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THE COMPARISON BIOINDICATION METHODS IN THE ASSESSMENT OF ENVIRONMENTAL POLLUTION WITH HEAVY METALS

PORÓWNANIE METOD BIOINDYKACYJNYCH W OCENIE ZANIECZYSZCZENIA ŚRODOWISKA METALAMI CIĘŻKIMI

Key words: biomonitoring, heavy metals, birch, Scots pine, moss bags.
Słowa kluczowe: biomonitoring, metale ciężkie, brzoza, sosna zwyczajna, moss-bag.

Summary

The research applied four methods frequently used in bioindication of environmental pollution with heavy metals: the determination of accumulation in the exposed Sphagnum fallax moss - the "moss-bag" transplant method, in growing Pleurozium schreberi moss, in Betula pendula (Roth.) leaves and Pinus sylvestris L. needles. The research was conducted in the region of Olkusz, influenced by high level emissions originating from long range transport and from local sources, among them a zinc and lead ore mine and smelter, with mounds of smelter wastes 25–30 m high, covering an area of 10.9 ha.
The objective of this work was to compare the four bioindicator methods in the evaluation of the environmental pollution with Cd, Pb, Zn, Cr, Fe, Cu, Ni and As. Among the factors differentiating the absolute result levels of metal accumulation were, first of all, the time of

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exposure deriving from the specifics of the bioindicator method applied, the source of pollution (air, soil), and the features (morphological build) of the indicator plant. The environmental pollution results were presented in the form of maps, with isolines differentiating the zones of pollution proportionately to the metal content levels found in specific bioindicators. The statistical analysis of the results indicated strong dependencies of the results on the applied methods in the case of high levels of pollution, and showed highly differentiated results concerning Cd, Pb, Zn, and As.

Streszczenie

W badaniach zastosowano cztery często stosowane metody bioindykacji zanieczyszczenia środowiska metalami ciężkimi: określenie akumulacji w eksponowanym mchu Sphagnum fallax – metoda transplantacyjna moss-bag, w rosnącym mchu Pleurozium schreberi i w liściach Betula pendula (Roth.) oraz igłach Pinus sylvestris L. Badania wykonano w rejonie Olkusza, będącego pod wpływem dużych emisji pochodzących z dalekiego transportu i lokalnych źródeł, m.in. z zakładu wydobycia i przetwórstwa rud cynkowo-ołowiowych oraz hałdy odpadów hutniczych wysokość 25–30m i powierzchni 10,9 ha. Badania miały na celu porównanie czterech metod bioindykacyjnych w ocenie zanieczyszczenia środowiska: Cd, Pb, Zn, Cr, Fe, Cu, Ni i As. Czynnikami różnicującym bezwzględny wynik poziomu akumulacji metali był przede wszystkim czas ekspozycji, wynikający ze specyfiki zastosowanej metody bioindykacyjnej, źródło zanieczyszczenia (powietrza, gleba) oraz cechy (budowa morfologiczna) rośliny wskaźnikowej. Wyniki zanieczyszczenia środowiska przedstawiono w formie map z izoliniami, różnicującymi strefy zanieczyszczenia proporcjonalnie do skali zawartości metali w poszczególnych bioindykatorach. Analiza statystyczna wyników wykazała silne zależności wyników zastosowanych metod przy wysokim poziomie zanieczyszczenia i dużym zróżnicowaniu wyników. Dotyczyło to: Cd, Pb, Zn, i As.

1. INTRODUCTION

Environmental hazards, brought about by environmental pollution, are accompanied by the attempts to establish control programs on the national, regional and global scales.

Deposition measurement requires long term sampling and high number of measurement points. The possibility of carrying out such measurements with the use of monitoring equipment is limited, mainly in view of high costs and lack of appropriately sensitive and inexpensive technologies, which would allow for the simultaneous measurement of many substances polluting the air [Pucket 1988].

In Poland, technical measurements of the air pollution deposition carried out in the framework of the National Environmental Monitoring, are mainly performed in towns. The number of measurement points within the agricultural and forest areas is marginal. The monitoring system is focused mainly on measurement of gaseous air pollution (sulphur dioxide and nitrogen oxides) and air borne dusts. Within the forest monitoring system the measurements are performed by passive methods for air pollution measurement, however, only measurements of gaseous pollutants are carried out [Wawrzoniak 2004].

Application of the bioindication methods offers an opportunity of a significant intensification of studies on air pollution, especially with the trace elements. Biomonitoring in a general sense can be defined as the use of plants and animals with the objective of gaining quantitative and qualitative information on certain characteristics of the biosphere [Wolterbeek 2002].

The goal of this work was to evaluate different bioindication methods that provide a biologically-based complementary information for the mandatory environmental monitoring of metals. Application of four bioindication methods allowed for a more complete determination of environmental pollution for an area of extreme historical contamination with metals, originating from long-range transport as well as local sources.

2. MATERIALS AND METHODS

The study area is a region around the town of Olkusz and is one of the oldest European centers for the metallurgic industry. It is situated between three large industrial regions. On the west and east, it borders the Upper Silesian Industrial Region and the Cracovian Region, respectively, and from the south, it is under the influence of emissions from Moravia (Czech Republic), reaching the area through the Moravian Gate Pass (Fig. 1). These three regions are characterized by high concentrations of plants belonging to industries that are particularly harmful to the environment (power generation, steel, and non-ferrous industrial extraction and processing, power production, mining, chemical and cement production). One of the most important industrial plants in the area is the mining and metallurgical Bolesław complex, located in the region of Bukowno near Olkusz. This plant started functioning in 1952. The study area encompasses, in particular, the location of the waste heap from the refining process of the zinc and lead ores. The dust from this heap (height of 25–30 m, area of 109 hectares), contributes to the contamination of the surrounding areas [Godzik 1993; Dmowski and Badurek 2002].

Four bioindicative methods were employed in the research. They encompassed the determination of the accumulation of metals in the following: exposed *Sphagnum fallax* moss [the moss bags method, Goodman and Roberts 1971], growing *Pleurozium schreberi* moss [Grodzińska et al. 1999], silver birch leaves *Betula pendula* Roth. [Dmuchowski 2005] and Scots Pine *Pinus silvestris* L. needle [Dmuchowski and Bytnerowicz 1995].

The materials used for the analyses (moss, needles and leaves) were collected in the second half of July. Bags with peat moss were left for 12 weeks. Reference samples consisted of Scots pine needles, silver birch leaves and *P. schreberi* and *S. fallax* mosses collected in the Augustów Primeval Forest, which is considered (in relative terms) the least polluted area in Poland.

The study area was situated to the east of Olkusz, and its western border reached the suburbs of the town (Fig. 1) and was 14 km x 14 km, divided into 49 smaller squares of 2 km x 2 km. The sampling locations were selected to meet two criteria: the appropriate pines, birches and mosses (within the distance of at most 100 m) had to be present and they had to be at least 300 m from the main roads with heavy traffic. Four additional monitoring sites were selected near Bolesław complex.

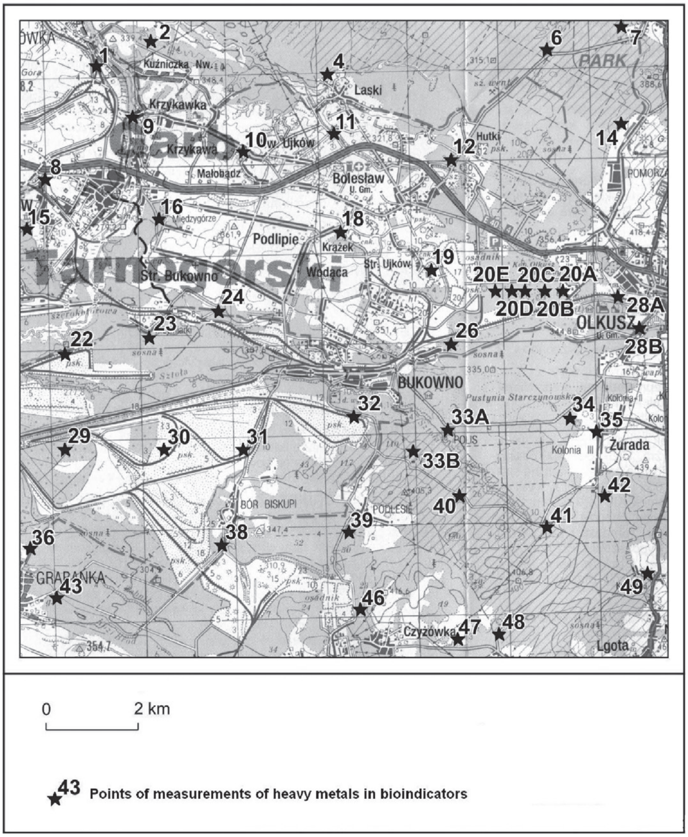

Fig. 1. Locations of the measurement points in the surroundings of Olkusz

Rys. 1. Lokalizacja punktów pomiarowych w okolicy Olkusza

Needles and leaves were washed for one minute in distilled water before being dried and ground. The powdered samples were dry-mineralized in a muffle oven. The analyses were performed by flame AAS (Perkin Elmer 1100A) and arsenic with generation of hydrides using the FIAS 200 countershaft [Perkin Elmer 1990].

The results are presented as maps of heavy metal pollution deposition. All of the maps were drawn digitally with the use of the specialized MapInfo software.

Basic statistical parameters (medians, min., max.) were calculated for examined variables.

Correlation coefficients were used for evaluation of relationships between four bioindication methods. Statistical analyses were performed using Statistica 7.0 (Statsoft inc.). Level of significance was set up at 0.05.

3. RESULTS AND DISCUSSION

The results of environmental pollution are presented in the form of maps with respective isoquants. These isoquants differentiate the zones of pollution in proportion to the scale of heavy metal concentration, accumulated in bioindicators.

Table 1 summarizes the properties of the applied bioindication methods, defining what source of contamination the results of the measurements reflect. The main factor that determines the absolute values of measurements in relation to the degree of environmental pollution, expressed through the level of accumulation of heavy metals in the bioindicator plants is the time of exposure. Level of heavy metal accumulation results also from the specific features of the bioindication method applied, the source of contamination (air, soil) and the properties (morphological structure) of the biomonitoring plant.

Table 1. Properties of the bioindication methods applied
Tabela 1. Właściwości zastosowanych metod bioindykacyjnych

Method	Period of exposure	Source of contamination
Moss-bags method	12 weeks	air
Accumulation in the moss *Pleurozium schreberi*	2–3 years	air
Accumulation in birch foliage	3 months	air and soil
Accumulation in pine needles	14 months	air and soil

Table 2 provides the ranking of the biomonitors in terms of the levels of metal accumulation from the lowest to the highest values. The comparison accounts for: the medians for all the measurements, the minimum and the maximum values, the reference material values (for the samples collected in the Augustów Primeval Forest) and ratio median/control. In the polluted area in the vicinity of Olkusz the levels of accumulation of metals were the highest, and this applied to almost all measured metals and all the biomonitors used.

The metals contained in the birch leaves and in the pine needles originate from the contamination of the air and the soil. The levels of accumulation of metals in the birch and pine foliage differed because of the difference in the duration of exposure and possibly due to differences between surface area/biomass ratio and pubescence of birch leaves help-

Table 2. Comparison of heavy metal concentration in the bioindicators in the polluted and the control area

Tabela 2. Porównanie zawartości metali ciężkich w bioindykatorach w obszarach zanieczyszczonych oraz obszarach kontrolnych

Heavy metal	Pb	Cd	Zn	Cu	Fe	Cr	Ni	As
Moss bags								
Median	17.3	1.33	143	3.6	425	1.64	1.2	0.48
Minimum	7.2	0.39	54	2.6	276	1.13	0.98	0.15
Maximum	461	18.67	2352	8.6	3630	4.59	3.47	17.1
Control	2.5	0.16	35	3.9	252	0.98	0.95	0.11
Median/control	6.9	8.3	4.1	0.9	1.7	1.7	1.3	4.4
P. schreberi								
Median	158	6.41	446	12.8	2710	6.38	4.45	1.47
Minimum	48.8	3.27	129	6.4	1176	2.37	1.5	0.23
Maximum	1096	17.7	1990	23.6	5431	22.5	12.3	19
Control	3.1	0.16	42	4.2	243	2.2	1.37	0.21
Median/control	51.0	40.1	10.6	3.0	11.2	2.9	3.2	7.0
Scots pine								
Median	13	1.22	101	3.5	207	0.9	0.84	0.45
Minimum	3.7	0.28	48	2.7	83	0.37	0.39	0.13
Maximum	255	5.78	548	5.9	614	3.31	3.27	9.05
Control	1.3	0.12	34	2.5	67	0.35	0.36	0.11
Median/control	10.0	10.2	3.0	1.4	3.1	2.6	2.3	4.1
Silver birch								
Median	14.8	2.88	838	5.9	214	1.28	1.37	0.37
Minimum	4.1	0.75	253	4.4	99	0.86	0.61	0.11
Maximum	255	8.61	2436	7.8	1398	3.99	7.15	6.44
Control	1.7	0.19	321	3	75	0.66	0.68	0.1
Median/control	8.7	15.2	2.6	2.0	2.9	1.9	2.0	3.7

ing more effective adsorption and absorption of metals. Birch leaves accumulated somewhat more of metals than the pine needles. This applied to the reference trees and to the minimum, maximum, and median values for the trees from the polluted area. Exception ally high accumulation was determined for zinc, whose concentration in birch leaves was 5–8 times higher than in the pine needles. Silver birch displays an exceptional association with zinc for the plant kingdom [Danny and Wilkins 1987; Dmuchowski et al. 2010]. The literature search has not shown any publication dealing with this phenomenon and explaining it. However the pine needles accumulated more arsenic than the birch leaves.

The metals found in the moss originated from air pollution. The period of exposure of moss *Pleurozium schreberi* growing in the field, was about three years longer than the exposure the moss *Sphagnum fallax* used in the moss-bags transplant method. Mainly this methodological difference resulted in the higher accumulation of metals in the growing moss *Pleurozium schreberi* than in the moss-bag exposed *Sphagnum fallax*. The differences observed were particularly pronounced in accumulation of lead, chromium and copper.

Table 3. Correlation coefficients between contamination for heavy metals in four bioindicators
Tabela 3. Współczynniki korelacji pomiędzy zawartością metali ciężkich w czterech bioindykatorach

Bioindicators	Moss bags	Silver birch	Scots pine
Pb			
Silver birch	**0.73**		
Scots pine	**0.87**	**0.84**	
P. schreberi	**0.71**	**0.94**	**0.80**
Cd			
Silver birch	**0.61**		
Scots pine	**0.81**	**0.72**	
P. schreberi	**0.68**	**0.49**	**0.65**
Zn			
Silver birch	**0.67**		
Scots pine	**0.73**	**0.62**	
P. schreberi	**0.65**	**0.44**	**0.78**
Cu			
Silver birch	0.14		
Scots pine	**0.47**	**0.35**	
P. schreberi	0.25	**0.43**	**0.47**
Fe			
Silver birch	**0.56**		
Scots pine	**0.56**	**0.60**	
P. schreberi	**0.42**	**0.66**	**0.58**
Cr			
Silver birch	0.04		
Scots pine	**0.35**	0.27	
P. schreberi	0.07	-0.04	0.11
Ni			
Silver birch	0.20		
Scots pine	0.27	**0.41**	
P. schreberi	0.04	0.06	0.03
As			
Silver birch	**0.51**		
Scots pine	**0.59**	**0.73**	
P. schreberi	**0.58**	**0.39**	**0.72**

Significant correlations at $P<0.05$ are in bold.

The periods of exposure of moss *Sphagnum fallax* in the moss-bag transplant method and of the birch foliage were similar, roughly three months. Birch foliage accumulated less lead, iron, chromium, nickel and arsenic, while it accumulated more zinc, and also cadmium and copper. This concerned all the values for the polluted area and for the reference samples.

The region of Olkusz, and especially the surroundings of the lead and zinc foundry in Bukowno, should be considered as extremely strongly contaminated with heavy metals, in particular – with lead, cadmium, zinc and arsenic.

Table 3 presents correlations coefficients between pairs of bioindication methods for each heavy metal separately. On the basis of these results we can observe very strong correlations for Pb, Cd and Zn i.e. metals which have high level of contamination. Correlations for heavy metals which are of lower level are weaker. The weakest correlations were observed between Cr and Ni.

Table 4. Mean values of correlation coefficients for all pairs of bioindicators for each heavy metals
Tabela 4. Średnie wartości współczynnika korelacji wszystkich par bioindykatorów dla średniej zawartości metali ciężkich

Heavy metals	Pb	Cd	Zn	As	Fe	Cu	Ni	Cr
Mean value of correlation coefficients	0.82	0.66	0.65	0.59	0.56	0.35	0.17	0.13

Table 4 presents the mean values of correlation coefficients between all bioindication methods in terms of determining contamination with particular heavy metals. The average values of the Pearson correlation coefficient and of the determination coefficient over all the pairs of the bioindication methods for the individual heavy metals, ranked according to the strength of relationships, are presented from the strongest to the weakest correlation.

Patterns of contamination for Pb and Ni for all bioindication methods are presented in the maps (Fig. 2). These two heave metals were chosen because they present quite opposite results for bioindication methods.

The pattern of contamination for Pb (Fig. 2A) for all bioindicatation methods is very similar and the level of contamination is very high while for Ni (Fig. 2B) pattern of contamination for each method is various and the level of contamination is very low.

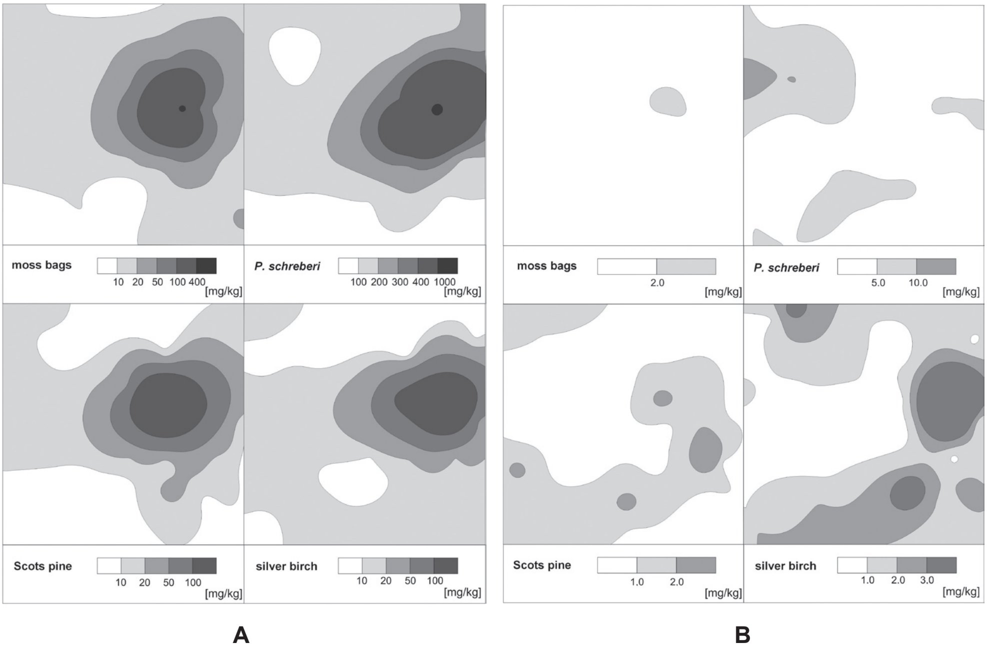

Fig. 2. Contamination of the environment with Pb (A) and Ni (B) based on the accumulated concentration of this element in the bioindicators

Rys. 2. Zanieczyszczenie środowiska Pb (A) i niklem (B) w oparciu o zakumulowane zawartości tych elementów w bioindykatorach

4. CONCLUSIONS

1. It was shown that the region of Olkusz, and especially the surroundings of the mining and metallurgic complex "Bolesław", should be considered as extremely strongly contaminated with lead, cadmium and zinc. The thresholds of toxicity for plants, which are less sensitive to zinc than humans, have been exceeded in this area.
2. The time period of exposure is the primary factor that determines the expression of the absolute level of environmental pollution through the concentration of accumulated heavy metals in the bioindicators. The level of accumulation also results from the specific features of the bioindication methods applied, followed by the source of contamination (air, soil) and the properties (morphological and anatomical structure) of the indicator species.
3. The accumulation of metals was the highest in the moss *Pleurozium schreberi*, as shown by all the statistical indices used (mean values for all measurements, medians, minimum and maximum values). The exception was the concentration of zinc, which was the highest in the birch foliage.

4. The strongest correlations between four bioindication methods were observed for the heavy metals with high contamination level (Pb, Cd). The weakest correlations were observed between heavy metals with low contamination level (Ni, Cr).
5. Relationships between rate median/control (median of contamination level divided by contamination in not polluted area) and mean values of correlation coefficients is strong and positive. The lower rate median/control was observed the lower correlation between results of four bioindication methods, and vice versa the higher rate median/control the higher correlation between the examined methods.

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