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HEAVY METALS CONTENT AND BIOCHEMICAL INDICATORS IN BIRCH LEAVES FROM POLLUTED AND CLEAN AREAS

ZAWARTOŚĆ METALI CIĘŻKICH ORAZ WSKAŹNIKI BIOCHEMICZNE W LIŚCIACH BRZOZY Z TERENÓW ZANIECZYSZCZONYCH I CZYSTYCH

Abstract: There were conducted studies concerning accumulation of heavy metals (Fe, Zn, Cd and Pb) in *Betula pendula* Roth. leaves, in surface soil within cities of Silesian and Małopolska District. Additionally, there was studied guaiacol peroxidase activity and content of -SH groups.

Concentrations of heavy metals in birch leaves in most cases did not exceed permissible values. There was noticed an increased Zn content (above 100 mg/kg d.m.) in *Betula pendula* Roth. leaves on the all studied stands of Silesia. Accumulation of Fe, Zn, Cd and Pb in soils of the Silesian cities often exceeded the permissible level. The conducted studies showed that there is a positive correlation between Zn accumulation in white birch leaves and its accumulation in surface soil. There were not noticed significant differences in content of non-protein -SH groups and guaiacol peroxidase activity in *Betula pendula* ROTH. leaves coming from Silesia and Małopolska District.

Keywords: *Betula pendula* Roth., heavy metals, guaiacol peroxidase, -SH groups

Introduction

Because of the increasing population and industrialization plants are affected by a wide array of substances, inter alia heavy metals, that contaminate air, water and soil [1].

Biological material such as lichens, tree bark, tree rings and leaves of higher plants had been used to detect the deposition, accumulation and distribution of metals [2]. Higher plants are usually not as suitable biomonitors as lichens and mosses are often missing, higher plants can act as biomonitors. In industrial and urban areas higher plants

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can give better quantifications for pollutant concentrations and atmospheric deposition than non-biological samples [3]. Foliage analysis has been used as a valid indicator of air pollution and in order to identify and possible polluted areas classification according to their pollution level [2].

Defending against heavy metal contamination plants developed defense mechanisms which allow them to grow and expansion in the contaminated environment. Within the confines of heavy metal tolerance plants separate toxic compounds in vacuole (sequestation), there are induced antioxidant enzymes *eg* superoxide dismutase, catalase and peroxidase [4], plants also release compounds complexing metals such as phytosiderophores, organic acids, free amino acids, peptides such as glutathione, phytohelatins and metallothioneins [5, 6]. Ecophsiological changes in the urban trees may be used as heavy metal stress biomarkers [1]. Activities of antioxidant enzymes in trees have been reported in the literature and used as in indicator in pollutions monitoring [7]. Similarly non protein compounds rich in -SH groups which are important factors determining plant tolerance to heavy metals ions [8].

Betula pendula Roth. is a common species found in urban parks in many cities in Poland. Also silver birch has proved to be a good bioindicator of heavy metals in contaminated environments of both natural and anthropogenic origin [9].

The aim of this work was evaluation of the heavy metals concentration in *Betula pendula* Roth. leaves and in soil in polluted area in Silesia in comparison to a potentially unpolluted area of Małopolska. Looking for indicators of stress caused by heavy metals we studied the activities of guaiacol peroxidase and non-protein -SH groups content.

Materials and methods

Material (*Betula pendula* Roth. leaves and soil) were collected in June and July 2008 from the eight parks localized on: Silesian area (Silesian Park of Culture and Recreation in Chorzow, urban park in Szopienice, Bytkowski Forest, Alfred's Park) recognized as a polluted area and from Malopolska District (urban park in Jaszowice, urban park in Brzeszcze, urban park in Bobrek and ethnographic park in Wygielzow) which was recognized as an area potentially free from heavy metal pollution.

Soil sampling

Soil Samples were taken from the two depths: 0–10 cm and 10–20 cm. The soil samples were air-dried and sieved through a 1 mm sieve. Metals were extracted with 10 % HNO_3 [10]. The concentration of metals (Zn, Cd, Pb and Fe) was measured with flame absorption spectrometry (Unicam 939 Solar). The quality of the analytical procedures was controlled using reference material (Certified Reference Material CTA-OTL-1 Oriental Tobacco Leaves).

Plants samples

Leaves of *Betula pendula* Roth. were collected randomly from ten trees in each of the parks.

Leaves were washed in tap and distilled water, dried at 105 ^{o}C to a constant mass and grounded to a fine powder, then dry mineralized at 450 ^{o}C and dissolved in 10 % HNO_3. After filtration the metal (Zn, Cd, Pb and Fe) contents was determined by the flame Atomic Absorption Spectrometry [10]. The quality of the analytical procedures was controlled using reference material (Certified Reference Material CTA-OTL-1 Oriental Tobacco Leaves).

To measure the contents of -SH groups, the plant material was homogenized in a 5 vol/g mixture containing 2 % 5-sulphosalicylic acid, 1 mM EDTA and 0.15 % sodium ascorbate. The extract was centrifuged at 20 000 × g for 10 min. The absorbance at 415 nm was read 1 min after addition DTNB (5,5'-dithio-bis(2-nitrobenzoic acid). The number of non-protein -SH groups was calculated from the standard curve prepared using L-cysteine and expressed as nmol -SH g^{-1} fresh mass [11].

Guaiacol peroxidase POD activity was measured according to Fang and Kao [12]. Leaf tissues were homogenized with 100 mM sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12000 × g for 20 minutes. The POD activity was measured in a reaction mixture that contained enzyme extract, phosphate buffer (pH 5.8), H_2O_2 and guaiacol. The increase in absorbance was recorded in the spectrophotometer at 470 nm and a unit of peroxidase activity was expressed as a change in absorbance per minute and per gram fresh mass of tissue.

Statistical analysis

The data were processed using software Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tuckey's multiple range test and to compute Pearson correlation coefficients.

Results and discussion

Contents of the studied heavy metals in soil from levels of 0–10 cm and 10–20 cm and in *Betula pendula* Roth. leaves are presented in the Table 1 and 2.

On the all studied sites localized in Silesia there was noticed an outpass of permissible values given by Kabata-Pendias and Pendias [13] for the studied elements. Heavy metal content in the parks of Malopolska was within limits recognized as normal values.

Pb content in soil closely depends on mineralogical and granulometric composition and derivation of soil bedrocks but simultaneously occurrence of this element in soil surface is, first of all, connected with anthropogenic factors. Pb content in unpolluted soils should amount to 20 mg/kg, however Gambus and Gorlach [14] increase this range to 25 mg/kg, giving also Pb content for the polluted soils within 4560 mg/kg. Permissible Pb concentration in soil is 100 μg/g [13]. Lukasik et al [15] in studies on

Table 1

The concentrations of heavy metals in fractions of the soils extracted with HNO_3 [$mg \cdot kg^{-1}$ air-dry mass], average ±SD

Site \ Metal	Fe [mg/kg]		Zn [mg/kg]		Cd [mg/kg]		Pb [mg/kg]	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
SPCR	3031.7 ± 134.6^{a}	2010 ± 152.3^{a}	1526.7 ± 66.1^{a}	1171.3 ± 112.4^{a}	15.7 ± 0.2^{a}	14.59 ± 0.1^{a}	619.9 ± 46.8^{a}	387.03 ± 8.5^{a}
Park in Szopienice	2341 ± 124.5^{b}	954.4 ± 52.5^{b}	1804.7 ± 74.3^{b}	714.4 ± 58.4^{b}	18.6 ± 0.7^{b}	8.2 ± 0.3^{b}	420.5 ± 23.7^{b}	209.9 ± 7.7^{b}
Bytkowski Forest	3550 ± 18.4^{c}	3779.3 ± 76.0^{c}	1926.0 ± 13.7^{c}	2181 ± 50.6^{c}	29.6 ± 1.6^{c}	43.2 ± 0.6^{c}	991.8 ± 50.1^{c}	1145.3 ± 72.9^{c}
Alfred's Park	3345 ± 135.5^{c}	3137.5 ± 91.2^{d}	1737.7 ± 29.1^{b}	1343.5 ± 51.6^{a}	14.3 ± 0.06^{a}	12.5 ± 0.3^{d}	437.1 ± 0.07^{d}	393.2 ± 0.3^{d}
Park in Jawiszowice	906.6 ± 71.0^{d}	1056.7 ± 120.1^{bc}	65.2 ± 2.6^{d}	47.1 ± 4.5^{d}	0.76 ± 0.02^{d}	0.5 ± 0.02^{e}	23.9 ± 0.2^{e}	20.9 ± 0.4^{e}
Park in Brzeszcze	992.2 ± 66.2^{d}	1214.3 ± 164.0^{be}	78.4 ± 0.7^{d}	157.1 ± 13.4^{e}	0.82 ± 0.02^{d}	1.98 ± 0.4^{g}	41.9 ± 1.3^{f}	103 ± 2.9^{f}
Park in Bobrek	1829.5 ± 243.9^{e}	2687 ± 136.8^{f}	55.5 ± 7.1^{d}	69.7 ± 4.2^{f}	1.3 ± 0.1^{d}	1.4 ± 0.07^{g}	46.7 ± 1.4^{f}	52.6 ± 1.4^{g}
Park in Wygielzow	696.2 ± 69.5^{f}	388.8 ± 47.0^{g}	94.2 ± 2.2^{e}	32.2 ± 11.5^{d}	2.4 ± 0.5^{d}	1.18 ± 0.07^{g}	83.7 ± 1.9^{g}	36.97 ± 0.9^{h}

* a, b – values with the same letter in one column are statistically the same for $p < 0.05$.

the area of Piekary Slaskie find 4–25-fold outpass of permissible standards. In surface soil there was noticed accumulation on the level of 1506,2 mg/kg. Nadgórska-Socha et al [16] in the vicinity of the Nonferrous Smelting Plant "Szopienice" noticed values reaching up to 1350 mg/kg. Kabata-Pendias, Pendias [13] state that in Upper Silesia Pb concentration can reach from 6000 to 8000 mg/kg of soil.

Table 2

The concentrations of heavy metals in the leaves of *Betula pendula* Roth. [$mg \cdot kg^{-1}$ dry mass]

Metal / Site	Fe		Zn		Cd		Pb	
	Average	SD	Average	SD	Average	SD	Average	SD
SPCR	20.8a	0.7	178.3c	0.8	0.8bc	0.1	3.4d	0.3
Park in Szopienice	84.1d	4.3	177.7c	1.0	1.2d	0.2	9.5e	0.5
Bytkowski Forest	31.4b	5.4	171.5d	4.3	0.6ab	0.2	6.5f	1.4
Alfred's Park	60.1c	3.9	166.8d	1.2	0.3a	0.1	9.1e	0.4
Park in Jawiszowice	39.8b	3.4	139.2a	2.7	0.4a	0.03	1.2a	0.1
Park in Brzeszcze	44.0bc	13.3	116.3b	0.4	0.2a	0.01	2.2b	0.1
Park in Bobrek	33.2b	6.2	131.2a	7.0	0.9cd	0.2	2.3b	0.1
Park in Wygielzow	30.8b	0.3	134.2a	0.3	0.5ab	0.004	1.8b	0.2

* a, b – values with the same letter in one column are statistically the same for $p < 0.05$.

To the most Cd polluted soils belong soils of southern Poland, especially Silesia and Malopolska Districts. From monitoring studies of Terelak et al [17] it results that soils of higher than natural Cd content make up 67.3 % in Silesia and 45.3 % in Malopolska. The range of mean Cd contents in world soils is within 0.2–1.05 mg/kg, for Poland it amounts to 0.2 mg/kg [13]. In soils of Silesian parks the stated Cd concentrations were higher than the permissible one (4 mg/kg) [13]. Many authors show that on polluted areas Cd content can strongly exceed the norms. Relatively high Cd content was reported by Nadgórska-Socha et al [16] in the closest vicinity of Nonferrous Smelting Plant "Szopienice", the zinc wastes heap and a former galmey site were Cd content was within 43.3–123 mg/kg.

Mean Zn concentration in soils of different world countries is within 30–120 μg/g. Permissible Zn content in soil is 300 mg/kg [13]. Lukasik et al [15] in 5 parks on the area of Piekary Slaskie noticed in surface soil 0–5 cm Zn content of 4937 mg/kg, 10–20 cm 4053.2 mg/kg. In Krakow region the mean Zn concentration in the studied surface soils was 104.2 mg/kg, varying in the range of 36.1–732.0 mg/kg [14].

Fe content in natural soil solutions amounts to 470 mg/kg on average [13]. In soils samples collected from the eight parks higher than natural content of Fe was found. The Fe concentrations obtained in this study are higher than the ones obtained by Jankiewicz and Adamczyk [18] in the soil samples collected in the area of the city of Lodz.

The order of concentration Zn > Fe > Pb > Cd was found in leaves of *Betula pendula* Roth. on the investigated areas.

In case of Cd, Pb and Fe obtained concentrations in birch leaves did not exceed values recognized as toxic ones (respectively for Cd 5–30 mg/kg d.m., for Pb 30–300 mg/kg d.m. and Fe 200–400 mg/kg d.m.). Only Zn content was within the range recognized as a toxic one (100–400 mg/kg d.m.) [13].

In case of Pb obtained values were considerably lower than concentrations noticed by Łukasik et al [15] in *Acer pseudoplatanus* L. leaves (13–51 mg/kg d.m.) and *Robinia pseudoacacia* L. (38–53 mg/kg d.m.) growing in urban parks in Piekary Slaskie. Comparable values to the ones obtained in parks of Malopolska were noticed by Nadgorska-Socha et al [2] where in *Philadelphus coronarius* L leaves from urban parks of Cracow and Zagaje Stradowskie Pb content was within 2.39–3.14 mg/kg d.m.

Cd is not an element which plants need for their development, however it is extremely easily collected by root system and leaves, in general, proportionally to concentration in the environment [13]. Cd values not exceeding toxic values and lower than the ones in the present study were noticed by Nadgorska-Socha et al [2] in *Philadelphus coronarius* L. leaves collected from urban parks of Malopolska.

Birch is not mentioned in literature as a hyperaccumulator, however it shows exceptional relationship with Zn, much bigger than other tree species [19]. Steinnes et al [20] determined Zn content in *Betula pubescens* Ehr. leaves from Kola peninsula as 210 mg/kg d.m. on average. Hrdlicka et al [21] notice Zn content in *Betula pendula* ROTH leaves growing in little polluted environment in Rudawy amounting to 189 mg/kg d.m on average, and in the vicinity of a nonferrous smelting plant in Bukowno near Olkusz – amounting to 750–930 mg/kg.

In comparison to results of other authors Zn accumulation in *Betula pendula* Roth. leaves on the studied sites is not high. Higher Zn concentrations in *Betula pendula* Roth. leaves in noted Lukasik et al [15] in *Acer pseudoplatanus* L. and *Robinia pseudoacacia* L. leaves in parks on the area of Ruda Slaska where Zn concentration was within 141–342 mg/kg d.m.

Fe content in plants changes considerably in vegetative period to a different extend in individual plant organs, the most often it is between 10–400 μg/g d.m. Petrova [22] on the area of the town of Plovdiv, in *Betula pendula* Roth. Leaves, noticed Fe content from 89.3 mg/kg to 248.8 mg/kg.

In the study there was not noticed an increase of guaiacol peroxidase activity (Fig. 2) or an increase of number of non-protein SH groups (Fig. 1) on areas of a high degree of heavy metal pollution in relation to areas recognized as potentially free from pollution. The both indicators were on the similar level, regardless of a study site which the studied birch leaves came from.

Guaiacol peroxidase belongs to a big group of peroxidases taking part in the system of antioxidant defense of a plant cell [23]. Peroxidase activity is often used as a potential indicator of metal toxicity [24] However, as in this study also Pongrac et al [25] reported no change in GPX activity in *Thalspsi praecox* and *T. caerulescens* in the presence of Cd and Zn, similar results obtained Gratao et al [26] in leaves, roots and fruits of tomato grown in conditions of cadmium contamination. Baycu et al [1], examining the activity of POD in the leaves of Acer and Alianthus growing in urban

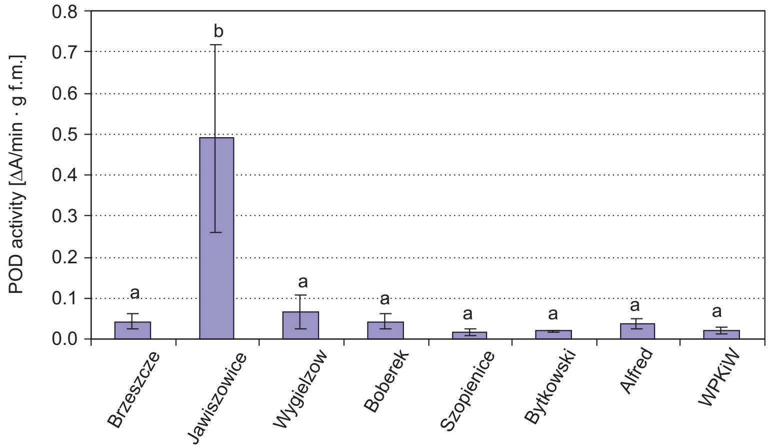

Fig. 1. Mean POD activity in leaves of *Betula pendula* Roth. Values with the same letter are statistically the same for $p < 0.05$

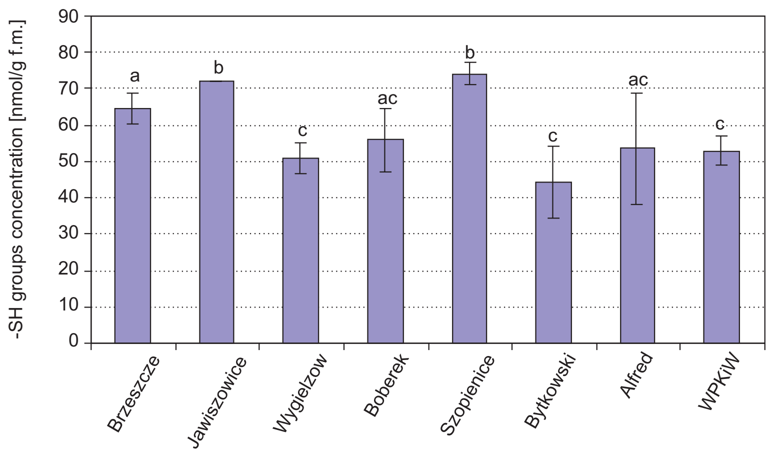

Fig. 2. Mean -SH groups concentration in leaves of *Betula pendula* Roth. Values with the same letter are statistically the same for $p < 0.05$

parks in Turkey, said both increase and decrease in peroxidase activity compared to control.

Many authors *eg* [8, 26,] reported an increase in number of non-protein-SH groups in response to elevated trace metal content which was not observed in the present study. Perhaps it was due to a small accumulation of metals studied in *Betula pendula* Roth. leaves or increased resistance of this species on these metals and the problem requires further study.

Conculsions

The obtained heavy metals concentrations in *Betula pendula* Roth. leaves did not exceed the levels considered as toxic for plants and because of this in the study we haven't observed the growth of non -SH groups level and peroxidase activity at contaminated sites. The environmental studies of ecophysiological parameters seem to be an essential in bioindication and also necessary for confirmation obtained results in controlled conditions and should be continued.

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ZAWARTOŚĆ METALI CIĘŻKICH ORAZ WSKAŹNIKI BIOCHEMICZNE W LIŚCIACH BRZOZY Z TERENÓW ZANIECZYSZCZONYCH I CZYSTYCH

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Abstrakt: Przeprowadzono badania dotyczące kumulacji metali ciężkich (Fe, Zn, Cd, Pb) w liściach brzozy brodawkowatej i w wierzchniej warstwie gleby na terenie miast Slaska oraz Małopolski. Dodatkowo w liściach *Betula pendula* Roth. badano aktywność peroksydazy gwajakolowej i zawartość niebiałkowych grup -SH.

Koncentracje metali ciężkich w liściach brzozy w większości przypadków nie przekraczały wartości dopuszczalnych. Odnotowano podwyższoną zawartość Zn (powyżej 100 mg/kg s.m.) w liściach *Betula pendula* Roth. na wszystkich badanych terenach Śląska. Kumulacja Fe, Zn, Cd, Pb w glebach miast Śląska często wielokrotnie przekraczała poziom dopuszczalny. Przeprowadzone badania wykazały, że istnieje dodatnia korelacja pomiędzy kumulacją Zn w liściach brzozy brodawkowatej a jego kumulacją w wierzchniej warstwie gleby. Nie odnotowano wyraźnych różnic w zawartości niebiałkowych grup -SH i aktywności peroksydazy gwajakolowej w liściach *Betula pendula* Roth. pochodzących ze Śląska i Małopolski.

Słowa kluczowe: *Betula pendula* Roth., metale ciężkie, peroksydaza gwajakolowa, grupy -SH