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. × bland, and S. elbursensis derived from willows planted in 1997 in coal-ash settling localities in the Krušně hory Mts., the Czech Republic, were determined. Plantlets micropropagated by organogenesis were grown in sterile hydroponic media supplemented with 0.5 mM Pb(NO₃)₂ or 0.5 mM Cd(NO₃)₂·4H₂O. 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

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. OC118.
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$_3$)$_2$.4H$_2$O (Kahle 1993; Kaliszová-Spirochová 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 µmol.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$_2$O$_2$ (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 $P < 0.05, n = 6$)**

<table>
<thead>
<tr>
<th>Clone</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb – root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>51,833$^{b,c}$</td>
<td>62,433$^b$</td>
<td>69,967$^{b,c}$</td>
<td>70,767$^b$</td>
</tr>
<tr>
<td>522</td>
<td>48,933$^b$</td>
<td>65,933$^{b,c}$</td>
<td>72,433$^c$</td>
<td>71,833$^b$</td>
</tr>
<tr>
<td>527</td>
<td>60,933$^c$</td>
<td>71,500$^c$</td>
<td>63,067$^b$</td>
<td>65,133$^b$</td>
</tr>
<tr>
<td>337</td>
<td>36,133$^a$</td>
<td>49,633$^a$</td>
<td>53,933$^a$</td>
<td>53,300$^a$</td>
</tr>
<tr>
<td></td>
<td>Pb – stem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>1,483$^b$</td>
<td>2,233$^b$</td>
<td>6,433$^b$</td>
<td>7,067$^c$</td>
</tr>
<tr>
<td>522</td>
<td>1,117$^{a,b}$</td>
<td>2,500$^b$</td>
<td>4,817$^b$</td>
<td>6,850$^c$</td>
</tr>
<tr>
<td>527</td>
<td>1,317$^{a,b}$</td>
<td>3,200$^a$</td>
<td>1,867$^a$</td>
<td>2,027$^b$</td>
</tr>
<tr>
<td>337</td>
<td>883$^a$</td>
<td>1,300$^a$</td>
<td>1,467$^a$</td>
<td>1,267$^a$</td>
</tr>
</tbody>
</table>
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. × bland* *a* 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 (NO₃)₂·4H₂O 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)**

<table>
<thead>
<tr>
<th>Clone</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cd – root</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>16,500⁺</td>
<td>20,200⁺</td>
<td>24,450⁺</td>
<td>25,600⁺</td>
</tr>
<tr>
<td>522</td>
<td>16,767⁺</td>
<td>16,733⁺</td>
<td>18,867⁺</td>
<td>21,833⁺</td>
</tr>
<tr>
<td>527</td>
<td>17,367⁺</td>
<td>17,400⁺</td>
<td>24,733⁺</td>
<td>24,850⁺</td>
</tr>
<tr>
<td>337</td>
<td>8,033⁺</td>
<td>11,167⁺</td>
<td>9,967⁺</td>
<td>9,900⁺</td>
</tr>
<tr>
<td><strong>Cd – stem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>717⁺</td>
<td>1,183⁺</td>
<td>2,410⁺</td>
<td>2,497⁺</td>
</tr>
<tr>
<td>522</td>
<td>1,520⁺</td>
<td>1,633⁺</td>
<td>1,850⁺</td>
<td>2,000⁺</td>
</tr>
<tr>
<td>527</td>
<td>1,380⁺</td>
<td>887⁺</td>
<td>2,733⁺</td>
<td>2,750⁺</td>
</tr>
<tr>
<td>337</td>
<td>360⁺</td>
<td>317⁺</td>
<td>420⁺</td>
<td>430⁺</td>
</tr>
</tbody>
</table>
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 et 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
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 (Assunção et al. 2003) or by the different cell wall permeability for ions, which both could influence the effectiveness 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.

References


Stoltz E., Gregor M. (2002): Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environmental and Experimental
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

---

Botany, 47: 271–280.

Received for publication June 15, 2009
Accepted after corrections September 30, 2009