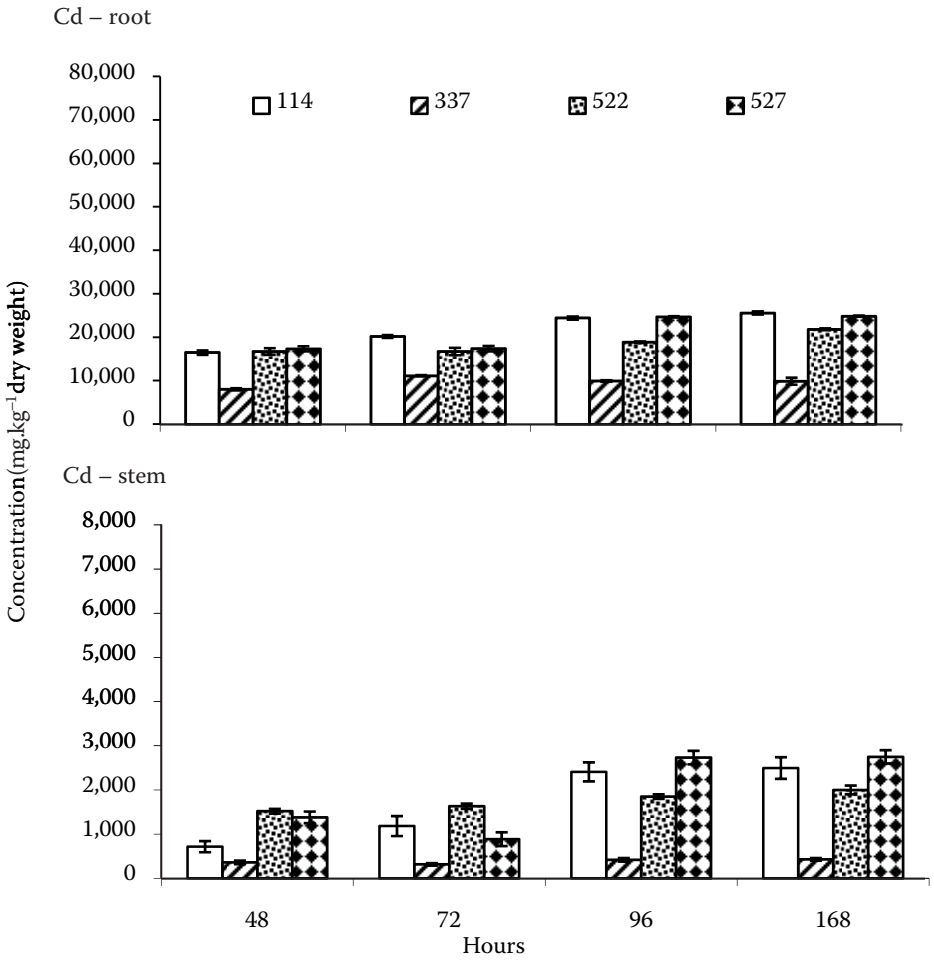

Fig 2. Means values (standard error in parentheses) of Cd ($mg.kg^{-1}$ dry matter) in aboveground parts and roots of willow clones grown in 0.5 mM of $Cd(NO_3)_2.4H_2O$ solution (n = 6). No Cd presence was detected in the control

pot experiment (Vandecasteele et al. 2005). Different translocation dynamics and accumulation capacity of plant tissues for heavy metals could be explained by the insufficient selectivity of various ion transporting channels (Assunçao et al. 2003) or by the different cell wall permeability for ions, which both could influence the effectivity of intracellular storage dynamics (Clemens et al. 2002). The prevailing accumulation of heavy metals in roots of several tested tree species could be explained by the formation of low soluble heavy metal compounds during metal detoxification processes and their storage in cells (Hall 2002; Ma et al. 2005).

Conclusively, the authors are aware that the short-time hydroponics could not reflect all the factors influencing the complexity of heavy metal accumulation dynamics. On the contrary, this method eliminates possible interference of soil chemical reactions and the activity and metabolic products of soil biota. The results obtained in this study sufficiently confirmed the clonal differences in the uptake and translocation of heavy metals in plant tissues. Short-time hydroponics is sufficiently effective and could be preferentially used for rapid and low-cost evaluation of the heavy metal accumulation capacity of various woody tree species especially of fast-growing willows used in heavily contaminated localities.

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Corresponding author:

RNDr. Jana Malá, CSc., Výzkumný ústav lesního hospodářství a myslivosti, v.v.i., Strnady 136, 252 02 Jíloviště, Česká republika
tel./fax: + 420 257 920 681, e-mail: mala@vulhm.cz