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PHYTOREMEDIATION OF ZINC, LEAD AND CADMIUM RICH POST-FLOTATION TAILINGS USING TREE CLONES

FITOREMEDIACJA ODPADÓW POFLOTACYJNYCH O DUŻEJ ZAWARTOŚCI CYNKU, OŁOWIU I KADMU Z WYKORZYSTANIEM KLONÓW ROŚLIN DRZEWIASTYCH

Abstract: It was tested the usefulness of *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Dabrowicka purple plum' and *Taxus baccata* clones in removal of metallic elements from post flotation tailings contaminated with cadmium, lead and zinc.

Obtained results indicated a certain potential in that respect of plants belonging to the genus *Prunus*, therefore it is recommended to monitor carefully the orchard farms located within impact of metalliferous dusts containing heavy metals. Examined clone of *B. pendula* expressed the ability to take up and accumulate relatively high amounts of Cd, Pb and Zn in roots with their further transfer to aboveground organs. Tested genotype of *Taxus baccata* proved to be inappropriate for this purpose. It can be only considered efficient to stabilize spoil shelves and slopes in order to prevent wind and water erosion.

Keywords: industrial wastes, zinc, lead, cadmium, phytoremediation, tree clones

Urban, industrial, and agricultural human activities are ever increasing source of environmental pollution, especially by both organic pollutants and heavy metals. In the Olkusz district, located on the border of the areas of Krakow-Czestochowa Jura and Silesia Upland, mining and metallurgical engineering have led to environmental degradation. During the exploitation of zinc and lead ore deposits the post-flotation tailings are formed, which constitute inappropriate substratum for plant growth because of low water capacity, the susceptibility to wind erosion, and elevated levels of lead, zinc and cadmium compounds. In settling ponds, with the surface of about 100 ha, 38 millions tons (38 Tg) of wastes are accumulated, containing about 1.0 % of Zn, 0.5 % of Pb, and 77 % of dolomite. They are the cause of dust pollution emission to the

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atmosphere. Presently, efficient technologies are needed that can provide the decrease of heavy metal content, mainly of lead and cadmium, up to the level allowing the use of stored dolomite in the agriculture [1–4]. The plant-assisted bioremediation of contaminants is environmentally friendly and cost-effective technique. The procedure, seen as a form of ecological engineering, depends on synergistic relationships among plants, microorganisms and the environment. Thus, phytoremediation is a promising pro-ecological method of air, soil or other substrata and groundwater detoxification. Actually it is tended towards exploiting vascular plants to increase effectiveness of *in situ* inorganic contaminants remediation. Tree species have range of features, which make them the possible candidates for application in phytoremediation techniques, especially *Salix* species, have the great potential for cadmium phytoextraction [5–9]. Another tree species that have been also shown to be promising plant material to clean-up toxic levels of metals are: *Acer pseudoplatanus* L., *Betula pendula* Roth., *Populus alba* L., *Populus deltoids* Bartr. ex Marsh, *Prunus virginiana* L. [10, 11].

In presented study candidate tree clones were selected to test remediation effectiveness from calamine waste heap rich in zinc, lead and cadmium compounds. Some deciduous trees, and the coniferous *T. baccata*, were included in the experimental design to assess their usefulness for zinc, lead and cadmium bioaccumulation.

Materials and methods

Plant material constituted clone B1of *Betula pendula* Roth (*Betula verrucosa* Ehrh.), clone E2 of *Prunus cerasus* L.'Tabel® Edabriz', clone WD3 of *Prunus domestica* L. 'Dabrowicka purple plum' and clone C5 of *Taxus baccata* L. Respective plant material was obtained *in vitro* from stabilized shoot cultures and acclimatized to *ex vitro* in the greenhouse conditions.

Experimental plots were established on the shelf of the settling pond formed from material disposed after zinc and lead ores flotation in Bukowno near Olkusz, at the altitude of 308 m a.s.l. (N 50^o 16.856′ E 19^o 30.204′), with eastern exposure. The three year experiment was carried out in three replications, using randomized blocks method. On the substratum, coming from the active settling pond, 30 two year old plantlets of each clone were planted without any preliminary treatments. A substratum control constituted the unplanted plot. Plants were spaced in the distance 1 × 1 m. They were placed into holes with the soil clod directly onto post-flotation substratum, covering with post-flotation materials. During the whole experiment plants were neither tended nor fertilized. At the end of the three vegetation periods experiment analyses of both plant material and substrata were performed.

From every replication one mixed plant sample, from 5 vigorously growing plants, separately for roots and shoots of each clone was taken. Plant samples, collected in May 2008, were washed with running water, and afterwards were rinsed thoroughly with distilled water, dried to constant mass, ground and dry-ashed at 450 ^{o}C. Simultaneously and from each replication, substratum samples were taken from 0–30 cm layer of a rooting zone of trees acquired for plant analyses, as well as from the same depth of the control plot. The substratum samples were air dried and the following analyses were performed: pH potentiometrically, total nitrogen and carbon contents with the use of TOC-TN 1200 Thermo Euroglas apparatus. The content of organic carbon was calculated as a difference between total and inorganic carbon. The total contents of Zn, Pb and Cd were determined in plant and substratum samples, after digestion in the mixture of nitric(V) and chloric(VII) concentrated acids, with the use of an atomic emission spectrophotometer with inductively coupled argon plasma ICP-AES JY 238 ULTRACE using ICP multi-element standard solution IV (Merck). The accuracy of analytical methods was verified with the reference to the certified reference material GSS-8 (GBW 07408 – State Bureau of Meteorology, Beijing, China). Chemical analyses of plants and substrata were performed in three replicates. The results were subjected to STATISTICA 6.1, ANOVA analysis and a posteriori Fisher's test was used to study differences between respective variants at the significance level $\alpha = 0.05$.

Results and discussion

Selected characteristics of substrata taken from the rooting zone of tree clones are shown in Table 1. In all studied samples pH values were equal or higher than 7.5, and they revealed low organic carbon contents ranging from 0.68 to 1.001 $g \cdot kg^{-1}$. They were statistically differentiated by nitrogen content, which varied from 0.029 $g \cdot kg^{-1}$ (mean value from control plots) to 0.049 $g \cdot kg^{-1}$ (plots planted with birch). Studied substrata were characterized by a very strong contamination with cadmium, zinc and lead (Table 2). Determined contents of these elements exceeded limiting levels defined by the Ministry of the Environment in the directive for industrial lands issued on 9 September 2002 [12] about 5 fold for Cd, 8 fold for Zn and from 5 to 8 fold for Pb. Substrata taken from the rooting zone of respective tree clones, analyzed after the period of plant cultivation, were diversified considering zinc and lead contents whereas contents of cadmium were still on the same level likewise in the substratum taken from not planted, control plots. Definitely the highest Pb content was found in the substratum sample from *P. domestica* 'Dabrowicka purple plum' plots, while contents of all studied heavy metals determined in the sample from plots planted with *Taxus baccata* were equivalent to the level of their content in the control plots.

Table 1

Mean values of pH, organic carbon and total nitrogen contents in studied substrata

Substratum	pH in 0.01 mol · dm^{-3} $CaCl_2$	Organic C contents [$g \cdot kg^{-1}$]	Total N contents [$g \cdot kg^{-1}$]
B1*	7.6^{a} **	0.788^{a}	0.049^{d}
E2	7.7^{a}	0.967^{a}	0.041^{d}
WD3	7.6^{a}	0.680^{a}	0.035^{abc}
C5	7.7^{a}	1.001^{a}	0.033^{ab}
K6	7.5^{a}	0.796^{a}	0.029^{a}

* Sample of substratum taken from rooting zone of plants from plots planted with: B1 – *Betula pendula*, E2 – *Prunus cerasus*, WD3 – *Prunus domestica* 'Dabrowicka purple plum', C5 – *Taxus baccata*, K6 – sample taken from the control plot, not planted from the depth of 0–20 cm; ** The different letters indicate statistically significant differences among mean values.

Table 2

Total contents of heavy metals in plant organs of studied tree clones and in substratum analyzed after the period of plants cultivation (mean ± standard deviation of the mean)

Material	Cd	Pb	Zn
	[mg · kg^{-1}]		
Roots – B1*	11.53 ± 0.95[a]	495.69 ± 9.31[ab]	1695.3 ± 96.9[b]
Roots – E2	12.40 ± 0.62[a]	691.59 ± 0.70[abc]	1750.7 ±52.7[b]
Roots – WD3	21.63 ±0.66[c]	1115.3 ± 0.82[c]	3875.9 ± 53.4[c]
Roots – C5	19.93 ± 1.16[b]	296.0 ± 5.70[a]	1148.9 ± 50.28[a]
Shoots – B1*	13.67 ± 0.86[c]	1365.1 ± 7.23[d]	1470.4 ± 16.7[d]
Shoots – E2	1.74 ± 0.55[b]	372.6 ± 0.91[c]	760.1 ± 1.57[c]
Shoots – WD3	2.51 ± 0.46[b]	212.5 ± 2.33[b]	493.0 ± 3.31[b]
Shoots – C5	0.16 ± 0.04[a]	19.9 ±1.29[a]	53.8 ± 1.93[a]
Substratum – B1**	72.79 ± 062[a]	3010.0 ± 40,2[ab]	8267.7 ± 111.1[b]
Substratum – E2	74.00 ± 2.35[a]	3819.0 ± 26,5[c]	8113.6 ± 59.1[b]
Substratum – WD3	75.43 ± 1.20[a]	4935.3 ± 53.3[d]	7825.5 ± 31.1[a]
Substratum – C5	73.62 ± 3.00[a]	3158.5 ± 201.6[b]	8197.0 ±112.1[b]
Substratum – K6	73.79 ± 0.76[a]	3179.2 ± 5.73[b]	8155.4 ± 171.7[b]

* Samples of plant organs taken from respective tree clones; **Sample of substrate taken from rooting zone of plants from plots planted with: B1 – *Betula pendula*, E2 – *Prunus cerasus*, WD3 – *Prunus domestica* 'Dabrowicka purple plum', C5 – *Taxus baccata*, K6 – sample taken from the not planted control plot; The different letters indicate statistically significant differences among mean values.

The results of cadmium, lead and zinc contents of the roots and shoots of B1 clone of *B. pendula*, E2 clone of *P. cerasus* L.'Tabel® Edabriz', WD3 clone of *P. domestica* 'Dąbrowicka purple plum', and C5 clone of *T. baccata* are given in Table 2. Comparison of heavy metals contents in plant clones resulted in significant differences in both underground and aboveground organs. The most effective in the accumulation of studied heavy metals in roots proved to be *Prunus domestica* clone. Contents determined in roots amounted to 21.63 mg Cd · kg^{-1} d.m., 1115.3 mg Pb · kg^{-1} d.m. and 3875.9 mg Zn · kg^{-1} d.m.. The lowest content of lead and zinc contents were determined in roots of *Taxus baccata* (Table 2). Considering aboveground plant parts, the highest levels of cadmium (13.67 mg · kg^{-1} d.m.), lead (1365.1 mg · kg^{-1} d.m.) and zinc (1470.4 mg · kg^{-1} d.m.) were found in shoots of birch. There were also determined relatively high contents of lead and zinc in shoots of *Prunus cerasus*, that is: 372.6 and 760.1 mg · kg^{-1} d.m., respectively. Surprisingly, the lowest contents of all studied elements were determined in shoots of *Taxus baccata.* Summing up, Table 2 shows that apart from *B. pendula*, in aboveground parts of plant material used in the experiment contents of cadmium, lead and zinc were noticeably lower than in roots.

High-biomass crops, comprising trees, are promising as phytoextractors. The biotechnological approaches, in this respect, are aimed at obtaining GMO trees, with the combinated trait of high uptake of heavy metals or high tolerance to such pollutants, to enhance plant survival on contaminated sites [7, 11, 13, 14]. Generated transgenic lines up till now have been tested under artificial conditions. As far as the field experiments with tree clones are concerned, only a material obtained in a traditional way is applied. Moreover, trees used in this kind of experiments belong to genera commonly used for reclamation such as: *Populus, Salix, Alnus*, *Acer, Betula, Fraxinus, Robinia, Quercus* [11, 15]. In order to check the usefulness of woody plants to heavy metal phytoextraction, in presented experiment were compared the effectiveness of *Betula,* frequently used for these purposes with E2 clone of *P. cerasus* and WD3 clone of *P. domestica*. As far representatives of *Rosaceae* family have not been tested under such extreme conditions. With the aim of obtaining different accumulation of heavy metals was also used *Taxus*, which, in contrast to the above-mentioned clones, is characterized by an insignificant biomass increase. It was proved that only the B1 clone of *Betula pendula* was efficient in transfer of cadmium and lead to aboveground parts. Rosselli et al [11], working with decidous tree clones on metal contaminated soil, have found that *Betula* transferred to leaves elevated amounts of zinc and cadmium. Whereas Meers et al [16] have determined as effective only the clones of *Salix dasyclados* 'Loden', *S. fragilis* 'Belgish Rood' and *S. schwerinii* 'Christina' out of five other tested. The other difficulty is how long period of time would be needed to get the ground purified. Nevertheless, even though the introduction of trees would not give immediately positive results in that respect, it is also of great importantce to stabilize in such a way spoil shelves and slopes in order to prevent wind and water erosion.

The great endanger is brought about by high cadmium levels, so this element is presently particularly studied, especially in respect of biological quality of edible crop organs [17–20]. Next to cadmium, lead is frequently reported to have the highest impact on organisms. According to Gaweda [21] lead accumulated in plant tissue significantly decreased the content of physiologically important components of cells. The mechanisms of this phenomenon are intensively studied [22, 23]. The presented data showed a certain potential for application of *Prunus domestica* and *P. cerasus* clones in phytoremediation. At the same time, it is worth underlined that the ability of heavy metals accumulation in aboveground organs poses a threat to transfer of metals to fruits. Thus there is a considerable risk that yield coming from orchard cultivations located within the range of the emitter impact needs to be carefully monitored.

Conclusions

1. *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Dabrowicka purple plum' and *Taxus baccata* varied widely when assessed for effectiveness of contaminant remediation.

2. The studied *Prunus* clones revealed ability to heavy metal accumulation in their organs therefore it is recommended to monitor of orchard farms located within the range of metalliferous dust impact.

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FITOREMEDIACJA ODPADÓW POFLOTACYJNYCH O DUŻEJ ZAWARTOŚCI CYNKU, OŁOWIU I KADMU Z WYKORZYSTANIEM KLONÓW ROŚLIN DRZEWIASTYCH

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Abstrakt: Testowano przydatność klonów *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Węgierka Dąbrowicka', i *Taxus baccata* do usuwania metali ciężkich z materiałów odpadowych zanieczyszczonych kadmem, ołowiem i cynkiem.

Uzyskane wyniki wskazują na pewien potencjał roślin z rodzaju *Prunus* w tym zakresie. Z tego względu należy prowadzić monitoring upraw sadowniczych zlokalizowanych w zasięgu oddziaływania metalonośnych pyłów zawierających metale ciężkie. Wykorzystany w badaniach klon brzozy wykazał zdolność pobierania i akumulowania względnie dużych ilości Cd, Pb i Zn w korzeniach i ich przemieszczania do pędów. Testowany klon *Taxus baccata* okazał się nieprzydatny do usuwania tych pierwiastków z odpadów poflotacyjnych i może być jedynie wykorzystany do stabilizacji półek i zboczy w celu zapobieżenia erozji eolicznej i wodnej.

Słowa kluczowe: odpady przemysłowe, cynk, ołów, kadm, fitoremediacja, rośliny drzewiaste