Polish J. of Environ. Stud. Vol. 18, No. 4 (2009), 553-561

Original Research

Phytoextraction of Cd, Ni, and Pb Using Four Willow Clones (*Salix* spp.)

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Received: 29 July 2008
Accepted: 27 December 2008

Abstract

In order to determine their phytoextraction potential, four clones of Salix (1. *Salix alba* – clone 68/53/1; 2. *Salix alba* – clone 106/54/0; 3. *Salix matsudana* – clone SM 4041; and 4. *Salix nigra* – clone 0408) were exposed to elevated concentrations of Cd, Ni and Pb-EDTA in a water culture solution. The translocation ratio to upper plant parts was very low for all applied heavy metals and, therefore, the metal uptake was restricted to the roots, especially regarding Pb. The ability of the clones to extract and translocate Cd, Ni and Pb differed depending on the quantity of metal content in the nutrient solution and of the willow genotype. The ability of the investigated clones to accumulate Cd in leaves is to our knowledge among the highest so far recorded compared to other hydroponic trials in literature. The preference for Cd-stimulated root growth was determined. This genotype-specific response could be part of a mechanism for Cd resistance.

Keywords: *Salix*, Cd, Ni, Pb, phytoextraction

Introduction

Rapid increase of global industrialization highlighted heavy metals as ubiquitous environmental pollutants. Heavy metals are elements that naturally occur in the biosphere, but human activities extract and concentrate these elements. Over the last five decades, the annual worldwide release of heavy metals reached 22,000 t for cadmium and 783,000 t for lead [1]. Common sources of heavy metals are activities such as metalliferous mining and smelting [2, 3], industry, agriculture [4], power plant wastewater sludge [5], fossil fuel combustion, emissions from brake linings and tires [6], military activities and warfare. Warfare in Serbia during 1999, for example, created several polluted sites, including oil drainage from the damaged and bombed refineries in Novi Sad and Pančevo, both densely populated towns.

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Phytoremediation is the use of plants and their associated microbes to remove, transfer, stabilize and/or degrade contaminants in soil, sediment, water and air [7, 8]. This is an efficient, environmentally friendly cleanup technology for a variety of organic and inorganic pollutants. Phytoremediation is significantly cheaper compared to the standard, more established remediation methods (soil washing, excavation, incineration, pump-and-treat systems), since it is performed *in situ* and exploits solar energy. Phytoextraction is a category of phytoremediation used for heavy metal contamination cleanup. It is the use of plants to extract pollutants and accumulate them in their tissues, followed by harvesting of the plant material. The purpose is to decrease heavy metal quantity in polluted soils to environmentally acceptable levels. Harvestable plant parts can later be used for non-food purposes (for example in the wood or cardboard industry, as a biofuel, etc.), recycling of the accumulated element (phytomining), or they can be ashed and disposed in a landfill [9].

Table 1. Shoot mass depending on Cd, Ni and Pb concentrations (g).

Treatment		Cd			Ni			Pb		
		Control	10^{-4} M/l	10^{-5} M/l	Control	10^{-4} M/l	10^{-5} M/l	Control	10^{-4} M/l	10^{-5} M/l
Clone	1	2.09 a	0.72 d	0.91 d	2.09 a	0.33 e	1.74 abc	4.75 b	5.42 a	4.24 bc
	2	1.66 b	0.31 e	0.84 d	1.66 bc	0.41 e	1.41 c	3.91 cde	3.75 cde	4.16 bcd
	3	1.47 b	0.92 d	1.05 cd	1.47 bc	0.38 e	0.92 d	3.56 de	2.28 f	3.35 e
	4	2.07 a	1.35 bc	1.46 b	2.07 a	0.85 d	1.81 ab	5.60 a	3.72 cde	4.30 bc

Statistical analyses were independent for each heavy metal. LSD values were: Cd – 0.349; Ni – 0.346; Pb – 0.592). Values followed by the same letter do not differ significantly at the level of $p<0.05$.

Different plant species are used for different phytotechnologies. Characteristics of a good phytoextractor are that it has to be a fast-growing species, to have a high biomass, be competitive, tolerant to a specific type of pollution with high levels of plant uptake, translocation and accumulation of heavy metals in harvestable plant tissues [7]. Although hyperaccumulator species are useful research models to understand the biological mechanisms involved in metal accumulation and tolerance, they are usually not suitable for phytoextraction, despite their high accumulation properties. They are often slow growing and attain low biomass. An alternative to the use of hyperaccumulator species are fast growing, high biomass species, that can extract significant amounts of heavy metals in spite of their low accumulation. Use of trees as vegetation cover for the phytoextraction of land contaminated by heavy metals does seem to have considerable potential [10]. Willow trees are promising energy crops that can extract substantial amounts of heavy metals from soil. *Salix* species attain high biomass and they are easy to propagate and can be frequently harvested by coppicing, yielding as much as 10-15 dry matter t ha^{-1} $year^{-1}$ [11]. Willow trees have extensive, spreading roots and invasive growth strategies expanding their use as *in situ* phytoextractor plant species [12]. The use of willows in remediation is well established in developed European and North American countries, where willows were used for phytoremediation of numerous organic and inorganic pollutants [7].

The aim of this study was to evaluate phytoextraction potential of three willow species (one with two clones) grown at different concentrations of Cd, Ni and Pb in hydroponic solutions. Among the heavy metals, Cd and Pb appear to be the most dangerous to the environment. Since there are few areas in Serbia with elevated Ni concentrations above certified limits, these three metals were chosen for screening. Although a significant amount of research has investigated willow remediation capacities [10, 13-19], there are no thorough data regarding specific species from this paper. Since genotype specificity has a hypothetically large significance, these species were chosen for a phytoremediation test. Hydroponic study was chosen in order to create controlled conditions, especially in the root growth medium. This experimental model can provide a selection between investigated clones that depend only on genotype properties.

Experimental Procedures

The experimental material consisted of four willow genotypes:

1. *Salix alba* – clone 68/53/1;
2. *Salix alba* – clone 106/54/0;
3. *Salix matsudana* – clone SM 4041; and
4. *Salix nigra* – clone 0408.

These genotypes were obtained from the Institute for Lowland Forestry and Environmental Protection in Novi Sad, Serbia. The genotypes chosen for screening are the result of a long period selection of genes that cause high biomass production. One of three selected species is native (autochthonous) in Serbia (*Salix alba*), for it can be important for ecological adaptation and the success of potential in vivo trials. Two clones of this species were taken in order to compare intra- and inter- genotype specificity. The willows were grown in a semi-controlled environment (greenhouse), in nutrient solutions using a water culture method. Temperature was kept under 30°C. Illumination was natural depending on outside light conditions. Solutions were permanently aerated in order to supply O_2 and proper mixing. Cuttings were placed on a drilled linoleum cover. The volume of each hydroponic pot was 40 liters. Eighteen willow woody cuttings, 20 cm in length, with one shoot per cutting, were grown in each of the pots. Each treatment consisted of 36 woody cuttings (two pots). Pot distribution was randomized and changed every two weeks. Solutions were also replaced every two weeks. Plants were treated with three different metals, separately (Cd, Ni and Pb), each in two concentrations (10^{-4} M and 10^{-5} M) in a full strength Hoagland solution. The figures of these concentrations are displayed in mass/volume unit (μg/ml): Cd – 11.24 μg/ml and 1.12 μg/ml; Ni – 5.87 μg/ml and 0.59 μg/ml; Pb – 20.72 μg/ml and 2.07 μg/ml. Control plants were grown in full strength Hoagland nutrient solution without metals. Because of precipitation of Pb with other salts from nutrient solution, Pb can be physically unavailable to the plant. Therefore, Pb was chelated with EDTA (ethylenediaminetetracetic acid) to form a soluble complex. The metals used were Cd (10^{-4} M and 10^{-5} M, supplied as $CdCl_2 \cdot H_2O$), Ni (10^{-4} M and 10^{-5} M, supplied as $NiSO_4 \cdot 6H_2O$) and Pb (10^{-4} M and 10^{-5} M, supplied as Pb-EDTA). pH solutions were kept at 5.0 level. The plants treated with Cd and Ni were measured, analyzed and harvested after 70 days. The plants

Table 2. Root mass depending on Cd, Ni and Pb concentrations (g).

Treatment		Cd			Ni			Pb		
		Control	10^{-4} M/l	10^{-5} M/l	Control	10^{-4} M/l	10^{-5} M/l	Control	10^{-4} M/l	10^{-5} M/l
Clone	1	1.00 c	1.29 ab	0.94 c	1,00 a	0.29 e	1.09 a	3.44 de	7.12 a	5.40 b
	2	0.77 de	0.48 g	0.68 ef	0.77 bc	0.29 e	0.81 b	4.18 c	4.26 c	4.49 c
	3	0.76 de	0.79 de	1.18 b	0.76 bc	0.35 e	0.63 cd	3.55 d	2.41 gh	3.02 ef
	4	0.59 fg	1.39 a	0.89 cd	0.59 d	0.61 d	0.73 bcd	2.74 fg	1.69 i	1.99 hi

Statistical analyses were independent for each heavy metal. LSD values were: Cd – 0.126; Ni – 0.141; Pb – 0.467). Values followed by the same letter do not differ significantly at the level of $p<0.05$.

treated with Pb were harvested and analyzed after 95 days. Plants treated with Cd and Ni were harvested 25 days earlier due to developed chlorosis and necrosis that endangered the viability of plants. Symptoms were evident on 10^{-4} M of applied Cd and Ni. Plant material was rinsed in deionized water, dried and prepared for analysis following standard methods for the examination of water and wastewater [20]. The concentrations of heavy metals were determined after mixing and heating with H_2O_2, drying at 450ºC and treatment with 25% HCl. Concentrations of Cd, Ni and Pb were determined from prepared solutions by employing atomic absorption spectrophotometry (AAS). The content of Cd, Ni and Pb was measured in roots, shoots and leaves. Shoot material consisted only from stems without leaves. Leaf samples were divided into two groups. "Upper leaves" consisted of the 7 youngest leaves at the top of the shoot, and the rest of the leaves formed the "Lower leaves" sample. Contents of Cd, Ni and Pb in control plants were under detection limits and therefore were not included in the statistical analyses.

Distribution of metals within the plant was expressed as the $[Element]_{root}$: $[Element]_{shoot}$: $[Element]_{lower\ leaves}$: $[Element]_{upper\ leaves}$ ratio, starting from the lowest concentration with given value 1, and is shown in Table 3.

Statistical analyses were conducted using Duncan's Multiple Range Test, at a level of significance of $p<0.05$, using 2-way factor analyses (factor one – clones; factor two – metal treatment). The average values shown in the tables and figures followed by the same letter did not differ significantly. Values decreased following the alphabetical order. The least significant difference (LSD), between the average values of treatments is shown in the table and figure legends. Standard error bars are shown in figures. In tables, a statistical analyses was conducted independently for each heavy metal.

Results

Homogenous chlorosis of younger leaves, at the moment of harvesting, was determined on plants treated with Cd (100% of plants) on higher concentrations applied, 10^{-4} M. Chlorosis was also evident on plants treated with 10^{-4} M of Ni on both young and old leaves (also in 100% of plants), together with necrosis that was first visible in the form of spots on the adaxial side, mostly on the leaf margins, and that was fast developed into clear patches. Plants treated with Pb-EDTA did not have any visible symptoms of treatment.

The control plants of all clones had higher shoot mass compared to both applied concentrations of Cd (Table 1). Decrease of shoot mass was more expressed on Cd concentrations of 10^{-4} M, although the difference in shoot mass between two Cd treatments (10^{-4} M and 10^{-5} M) was not significant for clones 1, 3 and 4. Clone 4 had the smallest decrease of shoot mass compared to control plants (only for one level of LSD), while clones 1 and 2 decreased their shoot mass over 60% (clone 1) and 80% (clone 2).

Although compared to control, Ni concentrations of 10^{-5} M decreased shoot mass in all cases, which was significant only for clone 3. Restriction of growth caused by 10^{-4} M of Ni was very strong, > 80% (clone 1), > 70% (clone 2 and 3) and > 50% (clone 4).

Pb-EDTA treatment caused a statistically significant decrease in shoot mass only for clones 3 (10^{-4} M) and 4 (both applied Pb concentrations) (Table 1). Furthermore, the growth of clone 1 was stimulated with 10^{-4} M of Pb.

Opposite the shoot mass, Cd treatment of clones 1, 3 and 4 showed a significant increase in root mass (Table 2). The roots of clones 1 and 4 increased in weight in the presence of higher Cd concentration (10^{-4} M) and clone 3 in the presence of lower Cd concentration (10^{-5} M).

The root mass for all clones treated with Ni concentration of 10^{-5} M, was not significantly different between control and treated plants (Table 2). However, the inhibition of root growth, influenced by 10^{-4} M concentrations of nickel, was evident and significant for all clones except No. 4.

Responses of root growth to Pb-EDTA treatment were clone-specific. Clone 1 had a significant increase in root mass for both Pb-EDTA concentrations, compared to control plants (Table 2). Clone 2 had no response to treatment, while clones 3 and 4 had a statistically important decrease in root mass of treated plants.

Cd concentrations in roots, shoots and leaves was significantly higher in the plants treated with 10^{-4} M of Cd, compared to a lower Cd concentration of 10^{-5} M (Figs. 1, 2 and 3).

The difference between Cd accumulations in clones was significant. The content of Cd in roots was several

Table 3. Root : Shoot : Lower leaves : Upper leaves ratios.

		Clone 1	Clone 2	Clone 3	Clone 4
Cd	10^{-4} M	23.8:1.0:1.4:1.3	73.7:6.5:2.8:1.0	25.6:1.0:1.4:1.5	8.9:1.0:1.5:1.2
	10^{-5} M	16.5:1.0:1.5:1.3	51.7:2.2:2.4:1.0	32.4:1.0:1.8:1.7	9.2:1.0:1.6:1.3
Ni	10^{-4} M	55:1.0:3.5:3.9	58:1.0:2.7:2.4	66.7:1.0:4.0:2.4	85.5:1.0:4.6:4.0
	10^{-5} M	119:1.0:2.8:4.0	105.2:1.0:1.7:2.3	128.1:1.0:2.8:3.4	202.5:1.0:2.2:2.4
Pb	10^{-4} M	239:1.0:2.1:1.2	179:1.2:1.7:1.0	1176:1.0:1.4:1.0	1744:1.3:2.8:1.0
	10^{-5} M	170:1.0:2.0:2.1	266:1.0:1.0:1.5	533:1.0:1.8:1.3	718:1.0:1.9:2.6

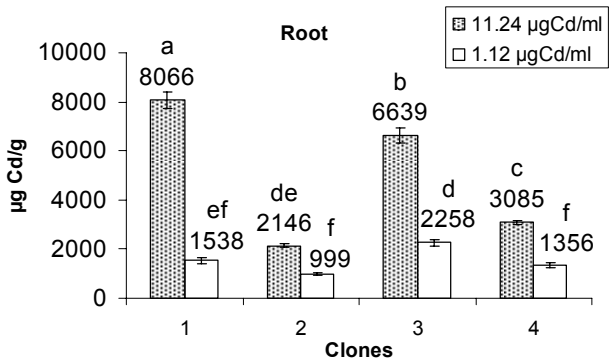

Fig. 1. Concentration of Cd in roots of the investigated clones (µg Cd / g of dry weight) (LSD = 715.20).

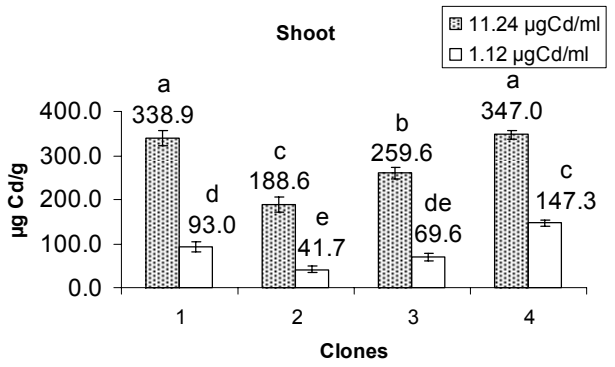

Fig. 2. Concentration of Cd in shoots of the investigated clones (µg Cd / g of dry weight) (LSD = 50.07).

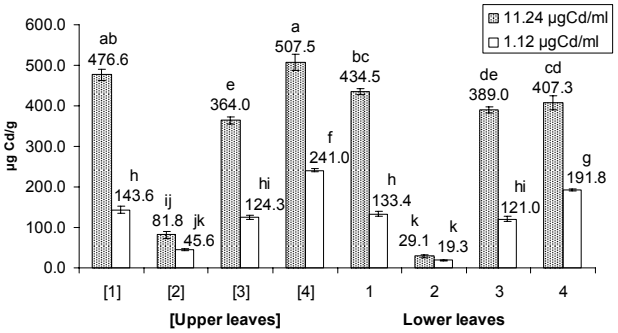

Fig. 3. Concentration of Cd in upper and lower leaves of the investigated clones (µg Cd / g of dry weight) (LSD = 42.47).

dozen times higher comparing to the shoot and leaf Cd concentrations. The ratio of Cd translocation from root to shoot was specific for each clone.

Clone 4 had the highest Cd translocation level from roots to leaves (Root:Leaf ratio below 10) for both applied Cd concentrations, and therefore the highest Cd content in leaves compared to the other clones (Table 3).

Clone 2 had the lowest translocation level (Root:Leaf ratio 51.7 and 73.7) and therefore the lowest leaf Cd content.

The difference in the accumulation of Ni, between nickel treatments (10^{-4} M and 10^{-5} M) in roots and shoots, was significantly lower compared to that of the Cd accumulation (Figs. 4, 5 and 6). The ratio between different concentrations, in roots and shoots (10^{-4} M:10^{-5} M,) for Cd was 2.1-6.2, and for Ni 1.0-3.0. Translocation of nickel from root to shoots was much lower compared to Cd translocation, except for clone 2 (Table 3).

The level of nickel translocation to the upper parts of the plant was concentration-dependent. It was two times higher in the plants grown on 10^{-4} M of Ni compared to the plants grown on 10^{-5} M of Ni.

The concentration of Ni in the shoots was a few times lower (1.7-4.6) compared to concentrations of Ni in the leaves. The uptake and translocation of nickel to the upper parts of plants for clones 1, 3 and 4 was significantly lower compared to cadmium translocation in those clones. Therefore, the concentration of Ni in the leaves (28.9-148.6 µgNi/g dry weight) was a few times lower compared to the Cd content in the leaves (121.0-507.5 µgCd/g dry weight).

Concentration of Pb in the roots, shoots and leaves was also significantly higher in plants treated with 10^{-4} M of Pb-EDTA, compared to the clones treated with 10^{-5} M of Pb-EDTA (Figs. 7, 8 and 9).

The differences between treatments were exceptionally high for clones 3 and 4, where the Pb concentration ratio (10^{-4} M:10^{-5} M) in the roots was 7.3 (clone 3) and 6.3 (clone 4). The quantity of Pb uptake and accumulation in the roots of clones 3 and 4 was several times higher compared to Cd and Ni content in the roots.

However, the transport of Pb to the upper parts of plant was significantly lower compared to Cd and Ni transport (Table 3). On 10^{-4} M of applied Pb-EDTA, higher concentrations of Pb were accumulated in lower leaves compared to the upper leaves. In general, the Pb concentrations in the

shoots and leaves were several times lower compared to the Cd and Ni concentrations in the shoots and leaves. For both Ni and Pb, the clone differences of metal accumulation in the shoots and leaves were not statistically significant, with only a few exceptions.

Discussion

The analysis of morphological parameters showed significant differences among clone growth reactions to treatment. A strong decrease in shoot mass of the plants treated with 10^{-4} M of Cd and Ni, with determined signs of chlorosis and necrosis, suggest that the toxic influence of these metals on this level of contamination leads to significant decrease in the viability of genotypes investigated. Plants treated with Pb-EDTA did not have any chlorosis signs, or any other symptoms of metal toxicity (hence the time period of hydroponic trial for Pb-EDTA was longer). Since the translocation of Pb to the green plant parts was very low, the shoots and leaves were protected from the toxic effect of Pb, which is the reason why the Pb-EDTA treatment did not have a significant influence on the shoot mass of the treated plants. Clone 4 can be distinguished as a genotype with the

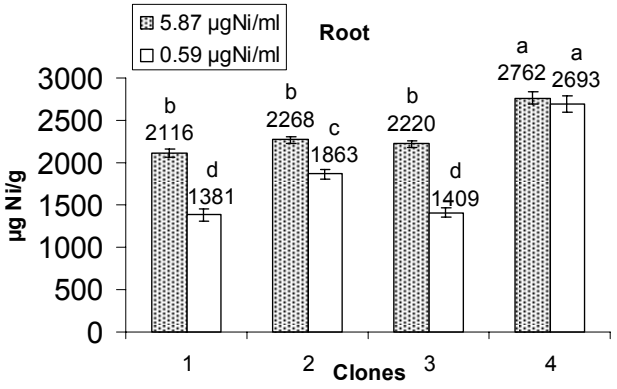

Fig. 4. Concentration of Ni in roots of the investigated clones (µg Ni / g of dry weight) (LSD = 245.60).

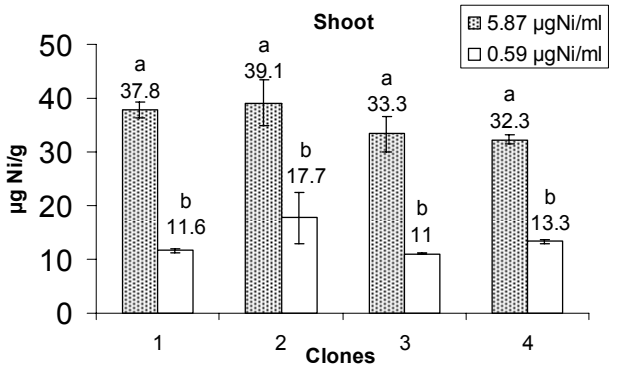

Fig. 5. Concentration of Ni in shoots of the investigated clones (µg Ni / g of dry weight) (LSD = 12.26).

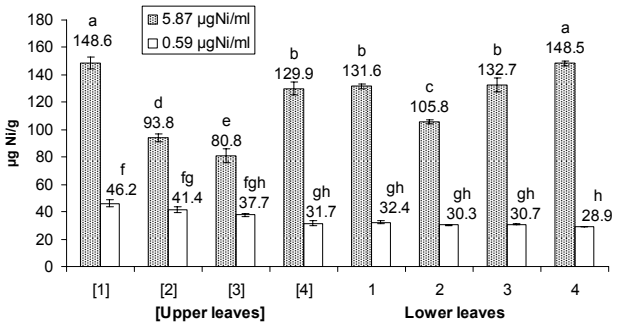

Fig. 6. Concentration of Ni in upper and lower leaves (c) of the investigated clones (µg Ni / g of dry weight) (LSD = 11.96).

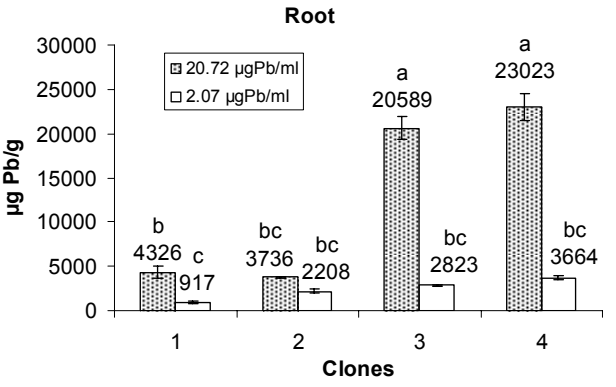

Fig. 7. Concentration of Pb in roots of the investigated clones (µg Pb / g of dry weight) (LSD = 3269).

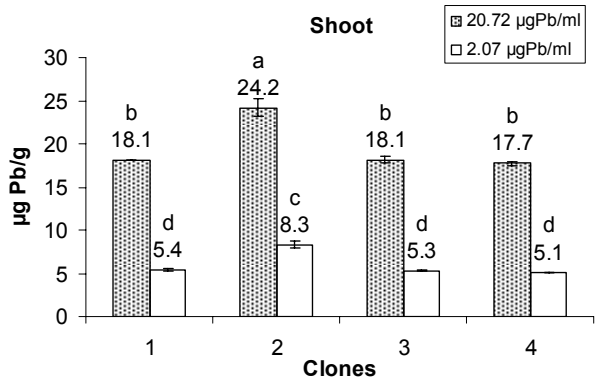

Fig. 8. Concentration of Pb in shoots of the investigated clones (µg Pb / g of dry weight) (LSD = 2.11).

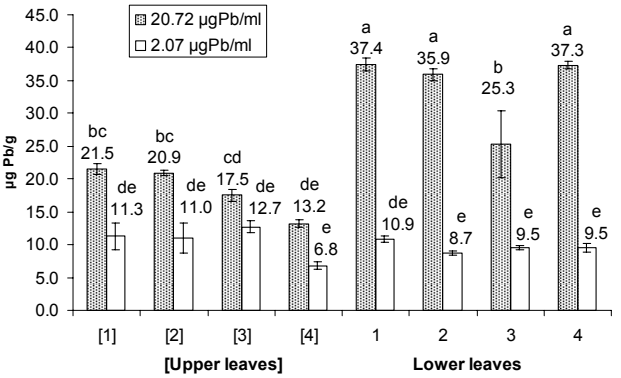

Fig. 9. Concentration of Pb upper and lower leaves (c) of the investigated clones (µg Pb / g of dry weight) (LSD = 6.66).

best bioproduction tolerance to treatments of Cd and Ni. This genotype had the highest shoot mass in general and displayed the highest bioproduction tolerance to Cd and Ni treatment. The difference between the plants was often greater within the same species (between clones 1 and 2 of the same species, *Salix alba*), than between different species. Therefore, reactions to the treatments were not only species-specific, but clone- or genotype-specific, which coincides previous research [13, 14]. The presence of Cd in the hydroponic solution stimulated root growth for clones 1, 3 and 4 on different concentrations of Cd. Similar results were obtained in trees by Gussarsson (1994) [21], although at lower Cd concentration. In that case, the author suggested that the accumulation of Cd in fine roots, combined with a preference for root growth, could be part of a mechanism for Cd resistance. In contrast, the Ni and Pb-EDTA treatments caused a reduction of root growth, except for clone 1 treated with Pb-EDTA. These results also suggest clone-specific responses of root growth to the Ni and Pb-EDTA treatments.

The concentrations of accumulated Cd, Ni and Pb in the roots, shoots and leaves were genotype specific. Other researchers similarly observed genetically determined reactions to metal stress. Landberg and Greger (1994) [22] used a hydroponic experiment to test 94 clones of *Salix viminalis* and *Salix dasyclados*. Variations in tolerance and metal uptake between clones was significant. In some cases, clones differed by a factor of 80 in metal uptake ability.

The difference between clones in metal accumulation ability, was expressed more in higher concentrations applied (10^{-4} M), for all metals (Cd, Ni and Pb). Variability in metal accumulation ability was higher at higher concentrations. These facts suggest that clonal specific ability for heavy metal accumulation depends on concentration of heavy metal in the growth medium. This assumption is confirmed by the fact that the translocation ratio of Ni was approximately 50% higher at higher Ni concentrations applied (10^{-4} M) as compared to lower concentrations (10^{-5} M). Galardi et al. (2007) [23] demonstrate that for *Alyssum bertolonii* the root and shoot respond differently to different Ni concentrations, so that with increasing nickel concentration in the medium the variation in root tolerance decreased, whereas variation in shoot tolerance increased.

Although roots where rinsed in deionized water that allows sorption of one portion of metals in the apparent free space, translocation of metals from the roots to the upper parts was very low for all applied heavy metals. Many metal-tolerant species have restricted translocation of metals to the shoot [24-26]. The suggested reason for restricted shoot and leaf metal uptake is the presence of exclusion mechanisms, probably for the protection of photosynthesis from toxic levels of heavy metals [3, 27]. The lowest transport level was determined for Pb. The translocation ratio of Pb was up to ten times lower compared to those of Cd and Ni, even though Pb was supplied chelated with EDTA, which should increase mobility of Pb. Several investigations suggest that some synthetic chelates have a potential to increase solubility, mobility and translocation of Pb associated to different soil fractions [28-36]. Huang et al. (1997) [30] indicated that the effectiveness of such chelates for Pb, in decreasing order, was EDTA > HEDTA > DTPA > EGTA > EDDHA. Hernández-Allica et al. (2007) [37] report that even considering the negative effects, proper management of the EDTA application can reduce metal phytotoxicity and increase the uptake of metals with low phytoavailability. In turn, EDTA-metal complexes can be toxic for plants and soil microorganisms, they have low degradability and high solubility so they can easily reach the groundwater, form high stability complexes with other heavy metals in soil, there for increasing their toxicity, with the consequent environmental damage, and so their use must be considered with caution [35, 36, 38].

Malkowski et al. (2004) [25] suggest that the mechanisms of Pb and Cd translocation from root to shoots are different, since their result also presented ten-fold higher accumulation of Pb in apical root segments of maize as compared to Cd, and higher Cd transport to shoot as compared to Pb. The authors proposed that higher amounts of Pb are binding in the root apoplast (cell walls) as compared to Cd. According to Free Ion Activity Model (FIAM), activity of a particular metal species in the soil solution is a major determinant to bioavailability [39]. Although, the use of soil amendments such as EDTA should increase metal ion activity, the success of its application is not guaranteed. One of the problems with the implementation of FIAM arises from the depletion of ions around roots, so that rhizosphere conditions may not reflect bulk soil conditions [40]. Therefore, after selection of genotypic references in hydroponic experiments, researched clones must be tested in outdoor soil conditions. Success of such application must be assessed depending on soil properties.

The concentrations of Cd in leaves of *S. alba* (476.6 μg/g of dry weight), *S. matsudana* (389.0) and *S. nigra* (507.5) and shoots of *S. alba* (338.9), *S. matsudana* (259.6) and *S. nigra* (347.0) are among the highest concentrations ever published, compared to other hydroponic screenings [15, 16]. According to Lux et al. (2002) [18] and Lunackova et al (2003) [19], Cd content in *Salix alba* varied between 2.0-62.1 in shoots and 3.0-160 in leaves and stems (μg/g of dry weight). The higher concentration of Cd in leaves of *Salix* species (519.0 and 584.0 μg/g) was reported only by Cosio et al. (2006) [15] on aplied concentrations of 50 μM in hydroponic solution. In that research, Cd content in leaves was smaller when increasing treatment to 100μM (10^{-4} M = 11.24 μg/ml) and 200 μM. Maximum tolerable concentrations of Cd in the Republic of Serbia (μg/g air-dried soil) is 3 μg/g. Research confirms that Cd usually does not exceed these limits more than 10-fold, rarely up to 20-fold [41, 42]. In the regions of Poland under strong anthropopresure, concentrations of Cd have varied between 4.6-64.0 μg/g d.w [42]. Cadmium accumulation in plants depends on the system, metal phyto-toxicity, concentration and bioavailability in soils. Nevertheless, investigated genotypes certainly have potential as accumulators of Cd.

Willow biomass production can reach as much as 10-15 dry t ha^{-1} $year^{-1}$ [11], the amount of metal extracted from polluted soil can be substantial. The difference between

hydroponic and soil phytoextraction can be substantial. For example, Klang-Westin & Eriksson (2003) [17] determined that net removal of Cd by *Salix* from different soil types varied between 2.6 and 16.5 g Cd ha^{-1} $year^{-1}$. Shoot and leaf accumulation ability of Cd in many *Salix* species is confirmed [15, 16]. The fluxes of heavy metals in *Salix* stands in field conditions are not well investigated and the net removal of metals from the soil is by far under these assumed limits. The good part of low metal translocation ratio from roots to upper plant parts is that metals cannot be easily dispersed in the environment through herbivores or at senescence. However, some researchers report that uptake and translocation ratio in field plants differs with that in hydroponics, so that *Salix* species had significantly higher metal translocation ratio *in situ*, compared to hydroponics [3, 16]. These differences may be due to the fact that root activity in field conditions (interactions between root-soil particles, root-bacteria and/or root-mycorrhiza) affects metal uptake and translocation [43-46]. Nevertheless, hydroponic experiments can still be used as a starting point in potential phytoextraction use of not yet investigated or applied plant genotypes [47]. Hydroponic screenings are faster compared to soil-grown trials and enable differentiation between clones which broadly correspond to those observed in the field [16, 48].

Since the cost of phytoextraction technology is significantly smaller compared to conventional methods, it certainly has future potential in a developing country like Serbia, where many polluted sites await remediation.

Conclusion

All investigated genotypes accumulated higher concentrations of Cd, Ni and Pb in their roots than they did in their aboveground tissues. Clone 4, *Salix nigra* – clone 0408, is the most promising genotype for potential use in Cd and Ni phytoextraction and deserves further attention in field validation. The toxic influence of Cd and Ni on the investigated clones at the applied concentration of 10^{-4} M was significant.

The ability of clones to extract and translocate Cd, Ni and Pb differs depending on the quantity of the metal load in the nutrient solution. Although the translocation ratio to the upper plant parts was low, if biomass production is not disturbed with toxic influence of heavy metals, the amount of extracted heavy metal can be significant.

Concentrations of Cd accumulated in leaves and shoots are among the highest ever recorded. Therefore, the potential of investigated clones for Cd phytoextraction is promising.

The increase of the root mass influenced by the presence of Cd in the hydroponic medium was detected. This preference for root growth could be a part of a mechanism for Cd resistance. Further analyses are required to determine the cause for this reaction of the roots.

Since willow phytoextraction potential is clone- or genotype-specific, the selection of specific clones rather than species based on environmental properties of each contaminated site should be performed. Results from this paper are the baseline for future decisions about what genotypes to deploy based on the element in need of remediation.

Serbia, as a developing country in economic transition, has a high potential for the possible use of not-yet-commercially-used phytoextraction, based on the relatively low cost of this environmentally friendly technology.

Acknowledgements

This study was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia in the area of Technology development as part of project No. TR20001.

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