

Heavy metals uptake by the hybrid aspen and rowan-tree clones

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ABSTRACT: Micropropagated plantlets derived from selected clones of the hybrid aspen (*Populus tremula* × *Populus tremuloides*) and the rowan-tree (*Sorbus aucuparia* L.) were used to determine the comparative study of uptake of the toxic, heavy metals Cd, Pb and the essential metal Mn. Samples of roots and aboveground parts (hypocotyl-derived tissues, leaves and stems) were taken from the plantlets grown for 24, 48, 96, or 168 hrs under aseptic conditions, in hydroponics with the toxic heavy metal and the essential metal salts. The concentration and distribution of the accumulated metals were determined using the ICP-OES method. The differences in the uptake capacity of hybrid aspen and rowan-tree clones for Cd, Pb and Mn were identified. Generally, the amounts of accumulated Cd and particularly Pb were much higher in the roots of both hybrid aspen and rowan-tree clones, than in their shoots, at all sample times. Conversely, the amounts of accumulated Mn were significantly lower than Cd and Pb in all plant parts of the hybrid aspen and rowan-tree samples. Patterns of Mn uptake were similar in the above-mentioned tissues of both clones, at all sample times. We concluded that the two clones of hybrid aspen and rowan-tree, lacking auxiliary soil microbiota, can accumulate large amounts of the toxic heavy metals Cd (800–1,500 mg/kg) and Pb (5,000–13,000 mg/kg) in roots and about 100 mg/kg of Cd was determined in aboveground part of hybrid aspen.

Keywords: phytoremediation; heavy metals; hybrid aspen; rowan-tree; micropropagation

Restoration of soils contaminated by heavy toxic metals constitutes a major component in the environmental policy of industrial countries (McGRATH 1998; SALT et al. 1998). The use of plants for this purpose represents an environmental-friendly and cost-effective approach (ARTHUR et al. 1995). Recent studies indicate that many plant species have a great potential to accumulate different xenobiotics, organic and inorganic compounds, including toxic heavy metals. This capability can be used for phytoremediation, treatment and stabilization of the soils (LOMBI et al. 2001). Identification of plant species accumulating large amounts of xenobiotics, so called hyperaccumulators, or species preferentially accumulating specific substances, e.g. toxic

heavy metals, is an impetus for the research in the phytoremediation field (BROWN et al. 1994). It could be supposed that, the plants, surviving for a long time in polluted regions, approximate to the above-mentioned attributes. Investigation of accumulation/detoxification capabilities of these species, their propagation by rapid biotechnological techniques and breeding of clones according to their specific selectivity for contaminants could accelerate implementation of the species, highly effective for soil detoxification (BAKER et al. 2000).

Hybrid aspen (*Populus tremula* × *Populus tremuloides*) and rowan-tree (*Sorbus aucuparia*) growing in the Ore Mountains (Krušné hory), represent fast-growing tree species even under extreme climatic

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Fig. 1. Multiapex culture of hybrid aspen growing in the Bank of explants FGMRI



Fig. 2. Multiapex culture of rowan-tree growing in the Bank of explants FGMRI

conditions and in poor soils, severely contaminated by industrial emissions or waste. As such, aspen and rowan-tree are the ideal candidates for phytoremediation (JOACHIM 1991; DIX et al. 1997).

In this work, we compared the capability and possible differences in accumulation of toxic heavy metals Cd, Pb, and essential Mn, in roots and in aboveground tissues of micropropagated plantlets, originating from selected hybrid aspen and rowan-tree plus trees, growing in trial plots in polluted areas of the Ore Mountains, the Czech Republic.

MATERIALS AND METHODS

Origin of hybrid aspen and rowan-tree plant material

In early May, 2004, dormant buds were collected from 24-year old plus trees of hybrid aspen and rowan-trees growing at four provenance plots in the emission areas of the Ore Mountains. The shoot tips from sterile dormant buds were used for induction of organogenesis. Micropropagation of both species was described by MALÁ et al. (2006). The micropropagated clones of hybrid aspen (No. 5) and rowan-tree (No. 26), both stored in the Bank of Explants of the Forestry and Game Management Research Institute (FGMRI), were used for the heavy metal uptake studies (Figs. 1 and 2).

Preparation of plantlets

Micro-cuttings from multi-apex cultures, growing in the explants bank, were rooted in Murashige-

Skoog agar medium (MS) (MURASHIGE, SKOOG 1962) (diluted three times MS amended with β -indolylbutyric acid – IBA 0.6 mg/l). The explants were grown at 24°C, and under white fluorescent light (30 $\mu\text{mol}/\text{m}^2/\text{s}$) and a 16h photoperiod. The rooted plantlets were cultured hydroponically in a 10% salts solution of MS medium. The plantlets (stems about 25 cm long; roots 30 cm long) were used for the heavy metal uptake studies.

Plantlet cultivation in salt solution of heavy metals

The plantlets were grown hydroponically under sterile conditions in 10 times diluted solution of MS salts (inclusive EDTA) amended with $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$, $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ or $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ metal salts, onto concentration 0.1mM or 0.5mM of appropriate heavy metals. The solutions were adjusted to pH 5.6 using 1.0M KOH (all substances Sigma-Aldrich Co.). The control plantlets were cultured in 10 times diluted solution of MS salts.

To determine the heavy metal content, six plantlets per concentration of Cd, Pb, and Mn from each clone and the control ones (i.e., 48 plantlets) were sampled after growing in the various treatments, for 24, 48, 96 or 168 hrs.

Determination of heavy metal content of the plantlets

The dried plant tissues were ground to a powder and ca. 0.5 g samples were digested in a 12 ml of mixture of 10 ml concentrated HNO_3 and 2 ml concen-

trated H₂O₂ (both Lach-Ner Ltd., Czech Republic), in digestion glass tubes, for 20 minutes. Next, the tubes were closed and digestion was completed in two phases by microwave equipment MDS 2000 (CEM, USA). Digestion protocol: I. phase – 5 min, 434 W of microwave power, pressure 275.8 kPa; 10 min, 434 W of microwave power, 827.4 kPa; 10 min, cooling. II. phase – 5 min, 602 W of microwave power, 275.8 kPa; 5 min, 602 W of microwave power, 551.6 kPa; 10 min, 602 W of microwave power, 827.4 kPa; 15 min, 602 W of microwave power, 1,172.2 kPa; 10 min, cooling. After cooling, deionized water was used to adjust the final volume to 50 ml. The concentrations of the metal ions were determined by ICP-OES (Varian, Australia).

Total concentrations of heavy metals in the deionized water washed and dried samples (80°C for 24 hrs) of aboveground parts and roots were determined, using an Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES). Limits of detection (LOD) are for Cd 0.1 mg/kg, for Mn 0.7 mg/kg and for Pb 2 mg/kg.

The data were subjected to using ANOVA UNISTAT v. 5.6. and two tailed test (the means of six plantlets for each sampling time).

RESULTS

Evaluations of plantlets

When sampled, plantlet growth and external morphology of both clones, grown in both concentrations of Cd, Pb and Mn salt solutions, were similar to control plantlets. None of the metals had changed the color of stems and leaves, or in root branching.

Determination of heavy metals in plantlets

Hybrid aspen uptake of Cd

Roots

The amounts of Cd increased linearly, in 0.1mM solution approximately 2.5 times, from 173.9 mg/kg to 436.2 mg/kg of dry matter, and in 0.5mM solution approximately 1.7 times, from 487.9 mg/kg to 862.3 mg/kg of dry matter (Table 1) during the time interval monitored. There were significant differences ($P = 0.05$) between the amounts of Cd in roots for the 0.1 and 0.5mM treatment. No Cd was detected in the roots of control plantlets.

Aboveground parts

Analogous to the roots, the amounts of Cd increased linearly, in 0.1mM solution approximately 23 times from 5.4 mg/kg to 126.4 mg/kg of dry matter, in 0.5mM solution and approximately 19 times from 4.6 mg/kg to 86.2 mg/kg of dry matter (Table 1). There were no significant differences between the amounts of Cd in aboveground parts for the 0.1 and 0.5mM treatments. No Cd was detected in aboveground parts of the control plantlets.

Hybrid aspen uptake of Pb

Roots

The amounts of Pb increased practically linearly, in 0.1mM solution approximately 2.6 times from 1,376.0 mg/kg to 3,568.9 mg/kg of dry matter, and in 0.5mM solution 2.4 times from 5,429.0 mg/kg to 13,051.4 mg/kg of dry matter (Table 2) during the time interval monitored. There were significant differences ($P = 0.05$) between the amounts of Pb in the roots, for the 0.1 and 0.5mM treatments.

Table 1. Mean values (standard errors in parentheses) of Cd (mg/kg dry matter) in aboveground parts and roots of hybrid aspen and rowan-tree grown on 0.1 and 0.5mM of Cd(CH₃COO)₂·3H₂O solutions ($n = 6$). No Cd presence was detected in the controls

	Concentration (mM)	Time (hrs)			
		24	48	96	168
Hybrid aspen					
Aboveground parts	0.1	5.4 ± 0.4	121.7 ± 1.2	50.8 ± 2.4	126.4 ± 5.3
	0.5	4.6 ± 0.5	9.8 ± 0.6	34.1 ± 1.8	86.2 ± 5.6
Roots	0.1	173.9 ± 8.6	211.7 ± 10.4	268.0 ± 8.9	436.2 ± 14.3
	0.5	487.9 ± 10.5	569.1 ± 12.7	735.1 ± 12.4	826.3 ± 10.9
Rowan-tree					
Aboveground parts	0.1	0.41 ± 0.05	0.65 ± 0.06	1.13 ± 0.2	1.8 ± 0.4
	0.5	0.6 ± 0.02	2.4 ± 0.3	2.8 ± 0.3	2.88 ± 0.5
Roots	0.1	83.0 ± 2.6	229.3 ± 10.6	292.0 ± 14.2	449.7 ± 12.9
	0.5	510.6 ± 13.8	1,012 ± 12.6	1,050.0 ± 18.5	1,514.0 ± 21.4

Table 2. Mean values (standard errors in parentheses) of Pb (mg/kg dry matter) in aboveground parts and roots of aspen and rowan-tree grown on 0.1 and 0.5mM of $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ solutions ($n = 6$). No Pb was detected in the control treatment plantlets

	Concentration (mM)	Time (hrs)			
		24	48	96	168
Hybrid aspen					
Aboveground parts	0.1	4.6 ± 0.6	14.6 ± 2.5	23.7 ± 2.9	49.8 ± 4.2
	0.5	10.9 ± 1.7	12.2 ± 2.3	33.65 ± 4.5	56.7 ± 3.8
Roots	0.1	1,376.0 ± 32.1	2,221.1 ± 36.1	2,592.3 ± 24.6	3,568.9 ± 20.0
	0.5	5,429.0 ± 57.8	8,724.5 ± 45.3	9,606.4 ± 32.8	13,051.4 ± 75.4
Rowan-tree					
Aboveground parts	0.1	< 1.5	< 1.5	1.8 ± 0.2	2.2 ± 0.2
	0.5	4.4 ± 0.5	1.7 ± 0.9	2.6 ± 0.7	4.8 ± 0.6
Roots	0.1	249.4 ± 12.7	444.6 ± 11.3	515.1 ± 14.2	625.0 ± 15.1
	0.5	2,577.0 ± 18.9	2,719.2 ± 26.8	3,824.0 ± 28.6	5,728.3 ± 33.5

No Pb was detected in the roots of control plantlets.

Aboveground parts

The amounts of Pb have also increased linearly, in 0.1mM solution approximately 10.8 times from 4.6 mg/kg to 49.8 mg/kg of dry matter, and in 0.5mM solution 5.2 times from 10.9 mg/kg to 56.7 mg/kg of dry matter (Table 2) during the time interval monitored. There were no significant differences between the amounts of Pb in aboveground parts for the 0.1 and 0.5mM treatments. No Pb was detected in the aboveground parts of control plantlets.

Rowan-tree uptake of Cd

Roots

Analogous to Pb, the amounts of Cd increased linearly, in 0.1mM solution approximately 5.4 times from 83.0 mg/kg to 449.7 mg/kg of dry matter, and in 0.5mM solution approximately 3 times from 510.6 mg/kg to 1,514.0 mg/kg of dry matter (Table 1) during the time interval monitored. There were significant differences ($P = 0.05$) between the amounts of Cd in the roots for the 0.1 and 0.5mM treatments. No Cd was detected in the roots of control plantlets.

Aboveground parts

The amounts of Cd have also increased linearly, in 0.1mM solution approximately 4.4 times from 0.41 mg/kg of dry matter at the beginning, to 1.8 mg per kg of dry matter, at the end of the experiment, and 4.8 times in 0.5mM solution, from 0.6 mg/kg to 2.88 mg/kg of dry matter (Table 1) during the time interval monitored. There were no significant differences between the amounts of Cd in the aboveground part,

for the 0.1 and 0.5mM treatments. No Cd was detected in the aboveground parts of control plantlets.

Rowan-tree uptake of Pb

Roots

The amounts of Pb have increased linearly, in 0.1mM solution approximately 2.5 times, from 249.4 mg/kg to 625.0 mg/kg of dry matter, and in 0.5mM solution approximately 2.2 times from 2,577.0 mg/kg to 5,728.3 mg/kg of dry matter (Table 2) during the time interval monitored. There were significant differences ($P = 0.05$) between the amounts of Pb in the roots for the 0.1 and 0.5mM treatments. No Pb presence was detected in the roots of control samples.

Aboveground parts

No Pb was detected in 0.1mM solution during 24 and 48 hrs; the amounts of Pb have reached 1.8 mg/kg and 2.2 mg/kg at 96 hrs and 168 hrs, respectively. The amounts of Pb in 0.5mM have varied approximately between 3.4 mg/kg (Table 2). There were significant differences ($P = 0.05$) between the amounts of Pb in the aboveground part for the 0.1 and 0.5mM treatments. No Pb presence was detected in the aboveground parts of control samples.

Hybrid aspen uptake of Mn

The amounts of Mn in the aboveground parts of hybrid aspen, cultured on the supplied hydroponic solutions, were on average and the data of all samples were comparable to those of the control samples and they remained unchanged during the experiment. The amounts of Mn in the roots of hybrid aspen

Table 3. Mean values (standard errors in parentheses) of Mn (mg/kg dry matter) in aboveground parts and roots of aspen and rowan-tree grown on 0.1 and 0.5mM of $\text{MnSO}_4 \cdot 1 \text{H}_2\text{O}$ solutions ($n = 6$). In control hybrid aspen hydroponic samples Mn was detected at an average concentration of 318.5 ± 30.4 (mg/kg dry matter) in aboveground parts while in roots the concentration was 78.0 ± 28.4 (mg/kg dry matter)

	Concentration (mM)	Time (hrs)			
		24	48	96	168
Hybrid aspen					
Aboveground parts	0.1	302.3 ± 25.4	284.7 ± 32.5	333.4 ± 25.6	338.4 ± 20.9
	0.5	328.0 ± 30.4	317.9 ± 26.8	319.2 ± 31.6	364.9 ± 30.8
Roots	0.1	101.5 ± 30.6	72.5 ± 35.1	140.9 ± 26.4	96.41 ± 30.5
	0.5	132.6 ± 21.5	152.5 ± 29.4	138.5 ± 36.4	177.2 ± 21.7
Rowan-tree					
Aboveground parts	0.1	382.1 ± 16.7	409.2 ± 20.4	553.6 ± 17.9	768.5 ± 23.5
	0.5	438.1 ± 12.4	535.9 ± 19.4	588.2 ± 24.6	613.7 ± 20.4
Roots	0.1	586.0 ± 15.7	476.8 ± 20.6	699.1 ± 28.1	917.6 ± 31.0
	0.5	539.2 ± 19.2	759.2 ± 31.4	761.7 ± 24.6	790.9 ± 34.1

grown on the hydroponic solutions, especially in the 0.5mM solution, increased significantly (almost a two fold increase after 48 hrs) compared to the control plants. The amounts of Mn in the roots were significantly lower than in the aboveground parts of hybrid aspen in all sample times, for both hydroponic solutions and the control samples. The difference was significant, at the 0.05 probability level (Table 3). In the control hybrid aspen hydroponic samples, Mn was detected of an average concentration of 318.5 ± 30.4 (mg/kg) in the aboveground parts, while in the roots the concentration was 78.0 ± 28.4 (mg/kg).

Rowan-tree uptake of Mn

At all sample data the amounts of Mn in rowan-tree aboveground parts of plantlets, grown in the 0.05mM solution of heavy metals, were significantly higher (nearly twice) than in the control plantlets. Similarly, the amounts of Mn in the roots of rowan-tree cultured in both solutions were increased significantly (at 168 hrs the increase was nearly 7 times), compared to the control plantlets. The amounts of Mn in the roots were not significantly different throughout the experiment, for both solutions (Table 3).

Presence of Mn was detected of the concentration of 333.6 ± 36.4 (mg/kg) in the aboveground parts and of the concentration of 144.6 ± 38.4 (mg/kg) in the roots, in control rowan-tree.

DISCUSSION

The use of fast growing woody plants with high capability to uptake and accumulate toxic compounds

forms an important objective of contemporary environmental politics of industrially developed countries (LOMBI et al. 2001). The interest is focused mainly on plants that have ability to accumulate heavy metals for prolonged periods without suffering any affliction or retardation of growth (TOMSETT, THURMAN 1988; RASKIN et al. 1997; LASAT 2002). At present, the pathophysiological influences of heavy metals have been determined for over 200 plant species (ZENK 1996). Fast growing pioneer trees such as hybrid aspen and rowan-tree could be the candidates for absorption and accumulation of environmental pollutants including heavy metals (DIX et al. 1997). They are amenable to effective micropropagation and could be advantageously utilized for phytoremediation plantings (MALÁ et al. 2006). STOLTZ and GREGER (2002) described utilization of *Salix* for such purposes. Recently FRENCH et al. (2006) demonstrated, based on a 3-year long phytoremediation trial, that phytoextraction by *Salix*, *Populus* and *Alnus* could reduce contamination hotspots of more mobile elements (Cd and Zn) within a 25–30 year crop cycle. In our study we compared the uptake capabilities of hybrid aspen and rowan-tree for toxic heavy metals Cd and Pb, and for the essential heavy metal Mn. The hybrid aspen and rowan-tree clones were originated from the plus trees growing in polluted provenance plots in the Ore Mountains. To avoid the possible influence of genotype, the plantlets were propagated by organogenesis *in vitro* (MALÁ et al. 2006). For exclusion of microedaphone influences, the micropropagated plantlets of both clones were cultured in the sterile hydroponic solutions (MARSCHNER et al. 1996; WHITING et al. 2001). Suitable concentrations of heavy metals and also the pH of the solution, which has a major effect on accumulation capacity (KAHLE 1993), were

established earlier (KALIŠOVÁ-ŠPIROCHOVÁ et al. 2003). The complex agent (EDTA) had supplied ion availability for the plantlets in solution.

Remarkable differences were observed in the uptake and translocation of the toxic heavy metals Cd and Pb, and the essential heavy metal Mn in hybrid aspen and rowan-tree. Cd and Pb were accumulated preferentially in the roots of both clones, whereas Mn was distributed in the aboveground parts. The accumulation patterns of Cd and Pb differed from those of Mn in both clones. Amounts of Cd and Pb increased nearly linearly from the beginning to the end of experiment, whereas amounts of Mn remained substantially unchanged throughout the experiment (Table 3). We supposed selective distribution of physiologically transferred Mn to the assimilation organs. In control hydroponics samples supplied by ten times diluted MS salt solution (containing basic dosages of Mn), only lower concentrations of Mn were detected in comparison with those in hydroponic samples supplied by higher concentrations of Mn.

The accumulation capacity for toxic heavy metals of hybrid aspen clone was significantly higher than that of the rowan-tree clone. Conversely, both clones accumulated more than 100 mg/kg toxic heavy metals in the roots. These results agree with the metal accumulation of the two *Salix* clones (VANDESCASTEELE et al. 2005), both clones exhibited high accumulation levels of Cd in the shoots, where as Pb was found mainly in the roots. Insufficient selectivity of the ion transport channels results in possible translocation of Cd and Pb to the root cells (ASSUNCAO et al. 2003). In our experiment, high concentration of heavy metals in the roots and higher amounts of heavy metals in aboveground parts (especially more mobile Cd) were also observed.

The two tested clones of rowan-tree and hybrid aspen can accumulate high concentrations of toxic metals Cd (800–1,500 mg/kg) and Pb (5,000 to 13,000 mg/kg) in the roots, in addition hybrid aspen accumulated more than 100 mg/kg Cd in the aboveground parts. The authors are aware of the fact that hydroponics cannot fully imitate the complexity of conditions in the soil environment. However described toxic metal uptake in hydroponics may serve as a valuable model system for basic, rapid and cheap evaluation and selection of suitable tree species for heavy metal accumulation.

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Absorpce těžkých kovů klony hybridní osiky a jeřábu ptačího

ABSTRAKT: Pro komparativní studii absorpce toxických těžkých kovů Cd, Pb a esenciálního těžkého kovu Mn byly použity výpěstky *in vitro* namnožené z vybraných klonů hybridní osiky (*Populus tremula* × *Populus tremuloides*) a jeřábu ptačího (*Sorbus aucuparia* L.). Vzorky z kořenové a nadzemní části byly odebrány z výpěstků *in vitro* pěstovaných 24, 48, 96 a 168 hodin v aseptických podmínkách v hydroponii v roztoku solí toxických těžkých kovů a esenciálního těžkého kovu. Obsahy kovů a jejich distribuce byly stanovovány pomocí ICP-OES metody. Byly zjištěny rozdíly v absorpční kapacitě klonů hybridní osiky a jeřábu pro Cd, Pb a Mn. Ve všech sledovaných časových intervalech bylo množství akumulovaného Cd a Pb vyšší v kořenech než ve stoncích, a to jak u klonu hybridní osiky, tak u jeřábu ptačího. Proti tomu množství akumulovaného Mn bylo prokazatelně nižší než množství akumulovaného Cd a Pb ve všech odebraných rostlinných vzorcích hybridní osiky i jeřábu ptačího. Způsob absorpce Mn byl podobný u všech zmíněných pletiv obou klonů ve všech intervalech. Lze shrnout, že klony hybridní osiky a jeřábu ptačího i při absenci půdní mikroflóry jsou schopny absorbovat v kořenech velké množství toxických těžkých kovů Cd (800 až 1 500 mg/kg) a Pb (5 000–13 000 mg/kg) a ve stonku hybridní osiky byl stanoven vyšší obsah Cd (přibližně 100 mg/kg).

Klíčová slova: fytořemediace; těžké kovy; hybridní osika; jeřáb ptačí; mikropropagace

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