Journal of Hazardous Materials 197 (2011) 109–118

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Comparison of four bioindication methods for assessing the degree of environmental lead and cadmium pollution

Wojciech Dmuchowski [a,b,*], Dariusz Gozdowski [b], Aneta Helena Baczewska [a]

[a] *Botanical Garden–Center for Conservation of Biological Diversity, Polish Academy of Science, Prawdziwka 2, 02-973 Warsaw, Poland*
[b] *Warsaw University of Life Sciences-SGGW, Faculty of Agriculture and Biology, 159 Nowoursynowska 159, 02-776 Warsaw, Poland*

ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form
14 September 2011
Accepted 16 September 2011
Available online 22 September 2011

Keywords:
Biomonitoring
Heavy metals
Birch
Scots pine
Moss bags

ABSTRACT

The purpose of this study was to assess the application of several bioindication methods for the monitoring of environmental pollution from Pb and Cd. The study area centered on the town of Olkusz, Poland, which is one of the oldest centers for the metallurgical industry in Europe. The assessment of environmental pollution due to metals was performed using four frequently used bioindication methods: moss-bag (*Sphagnum fallax*), determination of metal accumulation in *Pleurozium schreberi*, silver birch foliage, and Scots pine needles. The region of Olkusz, and especially the area surrounding the mining and metallurgical Bolesław complex, was extremely contaminated with Pb and Cd. The results of the investigations are presented as contamination deposition maps. Despite the application of various methods and the resulting diversity of the specific exposure periods for different biomonitors, the spatial distribution of contamination shown on the maps was similar, as confirmed by the statistical analysis of the results.

1. Introduction

Metals, such as Pb and Cd, are much more harmful to people than they are to plants. Pb and Cd belong to the group of elements with a high risk of disturbing chemical equilibrium in the biosphere because they are characterized by a high capacity for accumulation in the environment [1].

The application of bioindication methods provides a significant and intense study of pollution, especially pollution that is due to the trace elements. Generally, biomonitoring can be defined as the use of plants and animals to gain quantitative and qualitative information about certain characteristics of the biosphere [2].

Mosses are among the most frequently used monitors of environmental pollution. Often, their ability to effectively accumulate metals is utilized in this context. The very first studies on the use of mosses to assess metal pollution in air were carried out in Sweden in the 1960s [3]. In parallel to the biomonitoring studies, several methodological projects were conducted to determine the influence exerted by numerous factors on the level of metal accumulation in mosses [4–10].

Determination of the accumulation of pollutants in the living organisms, in spite of its various limitations is still considered to be a very good method of evaluating the condition of the environment. It was found particularly useful when evaluating the degree of pollution with metals. Simultaneous application of various biomonitors allows one to minimize the fundamental limitations of the bioindicative methods. 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 bag method), growing *Pleurozium schreberi* moss, Silver Birch leaves, and Scots Pine needles.

The assessment of atmospheric pollution using naturally growing mosses as biomonitors has been widely implemented as an effective method of evaluation of air contamination with metals. However, similar to other bioindication methods, this method has limitations caused by the difficulties in finding a given biomonitoring species over the entire study area. Such situations are common in significantly polluted or urbanized areas. In such situations, moss bag methods have been employed that involve the physical transfer of mosses from their natural areas to a studied location [11]. This method was based on the collection of moss in a relatively unpolluted area and its placement in nylon hairnets, which were later exposed to the contaminated study areas. The concentration of metals in the moss bags was correlated with atmospheric metal concentrations [12].

Studies involving the chemical analyses of pine needles for the biomonitoring of environmental pollution started in the 1970s. These studies confirmed the advantages of pine as an indicator species, resulting in an elaboration of the monitoring

* Corresponding author. Tel.: +48 22 7541426; fax: +48 22 7541426.
E-mail addresses: w.dmuchowski@obpan.pl (W. Dmuchowski), dariusz_gozdowski@sggw.pl (D. Gozdowski).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.09.062

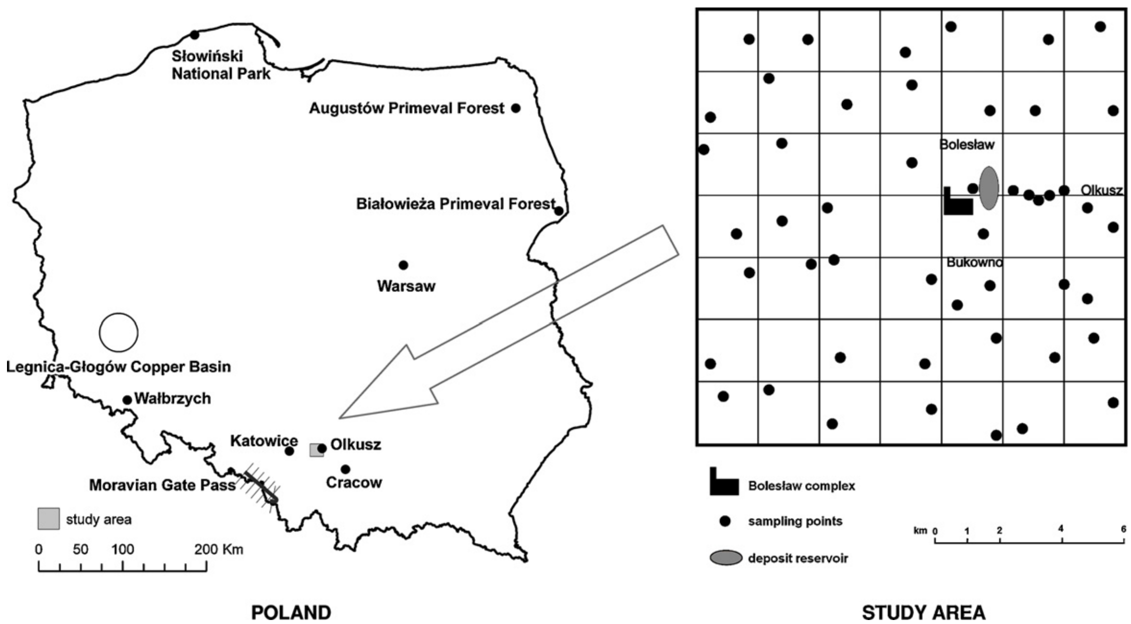

Fig. 1. Map of Poland indicating geographical features discussed in the article and the study area.

methodology and facilitation of the interpretation of the results from later investigations [13–17]. Silver birch has often been used as an environmental pollution biomonitor [18–24]. The reason there has not been a wide-scale use of silver birch (and Scots pine) as a monitor is the lack of fundamental methodological studies.

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. Study area

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 [25].

The study area encompassed 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 1.09 km^2) was transported by the wind and contributed to the contamination of the surrounding areas. In addition, dust emissions from the stacks of the Bolesław complex were transported over much longer distances. This pattern also applies to other industrial plants located outside of the study area, such as the Katowice Steel Works. Another problem comes from the secondary emissions from the contaminated uncovered bedding [26].

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

3. Materials and methods

The study was conducted using four common methods for the biomonitoring of environmental pollution due to metals determined by their accumulation in the following:

- the exposed *Sphagnum fallax* moss (the "moss bags" method);
- the growing moss *Pleurozium schreberi*;
- the leaves of silver birch(*Betula pendula* Roth) and
- the needles of Scots pine (*Pinus silvestris* L.) trees.

Table 1 summarizes the properties of the applied bioindication methods and defines what sources of contamination the measurements reflect. The main factor that determines the absolute values of measurements in relation to the degree of environmental pollution is the time of exposure, which is expressed through the level of accumulation of heavy metals in the biomonitors plants. The level of heavy metal accumulation is also impacted by 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 applied bioindication methods.

Method	Period of exposure	Source of contamination
Moss-bag 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
Comparisons of measured and certified concentrations of Pb and Cd (mg/kg) in certified reference material (NIST-USA).

Standard	Certified		Measured		Recovery (%)	
	Pb	Cd	Pb	Cd	Pb	Cd
Pine needles 1575	10.8 ± 0.5	<0.5[a]	10.5 ± 0.3	0.418 ± 0.031	97.2	–
Pine needles 1575a	0.167[a] ± 0.015	0.232 ± 0.004	0.18 ± 0.02	0.219 ± 0.010	107.8	94.4
Spinach leaves 1570a	0.20[a]	2.87 ± 0.07	0.192 ± 0.005	2.73 ± 0.04	96.0	95.1

[a] Uncertain values.

3.1. Sample collection

The materials used for the analyses (moss, needles and leaves) were collected in the second half of July in the year 2005. Bags with peat moss were left for 12 weeks in the same locations. Research areas were selected in each of the 49 squares.

The moss *S. fallax* (H. Klinggr.) was used in this study and was collected in the Augustów Primeval Forest (Fig. 1), which is considered an area of relatively low air pollution in Poland [14]. The moss was brought to the laboratory and cleaned by removing grass, leaves and litter by hand. Then the moss was washed with distilled water and dried at 50 °C. Samples were prepared for exposure by weighing about 1 g of air-dried moss and placing it into a plastic net with a small mesh and then submerging it in the distilled water to complete saturation. The moss was then formed into sphere-shaped bags of about 4–5 cm in diameter. These moss bags were then exposed for 12 weeks in the summer season.

Mosses are among the most commonly used monitors of environmental pollution due to heavy metals. In this study, the moss was used. *P. schreberi* sample collection and preparation was carried out in accordance with the European Program [27]. Moss was collected from the forest openings at a certain distance from the trees to avoid the filtering influence of the tree canopies, which could have a significant impact on the results. From a surface area of approximately 200 m^2, only gametophytes were collected without bedding, and only their green parts were for monitoring purposes (representing the last two to three years of growth).

The Scots pine needles and the silver birch leaves were collected from eight trees, growing side by side, with a single mixed sample made of an equal amount of biomass from each tree.

The needles of Scots pine were collected from the 2nd to 4th whorl from the top by cutting branches from the outer parts of the canopy according to the methodology presented by Dmuchowski and Bytnerowicz [14].

The silver birch leaves were collected from the upper part of the canopy at a height of about 3 m and on four sides of the tree circumference.

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 [14].

3.2. Chemical analyses

The materials collected were placed in linen bags and dried at 70 °C for 6 days. The dried materials were ground to a powder in a stainless steel impact mill (Fritsch 14702) and stored in tightly sealed plastic containers until the time of analysis. 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 (Naberthern L40/11/P320) using the following time/temperature procedure: 120 °C - 2 h, 200 °C - 1 h, 300 °C - 1 h, and 450° - 5 h. The ashes were digested in 30% HCl (Merck suprapur) and filtered through a filter paper [28].

The analyses were performed by flame AAS (Perkin Elmer 1100A), connected to deuterium background correction, hollow cathode lamps (HCL) and acetylene burner. Analytical wavelengths were 228.8 nm for Cd, 217 nm for Pb [29]. Three replicate subsamples of each sample were processed. Three blanks were run with each batch of samples; thus, each sample was blank corrected. The detection limits in a sample were: Pb - 0.03 mg/kg and Cd - 0.01 mg/kg.

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 [14].

To provide quality control (QC), the elemental content in the plant samples was determined using certified reference materials from the NIST- USA (Table 2). The obtained results were in good agreement with the certified values. The recovery range was from 90 to 95%.

3.3. Methodology of map generation

The results of this work are presented as maps of heavy metal pollution deposition. All of the maps were drawn digitally with the use of the specialized MapInfo software [30]. For spatial interpolation was used ordinary kriging based on an assumption that there is a definite correlation between the distance between points and the degree of their similarity, expressed through the semi-variogram [31]. Assuming that this statistical image of dependence is a good representation of the spatial interaction between the measurement points, it can be used in the forecasting of values at the interpolation points.

3.4. Statistical analysis

Basic statistical parameters were calculated for all examined variables. Statistical comparison of medians was performed using Kruskal–Wallis test (non-parametric one-way analysis of variance). Relationships between the accumulations of different metals in each of the biomonitors were evaluated using linear regression [32]. Because the variables did not follow the normal distribution, the log-transformation ($\log_{10}(X)$) was applied. Regression functions, determination coefficients and observed levels of significance (where p-value <0.05 indicates a significant relationship) were presented. Determination coefficients (R^2) closer to 1 (100%) indicated a stronger relationship between the metal concentrations in particular pairs of the indicator plants. These relationships expressed similarities between the patterns of contamination depicted on the isoquant maps, which were obtained through the application of different bioindication methods.

Multivariate evaluation of the similarity in heavy metal accumulations from the examined biomonitors was conducted using cluster analysis. Cluster analysis was carried out using the single linkage (nearest neighbor) algorithm based on squared Euclidean distances for the mean values of Cd and Pb of standardized log-tranformed variables [33]. The statistical analyses were performed using the Statistica 8.0 statistical package.

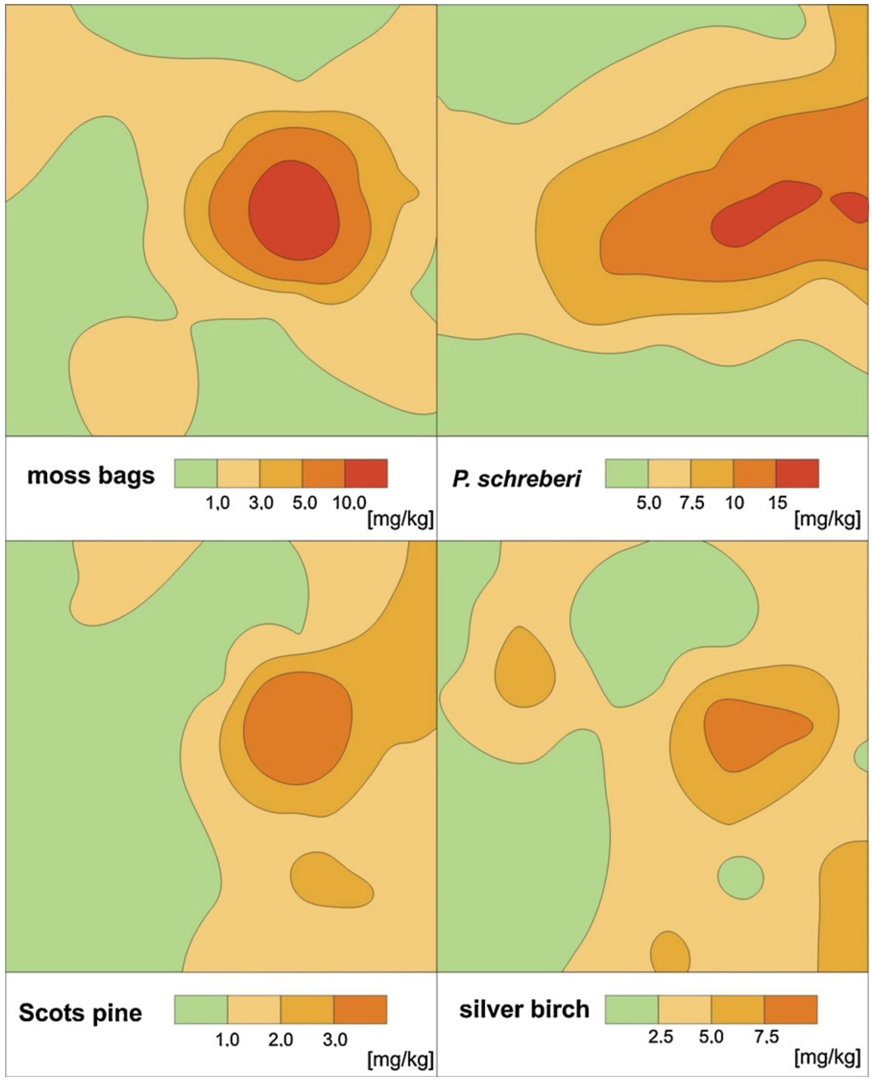

Fig. 2. Contamination of the environment with cadmium based on the accumulated concentration of this element in the biomonitors.

4. Results and discussion

The results of the environmental pollution analysis are depicted in maps with respective isoquants. These isoquants differentiate the zones of pollution in proportion to the scale of metal concentration in particular biomonitors. The maps showing the concentration of the Cd and Pb are based on results from 53 measurement points (Figs. 2 and 3). Table 3 presents the basic descriptive statistics of the results.

4.1. The moss-bag method

4.1.1. Cadmium

The average concentration of Cd (similar to the result of Pb) was higher than the reference level (0.16 mg/kg) in all analyzed samples. The results ranged from 0.39 to 18.7 mg/kg. The majority of the measurements, however, did not exceed 2 to 3 mg/kg, and only three samples that were extracted near the waste heap and the Bolesław complex showed distinctly higher Cd concentrations (between 10.5 and 18.7 mg/kg).

The Cd and contamination map (as well as Pb) shows the highest levels of pollution near the waste heap and stretching in the eastern direction due to the prevailing western direction of the winds. In the mosses exposed near Warsaw in 2004, values ranged between 0.32 and 0.60 mg/kg, while the highest value observed of 1.18 mg/kg during the entire study (between the years 1992 and 2004) was measured in 1992 near the steel mill [34].

4.1.2. Lead

The Pb concentrations in the moss after twelve weeks of exposure in the study area were higher than the reference sample (2.5 mg/kg) in all samples from all measurement points. These results varied significantly and ranged between 7.2 and 461 mg/kg. The measurements exceeded 10 mg/kg over almost the entire study area (Fig. 3). A high level of contamination was observed in the area around the Bolesław complex, while the area with the highest level of contamination was located near the processing waste dump of the foundry. On the western side of the dump, at a distance of about 500 m, the concentration of Pb was the highest and amounted to 461 mg/kg.

These results show high Pb contamination in the study area. For comparison, the results using the same method in Warsaw (Poland) between 1992 and 2004 ranged from 6.2 to 49.4 mg/kg [33]. This large variation in the results provides evidence for the existence of a point source with a relatively limited reach. In this case, this source could be the processing waste heap from the Bolesław complex. Despite sprinkling, dust from the waste heap is blown by wind and contributes to the contamination of the surrounding neighborhood.

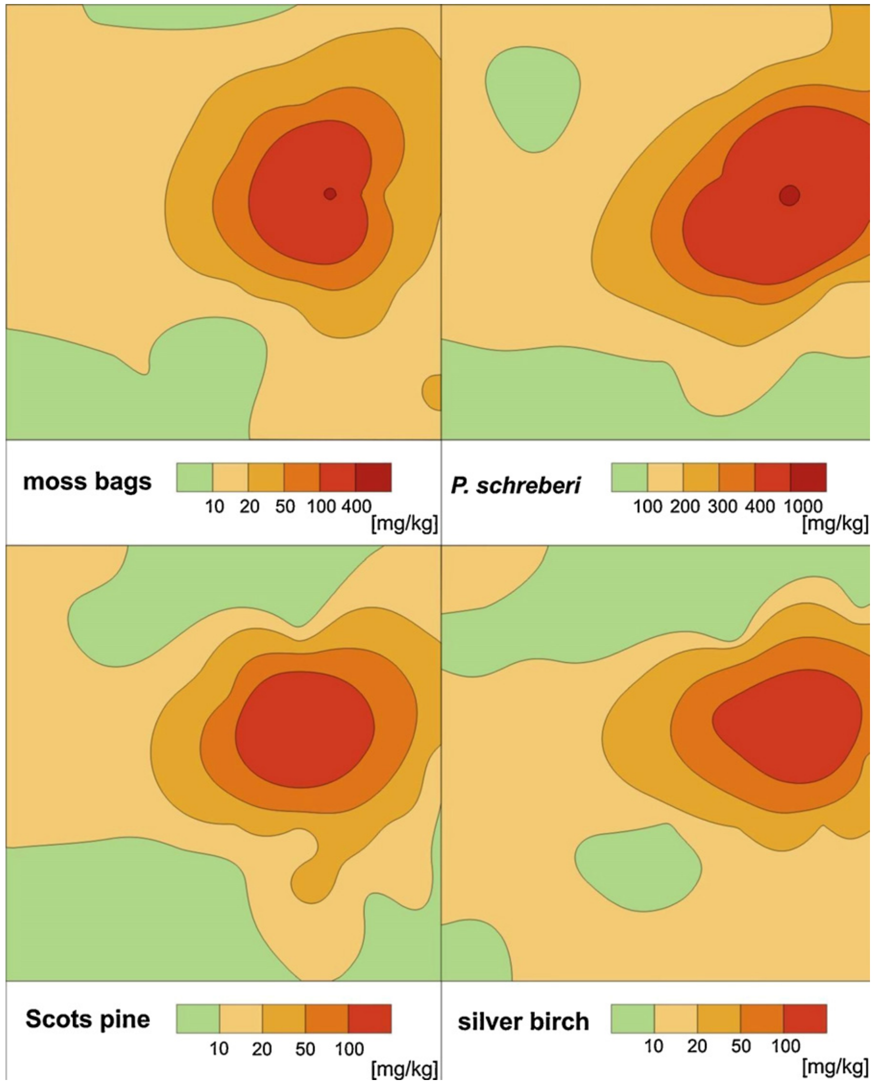

Fig. 3. Contamination of the environment with lead based on the accumulated concentration of this element in the biomonitors.

4.2. *Accumulation of metals in moss Pleurozium schreberi*

4.2.1. *Cadmium*

The concentration of Cd in the growing moss *P. chreberi* ranged between 3.27 and 17.7 mg/kg, while the reference value was 0.24 mg/kg. The spatial distribution of contamination showed that the highest level of contamination occurred in the areas of the Bolesław complex and the industrial waste dump (Fig. 2).

The average concentration of cadmium in the mosses from Greenland was about 0.1 mg/kg, while in the northern regions of Norway, Sweden and Finland, it was below 0.2 mg/kg [35]. Berg and Steinnes [36] determined a background content of Cd between 0.07 mg/kg in northeastern Norway to 0.43 mg/kg in southwestern Norway. In the Czech Republic, according to Sucharová and Suchara [37], cadmium concentrations from 250 measurement points ranged between 0.09 and 2.24 mg/kg. Poikolainen et al. [38] measured between 0.03 and 2.24 mg/kg of cadmium from 1608 measurement points in 1985 in Finland, and in 2000, they measured between 0.01 and 0.42 mg/kg from 895 measurement points. Finally, Galsomiés et al. [39] measured concentrations between 0.7 and 1.0 mg/kg in France in 1996.

According to Grodzińska et al. [40], Cd concentrations in Poland ranged between 0.03 and 0.14 mg/kg in the least polluted region (province of Podlasie). In the strongly polluted areas, such as the Upper Silesian and Cracovian Industrial Region, the concentration ranged between 0.60 and 16.8 mg/kg. Therefore, compared with the literature values and the background reference, the studied area featured an extremely high Cd contamination in the air.

4.2.2. *Lead*

Pb concentration in the moss *P. schreberi* ranged from 48.8 to 1096 mg/kg. The results were highly varied and were much higher than the reference level of 3.1 mg/kg.

When discussing these results, we should note that the use of unleaded fuels for motor vehicles significantly decreased Pb pollution. Consequently, between 1985 and 1990, the Pb concentration in the moss decreased by 60% in Denmark, by 40% in Norway, and by 30% in Sweden and Finland [41]. In Germany, the median Pb concentration in the moss decreased by 41% between 1990 and 1995 [42].

The Pb concentration in *P. schreberi* from relatively unpolluted areas in 1975 was about 33 mg/kg in the Białowieża National Park (Poland) [43]. In 1998, the Pb concentration in northeastern Poland (province of Podlasie) ranged from 5.6 to 105 mg/kg (average: 18 mg/kg) [40]. In 1985, contamination in the northern areas of Norway, Sweden and Finland was below 10 mg/kg [35]. In the Czech Republic, the minimum concentration decreased from 4.1 mg/kg in 1995 to 1.8 mg/kg in 2000 [37].

Table 3
Basic parameters of variability of heavy metal concentrations in the biomonitors around Olkusz (mg/kg).

	Pb	Cd
Moss bags		
Median	17.3[a*]	1.33[a]
Mean	42.7	2.49
Range	7.2–461	0.39–18.7
Lower quartile	11.9	0.76
Upper quartile	26.1	2.20
Control	2.5	0.16
Moss - *Pleurozium schreberi*		
Median	158[b]	6.71[c]
Mean	278	7.73
Range	48.8–1096	3.27–17.7
Lower quartile	107	4.39
Upper quartile	289	9.65
Control	3.1	0.24
Needles - Scots pine		
Median	13.0[a]	1.22[a]
Mean	36.8	1.53
Range	4.0–255	0.28–5.78
Lower quartile	9.5	0.74
Upper quartile	19.3	2.09
Control	1.3	0.12
Leaves - silver birch		
Median	15.0[a]	3.58[b]
Mean	40.5	3.87
Range	3.8–229	0.75–8.61
Lower quartile	9.9	1.83
Upper quartile	19.3	5.09
Control	1.7	0.19

*Different letters indicate significant differences between medians based on Kruskal–Wallis test.

The concentrations of Pb in the moss were much higher in the areas featuring significant air pollution with this metal. In the Ojców National Park in 1975, Pb content in the moss was, on average, 140 mg/kg, while it was 270 mg/kg in 1980. In 1998, Pb content in the moss in the Upper Silesian–Cracovian Industrial Region ranged between 8.8 and 499 mg/kg, [40]. In other European countries in 1995 to 1996, the maximum Pb concentrations in Germany, Finland and Lithuania were 78 mg/kg, 19.2 mg/kg, and 33 mg/kg [41], respectively, and it was 48 mg/kg in the Czech Republic in 2000 [37].

By comparing the results with the reference values and with those obtained from the literature, we can see that Pb pollution in the study area was high. The area with the highest Pb content was near the Bolesław complex. High Pb levels were observed over the entire study area, with a minimum value of 49 mg/kg.

4.3. Accumulation of metals in Scots pine needles

4.3.1. Cadmium

The Cd concentrations measured in the Scots pine needles collected near Olkusz ranged from 0.28 to 5.78 mg/kg, while the background reference value was 0.12 mg/kg.

Molski and Dmuchowski [44] measured a maximum concentration of 0.17 mg/kg in the needles from the northern edge of Finland. Dmuchowski and Bytnerowicz [14] obtained an average of 1.53 mg/kg of Cd (with maximum reaching 4.70 mg/kg) in the central part of the Upper Silesian Industrial Region.

The region of Olkusz, when considering the measurements presented here, can be considered significantly contaminated with Cd, especially in the area around the Bolesław complex. The distribution of isoquants on the map distinctly indicates the foundry as the source of emissions. Nearly 20% of the study area had Cd concentrations in the needles higher than 2 mg/kg.

4.3.2. Lead

The concentrations of Pb in the Scots pine needles collected within the study area ranged between 4.0 and 255 mg/kg. This range of measurements was significant, and even the lowest of the values was much higher than the reference value of 1.3 mg/kg measured in the Augustów Primeval Forest. The area located in the southwestern part of the study area featured much lower Pb contamination, with concentrations in the needles not exceeding 10 mg/kg.

Comparing the results of these investigations of Pb pollution in different years should help us understand the temporal dynamics of this element. In the last twenty years, the use of unleaded fuels for vehicles and the resulting emissions and deposition of Pb in the environment significantly decreased [34,45].

The maximum Pb concentrations measured in the Scots pine needles in the 1990s were much lower; for instance, the maximum concentration was 1.3 mg/kg in the needles collected in the Białowieża Primeval Forest [14]. In the needles of Scots pines growing on the Kola Peninsula in the neighborhood of copper and nickel foundries, the Pb concentrations were on average 5.8 mg/kg [20].

Dmuchowski and Bytnerowicz [14] measured a maximum value of 10.2 mg/kg of lead in the central part of the Upper Silesian Industrial Region (Poland). In northeastern Turkey in 2000, in the urbanized areas of Erzurum, the maximum Pb concentration in the Scots pine needles was 40 mg/kg, and it was 19 mg/kg in the suburban areas [15].

When comparing these results with those from the literature, we can state that a significant part of the study area is strongly contaminated with Pb. This area is primarily near the waste dump, foundry and some other nearby areas where the inhabitants have been relocated due to the high level of Pb contamination. In the latter area, the Pb concentrations in the needles exceeded 100 mg/kg.

4.4. Accumulation of metals in the silver birch leaves

4.4.1. Cadmium

The concentration of Cd in the silver birch leaves ranged between 0.75 and 8.61 mg/kg. In all of the analyzed samples, the Cd concentrations were much higher than in the reference samples, which had a concentration of 0.19 mg/kg.

According to Steinnes et al. [20], the concentration of Cd in the leaves of mountain birch (*Betula pubescens tortuosa* Ledep.) on the Kola Peninsula was on average 0.21 mg/kg. The relatively small number of polluted leaves from Lapland, central Finland, and southern England contained 0.27 mg/kg, between 0.74 and 2.3 mg/kg and 0.96 mg/kg, respectively. In Hancock County (Maine, USA), the average concentration of Cd was 1.14 mg/kg [46], and in Holy Cross Mountains (Poland), the average concentration was 0.5 mg/kg [47]. In the Rudavy Mountains (Czech Republic) in the industrial region of Bohemia, the average concentration of Cd in the leaves of silver birch was 0.3 mg/kg [18]. Piczak et al. [48] measured from 1.10 to 3.84 mg/kg in the agglomeration of Wrocław (Poland) and from 1.21 to 3.12 mg/kg in Wałbrzych.

From the above comparison of the respective measurements, it can be concluded that the environmental pollution with Cd near Olkusz was very high and encompassed a large share of the study area. In 63% of the study area, the Cd concentration in the leaves was higher than 2.5 mg/kg, and it was in excess of 5 mg/kg in 14% of the area. The highest contamination was observed near the Bolesław complex.

4.4.2. Lead

The concentration of Pb in the silver birch leaves was between 3.8 and 229 mg/kg, compared to 1.7 mg/kg in the reference sample. Thus, the variation in the study results was relatively high.

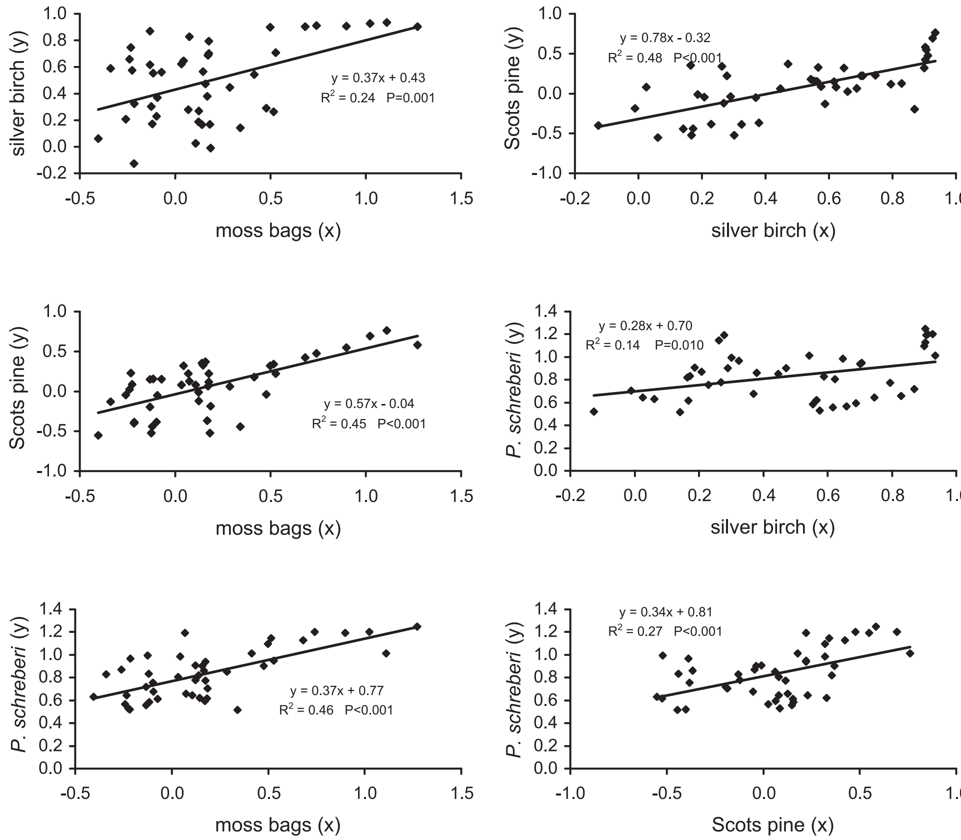

Fig. 4. Relationships between the concentrations of cadmium (mg/kg) in particular biomonitors (linear regressions function for log-transformed data).

In the contaminated area near the copper and nickel foundry on the Kola Peninsula, the average Pb concentration in the birch leaves was 1.17 mg/kg, while in the less contaminated areas, this concentration did not exceed 0.22 mg/kg [20,49]. In the urban environment, the silver birch leaves contained the following: in Wrocław (Poland), between 1.5 and 10 mg/kg; and in Wałbrzych, between 1.1 and 3.2 mg/kg [48]. Migeon et al. [50] found that near the biggest Zn and Pb production plant in Europe (northern France), the maximum Pb concentration in the leaves was less than 30 mg/kg. According to Hrdlička et al. [51], a normal Pb concentration in birch leaves ranges between 2 and 10 mg/kg; however, near Olkusz, the Pb concentrations averaged 19.9 mg/kg, and they were about 30 mg/kg in the area around Bolesław complex.

These results indicate extremely high lead contamination, especially near the foundry and waste dump in the Bolesław complex. In more than 25% of the study area, the Pb concentration in the birch leaves was higher than 20 mg/kg, and it exceeded 100 mg/kg in more than 6% of the area.

4.5. Comparison of the four bioindication methods for assessing the degree of environmental pollution

Table 3 provides the levels of metal accumulation. The comparison accounts for the average values of all of the measurements, the medians, the minimum and the maximum values, and the reference material values. In the polluted area near Olkusz, the accumulation of metals was the highest for almost all of the measured metals and all of the examined biomonitors.

The metals contained in the birch leaves and in the pine needles originated from the contamination of the air and the soil. The levels of metal accumulation differed because of differences in the exposure duration, the surface area/biomass ratio and the pubescence of birch leaves, which allows for more effective adsorption and absorption of metals. Birch leaves accumulated somewhat more metals than the pine needles; significant difference was only for accumulation of Cd (Table 3). This is reflected in the results for reference trees and to the minimum, maximum, median and mean values for the trees from the polluted area.

The metals found in the moss originated from air pollution. The period of exposure for *P. schreberi* growing in the field was about three years longer than the exposure of *S. fallax* used in the moss-bag transplant method. This methodological difference resulted in the higher accumulation of metals in the growing *P. schreberi* than in the moss-bag-exposed *S. fallax* as well in comparison with other two biomonitors (Scots pine and silver birch) for both metals. The differences observed were particularly pronounced for the accumulation of Pb.

The periods of exposure for *S. fallax* in the moss-bag transplant method and of the birch foliage were similar (roughly three months). The birch foliage accumulated more Cd and the difference was significant (Table 3) but the difference for Pb was not significant.

Despite the application of various bioindication methods with different monitoring plants, the spatial distributions of heavy metal contamination in the presented maps were similar. While the absolute levels of contamination differed, the overall patterns were similar. The Bolesław complex, which processed ores of these metals, and the waste dump from the foundry were identified as the largest sources of contamination in the study area.

The statistical analysis determined the relationships between the concentrations of a given metal in the particular monitoring plants. The linear regression method based on log-transformed data was applied, and the coefficients of determination were used as measures of the relationships [32]. The results of the regression analyses are presented in Fig. 4 for Cd and Fig. 5 for Pb. The contamination of the environment with Pb was very high and

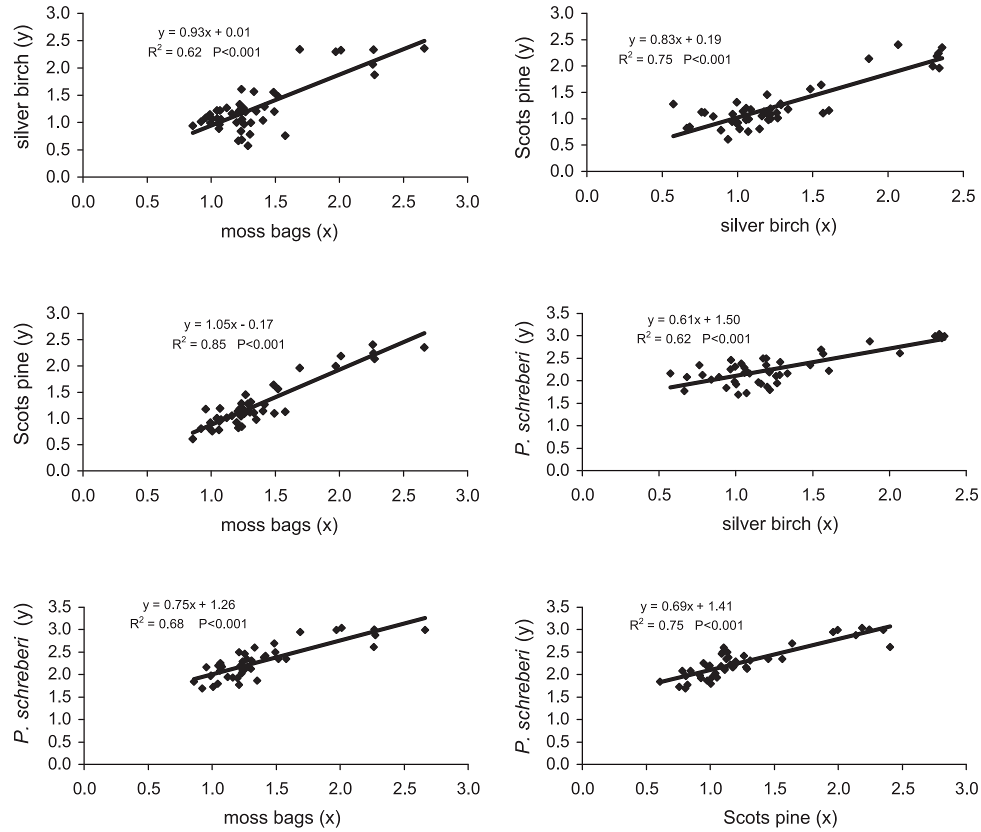

Fig. 5. Relationships between the concentrations of lead (mg/kg) in particular biomonitors (linear regression functions for log-transformed data).

varied significantly near Olkusz. The deposition maps represent the contamination results from the four bioindication methods. The relationship, expressed through the regression function parameters, was positive and significant ($p < 0.05$) for all pairs of the applied bioindication methods. The value of the determination coefficient was higher than 0.6 (60%) in all cases for Pb, which implied similarity in the patterns of pollution deposition. For the examined biomonitors, the relationships between the contamination levels of Cd were significant but weaker (R^2 between 0.14 and 0.48) than those for Pb. The contamination of the environment with Cd was significant and varied, much like the Pd contamination. The patterns of deposition associated with particular metals were similar and strongly interrelated, as confirmed by the statistical analysis of the results. The strongest relationship for Pb was observed between the results with the moss-bag method and the chemical analysis of the Scots pine needles ($R^2 = 0.85$). Very strong relationships were observed between silver birch and Scots pine as well as between Scots pine and *P. schreberi* (R^2 for both pairs was equal to 0.75). Regression equations for these relationships can be useful for conversion of the Pb content from one biomonitor to the other. Weaker correlations were observed for the Cd content in the examined biomonitors. The weakest association was observed between the measurements of Cd concentrations in the growing moss *P. schreberi* and in the foliage of the silver birch ($R^2 = 0.14$), while the strongest was observed between silver birch and Scots pine ($R^2 = 0.48$). Because of these weaker correlations the conversion of Cd content from one biomonitor to other is less accurate.

In other studies most of the authors compare metal concentration between various mosses, however, those studies lack comparisons between mosses and foliage of trees [36,38]. Knowledge about relationships between metal content in mosses and tree foliage could be important for other biomonitoring studies.

Patterns of accumulation for both metals (Pb and Cd) for the same biomonitors were very similar, because all correlations were positive and significant (results not presented in the manuscript).

To assess the multivariate relationships between the accumulation of heavy metals by four different plant indicators, a cluster analysis was performed using the single linkage (nearest neighbor) algorithm [33]. The squared Euclidean distances for the mean values of Cd and Pb of the standardized log-transformed variables were calculated, and the results of this analysis are presented in the form of dendrogram (Fig. 6).

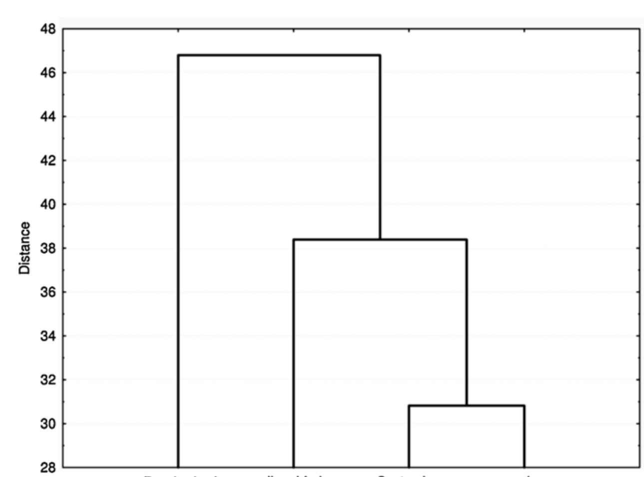

Fig. 6. Dendrogram obtained from cluster analysis, illustrating the similarities between the biomonitors in terms of the accumulation of heavy metals (Cd and Pb together).

Based on the dendrogram, we can assess which of the biomonitors display a similarity in terms of the accumulation of the examined heavy metals and which ones are characterized by a different level of accumulation under various contamination conditions. The accumulation of metals was most similar between the *S. fallax* in the moss bags method and the Scots pine needles. Pattern of accumulation observed for *P. schreberi* was much different than for the remaining biomonitors.

The European moss survey conducted in 15 states indicated that in the growing mosses during the years 1990–2005 there was a decrease in the average contents of Pb by 72% and Cd by 52% [52]. However the studies by Dmuchowski and Bytnerowicz [14] of pine needles in the region of Olkusz in 1995, and by Grodzińska et al. [39] of the growing moss *P. schreberi* in the year 1998, demonstrated that the contents of these metals were at the same levels as quoted in this publication. This confirms the continuing high regional and local emission and the historical contamination of the environment in the region of research.

5. Conclusions

The period of exposure is the primary factor that determines the expression of the absolute level of environmental pollution through the concentration of accumulated Pb and Zn in the biomonitors. The level of accumulation also results from the specific features of the bioindication method applied, followed by the source of contamination (air, soil) and the morphological and anatomical properties of the indicator species. The accumulation of metals was highest in the moss *P. schreberi* and confirmed by the statistical indices (median, minimum and maximum values).

The region of Olkusz and the area around the mining and metallurgical Bolesław complex should be considered extremely contaminated with lead and cadmium. The results of the investigations were presented as contamination deposition maps. Despite the application of various methods and the resulting diversity of the specific exposure periods for different biomonitors, the spatial distribution of contamination was similar, as confirmed by the statistical analysis.

The assessment of air pollution based on the metal accumulation in moss *S. fallax* using the moss-bag method provided a spatial distribution of contamination similar to that of other methods that were characterized by longer exposure periods. By employing transplantation, this particular method allowed for adequate coverage of the investigated areas and was free from the difficulties associated with finding the same native indicator plant species over the entire study area. The results presented in the paper (e.g. regression equations) make possible the comparison of various research data of contamination as evaluated by one or more of the bioindication methods.

It was especially so for Pb where correlations between all the methods were very strong, but it was true to a lesser degree for Cd where correlations were much weaker.

References

[1] M.C. Newman, H. Clements, Ecotoxicology - A Comprehensive Treatment, CRC Press, Boca Raton, 2008.

[2] B. Wolterbeek, Biomonitoring of trace element air pollution: principles: possibilities and perspectives, Environ. Pollut. 120 (2002) 11–21.

[3] Å. Rühling, G. Tyler, An ecological approach to the lead problem, Botaniska Notiser 122 (1968) 248–259.

[4] K. Breuer, A. Melzer, Heavy metal accumulation (lead and cadmium) and ion exchange in three species of *Sphagnaceae*, Oecol 82 (1990) 461–467.

[5] H.M.C.F. Tavares, M.T.S.D. Vasconcelos, Comparison of lead levels collected by *Sphagnum auriculatum* and by a low-volume aerosol sampler in the urban atmosphere of Oporto, Toxicol. Environ. Chem. 54 (1996) 195–209.

[6] D. Čeburnis, J. Šakalys, K. Armolaitis, D. Valilis, K. Kvietkus, In-stack emissions of heavy metals estimated by moss biomonitoring method and snow-pack analysis, Atmos. Environ. 36 (2002) 1465–1474.

[7] C. Reimann, A. Arnoldussen, R. Boyd, T.E. Finne, Ø. Nordgulen, T. Volden, P. Englmaier, The influence of a city on element contents of a terrestrial moss (*Hylocomium splendens*), Sci. Total Environ. 369 (2006) 419–432.

[8] C.C. Fernandez, J.R. Shevock, A.N. Glazer, J.N. Thompson, Cryptic species within the cosmopolitan desiccation-tolerant moss *Grimmia laevigata*, Proc. Natl. Acad. Sci. USA 103 (2006) 637–642.

[9] M. Aničić, M. Tomašević, M. Tasić, S. Rajšić, A. Popović, M.V. Frontasyeva, S. Lierhagen, E. Steinnes, Monitoring of trace element atmospheric deposition using dry and wet moss bags: accumulation capacity versus exposure time, J. Hazard. Mater. 171 (2009) 182–188.

[10] S. Dragović, N. Mihailović, Analysis of mosses and topsoils for detecting sources of heavy metal pollution: multivariate and enrichment factor analysis, Environ. Monit. Assess. 157 (2009) 383–390.

[11] G.T. Goodman, T.M. Roberts, Plants and soil as indicators of metals in the air, Nature 231 (1971) 287–292.

[12] H.G. Zechmeister, K. Grodzińska, G. Szarek-Łukaszewska, Bryophytes, in: B.A. Markert, A.M. Breure, H.G. Zechmeister (Eds.), Bioindicators and Biomonitors, Elsevier Science Ltd., New York, 2003, pp. 329–375.

[13] B. Maňkowska, Accumulation of As, Sb, S and Pb in soil and pine forest, Ekológia (ČSSR) 5 (1986) 71–79.

[14] W. Dmuchowski, A. Bytnerowicz, Biomonitoring of the environmental pollution in Poland using chemical analysis of Scots pine (*Pinus silvestris* L.) needles, Environ. Pollut. 87 (1995) 87–104.

[15] S. Yilmaz, M. Zengin, Monitoring environmental pollution in Erzurum by chemical analysis of Scots pine (*Pinus sylvestris* L.) needles, Environ. Int. 29 (2004) 1041–1047.

[16] T.M. Nieminen, J. Derome, A. Saarsalmi, The applicability of needle chemistry for diagnosing heavy metal toxicity to trees, Water Air Soil Pollut. 157 (2004) 269–279.

[17] M.M. Al-Alawi, K.L. Mandiwana, The use of Aleppo pine needles as a biomonitor of heavy metals in the atmosphere, J. Hazard. Mater. 148 (2007) 43–46.

[18] P. Hrdlička, E. Kula, Element content in leaves of birch (*Betula verrucosa* Ehrh.) in an air polluted area, Trees 13 (1998) 68–73.

[19] M.V. Kozlov, E. Haukioja, A.V. Bakhtiarov, D.N. Stroganov, Heavy metals in birch leaves around a nickel-copper smelter at Monchegorsk, northwestern Russia, Environ. Pollut. 90 (1995) 291–299.

[20] E. Steinnes, N. Lukina, V. Nikonov, D. Aamlid, O. Royset, A gradient study of 34 elements in the vicinity of a copper-nickel smelter in the Kola Peninsula, Environ. Monit. Assess. 60 (2000) 71–88.

[21] A. Dolgopolova, D.J. Weiss, R. Seltmann, P. Dulski, Dust dispersal and Pb enrichment at the rare-metal Orlovka–Spokoinoe mining and ore processing site: insights from REE patterns and elemental ratios, J. Hazard. Mater. 132 (2006) 90–97.

[22] C. Reimann, R.T. Ottesen, M. Anderson, A. Arnoldussen, F. Koller, P. Englmaier, Element levels in birch and spruce wood ashes - green energy? Sci. Total Environ. 393 (2008) 191–197.

[23] W.H.O. Ernst, H.J.M. Nelissen, Bleeding sap and leaves of silver birch (*Betula pendula*) as bioindicators of metal contaminated soils, Int. J. Environ. Pollut. 33 (2008) 160–172.

[24] A. Samecka-Cymerman, K. Kolon, A.J. Kempers, Short shoots of *Betula pendula* Roth. as bioindicators of urban environmental pollution in Wrocław (Poland), Trees 23 (2009) 923–929.

[25] W. Dmuchowski, Use of plant bioindicators in assessment of environmental contamination with heavy metals. The Botanical Garden PAS, 2005.

[26] K. Dmowski, M. Badurek, Thallium contamination of selected plants and fungi in the vicinity of the Bolesław zinc smelter in Bukowno (S. Poland), Preliminary Study, Acta Biol. Cracoviensia Ser. Botanica 44 (2002) 57–61.

[27] K. Grodzińska, G. Szarek-Łukaszewska, B. Godzik, Survey of heavy metal deposition in Poland using mosses as indicators, Sci. Total Environ. 229 (1999) 41–51.

[28] S.E. Allen, H.M. Grishaw, J.A. Parkinson, C. Quarmby, Chemical Analysis of Ecological Materials, Blackwell, Osney Mead, Oxford, 1974.

[29] D. Roberts, ATI Unicam, Atomic Absorption Spectrometry–Methods Manual, Issue 2, Unicam LTD, 1991.

[30] L. Daniel, P. Loree, A. Whitener (Eds.), Inside Mapinfo Professional, World Press, Albany, 2002.

[31] R. Haining, Spatial Data Analysis. Theory and Practice, Cambridge University Press, 2003.

[32] R.R. Sokal, F.J. Rohlf, Biometry: The Principles and Practice of Statistics in Biological Research, 3rd ed., W.H. Freeman and Company, New York, 1995.

[33] B.S. Everitt, S. Landau, M. Leese, Cluster Analysis, Edward Arnold, London, 2001.

[34] W. Dmuchowski, A. Bytnerowicz, Long-term record of deposition of airborne heavy metals to lichens and trees in Warsaw, Poland: 1992–2004, Environ. Pollut. 157 (2009) 3413–3421.

[35] Å. Rühling, L. Rasmussen, K. Pilegaard, A. Mäkinen, E. Steinnes, Survey of atmospheric heavy metal deposition in the Nordic countries in 1985 - monitored by moss analyses, NORD 21 (1987).

[36] T. Berg, E. Steinnes, Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute deposition values, Environ. Pollut. 98 (1997) 61–71.

[37] J. Sucharová, I. Suchara, Current multi-element distribution in forest epigeic moss in the Czech Republic - a survey of the Czech national biomonitoring program 2000, Chemosphere 57 (2004) 1389–1398.

[38] J. Poikolainen, E. Kubin, J. Piispanen, J. Karhu, Atmospheric heavy metal deposition in Finland during 1985–2000 using mosses as bioindicators, Sci. Total Environ. 318 (2004) 171–185.
[39] L. Galsomiès, S. Ayrault, F. Carrot, C. Deschamps, M.A. Letrouit-Galinou, Inter-species calibration in mosses at regional scale-heavy metal and trace elements results from Ile-de France, Atmos. Environ. 37 (2003) 241–251.
[40] K. Grodzińska, M. Frontasyeva, G. Szarek-Łukaszewska, M. Klich, A. Kucharska-Fabiś, S.F. Gundorina, Trace element contamination in industrial regions of Poland studied by moss monitoring, Environ. Monit. Assess. 87 (2003) 255–270.
[41] Å. Rühling, E. Steinnes, Atmospheric heavy metal deposition in Europe 1995–1996, NORD Environ. (1998).
[42] U. Siewers, U. Herpin, Schwermetalleinträge in Deutschland. Teil 1: Moos-Monitorring 1995/1996, Sonderhefte Reihe D - Geol Jahrb, Stuttgart, 1998.
[43] K. Grodzińska, Mosses as bioindicators of heavy metal pollution in polish national parks, Water Air Soil Pollut. 9 (1978) 83–97.
[44] B. Molski, W. Dmuchowski, The comparison of environmental pollution in Northern Finland near Kevo and in Poland with the use of *Pinus silvestris* L. as bioindicator, Rep. Kevo Subarctic. Res. Stat. 21 (1990) 27–30.
[45] F. Herman, S. Smidt, S. Huber, M. Englisch, M. Knoflacher, Evaluation of pollution-related stress factors for forest ecosystems in central Europe, Environ. Sci. Pollut. Res. 8 (2001) 231–242.
[46] C.J. McGee, I.J. Fernandez, S.A. Norton, C.S. Stubs, Cd, Ni, Pb, and Zn concentrations in forest vegetation and soils in marine water, Air Soil Pollut. 180 (2007) 141–153.
[47] Z.M. Migaszewski, A. Gałuszka, A. Świercz, J. Kucharzyk, Element concentrations in soils and plant bioindicators in selected habitats of the holy cross mountains, Poland, Water Air Soil Pollut 129 (2001) 369–386.
[48] K. Piczak, A. Leśniewicz, W. Żernicki, Metal concentration in deciduous tree leaves from urban areas in Poland, Environ. Monit. Assess. 86 (2003) 273–287.
[49] C. Reimann, F. Koller, G. Kashulina, H. Niskavaara, P. Englmaier, Influence of extreme pollution on the inorganic chemical composition of some plants, Environ. Pollut. 115 (2001) 239–252.
[50] A. Migeon, P. Richaud, F. Guinet, M. Chalot, D. Blaudez, Metal accumulation by woody species on contaminated sites in the North of France, Water Air Soil Pollut. 204 (2009) 89–101.
[51] P. Hrdlička, E. Kula, J. Załecka, Zawartość wybranych pierwiastków w liściach brzozy (*Betula verrucosa* Ehrh.) rosnącej na terenach znajdujących się pod wpływem imisji przemysłowych w okolicy Olkusza, Sylwan 145 (2001) 101–110 (in Polish).
[52] H. Harmens, D.A. Norris, E. Steinnes, et al., Mosses as biomonitors of atmospheric deposition: spatial patterns and temporal trends in Europe, Environ. Pollut. 158 (2010) 3144–3156.