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THE USEFULNESS OF COMMON BIRCH TO STABILIZATION OF GROUNDS STRONGLY CONTAMINATED BY HEAVY METALS

PRZYDATNOŚĆ BRZOZY BRODAWKOWATEJ DO STABILIZACJI GRUNTÓW SILNIE ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

Słowa kluczowe: materiały poflotacyjne, fitostabilizacja, metale ciężkie, *Betula pendula.*
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W pracy badano możliwość zastosowania brzozy brodawkowatej do stabilizacji gruntów silnie zanieczyszczonych metalami ciężkimi, a także wpływ niesprzyjających warunków uprawy, takich jak: duża zawartość w podłożu cynku, ołowiu i kadmu, niewielka zawartość węgla i azotu oraz mała retencja wodna podłoża na budowę morfologiczną roślin brzozy i budowę anatomiczną liści.

Rezultaty przeprowadzonego doświadczenia założonego na hałdzie materiałów odpadowych po flotacji rud cynku i ołowiu potwierdziły duże możliwości adaptacyjne brzozy do niekorzystnych warunków uprawy, a tym samym jej przydatność do fitostabilizacji gruntów zanieczyszczonych metalami ciężkimi. Stwierdzono ponadto, że budowa anatomiczna liści brzozy może być biomarkerem skażenia metalami ciężkimi, niedoboru wody i składników odżywczych.

1. INTRODUCTION

Mining and metallurgy of non-ferrous metals usually constitutes serious environmental hazard. Anthropogenic deposits such as settling ponds, where waste material is disposed after the flotation process, contribute to the degradation of adjacent areas causing air pollu-

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tion and water contamination, as they usually contain toxic levels of heavy metals [Dmowski et al. 2002, Wysocka et al. 2005]. The problem could be overcome by introducing a protective layer of vegetation in order to stabilize the waste material. Application of trees with the aim of stabilizing the ground is also: on one hand the ecologically friendly method for increasing biomass production and on the other a form of heavy metal immobilization within the rhizosphere [Brunner et al. 2008, Mendez and Maier 2008]. However, in the case of settling ponds it is quite an uneasy task, as post-flotation tailings are characterized by unsuitable physicochemical conditions for plants and are prone to wind and water erosion [Panasiuk et al. 2007, Pietrzykowski 2005].

Specific aims of the paper were to:

- evaluate the phytostabilization potential of common birch introduced on the shelf of the settling pond formed from the material disposed after zinc and lead ores flotation,
- verify plant morphology and anatomical features of leaves taken from plants exposed to experimental treatments.

2. MATERIALS AND METHODS

A non-ferrous metal smelter located in Bukowno near Olkusz by the processing of zinc and lead ores disposed post-flotation materials in the form of settling ponds with the surface of about 109 ha and elevated 25 m above the level of the ground. First experimental plot (PD1) was established on the shelf of the active pond formed from the post-flotation materials. Second experimental plot (PD2) was located on shelf of pond, in which substrate was formed from post-flotation materials mixed with waste dolomite rock. The disposal of those wastes finished in 1979 and afterwards grass community has been established along with common birch (*Betula pendula*) and pine (*Pinus sylvestris* L.) amounted to 15% of the surface. Third, control plot (PD3), was located in the distance of 40 km from the settling ponds in the experimental plot of University of Agriculture in Kraków where seedlings were planted into the garden plant soil. In April 2004 two year old seedlings of *Betula pendula* Roth (*Betula verrucosa* Ehrh, *Betulaceae* A. Gray) were planted directly into substrate on each of the three experimental plots. Fifty plants were spaced in the distance 1·1 m. 30 cm deep holes were filled up with the respective substratum. During the whole experiment, that is from April 2004 to the end of October 2008, plants were neither tended nor fertilized.

Before starting the experiment from each of experimental plots ground samples were taken from a depth 0–30 cm from 5 points of the plot and mixed to obtained a representative sample. In air dried samples following analyses were performed: the soil reaction by potentiometer in 0.01 mol·dm^{-3} $CaCl_2$ suspension, the level of total nitrogen and carbon with the use of TOC-TN 1200 Thermo Euroglas apparatus. The level of organic carbon was calculated as a difference between total and inorganic carbon. The total contents of Cd, Pb and Zn were determined after soil digestion in the mixture of nitric and perchloric concentrated acids. Contents of the elements in solutions were assessed with the use of the atomic emission spectrometer with inductively coupled argon plasma ICP-AES JY 238 ULTRACE.

Plant samples taken after 4 years of cultivation were washed with running water to remove substrate particles from roots. After separation into aboveground and underground parts samples were rinsed with distilled water, oven dried at 105ºC to constant mass, ground and dry-ashed at 450ºC. The 2 g samples were digested in the mixture of nitric and perchloric concentrated acids. The analyses of the elements were performed in the solutions with the use of the atomic emission spectrometer 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 Metorology, Beijing, China). Studied heavy metal concentrations were analyzed in three replicated samples per plant part and variant of the experiment. The results were subjected to one way STATISTICA 7.1, ANOVA analysis and a posteriori Fisher's test was used to determine the significance of differences between studied objects with the significance level $p = 0.05$.

Three young fully developed leaves were taken from ten randomly selected plants in every treatment. Leaves were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH=7.2). After dehydratation in a graded ethanol series, the material was immersed in acetone and then embedded in Epon 812 resin. Serial sections 1 mm thick were cut on Tesla 490A rotary ultramicrotome and stained with 0.1% methylene blue. The sections were examined under Nicon Eclipse C400 microscope.

3. RESULTS AND DISCUSSION

The post-flotation material taken from the first experimental plot (PD1) was characterized by neutral reaction with pH value amounted to 7.1, and low contents of organic carbon, that was 0.72 $g \cdot kg^{-1}$, and nitrogen (0.04 $g \cdot kg^{-1}$). The substratum of the second experimental plot (PD2) revealed alkaline reaction (pH = 8.0) and a bit higher organic carbon and total nitrogen contents than the substratum of the first experimental plot, amounting respectively to 1.52 and 0.17 $g \cdot kg^{-1}$. The soil of control plot had slightly acid reaction (pH = 5.5) with the level of organic carbon amounted to 227.2 $g \cdot kg^{-1}$, and of nitrogen to 13.93 $g \cdot kg^{-1}$. Both substrates from the region of settling pond were characterized by a strong contamination with cadmium, lead and zinc (Tab. 1), exceeding manifold limits defined by the Ministry of the Environment in the directive for industrial lands regarding the soil quality and the ground quality standards [2002], but the post-flotation material from the first experimental plot was 3-fold more polluted with Zn, 7-fold with Pb and 4-fold with Cd than substrate from the second experimental plot. Contents of zinc, lead and cadmium determined in the garden plant soil of the control plot (PD3) were the lowest and within limits defined by the Ministry of the Environment for agricultural and waste lands [2002].

Table 1. Selected properties of substrates determined before the experiment
Tabela 1. Wybrane właściwości substratów przed założeniem doświadczenia

Substrates	Org. C	Tot. N	pH 0.01 $CaCl_2$	Zn	Pb	Cd
	$g \cdot kg^{-1}$			$mg \cdot kg^{-1}$		
First experimental plot (PD1), post-flotation material	0.72	0.04	7.1	10 824	6090	85.6
Second experimental plot (PD2), post-flotation material + waste rock	1.52	0.17	8.0	3980	800.7	21.4
Control plot (PD3) garden plant soil	227.2	13.93	5.5	295.2	99.2	1.4

Zinc, lead and cadmium contents determined in birch roots were statistically significantly higher than in shoots. The highest contents of zinc, lead and cadmium amounting respectively to 872.87 $mg \cdot kg^{-1}$ d.m., 239.18 $mg \cdot kg^{-1}$ d.m. and 5.22 $mg \cdot kg^{-1}$ d.m. were determined in roots of plants cultivated on the first plot situated on the shelf of the active settling pond (Tab. 2). Contents of studied trace metals determined in roots of plants cultivated in post-flotation waste material (PD1) were about 2-fold higher than those determined in roots of birch grown on post-flotation material mixed with waste dolomite rock (PD2) and about 4-fold for Zn, 10-fold for Pb and 5-fold for Cd higher than in roots of birch cultivated in the garden plant soil.

Table 2. Heavy metals contents in vegetative plant organs of *Betula pendula* cultivated on respective experimental plots after the experiment
Tabela 2. Zawartość metali ciężkich w organach wegetatywnych *Betula pendula* uprawianych na odpowiednich poletkach doświadczalnych po zakończeniu eksperymentu

Stands	Studied plant material	Zn	Pb	Cd
		$mg \cdot kg^{-1}$ d.m.		
First experimental plot PD1	root	872.9[e]*	239.2[d]	5.22[d]
	shoot	779.8[d]	128.5[c]	4.73[c]
Second experimental plot PD2	root	476.2[c]	130.2[c]	4.11[c]
	shoot	160.3[a]	25.96[b]	2.94[b]
Control plot PD3	root	223.2[b]	23.34[b]	1.01[a]
	shoot	188.6[a]	13.01[a]	1.03[a]

* The same letters indicate a lack of statistically significant differences among mean values.

Despite of the strong contamination level with heavy metals, low contents of macro-elements and very low water capacity of the bare ground made of post-flotation wastes about 67 percent of planted plants survived on the first experimental plot (PD1), which proves the opinion that common birch belongs to species of broad ecological scale and easily adapting to unfavorable conditions [Asmus 2005, Karczewska and Król 2007, Krzaklewski et al. 1994, Prus-Głowacki et al. 2006]. On the second experimental plot occurred a bit more favorable conditions for plant growth than on the first one. They resulted from higher carbon and nitrogen contents, lower heavy metal contamination level and also better water retention capacities of the substratum covered with grass than of bare one. The above mentioned properties of substratum from the second plot (PD2) reflected the higher survival abilities of material planted on that plot, which amounted to 75%. In optimal growth conditions created on the control plot (PD3) 45 seedlings out of 50 planted survived, which makes 90%.

By examinations of plants cultivated on both plots situated on settling ponds and on the control plot it was found out that above ground parts of plants were morphologically comparable. Leafy shoots were well-shaped, with regular internodes. Nevertheless observations of underground parts revealed some differences in the structure of root systems among plants from different treatments. Birches cultivated on the first experimental plot (PD1) had well developed root systems with the most numerous lateral roots and the longest main root whereas root systems of plants grown on the second plot (PD2) were usually smaller and more similar to those of plants from the control plot (PD3). These differences can be caused mainly by differentiated water conditions of the three experimental plots. A formation of well developed root systems of plants cultivated on the first experimental plot (PD1) confirmed again the suitability of common birch for phytostabilization of mine tailings especially as it is a native species, drought-, and metal-tolerant [Mendez and Maier 2008]. However, it was stated that the cultivation of plant material in substratum containing post-flotation wastes induced a number of degenerative changes in leaf anatomical features, regardless the form of substratum (PD1, PD2) used during the experiment. The leaf structure was poorly developed and there were noticed dark deposits, especially in the upper epidermis of leaves taken from plants cultivated in these materials. The microscopic observations also revealed that the leaf vascular system of trees cultivated on both plots established on settling ponds was reduced in comparison to that of plant grown on the control plot.

It should be emphasized that both substrata contaminated with heavy metals had neutral or alkaline soil reaction, thus an important share of the total content of heavy metals occurred in insoluble forms, less toxic and not making a present hazard to plants growing on them [Asmus 2005, Brunner et al. 2008, Karczewska and Król 2007, Krzaklewski et al. 1994]. In such conditions the expected phytostabilization potential of common birch refers more to reduce exposure of the ground to wind, water and direct contacts with humans than to accumulation of heavy metals in plant tissues and in the ground around roots making them insoluble and/or immobilized on soil components. Therefore, it can be said that the inappropriate substrata properties resulted in a similar degree from a high heavy metal contamination likewise poor water conditions and lack of nutrients. That is why, a well known broad ecological amplitude of common birch, especially concerning nutrients, light and water requirements which makes of it a pioneer forest species, has become so essential [Asmus 2005, Karczewska and Król 2007]. The results of the study confirmed that common birch is able to grow over areas where other less resistant plants can’t survived and to act as a barrier to erosion and exposure of the contaminated ground. A statement is proved even by a rather moderate capacity of common birch to extract cadmium, zinc and lead from the substratum and accumulate them into shoots, as unlike phytoextraction, or hyperaccumulation of metals into root and shoot tissues phytostabilization primarily focuses on sequestration of the metals within the rhizosphere but not in plant tissues [Mendez and Maier 2008]. In that way a common birch meets the requirements of phytostabilization which is in accordance with researches and previous preliminary experiments conducted for a number of years [Krzaklewski et al. 1994, Pietrzykowski 2005, Prus-Głowacki et al. 2006].

4. CONCLUSIONS

1. Common birch meets the requirements of phytostabilization of post-flotation tailings in view of : high survival abilities, undisturbed plant morphology as well as moderate capacities to extract heavy metals.
2. Anatomical features of common birch leaves can be treated as biomarker of substrata properties such as heavy metal contamination, nutrient level or water availability.

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